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Chromosomal polymorphism of *Chironomus plumosus* Linnaeus and *Ch. entis* Shobanov (Diptera: Chironomidae) of the South Transural region

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Abstract. Species composition and chromosomal polymorphism in three natural populations of *Ch. plumosus* Linnaeus and *Ch. entis* Shobanov from the South Transural region are analysed. Six inversion disk sequences were detected in 5 out of 7 chromosome arms of *Ch. plumosus*. The number of disk sequences varied in different arms: 2 – in arm A, 3 – in arm B, 2 – in arm C, 2 – in arm D, 2 – in arm E, and 1 – in arms F and G respectively. Six inversion disk sequences were also detected in *Ch. entis*, but only in 2 out of 7 chromosome arms. The number of disk sequences is unevenly distributed along chromosome arms: 6 – in arm A, 2 – in arm C, and 1in each of the arms B, D, E, F, and G. The level of chromosomal and genomic polymorphisms in examined populations is relatively low in comparison with other populations from adjacent and other regions.

Key words: Diptera, Chironomidae, karyotype, chromosomal polymorphism.

Introduction

In-depth study of zoobenthos in South Transural region with a goal of estimating the nutritive base for the fish-farm ponds was started in the 1930s. Hydrobiological studies of Lakes Kamenskoe, Pesvyannoe, Volch'e, Glubokoe, Shadrino, Lihanovskaya Pereyma, Surgoyak, Duvankul, and Shchuch'e in Kurgan Province revealed some chironomids resistant to oxygen starvation, among which are larvae of the genus *Chironomus* (Kovalkova, 1982).

Ch. plumosus Linnaeus, 1758 and *Ch. entis* Shobanov, 1989 have been karyologically detected in several lakes of Kurgan Province (Kiknadze et al., 1991a; Gunderina et al., 1999). Study of the chromosomal polymorphism of *Ch. entis* has shown significant differences between populations from Western Siberia, the Transurals and Europe (Kiknadze et al., 1991a; Gunderina et al., 1999). Data on the inversion polymorphism in *Ch. plumosus* and *Ch. entis* were used for producing dendrograms of cytogenetic distances between populations (Golygina, 1999).

The present paper reports investigation of the chromosomal polymorphism in populations of *Ch. plumosus* and *Ch. entis* from Lakes Berkut and Krivskoe situated in the north of the South Transural region.

Material and methods

We have studied karyotypes of fourth-instar larvae of *Ch. plumosus* and *Ch. entis* collected at depths of 2-2.5 meters in central ooze of Lakes Berkut (samples taken on 14.02.2005 and 12.03.2006), Krivskoe (17.05.2006) and Belikul' (14.02.2005). Numbers of larvae examined: 136 of *Ch. plumosus* (75 from Berkut Lake, 60 from Krivskoe Lake and 1 from Belikul' Lake) and 150 of *Ch. entis* (115, 34 and 1 respectively). Larvae were washed, cleaned from soil and preserved in

Carnoy's fixative (3:1). Squash preparations of polytene chromosomes were made from cells of salivary glands using an ethyl-orcein method (Dyomin, Shobanov, 1990). Salivary glands were stained with 1.5-2% ethyl-orcein for 2 days, then the redundant orcein has been washed off with 70% ethyl alcohol, and glands were put in a drop of 20% lactic acid for 2-3 hours. After that, glands were cleaned off secretion and covered by a coverslip using a gentle pressure. Chromosomes were analysed with the help of Ergaval microscope with magnification of 7 x 40 objective, and preparations were photographed on to microfilm. Chromosomal analysis was performed on temporary preparations, and chromosomes with an average rate of polyteny were selected. Cytophotomaps were constructed from chromosomes taken from the same cell of a salivary gland. The standard cytophotomap by Maksimova (1976) detailed by Schobanov (1994) has been used for mapping chromosomes of *Ch. plumosus.* For *Ch. entis,* cytophotomaps and other data presented by a number of authors (Kerkis et al., 1989; Dyomin, Shobanov, 1990; Kiknadze et al., 1991a, b; Belyanina et al., 1992; Petrova, Klishko, 2005) were used.

Results

During the study of the inversion pool of a population of *Ch. plumosus* inhabiting Berkut Lake (75 larvae examined), six types of heterozygous inversions were found. Inversions were detected in arms A, B, C, D and E, with two types revealed in arm B and one type in other arms. Arms F and G displayed no changes in disk structure in comparison with standard disk sequences. Arm A appeared to be the most variable. 26.7% examined specimens have a heterozygous inversion *plu* A2 (10i-4k). Arm A was monomorphic in the rest of specimens. Arm B has heterozygous inversions *plu* B1 (24-23) and *plu* B2 (24-15c), occurring in 3.3% and 1.5% cases respectively. Arm C has inversion *plu* C2 (24-16) in 11% specimens. Inversion *plu* D3 (4-2) (arm D) and inversion *plu* E4 (9-7) (arm E) both occur in 3.3% cases. The number of heterozygous inversions per specimen equals 0.6. Genomic polymorphism for B-chromosomes is observed in 5% specimens. In Krivskoe Lake, 35% larvae (a total of 60) had heterozygous inversion *plu* A2, so that the number of heterozygous inversions per specimen in this population equals 0.4. Genomic polymorphism for B-chromosomes was observed in 1.7% specimens in the sample from Krivskoe Lake. Larvae collected in Belikul' Lake were not studied in respect of chromosomal and genomic polymorphisms.

