

ANIMAL
GENETICS

Chromosomal Polymorphism of the Unique Baikal Deep-Water Population of *Sergentia flavodentata* (Diptera, Chironomidae) from the Region of Hydrothermal Venting

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Received February 25, 2004; in final form, June 8, 2004

Abstract—The subject of this study is chromosomal polymorphism of a population of Baikal endemic species *Sergentia flavodentata* (Diptera, Chironomidae) from Frolikha Bay region of hydrothermal venting at a depth of 420–430 m. In 35–67% of larvae, six heterozygous inversions were found to be preserved for 13 years (materials of 1991, 1994, 1996, 1999, 2003). The number of zygotic combinations reached 14; 0.5–0.7 inversions per individual was observed. It is suggested that stable chromosomal polymorphism characteristic of this species is nonrandom, being a mechanism of the population adaptation to the specific conditions of hydrothermal venting community based on bacterial biochemosynthesis.

INTRODUCTION

Long-term karyologic studies of chironomid species within their area in various water bodies, including those exposed to a strong anthropogenic impact, showed that chironomids represent a useful model that adequately reflects the environmental influence on the hereditary apparatus of hydrobionts. In most natural populations, a high level of chromosome polymorphism was found, which promoted species adaptation to various environmental changes [1–11]. Karyotyping and analysis of chromosome polymorphism of chironomids from Lake Baikal have been initiated more than 20 years ago. The most studied populations of the genus *Chironomus* group *plumosus* are abundant in the littoral-sor zone [12, 13]. Conversely, the endemic Baikal species inhabit the open Baikal area and large bays. The population density of these species is typically low; they are unevenly distributed on the ground and difficult to access because of great depth and complex shape of the sea bottom. Thus, the material collection and obtaining representative samples become problematic. Of interest is the fact that among all groups of insects inhabiting Baikal, only chironomids of the genus *Sergentia* Kieffer inhabit both the minimum and greatest depths [14, 15]. During the 25-million-year history of their evolution, these chironomids have adapted to diverse conditions of the unique lake and created a flock of endemic species [16, 17].

Among *Sergentia*, *S. flavodentata* occupies a special place. This is the only eurybathic chironomid species that inhabits Baikal at a depth from 13 to 1400 m [18]. In addition, the population density and biomass of this species is the highest in the most unusual benthic com-

munities that were recently discovered in Baikal: in the regions of hydrothermal venting in Frolikh Bay, in those of the surface gas hydrate bedding, and on the “cemetery of benthic animals” in the Cape Bol. Solontsovyi [19–21].

Chromosomal polymorphism of Baikal *Sergentia* is still poorly studied because, as mentioned above, large samples of four-instar larvae suitable for karyologic analysis are inaccessible. The populations of narrowly adapted homosequential species *S. rynocephala*, *S. rara*, and *S. affinis* inhabiting Baikal littoral were chromosome monomorphic. No chromosome rearrangements were found in the deep-water species *S. assimilis*. Conversely, nearly all studied populations of the *S. baicalensis* larvae, frequently occurring in the littoral, displayed chromosomal polymorphism; two heterozygous inversions in chromosomes I and II were the most frequent. Numerous heterozygotes were recorded in a population of *S. nebulosa* at a depth of 30 m in Southern Baikal. In a sample containing 21 larvae, 70% were heterozygous and one inversion per individual was estimated. Only two types of inversion sequences were found in chromosomes II and III.

Three types of heterozygous inversions are identified by now in the karyotype of *S. flavodentata* from various regions of Middle and Southern Baikal: paracentric inversions in the regions 3–10 and 11–19 of chromosomes I and III, respectively (in some samples, they are recorded in 10–50% of larvae) and pericentric inversions in the regions 10–14 of chromosome I that were identified only in two cases. Although the content of heterozygous individuals is difficult to determine in separate populations because of the low sample size

(10–12 larvae), in general, the low diversity of inversion sequences testifies to the low level of chromosomal variation in *S. flavodentata* from the above Baikal regions.

