ЮБИЛЕЙНА НАУЧНА КОНФЕРЕНЦИЯ ПО ЕКОЛОГИЯ (СБОРНИК С ДОКЛАДИ)
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GENOME ALTERATIONS IN CHIRONOMIDS (DIPTERA) SUBJECT TO DIFFERING DEGREES OF TRACE METAL POLLUTION

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Abstract. The structure and function of the salivary gland polytene chromosomes of the chironomid *Chironomus luridus* from an unpolluted site in Bulgaria was described and compared to our previously published work on the closely related species *C. acidophilu*s from a river subject to long term acid (pH<3.0) mine drainage containing trace metals. Somatic heterozygous inversions in chromosome arms C, D and F occurred at a very low frequency in *C. luridus*. Intermediate and high activity of the nucleolar organiser was also observed. The Baliani rings BR1/BR2 occurred in seven states of activity but the state +/++ was greatest. Comparative analysis showed markedly higher structural and functional aberrations in *C. acidophilus* than in *C. luridus.*The aberrations in the *C. acidophilus* genome are probably a result of long term exposure to trace metals.

Keywords: Chironomidae; polytene chromosomes; rearrangements; trace metals; acid mine drainage

INTRODUCTION

Chironomids are good indicators of pollution as they are very responsive to environmental change and inhabit every type and condition of aquatic habitat (WARWICK, 1990). Their salivary gland polytene chromosomes are particularly sensitive to environmental change, including from chemicals such as pesticides (MEREGALLI *et al.*, 2002) and trace metals (MICHAILOVA *et al*., 1996; 1998; 2000; 2003), plus physical stressors such as radioactivity (PETROVA & MICHAILOVA, 1996).

A previous study revealed marked chromosomal aberrations in *Chironomus acidophilus* collected from a mine waste site subject to long-term (approx 250 years) pollution by trace metals (MICHAILOVA *et al*., submitted). However, *C. acidophilus* is not found in non-acidic water bodies and therefore comparison could not be made with the same species from an unpolluted site. *Chironomus luridus* is cytogenetically closely related to *C. acidophilus* as both species belong to the same cytocomplex

"pseudothummi" (KEYL, 1962). Therefore comparison was made between *C. acidophilus* and *C. luridus* collected from a non-acidified site in Bulgaria.

Cytogenetic analysis was performed on *C. luridus* in relation to trace metal concentrations in the water column and sediment from the same site. We hypothesize that the response of the genome of these two closely related species will differ due to different level of environmental trace metal pollution.

MATERIALS AND METHODS

Study sites, trace metal concentrations in the water and sediment

C. luridus was collected from South Park Lake (lat 42^0 40' 26.60''N long $23^0$18'36.77''E), Sofia, Bulgaria. This site was selected as it is non-acidified and is thought to be unpolluted.

The water column and sediments were sampled for copper, zinc and aluminium as our previous study (MICHAILOVA *et al*., submitted) has shown high levels of contamination by these metals in the polluted site inhabited by *C. acidophilus*. Iron and manganese were also analyzed as they are important in the partitioning in the water column and transport of trace metals (BOULT *et al*., 1994).

Five water and sediment samples were taken from in September 2005. Water samples were filtered (0.45 μm filter, Millipore, Billerica, Massachusetts) and acidified (pH 2.0). Sediment collected using a plastic scoop was dried, disaggregated and passed through a 250 μm sieve. The sieved sediment was digested in 10 of HNO_3 ('Aristar'-grade: BDH, Poole) for 1 hr. The trace metals were measured by inductively-coupled plasma optical emission spectroscopy (ICPOES) following 1 in 3 dilution with deionised water. Sediment was analysed by flame atomic adsorption spectroscopy (AAS) following dilution to 10 ml with deionised water. Calibration of both ICPOES and AAS was by internal standards.

