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Chromosomal variation of populations of *Chironomus plumosus* Linnaeus (Diptera: Chironomidae) from lakes of Kaliningrad, Russia

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Abstract. Polymorphism of natural populations of *Chironomus plumosus* from lakes of Kaliningrad with the presence of heavy metals (Pb, Cd, Fe, Zn, Cu, Mn, Ni, Cr, Co) in the ground was investigated. An increase in the number of heterozygous inversions per individual in the reservoir with the highest concentration of heavy metals is revealed. The rare inversions pluA6 and pluF5 were found in the most polluted reservoirs.

Key words: *Chironomus plumosus*, polymorphism, heavy metals.

Introduction

Water ecosystems within the city limits are in an unfavourable condition. Quality control of the environment with live testing systems (including chironomids) is therefore necessary. It is well-known that these invertebrate animals have polytene chromosomes allowing exact species identification, and chromosomal aberrations show the influence of various environmental factors. Moreover, the chironomid fauna of reservoirs of the Kaliningrad Prov. is insufficiently studied. In the present paper the results of study of population polymorphism of *Chironomus plumosus* L., 1758 from three lakes of Kaliningrad are presented.

Material and methods

Populations of *Chironomus plumosus* from lakes Shkolnoe, Karasevka, and Verkhnee (Kaliningrad) were studied (Table 1). The material was collected and fixed in alcohol acetic mixture (3 : 1, 96% ethanol : glacial acetic acid) during the years 2002 - 2006. In total, 275 larvae have been investigated.

Temporary cytological preparations of polytene chromosomes from salivary glands were made according the standard aceto-orcein method (Chubareva, Petrova, 1980). An analysis of cytogenetic characteristics of polytene chromosomes from salivary glands of fourth-instar larvae in two populations was performed: presence, localization and frequency of inversions were defined. In addition, land in which the studied reservoirs lie was analyzed for the presence of heavy metals. Chromosomes, their arms, borders of chromosomal rearrangements were identified on the basis of chromosome maps of *Ch. plumosus* presented by Kiknadze et al. (1991). Inversion borders were delimited in accordance with Golygina (1999). Average data on the polymorphism of natural populations of *Ch. plumosus* in the Palearctic were used in the analysis.

The following parameters of cytogenetic structure of the populations were studied: average number of heterozygous inversions per individual, quan-

Table 1. Sampling sites and amount of the collected material. n.d. - sex not determined.

Reservoir	Sampling year	Sampling month	Number of individuals	Male/Female
Lake Shkolnoe	2002	February	55	-
		June	38	-
Lake Karasevka	2002	November	20	-
	2005	January	40	20♀, 17♂, 3 n. d.
	2006	March	40	32♀, 8♂
Lake Verkhnee	2004	February	40	-
		June	20	18♀, 1♂, 1 n. d.
	2005	February	22	17♀, 2♂, 3 n. d.

Table 2. Concentration of heavy metals in the silt from the reservoirs of Kaliningrad. IHM – ions of heavy metals. * after Krotov et al. (2000).

IHM	Shkolnoe	Karasevka	Verkhnee	MCL*
Pb	3.5	12.25	2.8	32.0
Cd	0.001	<0.007	0.017	-
Fe	433.73	1.43	78	-
Zn	34.29	7.39	40.7	23.0
Cu	211.00	0.02	1.49	3.0
Mn	4.1	9.54	17.84	-
Ni	<0.015	0.32	<0.015	4.0
Cr	<0.01	<0.01	<0.01	6.0
Co	<0.01	<0.01	<0.01	5.0

tity of standard and inversion sequences, number of genotypic combinations of disks sequences. Measurements and micrographs were made using the microscope MBI-3 (10 x 20, 10 x 40) and the digital camera Canon Digital IXUS 750. Chemical analysis of the silt for the presence of heavy metals was carried out with the atomic-adsorption spectrograph in the laboratory of the sanitary-hygienic station.