This study presents the results of karyologic analysis of larvae from Frolikh Bay (Northern Baikal), which is a region of increased hydrothermal venting caused by a release of subaquatic hydrotherm [22]. In 1990, this region was examined with man-operated submersible *Pisces* at a depth of 420–430 m. As a result, a new, previously unknown unique benthic community was discovered, the metabolism of which is based on biogenic methane generation. The discovery of a community that exists mostly at the expense of bacterial chemosynthesis and is similar in structure and functional organization to the oceanic siping-type communities was a striking scientific event [23]. Apart from an extremely high number of other benthic invertebrates (sponges, amphipoda, planaria, oligochaete) and fish (golomyankas, sculpins), the large continuous areas were occupied with *S. flavodentata* larvae, whose population density was as high as 20–30 specimen per square decimeter [24]. This population was extensively studied in 1991 through 2003 with regard to the state of its karyological composition. The life cycle of most *S. flavodentata* larvae from this biotope is two years, whereas that of the others reaches even three years [25]. Therefore, about seven generations have elapsed from the beginning of our studies to the present time.

MATERIALS AND METHODS

The four-instar larvae examined in this study were collected in 1991 through 2003 in Frolikh Bay of Lake Baikal in the hydrotherm underwater region at a depth of 420–430 m. In 1991, the material was collected by man-operated submersible *Pisces* during a joint research expedition of the Institute of Oceanology and the Limnologic Institute, Russian Academy of Sciences. In 1994, 1999, and 2003, the material was collected by an Ocean dredger and in 1996, by a clam-shell dredger.

The procedure used to obtain the karyologic preparations of Baikal *Sergentia* as well as the principles of chromosome mapping have been previously described in detail [19]. The band sequence designation first gives the species abbreviation (fla) followed by the chromosome arm and the sequence number. In zygotes, standard sequences and inverted sequences of large chromosomes that contain only one inversion are designated 1 and 2, respectively; the inverted sequences of the short G arm that displayed three homologous variants are denoted as 2 and 3. The following parameters were studied to determine the dynamics of the inversion polymorphism: the total number of heterozygous individuals in a population; the number of inversions per individual; the frequency of band sequences in chromosome arms. The equilibrium of frequencies was recorded using χ^2 test [26].

RESULTS AND DISCUSSION

As noted above, the Baikal endemic chironomids of the genus *Sergentia* display high banding homology in all chromosome arms: three species were homosequential, of which one (*S. rara*) was chosen as a standard for chromosome mapping [17, 19]. The karyotype of homozygous *S. flavodentata* individuals from Frolikh Bay, $2n = 8$, is shared by those from the other Baikal regions (Fig. 1). The clear-cut chromosome banding patterns were observed. The nucleolar organizers were in chromosomes I and IV (regions 16 of arm B and region 1 of arm G, respectively). Unlike the standard, *S. flavodentata* has two homozygous inversions in arms A and E (in regions 6–9 and 3–7, respectively).

Chromosomal polymorphism of the population examined was different from that of other Baikal chironomid populations of the genera *Sergentia* and *Chironomus*, which have been so far studied in most detail. The main distinguishing feature is a wide spectrum of intrachromosomal rearrangements (Table 1). Unlike *S. nebulosa* and *S. flavodentata* from the Middle and Southern Baikal, which mostly contain two types of inversion sequences, the population from hydrothermal venting region contained six variants of chromosome structural rearrangements. These are five heterozygous paracentric inversions in five chromosome arms (band sequences IA (9–6): flaA2, IB (16–24): flaB2, IIC (2–9): flaC2, IID (16–23): flaD2, IIIF (11–19): flaF2) and heterozygosity for the centromere and nucleolar organizer that are localized terminally and alongside on chromosome IVG (Fig. 2). In the latter case, two types of homologs are observed: a homolog lacking nucleolus but containing centromere (flaG2) and a homolog with nucleolus but without centromere (flaG3). In addition, no pairing between homologs sometimes up to the seventh region occurred in heterozygotes carrying simultaneously two new sequences constituting a new genotypic combination flaG2.3. We suggest that a heterozygous inversion is located in region 3, the boundaries of which are not clearly defined so far.

Note that in the genus *Chironomus* populations inhabiting Olkhon Island (*C. plumosus*) and the littoral zone of Baikal (*C. entis* in Chivyrkuy Bay and Rangatui Lake-Sor, *C. agilis* in Proval Bay, Posolsky and Istoksy litters), the number of heterozygous inversions was as low as two to four though these populations differed in the level of chromosomal polymorphism (0.30–1.63 inversions per individual) [13]. Even in the population from Proval Bay with the highest index of inversions (1.63 per individual), which was the most saturated with chromosome rearrangements and contained only heterozygous larvae, the number of heterozygous inversion types was as low as four.