Animals

The forth larval stage (phase 6-7^{th}; WÜLKER & GÖTZ, 1968) were sampled in September, 2005. *C. luridus* was identified cytotaxonomically on the basis of the band sequence on the salivary gland polytene chromosomes using data of (KEYL & KEYL, 1959; KEYL, 1962; MICHAILOVA, 1989; KIKNADZE *et al*., 1991).

Cytogenetic analysis

Chironomid larvae were fixed in alcohol/acetic acid (3:1) and chromosome preparations were done according to MICHAILOVA (1989). Twenty five individuals and 575 cells of *C. luridus* were analysed. We analysed structural and functional alteration of *C. luridus* in comparison with the standard chromosome maps (MICHAILOVA, 1989; KIKNADZE *et al*., 1991) and with the alterations with *C. acidophilus* (MICHAILOVA *et al*., submitted).

We examined inherited and somatic structural chromosome aberrations in *C. luridis* gemone using the descriptions of SELLA *et al*. (2004). The level of functional activity was evaluated from changes in the activity two key cell structures in chromo-

some G, the Balbiani rings (BRs) and nuceolar organizer (NOR). The level of activity is according to BEERMANN (1971).

Statistical analysis

Inter-site differences in amounts of trace metals in water and sediments between South Park Lake and data in MICHAILOVA *et al*. (submitted) were compared using a Student's t-test. Functional activity of BRs and NOR in chironomids in *C. luridus* were compared to *C. acidophilus* (MICHAILOVA *et al*., submitted), again using the Student's t–test. The frequency of chromosome rearrangements in each chromosome of *C. luridus* and *C. acidophilus* as well as the total frequency of rearrangements were compared by the G test (SOKAL & RHOLF, 1995). A probability of $P<0.05$ was taken as significant in all cases.

RESULTS AND DISCUSSION

Metal concentrations in water and sediments

Table 1 shows the average, maximum and minimum concentration of soluble (< 0.45 μm) iron, zinc, copper, manganese and aluminium in the water column and associated with the sediment at South Park Lake. Concentrations of all four metals, including aluminium, in South Park are indicative of a non-metal polluted site, both according to EU standards and analysis of a number of non-polluted sites by FORSTNER & SALOMONS (1980). Comparison with data on the polluted Afon Goch in MICHAILOVA *et al*. (submitted) reveal that concentrations of iron, zinc, copper, manganese and aluminium were significantly higher ($P<0.05$) compared to South Park Lake. Deposited particulate (sediment) concentrations of iron, zinc, copper and manganese were higher ($P<0.05$) at the polluted site on the Afon Goch compared to the unpolluted site. Particulate aluminium was lower ($P<0.05$) at the acidified polluted site compared the non-acidified control.

Table 1. *Average, maximum and minimum concentration (n = 3) of soluble (s) (mg L^{-1};< 0.45 μm) iron, zinc, copper, manganese and aluminium in the water column and associated with the sediment (p) (mg dry wt^{-1}) in South Park Lake, Bulgaria.*

	pH	Iron		Zinc		Copper		Manganese		Aluminium	
		s	p	s	p	s	p	s	p	s	P
Min	7.6	0.17	8.3	0.05	0.01	0.01	0.01	0.28	0.19	0.58	6.1
Mean	7.6	0.42	11.11	0.068	0.041	0.172	0.056	0.83	0.257	1.716	17.55
Max	7.6	0.99	17.4	0.12	0.07	0.82	0.13	1.2	0.34	3.44	26.98

* Data from MICHAILOVA *et al*., submitted

Cytogenetic characteristics of the polytene chromosomes of C. luridus

Chironomus luridus has a chromosome set 2n = 8. It belongs to the "pseudothummi" cytocomplex (KEYL, 1962) with chromosome arm combinations AE (submetacentric), BF, CD (metacentric), G (acrocentric) (Fig. 1a-d). Chromosome G had a nucleolar organiser (NOR) and two Balbiani rings (BRs).

