Specific Features of the Cytogenetic Structure of the Population of Chironomus plumosus (L.) (Diptera, Chironomidae) in a Small Creek (Udmurt Republic, Russia)

Article *in* Inland Water Biology · December 2019
DOI: 10.1134/S1995082919060087

Roza Lozhkina
Russian Academy of Sciences

Viktor V. Bolshakov
Russian Academy of Sciences

ISSN 1995-0829, Inland Water Biology, 2019, Vol. 12, Suppl. 2, pp. S119–S122. © Pleiades Publishing, Ltd., 2019.
Russian Text © The Author(s), 2019, published in Biologiya Vnutrennykh Vod, 2019, No. 4, part 2, pp. 98–101.

BRIEF COMMUNICATIONS

Specific Features of the Cytogenetic Structure of the Population of *Chironomus plumosus* (L.) (Diptera, Chironomidae) in a Small Creek (Udmurt Republic, Russia)

R. A. Lozhkina[a, *], N. A. Shobanov[a, †], I. A. Antipov[b], and V. V. Bolshakov[a]

[a] *Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Nekouzskii raion, Yaroslavl oblast, Russia*
[b] *Yaroslavl State Agricultural Academy, Yaroslavl, Russia*
**e-mail: Lozhkina.roza@yandex.ru*

Received June 19, 2018; revised March 13, 2019; accepted April 9, 2019

Abstract—Chromosomal polymorphism is investigated in samples of *Chironomus plumosus* L., (1758) (Diptera, Chironomidae) from a pond on the Chemoshur creek in Udmurtia for the first time. The pool of polytene chromosome banding sequences of this species includes 12 banding sequences forming 17 zygotic combinations. The pluF2 sequence is found with a high frequency of 0.17. The cytogenetic distance between the known populations is determined.

Keywords: Chironomus plumosus, polytene chromosomes, polymorphism, adaptation, inversion, Udmurt Republic

DOI: 10.1134/S1995082919060087

Larvae of *Chironomus plumosus* (L., 1758) are the subject of many cytogenetic studies. Having a high level of variability at the morphological, biochemical, and cytogenetic levels (Butler et al., 1999; Belyanina, 2015; Kiknadze et al., 2016), *Ch. plumosus* larvae are able to populate a wide variety of water bodies en masse. It has been shown that the frequency distribution of the inversion variants of chromosomal sequences over the distribution range is uneven and depends primarily on the habitat conditions in a particular water body (Gunderina et al., 1999). This effect is observed even in the conditions of the same water body, with individual sequences confined to its different regions.

For a complete understanding of the adaptation mechanism of chironomids to various environmental conditions, data on the cytogenetic structure of populations living in poorly studied regions are of obvious interest. These regions include water bodies of the Udmurt Republic, which is located in the inland climate zone with hot summers and cold snowy winters. The region has a dense complex river network with a large number of underground water sources; as a result, unique conditions are formed, including habitat conditions for invertebrate animals.

Material for the study comprised 78 *Ch. plumosus* larvae collected in mid-April of 2013 from a pond on the Chemoshur creek (58°06′12″ N, 52°30′16″ E) using a hydrobiological scraper from a depth of 1.5 m; the soil type was gray silt with plant residues. In May of 2012, total dissolved solids of the water was 266 mg/L, pH 8, total hardness 4.8 mEq/L, silicon content 17 mg/L, calcium content 25 mg/L, and magnesium content 41 mg/L.

The Chemoshur creek (a fourth-order tributary of the Kama River) is in the Glazovsky district in the northwest of the republic. Its total length is ~3 km; an artificial pond was created on the stream 1 km from the mouth with a water-surface area of 10.000 m^2 and an average depth of 3 m. The pond is fed by both creek and ground waters, which is why the temperature in it is ≤15°C even during summer months.

The collected larvae were fixed in a mixture of alcohol (3 parts) and glacial acetic acid (1 part). Squash karyological preparations of polytene chromosomes were made according to the standard ethylorcein method (Gunderina et al., 1999). The preparations were analyzed using a Jenaval microscope at a magnification of 400× and photographed using a Canon Power Shot A470 camera. Chromosome mapping was performed according to the modernized Maximova system (Maksimova, 1976; Shobanov, 1994a, 1994b); inversion variants of chromosome arms were designated according to Shobanov (Shobanov, 1994a, 1994b). Mapping of the F arm was performed according to the updated cytophotomaps (Kiknadze et al., 2016). We used Nei's index (Nei, 1972) to calculate the cytogenetic distances; correspondence to the Hardy–Weinberg distribution was

† Deceased.