Chromosomal polymorphism of *S. flavodentata* population from the hydrovent community is unique because six out of seven sequences found in heterozygous larvae (flaA2, flaB2, flaC2, flaD2, flaG2, flaG3) were absent in other populations. Only one sequence,

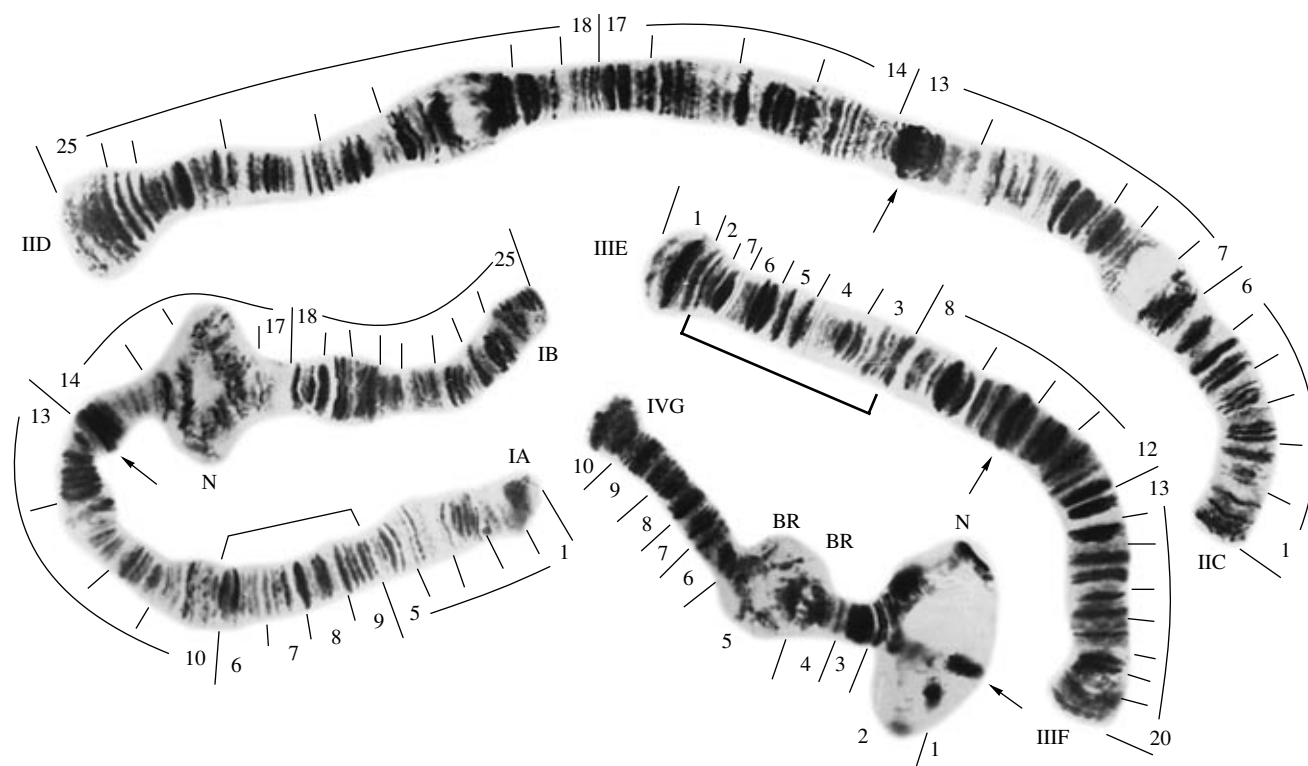


Fig. 1. Karyotype of *Sergentia flavodentata* from Frolikh Bay. IA, IB, IIC, IID, IIIE, IIIF, and IVG, chromosome arms; N, nucleolar organizer; BR, Baltiani ring; arrows indicate centromeres; the regions with homozygous inversions are set out in bold.