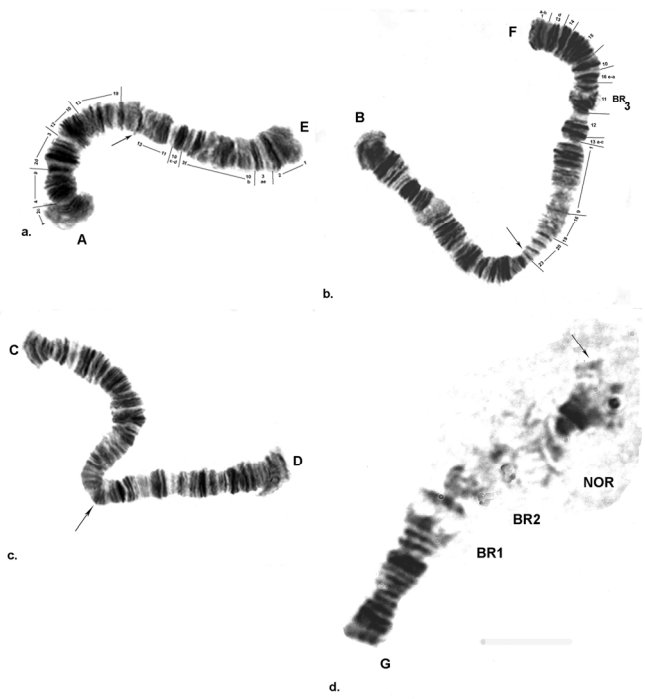

Fig. 1. *Polytene chromosome set in Chironomus luridus from South Park Lake, Sofia, Bulgaria. a. Chromosome AE; b. Chromosome BF; c. Chromosome CD; d. Chromosome G; BR – Balbiani ring; NOR-Nucleolar organizer. The arrow shows the localization of the centromere region. Scale bar: 10 µm*

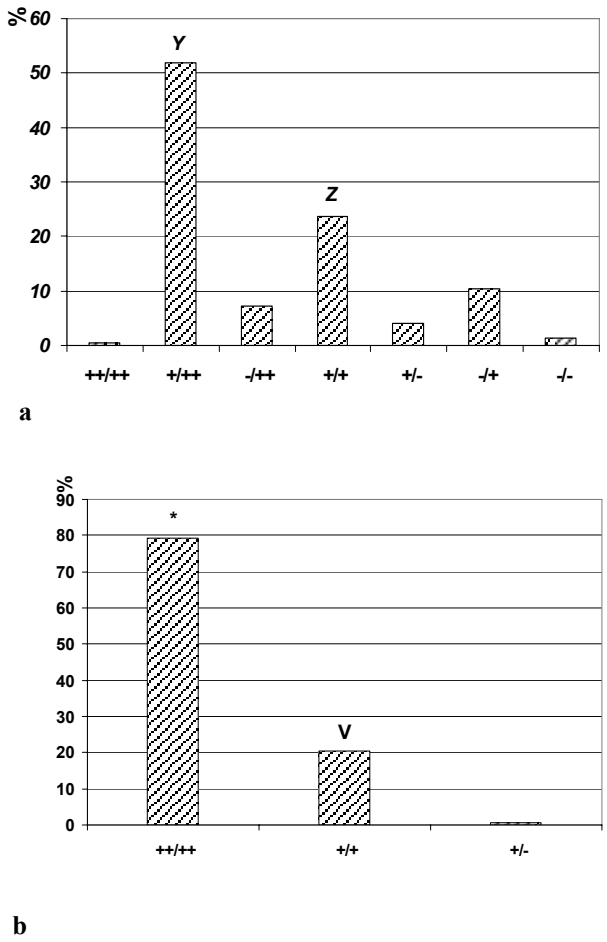

Fig. 2. *Activity of Balbiani rings (BR1/BR2)* (a) *and NOR* (b) *in C. luridus. a. Y – BR1/BR2 (+/++; P<0.05) compared with BR1/BR2 of all other states; Z – BR1/BR2 (+/+; P<0.05) compared with BR1/BR2 (++/++; -/++; +/-; -/+; -/-). b. V- activity (+/+; P<0.05) compared with state (+/-).*- Activity (++/++; P<0.05) compared with states (+/+; +/-).*