Results

Chemical analysis of the silt has indicated the presence of 9 ions of heavy metals in all the ponds investigated (Table 2). The concentration of copper in the silt of Lake Shkolnoe exceeded the maximum concentration limit (MCL) for the ground 70 times, and that of zinc exceeded it 1.5 times. In a less polluted Lake Verkhnee the content of zinc cations exceeded the MCL for the ground 1.8 times. In addition, very high content of iron cations was revealed in Lake Shkolnoe (Krotov et al., 2000). Despite the MCL for iron was not designated, data obtained during the last years show the negative influence of high concentrations of iron on living organisms (our data, unpublished).

The following data were obtained for larvae of *Chironomus plumosus* from Lakes Shkolnoe, Verchnee and Karasevka: the amount of heterozygous inversions per individual was 0.38 to 1.65, number of inversion sequences was 9 to 14, number of genotypic combinations was 7 to 37 (Table 3). 19% first-instar larvae in the most polluted reservoir, Lake Shkolnoe, carried rare inversions A6 and F5 (Table 4). Sequence B2 prevailing in the arm B is a so-called "ecological" inversion, because it was shown that the increase in frequency of the heterozygous inversion h'pluB2 depended on the concentration of water-dissolved oxygen

Table 3. Parameters of polymorphism of natural populations of *Ch. plumosus* of the lakes of Kaliningrad.

Parameters	Shkolnoe		Karasevka			Verkhnee		
	February 2002	June 2002	November 2002	January 2005	March 2006	February 2004	June 2004	February 2005
Number of individuals studied	56	38	20	40	40	40	20	22
Number of individuals with standard karyotype	0	4	1	21	26	10	1	5
Number of heterozygous inversions per individual	1.65	1.2	1.2	0.48	0.38	0.77	1.55	0.64
Number of heterozygous and homozygous inversions per individual	2.4	1.4	2.0	0.6	0.4	1.3	2.3	1.3
Number of inversion sequences	14	11	10	11	12	9	11	9
Number of genotypic combinations	38	14	17	8	7	19	14	14
Frequency of B-chromosomes, %	3.6	0	0	0	0	2.5	0	0

Table 4. General frequency of inversions in populations of *Ch. plumosus*. The number of individuals is given in brackets.

Inversion	Shkolnoe (93)	Karasevka (100)	Verkhnee (82)
plu A2	81.7	99	87.8
plu A1	17.2	1	12.2
plu A6	1	0	0
plu B1	34.4	61	52.4
plu B2	65.6	39	45.1
plu B3	0	0	2.5
plu C1	80.6	96	74.4
plu C2	19.4	4	25.6
plu D1	74.2	85	76.8
plu D2	25.8	13	23.2
plu D6	0	2	0
plu E1	94.6	100	90.2
plu E2	5.4	0	9.8
plu F1	80.7	100	97.6
plu F5	19.3	0	0
plu F2	0	0	2.4
plu G1	100	100	100

(Il'inskaja et al., 1999). All investigated populations had various chromosomal aberrations (asynapsis of homologous polytene chromosomes, amplification of centromeric chromatin); and populations from Lakes Shkolnoe and Verkhnee carried additional B-chromosomes.

Discussion

The population of *Chironomus plumosus* from Lake Shkolnoe has been investigated in winter and summer. Higher levels of *Ch. plumosus* polymorphism in winter are probably due to an increase of the contents of heavy metals in the ground during the cold season (Budnikov, 1998). Lake Shkolnoe was exposed to anthropogenic pollution for a number of years, and the investigated population of *Chironomus* adapts to extreme living conditions through accumulation of the greater number of sequences of disks and genotypic combinations. Thus, in the populations investigated 17 sequences of chromosome disks were found out of 35 known in the Palearctic. Of them, 12 sequences were

common for the three populations, whereas sequences p'pluA6 and p'pluF5 were detected only in the larvae from Lake Shkolnoe, and sequences pluB3 and pluF2 were found only in the population of Lake Verkhnee. In Kaliningrad, the level of natural polymorphism in populations of *Ch. plumosus* is exceeded in Lake Shkolnoe 1.5 times, in Lake Karasevka – 1.2 times (in 2002), in the summer sample of Lake Verkhnee – 1.3 times, and the average number of heterozygous inversions per individual in the European populations of *Ch. plumosus* is 0.95 (Golygina, 1999; Golygina, Kiknadze, 2001).

We can therefore conclude, that the increased frequency of certain chromosomal aberrations can reflect environmental conditions within reservoirs.

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