Table 1. Frequency of occurrence of banding sequences of chromosomes in *Chironomus plumosus* from the Chemoshur creek

A1	*A2*	*B1*	*B2*	*C1*	*C2*	*D1*	*D2*	*E1*	*F1*	*F2*	*G1*
0.93	0.07	0.99	0.01	0.74	0.26	0.19	0.81	1.00	0.83	0.17	1.00

Table 2. Frequency of occurrence of genomic combinations in the studied larvae of *Chironomus plumosus*

Genomic combination						Frequency, %
A	*B*	*C*	*D*	*E*	*F*	
1.1	1.1	1.1	1.2	1.1	1.1	6.40
1.1	1.1	1.1	2.2	1.1	1.1	17.90
1.1	1.1	1.1	2.2	1.1	1.2	2.60
1.1	1.1	1.1	2.2	1.1	1.2	12.80
1.1	1.1	1.2	1.1	1.1	1.1	2.60
1.1	1.1	1.2	1.2	1.1	1.2	3.80
1.1	1.1	1.2	1.2	1.1	1.1	12.80
1.1	1.1	1.2	2.2	1.1	1.1	14.10
1.1	1.1	1.2	2.2	1.1	1.2	10.30
1.1	1.1	2.2	2.2	1.1	1.1	1.30
1.1	2.2	1.1	1.1	1.1	1.1	1.30
1.2	1.1	1.2	2.2	1.1	1.1	2.60
1.2	1.1	1.1	1.2	1.1	1.2	3.80
1.2	1.1	1.1	2.2	1.1	1.1	3.80
1.2	1.1	1.1	1.1	1.1	1.1	1.30
1.2	1.1	2.2	2.2	1.1	1.1	1.30
1.2	1.1	1.2	1.2	1.1	1.1	1.30

determined using GenAIEx 6.5 software package (Peakall and Smouse, 2006, 2012).

In the course of analysis, we found 12 banding sequences of polytene chromosomes in *Ch. plumosus* (Table 1) forming 17 genomic combinations (Table 2). The level of heterozygosity is relatively high, 1.7 heterozygotes per individual.

Chromosome I (*AB*). Two banding sequences were found in the *A* arm. The dominant sequence is (*1a–12u*. Standard) (Kiknadze and Kerkis, 1986; Maksimova, 1976; Shobanov, 1994a), corresponding to pluA2 in the studies that use the Keyl system (*1a–2c.10a–12a.13ba.4a–c.2g–d.9e–4d.2h–3i.12cb.13c–14f.15a–14g.15b–19f*) (Keyl, 1962; Kiknadze et al., 2016). The pluA2 sequence (*1a–4j.10i–4k.10j–12u*) (Kiknadze and Kerkis, 1986; Maksimova, 1976; Shobanov, 1994a), which in the Kyle system corresponds to pluA1 (*1a–2c.10a–12c.3i–2h.4d–9e.2d–g.4c–a.13a–14f.15a–14g.15b–19f*) (Keyl, 1962; Kiknadze et al., 2016), occurs only as heterozygote with pluA1.

Two sequences were detected in the *B* arm. Sequence pluB1 (*12u–25s*. Standard) in the homozygous state is predominant in the population; pluB2 (*12u–15f. 23–15g. 23f–25s*) was identified only as a homozygote in one individual.

Chromosome II (*CD*). Two sequences were found in the *C* arm: pluC1 (*14o–25q*. Standard) and pluC2 (*14o–16h. 22f–16i. 22g–25q*); the latter is found both in the heterozygote with pluC1 and in the homozygous state.

Two sequences were found in the *D* arm: pluD1 (*1a–14o*. Standard) is found only in a heterozygous state and pluD2 (*1a–2i.7i–2j.7j–14o*) is found both in the homozygous state and in heterozygote with pluD1.

Chromosome III (*EF*). We found pluE1 sequence (*1a–11b*. Standard) in the *E* arm. Two sequences were found in the *F* arm: pluF1 (*11b–22m*. Standard) and pluF2 (*11b–13d.16k– 13e.16–22m*); the latter was found only in heterozygote with pluF1.

Chromosome IV. The *G* arm is monomorphic and is represented by the standard pluG1 sequence (*1a–8z*). Homologues are not always paired.