flaF2, was the most frequently recorded in other Baikal regions during almost all years of observation, whereas the rare flaD2 sequence was not recorded in 1994, 1996, and 1999, presumably because of the small sample size. In addition, the karyotype of the studied population was saturated with inversion sequences. The number of heterozygotes with inversions was as high as 35–67%, whereas in *S. flavodentata* from the Middle and Southern Baikal, only 20 to 50% of the total num-

ber of larvae were heterozygous. As judged from the largest samples (1991 and 2003), the level of chromosomal polymorphism remained unchanged and about a half of the population, 44 and 49% of larvae, contained chromosomal rearrangements. Similarly, the number of inversions per individual ranged from 0.61 to 0.70 in all years except 1996. At the same time, their relative content varied in the population as can be seen from the percentages of certain genotypic combinations. Thus,

Table 1. Chromosomal polymorphism of *S. flavodentata* population

Genotypic combination	1991 (<i>N</i> = 70)		1994 (<i>N</i> = 20)		1996 (<i>N</i> = 14)		1999 (<i>N</i> = 12)		2003 (<i>N</i> = 73)	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
flaA1.2	5	11.6	1	7.1	1	14.3	1	12.5	2	4.1
flaB1.2	5	11.6	2	14.3	1	14.3	3	37.5	17	34.7
flaC1.2	5	11.6	2	14.3	1	14.3	1	12.5	6	12.2
flaD1.2	2	4.7	–	–	–	–	–	–	4	8.2
flaF1.2	19	44.2	5	35.7	3	42.9	2	25.0	17	34.7
flaG2.3	7	16.3	4	28.6	1	14.3	1	12.5	3	6.1
Total number of inversions	43		14		7		8		49	
Number of heterozygotes with inversions, %	44.3		35.0		35.7		66.7		49.3	
Number of inversions per individual	0.61		0.70		0.50		0.67		0.67	

Note: *N*, the number of examined larvae, *n*, the number of heterozygous individuals with inversions.