Structural and functional differences in C. luridus and C. acidophilus chromosomes

As in *C. acidophilus*, we found both inherited and somatic chromosome rearrangement in *C. luridus*. However, all somatic rearrangements were significantly higher in *C. acidophilus* (MICHAILOVA *et al*., submitted) in comparison with *C. luridus* (Table 2). Somatic paracentric heterozygous inversions are found in *C. luridus*, but at a low frequency: arm C, 0.52%; arm D, 0.17%; arm F, 0.17%.Also, no deficiencies, deletions and asysnapsis were detected in the *C. luridus* genome. In *C. luridus*, seven states of activity of the Balbiani rings (BRs) were observed. The states BR1/BR2: +/++; +/+ were significantly higher (t=2.04, P<0. 05) in comparison with all other states. As in the normal karyotype, *C. luridus* shows significantly higher activity of NOR (Table 2; Fig. 2a, b). The specific "puff" at the telomere region of chromosome G occurred in *C .luridus* only very rarely (Table 3; Fig. 3).

Table 2. *Comparative analysis of structural and functional changes in the chromosomes of C. luridus and C. acidophilus*.*

Changes in chromosomes	*C. acidophilus** n = 705	*C. luridus* n= 575	G-test value	Significance
1. *Structural changes*				
Aberrations in chromosome AE	29	-	G = 34.5	P<0.001
Aberrations in chromosome BF	21	5	G= 7.6	P<0.05
Aberrations in chromosome CD	6	6	G = 0.12	P>0.1
Aberrations in chromosome G	4	-	G = 4.2	P<0.05
Total chromosome aberrations	60	11	G = 29.2	P<0.0001
Asynapsis, chromosome AE	57	-	G = 69.5	P<0.001
Asynapsis, chromosome BF	37	-	G = 44.4	P<0.001
Asynapsis, chromosome CD	29	-	G = 34.5	P<0.001
Asynapsis, chromosome G	90	6	G = 76.4	P<0.001
Total asynapsis	213	6	G = 240.4	P<0.001
2. *Functional changes*				
Chromosome "puff" at the telomere region	90	10	G = 62.2	P<0.001

* Data from MICHAILOVA *et al*., submitted; n – number of cells

Table 3. *Comparative analysis of functional changes in key chromosome structures (Balbiani rings, BR; Nucleolar organizer, NOR) of C. luridus and C. acidophilus. In all cases differences significant at P>0.05)* * Data from MICHAILOVA *et al*., submitted

Functional characteristic	*C. acidophilus**	*C. lirudu*s
BR1/BR2 activity	++/++, +/-	+/++, -/+
NOR activity	+/+, +/-	++/++