It should be noted that no individuals with a standard genomic combination were found in the studied population; three combinations were the most typical for the population: A1.1B1.1C1.1D2.2E1.1F1.1, A1.1B1.1C1.2D2.2E1.1F1.1, and A1.1B1.1C1.1D2.2E1.1F1.2, which were detected in 48% of the specimens (Table 2). In addition to the "standard" sequences, the following sequences were found in the population with a high frequency: pluC2 (50% of individuals); pluD2 (95%); and, for the first time for the studied populations of *Ch. plumosus*, pluF2 (33%) (likely due to the habitat conditions). This is indicated by a deviation from the Hardy–Weinberg distribution in the *B* and *C* arms (with $p < 0.05$). Predicted frequency of the pluB1.2 combination is 2.5%, but it was not found in any individual. The observed value of pluC1.1 is 50% (expected +4.5%), pluC1.2 is 47.5% (–8.5%), and pluC2.2 is 2.5% (+4.5%).

Using the data on the cytogenetic structure of population (Golygina, 1999; Shobanov and Bolshakov, 2011), we calculated the distance to the already-studied populations. The greatest cytogenetic distances were found to the populations in Lake Golodnaya Guba in Arkhangelsk oblast (Nei's index value is 0.131); Rybinsk Reservoir in Yaroslavl oblast (0.105); Lake Yalpug in Odessa oblast, Ukraine (0.094); and Lake Beloe, Yakutia (0.092). The closest were populations from the Karpysak River (0.013), channel 3 of the Ob River (0.021), and Rechport (0.026) in the vicinity of the city of Novosibirsk, as well as the water body in the town of Veliky Ustyug, Vologda oblast (0.014) (Golygina, 1999). They all have high frequency of occurrence of the pluA1 (0.66–0.94), pluB1 (0.80–1.0), pluD2 (0.26–0.80), and pluE1 sequences (0.99–1.00), as well as pluF2 in the two closest populations (0.036, the Kaprysak River; 0.031, water body in the town of Veliky Ustyug); the highest frequency of

this sequence (0.17) was recorded in the population we studied.

Deviations from the Hardy–Weinberg equilibrium found in the population indicate the presence of pressure that facilitates the selection of pluC1.2 heterozygotes. The presence of one individual with a pluB2.2 combination and the complete absence of pluB1.2 heterozygotes is possibly associated with the migration process from receiving streams and their floodplain systems (Shobanov and Bolshakov, 2011). The high frequency of occurrence of pluC1.2, pluD1.2, pluD2.2, and pluF1.2 combinations, as well as the level of heterozygosity, which is known to create a genetic reserve of the species, provide an advantage for the carrier specimens (Dubinin, 1966). This is likely due to a slightly increased content of silicon in the water, as well as calcium and magnesium, since these elements are thought to play an important role in the metabolism of chironomid larvae (Berezina, 2017; Martemyanov and Markiyanova, 2018). Unfortunately, it is not possible to assess the correlation of the cytogenetic structure with the chemical composition of water due to the lack of literature data on the chemical analysis in other water bodies. It is also worth noting that the Chemoshur creek is characterized by a smaller range of water-temperature fluctuations throughout the year.

The cytogenetic distances to the three nearest populations, despite them being geographically distant, correspond to intrapopulation variability of the species: 0.016 ± 0.006 (Shobanov and Bolshakov, 2011), in our case 0.013, 0.014, and 0.021. It should be noted that the nearest sites with a minimal cytogenetic distance can be 500–1900 km away, but the difference in latitude is only ~4° (~440 km); vice-versa, populations with the greatest cytogenetic distances are located at a greater distance in latitude. An exception is the population from the Rybinsk Reservoir, in which the pluB2 and pluC2 sequences are predominant. With a distance of ~800 km, the difference in latitude is <20 km, which again confirms the significance of environmental factors for the formation of the population cytogenetic structure.

CONCLUSIONS

The karyopool of *Ch. plumosus* population from the Chemoshur creek is represented by 12 banding sequences of polytene chromosomes forming 17 zygotic combinations. We found a high frequency of occurrence for the *F*2 sequence in the studied population: 0.17. The results of the analysis of the cytogenetic structure of *Ch. plumosus* population from the Chemoshur creek confirm the assumption that certain sequences of chromosome bands and their combinations are confined to specific environmental conditions.

ACKNOWLEDGMENTS

We are grateful to A.V. Krylov (Institute of Biology for Inland Waters, Russian Academy of Sciences) and I.V. Pozdeev (Perm Branch of the Federal State Budget Scientific Institution Russian Federal Research Institute of Fisheries and Oceanography) for valuable advice and recommendations in preparing the manuscript, as well as S. Pozdeev (resident of the village of Chura) for his assistance in collecting the material.