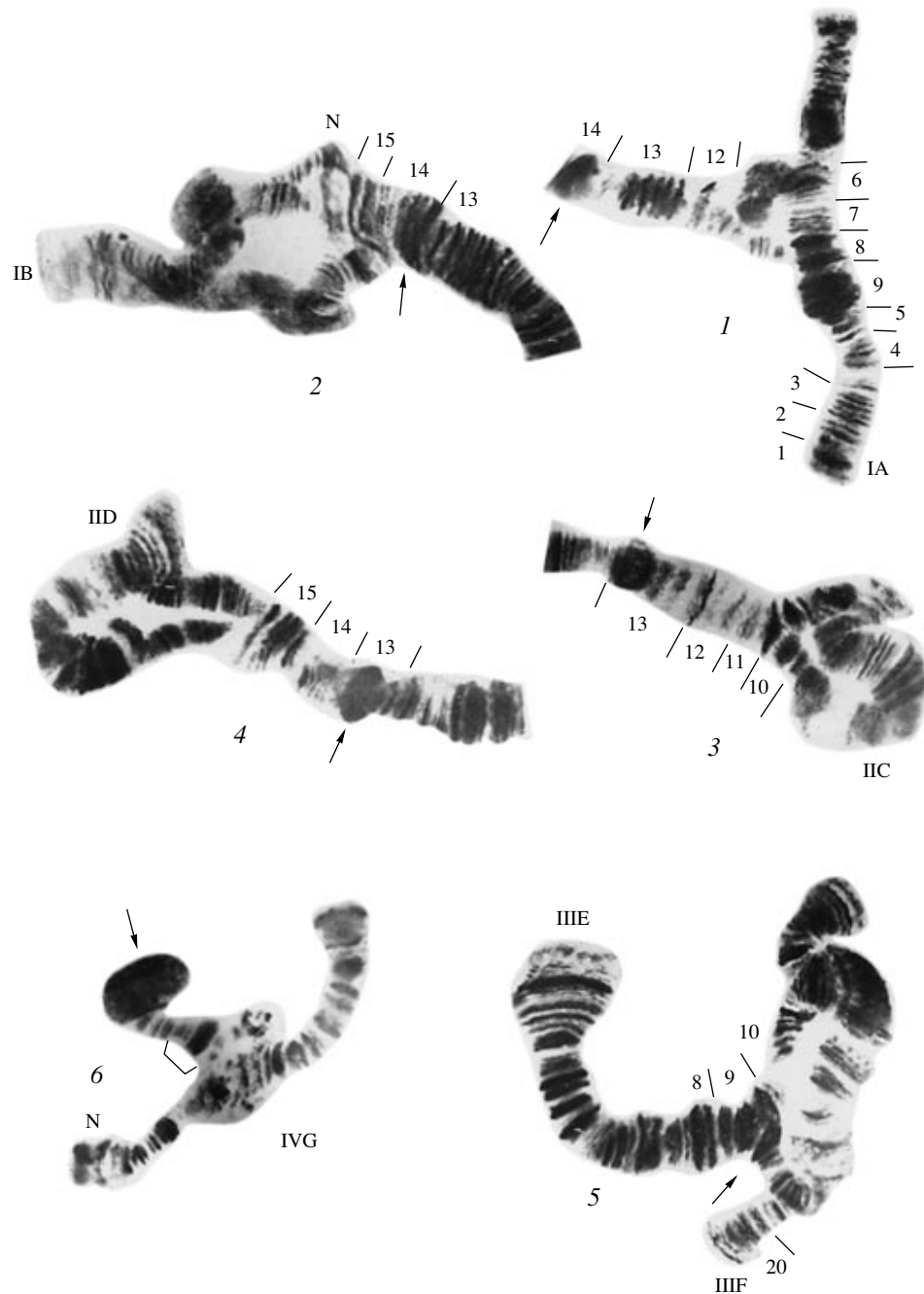


Fig. 2. Heterozygous inversions in *Sergentia flavodentata* from Frolikh Bay. 1, flaA1.2; 2, flaB1.2; 3, flaC1.2; 4, flaD1.2; 5, flaF1.2; 6, flaG2.3. Symbols are the same as in Fig. 1.

the number of heterozygotes with flaG2.3 sequences decreased almost five times from 1994 to 2003; conversely, the number of flaB1.2 heterozygotes increased three times in the recent years. The proportion of genotypic combinations flaC1.2 and flaF1.2 remained almost unchanged, whereas the relative number of flaA1.2 heterozygotes either increased or decreased with time. Thus, although the frequencies of different genotypes varied within a population, a certain level of chromosomal polymorphism reached under the con-

crete conditions of the population existence was maintained. In the studied population, 14 variants of heterozygotes carried from one to four inversion sequences (Table 2), among which heterozygotes with one and two inversions were the most frequent, whereas only one larva contained four inversions. In the large samples of 1991 and 2003, about one-third of heterozygous larvae (29 and 28%, respectively) carried a combination of several inversions, which is presumably of adaptive significance. Of interest is the fact that in the

Table 2. Genotypic diversity in *S. flavodentata* population

Year of experiment (sample size)	Number of inversion sequence (IS) variants and the number of heterozygotes with inversions							
	IS variants	variants of heterozygotes with IS	heterozygotes				total number of heterozygotes with IS	heterozygotes with IS combinations*
			with 1 IS	with 2 IS	with 3 IS	with 4 IS		
1991 (70)	6	14	22	6	3	–	31	29.0
1994 (20)	5	6	3	2	1	1	7	57.1
1996 (14)	5	5	3	2	–	–	5	40.0
1999 (12)	5	6	7	1	–	–	8	12.5
2003 (73)	6	13	26	8	2	–	36	27.8

* From total number of heterozygotes.

smaller samples of 1994 and 1996, where the lower number of heterozygotes (35 and 36%) was recorded, the number of inversion combinations was even higher, 57 and 40%.

The combinations of inversion sequences identified in heterozygous individuals are shown in Table 3. The table shows that there was no definite trend in inversion combinations in heterozygotes. The combinations of inversion sequences were individual and apparently random. Analysis of the band sequence frequencies

showed that throughout the observation period, the Hardy–Weinberg equilibrium was maintained, and no significant difference was found between the observed and expected genotype frequencies ($P < 0.95$, Table 4). Thus, no significant increase in frequencies of the standard (*flaA1*, *flaF1*, *flaG1*) and inversion sequences (*flaB2*, *flaC2*, *flaD2*) occurred with time, which suggests no changes in the population genetic structure and the ratio between frequencies of different sequences remained stable in generations.