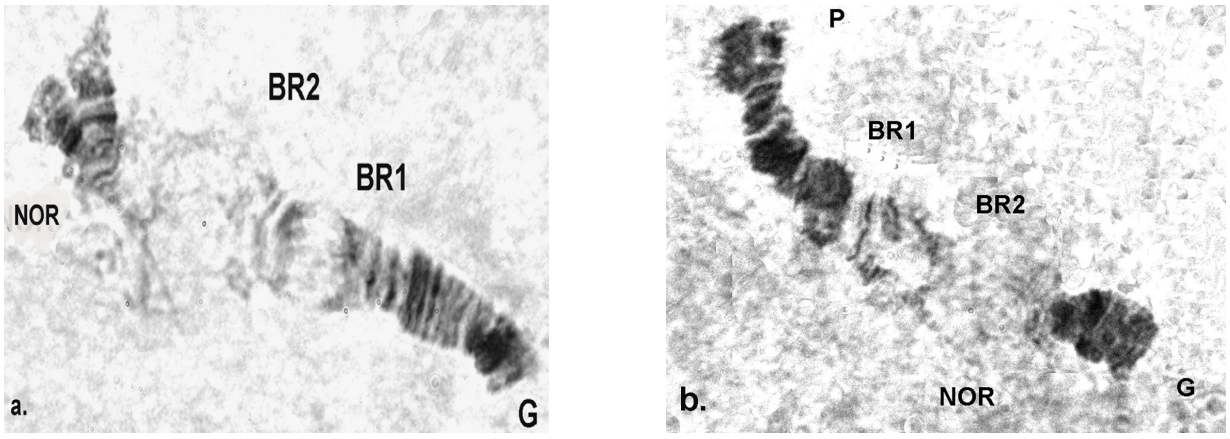

Fig. 3. *Activity of BRs in Chromosome G of C. luridus. a: Intermediate activity of BR1 and high activity of BR2. b: Intermediate activity of BR1/BR2 and a puff (P) at the telomere region; BR – Balbiani Ring; NOR – Nucleolar organizer. Scale bar: 10 µm*

The structural and functional alterations observed in *C. luridus* occurred at a very low frequency which strongly suggests that this is a random phenomenon and is not due environmental stress BENTIVEGNA & COPPER (1993) suggested that a reduction in NOR puff activity may be used as a biomarker of exposure to genotoxic substances inhibiting RNA synthesis. However, both NOR and the Balbiani rings retain their high state of activity in *C. luridus* suggesting that their function is not disturbed by contaminants. The specific buff appeared at the end of the telomere of chromosome G which occurred at a high frequency in *C. acidophilus* whereas in activity was low *C. luridus*. It is therefore unlikely that the appearance of this puff is connected to the synthesis of proteins important for their survival in polluted environments.

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ГЕНОМНИ ИЗМЕНЕНИЯ В ПОЛИТЕННИ ХРОМОЗОМИ НА ВИДОВЕ ОТ СЕМ. CHIRONOMIDAE (DIPTERA), ОБИТАВАЩИ ВОДОЕМИ С РАЗЛИЧНА СТЕПЕН НА МЕТАЛНО ЗАМЪРСЯВАНЕ

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(Резюме)

Проследена е структурно – функционалната изменчивост в политенни хромозоми от слюнчени жлези на видове от сем. Chironomidae, Diptera (*Chironomus acidophilus* Keyl и *Chironomus luridus* Strenzke**)**, срещащи се във водоеми с различна степен на замърсяване с йони на метали. Анализираните видове са филогенетично близки, имат диплоиден набор хромозоми 2n = 8, отнасят се към цитокомплекса “lacunarius”, с комбинация на хромозомни рамена AE, CD, BF и G. Изследвани са водоеми в Англия (Red river), характеризиращи се с ниско pH (<3.0) и високи концентрации на йони на метали (желязо, цинк, мед, манган, алуминий). Изследваните райони от България не се различават от референтите стойности на замърсявания с тежки метали. Особено чувствителен е геномът на *Chironomus acidophilus*, обитаващ водоемите в Англия. Установени са множество соматични структурно хромозомни изменения (инверсии, делеции, дефишънси). Изключително чувствителни са важни клетъчни структури: ядърцев организатор (NOR) и Балбиановите пръстени (BRs) при *C. acidophilus,* чиято функционална активност силно се понижава. Като компенсаторен механизъм се активират множество сайтове в политенните хромозоми на вида, което се манифестира чрез появата на специфични пуфи. Соматичната структурно хромозомна изменчивост при *C. luridus*, обитаващ водоеми в България е значимо по-ниска от тази на *C. acidophilus* (G тест- 29.201,df = 1, P < 0.001). С висока функционална активност се характеризира ядърцевият организатор и един от Балбиановите пръстени (BR_2) на *C.luridus*. Обсъждат се причините за различната геномна реакция на двата близки вида.