Larvae of *Ch. entis* were found in all three investigated lakes. Most of the examined specimens had sequence *ent* A44 in arm A. Percentage of those larvae equals 74% in the sample from Berkut Lake and 62% in the sample from Krivskoe Lake. Kiknadze et al. (1991a) points that disk sequence *ent* A44 is specific to populations of Kurgan Prov. We observed five types of heterozygous inversions in arm A: *ent* A12 (8% larvae from Berkut Lake and 12% from Krivskoe Lake), *ent* A14 (9% and 15% respectively), *ent* A15 (9% and 0%), *ent* A16 (8% and 12%), *ent* A17 (9% and 0%). Arm B had the sequence *ent* B11 in all studied specimens from all populations. Arm C had the disk structure *ent* C22 in most specimens examined (91% from Berkut Lake and 100% from Krivskoe Lake). 9% larvae from Berkut Lake had heterozygous inversion *ent* C12 in arm C. Arms D, E, F and G had standard disk sequences. A total of 13 disk sequences were observed in studied populations of *Ch. entis*. Number of heterozygous inversions per specimen was 0.35 in Berkut Lake and 0.47 in Krivskoe Lake. B-chromosomes were found in 2.6% specimens from Berkut Lake and 2.9% specimens from Krivskoe Lake. Larvae collected in Belikul' Lake were not studied in respect of chromosomal and genomic polymorphisms.

Discussion

Species diagnosis of larvae using cytogenetic methods revealed two closely related sympatric species, *Ch. plumosus* and *Ch. entis*, in the Lakes Belikul', Berkut and Krivskoe of the South Transural region. Genotypic variability in terms of chromosomal rearrangements and occurrence of B-chromosomes has been analysed in these water bodies except for Belikul' Lake. Both species have the greatest variability in arm A. *Ch. plumosus* has the only type of heterozygous inversion in arm A, but it clearly prevails (26.7-35%) in comparison with heterozygous combinations in other arms. In *Ch. entis* arm A is more varied and includes five types of heterozygous inversion. Overall percentage of larvae with heterozygous inversions in arm A is from 37% to 43% in different water pools. Chromosome arms B, D and E of *Ch. entis* are monomorphic. Larvae of *Ch. plumosus* have two types of heterozygous inversions in arm B and single types of heterozygous inversions in arms D and E. Examined populations of both species have monomorphic arms F and G. Larvae of *Ch. entis* from the Lakes Berkut and Krivskoe located in the north of Kurgan Prov. were shown to have 13 disc sequences in their karyotypes. Our data correlate well with evidence provided by Gunderina et al. (1999), who found 13 and 23 different disk sequences respectively in samples of this species from the water pools located in the north and in the south of Kurgan Prov.

Levels of chromosome polymorphism in *Ch. entis* and *Ch. plumosus* are insignificant in the examined samples. The number of heterozygous inversions per specimen varies from 0.4 to 0.6 in *Ch. plumosus* and from 0.35 to 0.47 in *Ch. entis*. Rates of heterozygous inversion of *Ch. plumosus* from Lakes Berkut and Krivskoe appeared to be lower than for adjacent territories. Acccording to Belyanina et al. (1983), number of heterozygous inversions per specimen equals 0.7, and 63% larvae have heterozygous inversions in the pools of the South Ural region. Also, there are 0.9-1.6 heterozygous inversions per specimen, and 60.9-90.3% specimens with heterozygous inversions in the pools of the Middle Urals (Philinkova, 1992; Belyanina, Loginova, 1993). For example, an average number of heterozygous inversions per specimen is equal to 0.95 for populations of *Ch. plumosus* in the Palaearctic region (Golygina, 1992; Golygina, Kiknadze, 2001). Our results on inversion polymorphism of *Ch. entis* correspond with those on other populations of South Transural region. There are 0.5 heterozygous inversions per specimen in a lake near Yurgamish city and 0.3 heterozygous inversions in a lake near Kizilbay village (Kiknadze et al., 1991a). The mean numbers of heterozygous inversions per specimen of *Ch. entis* are 0.66 for the South Ural region and 0.63 for the Middle Ural region (Belyanina et al., 1992). Up to the present, more than 20 populations of *Ch. entis* have been studied, and the level of heterozygosis in these populations varies widely – from 0.15 to 1.6 heterozygous inversions per specimen (Kerkis et al., 1989; Petrova, Klishko, 2005).

According to our data, in Kurgan Prov. the rate of genomic polymorphism for B-chromosomes varies from 1.7% to 5% for *Ch. plumosus* and from 2.6% to 2.9% for *Ch. entis*. This is comparable with the data on Middle Ural water pools where from 0.8% to 2.9% specimens of *Ch. plumosus* have B-chromosomes (Belyanina et al., 1992; Philinkova, 1992).

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