Thus, cytogenetic features of *S. flavodentata* from Frolikh Bay suggest that the unique and stable chromosomal polymorphisms of this population as well as high larval density are not accidental. These features presumably ensure successful population adaptation to specific conditions of the community of organisms in the region of underwater hydrothermal venting. Our data support an earlier suggestion that the regions like Frolikha Bay hydrovent were extremely important for preserving unique fauna complexes in Baikal with its long history (over 20 Myr) and unfavorable climatic periods [27]. These hydroventing regions with the communities only slightly depending on photosynthesis, whose metabolism is based on methane biogenesis, might serve as specific refugiums acting as sources of species radiation under favorable climatic conditions.

Karyologic analysis shows that exactly *S. flavodentata* has played an important role in the evolution of Baikalian *Sergentia*. In the hypothetic scheme of karyotype evolution, this species have a special place [17]. Having only two fixed inversions it is the nearest to the narrow-adapted homosequential species *S. rara*, *S. affinis*, and *S. rynocephala* inhabiting the Baikal littoral. The remaining species like those from the deep zones (*S. nebulosa*, *S. assimilis*) and *S. baicalensis* widely occurring in the littoral, preserved inversions that were first found in *S. flavodentata* along with the species-specific inversions. Hence, due to high genetic plasticity of *S. flavodentata* and other physiological proper-

Table 3. Combinations of zygotic inversion sequences in *S. flavodentata* population in 1991 and 2003

Band sequence	Inversion sequence					
	flaA2	flaB2	flaC2	flaD2	flaF2	flaG2, flaG3
flaA1	2/1					
flaB1		3/11				
flaC1			1/3			
flaD1				2/0		
flaF1					13/10	
flaG1						1/0
flaA2			1/0	0/1	1/0	
flaB2				0/1	1/3	0/1
flaC2					0/1	1/1
flaD2					0/1	
flaF2						2/0
flaA2, flaC2						1/0
flaB2, flaF2						1/1
flaC2, flaF2				0/1		1/0

Note: The first figure, 1991; the second figure, 2003.

Table 4. The frequencies of band sequences and heterozygosity in the *S. flavodentata* population

Band sequence	1991	1994	1996	1999	2003
flaA1	0.964	0.975	0.964	0.958	0.986
flaA2	0.036	0.025	0.036	0.042	0.014
H_{ob}	0.071	0.050	0.071	0.083	0.028
H_{ex}	0.069	0.049	0.069	0.080	0.027
flaB1	0.964	0.950	0.964	0.875	0.884
flaB2	0.036	0.050	0.036	0.125	0.116
H_{ob}	0.071	0.100	0.071	0.250	0.233
H_{ex}	0.069	0.095	0.069	0.219	0.206
flaC1	0.964	0.950	0.964	0.958	0.959
flaC2	0.036	0.050	0.036	0.042	0.041
H_{ob}	0.071	0.100	0.071	0.083	0.082
H_{ex}	0.069	0.095	0.069	0.080	0.079
flaD1	0.986	1.000	1.000	1.000	0.973
flaD2	0.014	0	0	0	0.027
H_{ob}	0.029	0	0	0	0.055
H_{ex}	0.028	0	0	0	0.053
flaE1	1.000	1.000	1.000	1.000	1.000
H_{ob}	0	0	0	0	0
H_{ex}	0	0	0	0	0
flaF1	0.864	0.875	0.893	0.917	0.884
flaF2	0.136	0.125	0.107	0.083	0.116
H_{ob}	0.271	0.250	0.214	0.167	0.233
H_{ex}	0.235	0.219	0.191	0.152	0.206
flaG1	0.900	0.900	0.964	0.958	0.979
flaG2	0.050	0.050	0.018	0.021	0.011
flaG3	0.050	0.050	0.018	0.021	0.010
H_{ob}	0.095	0.200	0.071	0.083	0.041
H_{ex}	0.095	0.180	0.069	0.080	0.040

Note: H_{ob} , observed heterozygosity; H_{ex} , expected heterozygosity.

ties, it might be the species that has initiated *Sergentia* speciation as well as their colonization of various depths and biotopes of Baikal.

ACKNOWLEDGMENTS

This study was conducted on the material collected with the assistance of our colleagues from the Limnological Institute. We are grateful to N.G. Sheveleva, O.A. Timoshkin, O.A. Novikova, T.I. Zemskeya, and I.G. Nikulina for providing larvae. Our special gratitude is to B. Goddeeris (Belgium) for organization of expeditions to Baikal in 1994–1996 supported by the

INTAS-94-4465 grant. We are grateful to I.I. Kiknadze (Institute of Cytology and Genetics, Russian Academy of Science) for discussion of our study and valuable comments.