FUNDING

This study was supported within the framework of the state assignment of the Ministry of Science and Higher Education of the Russian Federation "Physiological, Biochemical, and Immunological Reactions of Aquatic Organisms under the Influence of Biotic and Abiotic Environmental Factors" (state registration no. AAAA-A18-118012690123-4).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

REFERENCES

Belyanina, S.I., Chromosomal variation in *Chironomus plumosus* (L.) (Diptera, Chironomidae) from populations of Bryansk region, Saratov region (Russia) and Gomel region (Belarus), *Russ. J. Genet.*, 2015, vol. 51, no. 2, p. 138. https://doi.org/10.1134/S1022795415020040

Berezina, N.A., Tolerance of freshwater invertebrates to changes in water salinity, *Russ. J. Ecol.*, 2003, vol. 34, no. 4, p. 261. https://doi.org/10.1023/A:1024597832095

Butler, M.G., Kiknadze, I.I., Golygina, V.V., et al., Cytogenetic differentiation between Palearctic and Nearctic populations of *Chironomus plumosus* (L.) (Diptera, Chironomidae), *Genome*, 1999, vol. 42, no. 5, p. 797.

Dubinin, N.P., *Evolyutsiya populyatsii i radiatsiya* (Evolution of Populations and Radiation), Moscow: Atomizdat, 1966.

Golygina, V.V., The divergence of karyotypes of Holarctic species of the Chironomus plumosus group in the Palearctic and Nearctic: Diptera, Chironomidae, *Cand. Sci. (Biol.) Dissertation*, Novosibirsk, 1999.

Gunderina, L.I., Kiknadze, I.I., and Golygina, V.V., Intraspecific differentiation of the cytogenetic structure in natural populations of *Chironomus plumosus* L., the central species in the group of sibling species (Chironomidae: Diptera), *Russ. J. Genet.*, 1999, vol. 35, no. 2, p. 142.

Keyl, H.G., Chromosomen evolution bei Chironomus. II Chromosomenum bauten und phylogenetische Beziehungen der Atten, *Chromosoma*, 1962, vol. 13, no. 4, p. 464.

Keyl, H.-G. and Keyl, I., Die cytologische Diagnostik der Chironomiden I, *Arch. Hydrobiol.*, 1959, vol. 56, nos. 1/2, p. 43.

Kiknadze, I.I. and Kerkis, I.E., Comparative analysis of polythene chromosome banding patterns in sibling species of chironomids of the PLUMOSUS group of Western Siberia, *Tsitologiya*, 1986, vol. 28, no. 4, p. 430.

Kiknadze, I.I., Istomina, A.G., Golygina, V.V., and Gunderina, L.I., *Karyotypes of Palearctic and Holarctic Species of the Genus Chironomus*, Novosibirsk: GEO, 2016.

Maksimova, F.L., On the problem of the karyotype of Chironomus plumosus L., *Tsitologiya*, 1976, vol. 18, no. 10, p. 1164.

Martemyanov, V.I. and Markiyanova, M.F., Threshold concentrations of cations in the water necessary for maintaining the ionic balance between organism of *Chironomus balatonicus* Devai et al. larvae and environment, *Inland Water Biol.*, 2018, vol. 11, no. 1, p. 81.
https://doi.org/10.1134/S1995082917040071

Nei, M., Genetic distance between populations, *Am. Nat.*, 1972, vol. 106, p. 283.

Peakall, R. and Smouse, P.E., GENALEX 6: genetic analysis in excel. population genetic software for teaching and research, *Mol. Ecol. Notes*, 2006, vol. 6, p. 288.

Peakall, R. and Smouse, P.E., GENALEX 6.5 genetic analysis in Excel. Population genetic software for teaching and research—an update, *Bioinformatics*, 2012, vol. 28, p. 2537.

Shobanov, N.A., Karyofund of *Chironomus plumosus* (L.) (Diptera, Chironomidae). I. Standardization of polytene chromosome discs in the system of Maksimova, *Tsitologiya*, 1994a, vol. 36, no. 1, p. 117.

Shobanov, N.A., Karyofund of *Chironomus plumosus* (L.) (Diptera, Chironomidae). II. Inversion variants of chromosomal arms, *Tsitologiya*, 1994b, vol. 36, no. 1, p. 123.

Shobanov, N.A. and Bolshakov, V.V., Karyofund of *Chironomus plumosus* (L.) (Diptera, Chironomidae): V. Terminal and interstitial populations, *Cell Tissue Biol.*, 2011, vol. 5, no. 2, p. 196.
https://doi.org/10.1134/S1990519X11020155

Translated by A. Lisenkova