This study was supported by the programs “Biodiversity and Origin of the Baikal Fauna” and “The Study of Biogeochemical Processes in Baikal Littoral: Benthic Biodiversity, Hydrobiont Association with Minerals, Mechanisms of Biodestruction, Key Benthic Communities and Their Interaction with the Environment” (headed by O.A. Timoshkin), and the Program 13.19 of the Presidium of Russian Academy of Sciences (headed by M.A. Grachev and G.A. Zavarzin).

REFERENCES

1. Belyanina, S.I., Karyotypic Analysis of Chironomids (Chironomidae, Diptera) from Fauna of the Soviet Union, *Doctoral (Biol.) Dissertation*, Moscow: Inst. Evol. Morphol. Ecol. Anim., 1983.
2. *Motyl' Chironomus plumosus L. (Diptera, Chironomidae): sistematika, morfologiya, ekologiya, produktiya (Chironomus plumosus L. (Diptera, Chironomidae): Systematics, Morphology, Ecology, Production)*, Moscow: Nauka, 1983.
3. Petrova, N.A., Characterization of the Karyotype in Chironomids (Diptera, Chironomidae) from Global Fauna: II. Subfamily Chironominae, *Entomol. Obozr.*, 1990, vol. 69, no. 1, pp. 193–215.
4. Petrova, N.A., Chromosome Rearrangements in Three Chironomid (Diptera, Chironomidae) Species from the Chernobyl Region, *Genetika* (Moscow), 1991, vol. 27, no. 5, pp. 836–848.
5. Petrova, N.A., Il'inskaya, N.B., and Kaidanov, L.Z., Adaptiveness of Inversion Polymorphism in *Chironomus plumosus* (Diptera, Chironomidae): Spatial Distribution of Inversions over Species Range, *Rus. J. Genet.*, 1996, vol. 32, no. 12, pp. 1417–1430.
6. Gunderina, L.I., Kiknadze, I.I., Aimanova, K.G., *et al.*, Cytogenetic Differentiation of Natural and Laboratory Populations of *Camptochironomus tentans* (Fabricius) (Diptera, Chironomidae), *Rus. J. Genet.*, 1996, vol. 32, no. 1, pp. 45–57.
7. Kiknadze, I.I., Istomina, A.G., Gunderina, L.I., *et al.*, *Kariofondy khironomid kriolitozony Yakutii* (Karyotype Pools of Chironomids from the Cryozone of Yakutia), Novosibirsk: Nauka, 1996.
8. Il'inskaya, N.B., Petrova, N.A., and Matena, I., The Relationship between the Level of Inversion Polymorphism and the Type of Water Body, the Season, and the Year Observation in *Chironomus plumosus* L. (Diptera, Chironomidae), *Rus. J. Genet.*, 1999, vol. 35, no. 8, pp. 908–917.
9. Kiknadze, I.I. and Istomina, A.G., Karyotypes and Chromosome Polymorphism of Siberian Species of Chironomids (Diptera, Chironomidae), *Sib. Ekol. Zh.*, 2000, vol. 7, no. 4, pp. 445–460.
10. Aimanova, K.G., Kiknadze, I.I., Andreeva, E.N., and Seisebaev, A.T., Cytological Identification of Chironomid Species from Water Bodies of the Former Semipal-

- atinsk Testing Site, *Sib. Ekol. Zh.*, 2000, vol. 7, no. 4, pp. 503–509.
11. Miseiko, G.N., Zoocenoses in the System of Diagnostic Monitoring of the Ecological State of Various Water Bodies of Southwestern Siberia, *Doctoral (Biol.) Dissertation*, Ul'yanovsk: Ul'yanovsk. State Univ., 2004.
 12. Proviz, V.I., Specific Structural Features of Polytene Chromosomes in *Chironomus plumosus* L. Larvae from Posol'skii Sor, in *Krugovorot veshchestva i energii v vodoemakh* (Turnover of Matter and Energy in Water Bodies), Irkutsk, 1981, issue 1, pp. 157–158.
 13. Proviz, V.I., Ecological, Morphological, and Karyotypic Characterization of *Chironomus plumosus* L. (Diptera, Chironomidae) from the Coastal Zone of the Baikal Lake, *Cand. Sci. (Biol.) Dissertation*, Irkutsk: Irkutsk. State Univ., 1988.
 14. Linevich, A.A., *Khironomidy Baikala i Pribaikal'ya* (Chironomids of Baikal and Adjacent Regions), Novosibirsk: Nauka, 1981.
 15. Takhteev, V.V., Snimshchikova, L.N., Okuneva, G.L., *et al.*, Characterization of Benthic Populations from Deep Regions of the Baikal Lake, *Ekologiya*, 1993, no. 6, pp. 60–68.
 16. Papousheva, E., Proviz, V., Lambkin, C., *et al.*, Phylogeny of the Endemic Baikalian *Sergentia* (Chironomidae, Diptera), *Mol. Phylog. Evol.*, 2003, vol. 29, pp. 120–125.
 17. Proviz, V.I. and Proviz, L.I., Structure and Evolution of the Karyotype in a Group of Endemic Chironomid Species of the Genus *Sergentia* (Diptera, Chironomidae) from the Baikal Lake, *Zool. Zh.*, 1992, vol. 71, no. 6, pp. 60–70.
 18. Proviz, V.I., Chironomids (Diptera: Chironomidae), in *Annotirovannye spiski vidov fauny ozera Baikal i ego vodosbornogo basseina* (Annotated Lists of Species from Fauna of the Baikal Lake and Its Drainage Area), vol. 1: *Ozero Baikal* (Baikal Lake), Novosibirsk: Nauka, 2001, pp. 878–924.
 19. Proviz, V.I. and Proviz, L.I., *Atlas i opredelitel' lichinok khironomid roda Sergentia iz ozera Baikal* (Guide and Key to Chironomid Larvae of the Genus *Sergentia* from the Baikal Lake), Novosibirsk: OIGGM, 1999.
 20. Namsaraev, B.B., Zemskaya, T.I., Dagurova, O.P., *et al.*, Biological Communities in the Sediments: Regions of Hydrothermal Venting (Northern Baikal) and Near-Surface Occurrence of Gas Hydrates (Southern Baikal), *VI Int. Conf. of Gas in Marine Sediments: Abstracts Book*, St. Petersburg, 2000, pp. 102–103.
 21. Takhteev, V.V., Okuneva, G.L., Proviz, V.I., *et al.*, To the Characterization of Benthic Fauna and Zoobenthic Communities of Northern Baikal in the Region of the Baikal–Lena Nature Reserve, in *Issledovaniya vodnykh ekosistem Vostochnoi Sibiri: Bioraznoobrazie Baikalskogo regiona* (Studies of Water Ecosystems of Eastern Siberia: Biodiversity of the Baikal Region), Irkutsk: Irkutsk. Gos. Univ., 2000, issue 3, pp. 21–41.
 22. Golubev, V.A., Heat Flow through the Baikal Valley, *Dokl. Akad. Nauk SSSR*, 1979, vol. 245, no. 6, pp. 1333–1336.
 23. Kuznetsov, A.P., Strizhov, V.P., Kuzin, V.S., *et al.*, New Findings in the Nature of Baikal: A Community Based on Bacterial Chemosynthesis, *Izv. Akad. Nauk SSSR, Ser. Biol.*, 1991, vol. 5, pp. 766–772.
 24. Namsaraev, B.B. and Zemskaya, T.I., *Mikrobiologicheskie protsessy krugovorota ughleroda v donnykh osadkakh ozera Baikal* (Microbiological Processes of Carbon Turnover in Benthic Sediments of the Baikal Lake), Novosibirsk: Sib. Otd. Ross. Akad. Nauk, 2000.
 25. Goddeeris, B., Proviz, V., and Blinov, A., Life Cycle Characteristics of *Sergentia flavodentata* Chernovskij (Diptera, Chironomidae) in Lake Baikal, *Abstracts 13th Int. Symp. On Chironomidae*, Freiburg, 1997, p. 37.
 26. Lakin, G.F., *Biometriya* (Biometrics), Moscow: Vysshaya Shkola, 1968.
 27. Grachev, M., Fialkov, V., Nakamura, T., *et al.*, Extant Fauna on Ancient Carbon, *Nature*, 1995, vol. 374, pp. 123–124.