

## Karyotypic Features of *Chironomus entis* and *Chironomus borokensis* (Diptera, Chironomidae) from Lake Kotokel (Lake Baikal Basin)

V. I. Proviz<sup>a</sup> and N. V. Bazova<sup>b</sup>

<sup>a</sup>Limnological Institute, Siberian Branch, Russian Academy of Sciences, Irkutsk, 664033 Russia

<sup>b</sup>Institute of General and Experimental Biology, Siberian Branch, Russian Academy of Sciences, Ulan-Ude, 670047 Russia  
e-mail: proviz@lin.irk.ru

Received March 15, 2011

**Abstract**—Karyotypes and chromosomal polymorphism of sympatric sibling species *Chironomus entis* and *Chironomus borokensis* from Lake Kotokel were studied. Five inversion banding sequences were found in *C. entis*, including p'entA16, p'entB9, and p'entF6 that are new for the species; in *C. borokensis*, four inversion banding sequences were revealed, borA4 being new for the species. The level of chromosome polymorphism in *C. entis* and *C. borokensis* can be considered similar: 54.2 and 53.8% heterozygosity, 0.67 and 0.73 inversions per individual, respectively. The new data are compared with those on *C. entis* population from Lake Kotokel obtained in 1981 and those of *C. borokensis* from Lake Dukhovoe (Lake Baikal basin) obtained in 1983.

**DOI:** 10.1134/S0013873813010065

*Chironomus entis* Shobanov, 1989 and *Chironomus borokensis* Kerkis, Filippova, Shobanov, Gunderina et Kiknadze, 1988 (Diptera, Chironomidae) are well-studied sibling species from the group *plumosus* which now includes 14 species (Golygina et al., 2007). At the larval stage the species differ in the degree of development of ventral processes on abdominal segment VIII, the ventral coloration of the head capsule, the morphology of denticles on the mentum, and the shape and proportions of antennal segments (Shobanov, 1989, 2000); however, their reliable identification requires examination of species-specific characters of the polytene chromosomes from their salivary glands. *C. entis* has a vast geographic range. Its karyotypes and chromosome polymorphism have been studied in many Palaearctic and Nearctic (North American) populations, revealing specific banding sequences and specific regional traits in karyotype divergence (Demin and Shobanov, 1990; Belyanina et al., 1992; Kiknadze et al., 1991, 1996, 2000, 2004; Golygina, 1999; Kiknadze and Istomina, 2000; Petrova and Klishko, 2005; Golygina et al., 2007). *C. borokensis* is presently known only from Russia, where it occurs in Yaroslavl, Novosibirsk, Irkutsk, Chita Provinces, Khabarovsk and Primorye Territories, and Yakutia; populations from different parts of its range also demonstrate specificity of karyotype structure and chromosome polymorphism (Kerkis et al., 1988; Kiknadze

et al., 1991, 1996, 2004; Proviz, 1993; Kiknadze and Istomina, 2000; Siirin et al., 2003; Petrova and Klishko, 2005; Golygina et al., 2003, 2007).

Karyological studies of chironomid midges in the Baikal region were started by Bukhteeva (1974) who described the karyotype and inversion polymorphism of *Chironomus plumosus* from Iskrinskii pond in the environs of Irkutsk. This population consisted of the larval form *C. plumosus* f. l. *semireductus*; in the recent taxonomy, this form corresponds to the distinct species *C. entis* (Shobanov, 1989). In our previous research carried out in the 1980s in some reservoirs of the Baikal region and in the shallow nearshore zone of Lake Baikal proper, the species *C. entis* (Lake Kotokel, Chivyrkui Bay of Baikal, Lake Rangatui) and *C. borokensis* (Dukhovoe and Shantalyk lakes) were also differentiated by their larvae, as *C. plumosus* f. l. *semireductus* and *C. plumosus* f. l. *plumosus* (Linevich and Proviz, 1987; Proviz, 1988, 1989). Earlier studies of East Siberian waters showed that in most cases, only one species of the group *plumosus* was found in each of the typical habitats, this situation being stable for many years in large eutrophic lakes with a distinct profundal zone. In particular, only *C. plumosus* f. l. *semireductus* (= *C. entis*) was recorded in 1935–1981 in Lake Kotokel, the largest lake in the Baikal shore area (70 km<sup>2</sup>). Examination of a smaller Lake Dukho-

voe (6.16 km<sup>2</sup>), also located on the east Baikal shore, revealed only *C. plumosus* f.l. *semireductus* (= *C. entis*) in 1932–1937, and only *C. plumosus* f.l. *plumosus* (= *C. borokensis*) in 1983 (Linevich, 1981; Linevich and Proviz, 1987).

In 2008, Lake Kotokel attracted the attention of researchers due to cases of human and animal poisoning with fish from this lake. Hydrobiological studies demonstrated prolonged depression of the bottom biocenoses of the lake, which was first noticed in the 1990–2000s (Sokolova, 2001). Studies carried out in 2007–2008 showed that the depression continued while the diversity and quantitative parameters of the zoobenthos also declined (Bazova et al., 2010). The lake was described as a hypertrophic reservoir at the extreme dystrophic stage.

One of the possible factors which determined the catastrophic changes in the lake ecosystem was the presence of the introduced waterweed *Elodea canadensis* Michx which appeared in the lake in 1986, proliferated greatly in 1992, and died off en masse in 2000. According to the recent studies, the phytoplankton species composition shows clear signs of eutrophication, which is largely manifested in mass development of cyanobacteria, including toxic ones of the genus *Microcystis* (Belykh et al., 2010, 2011). Similar conclusions were made based on most parameters of the zooplankton (Sheveleva and Krivenkova, 2010).

Studies of the chironomid species composition have also revealed some differences during the period in question. Besides *C. entis*, one more dominant species of the *plumosus* group, *C. borokensis*, has appeared in Lake Kotokel. Now the two species occur sympatrically in black and gray clay-sand bottom areas. Morphologically, their larvae can be easily distinguished by the length of the ventral processes, allowing their relative abundance (%) to be determined. Our analysis of quantitative samples of zoobenthos taken in 2009 showed *C. entis* to be the dominant species in the central part of the lake. On average for the entire season, this species outnumbered *C. borokensis* at the depths 4.0–4.4 m, comprising about 70% of the total abundance. The fraction of *C. entis* was still greater at a depth of 14 m, reaching 96%. On the contrary, larvae of *C. borokensis* prevailed in the southern hollow of the lake at the depth 4.1 m, comprising 65%.

This communication reports the results of comparative karyological analysis of *C. entis* and *C. borokensis* populations from Lake Kotokel during the period of

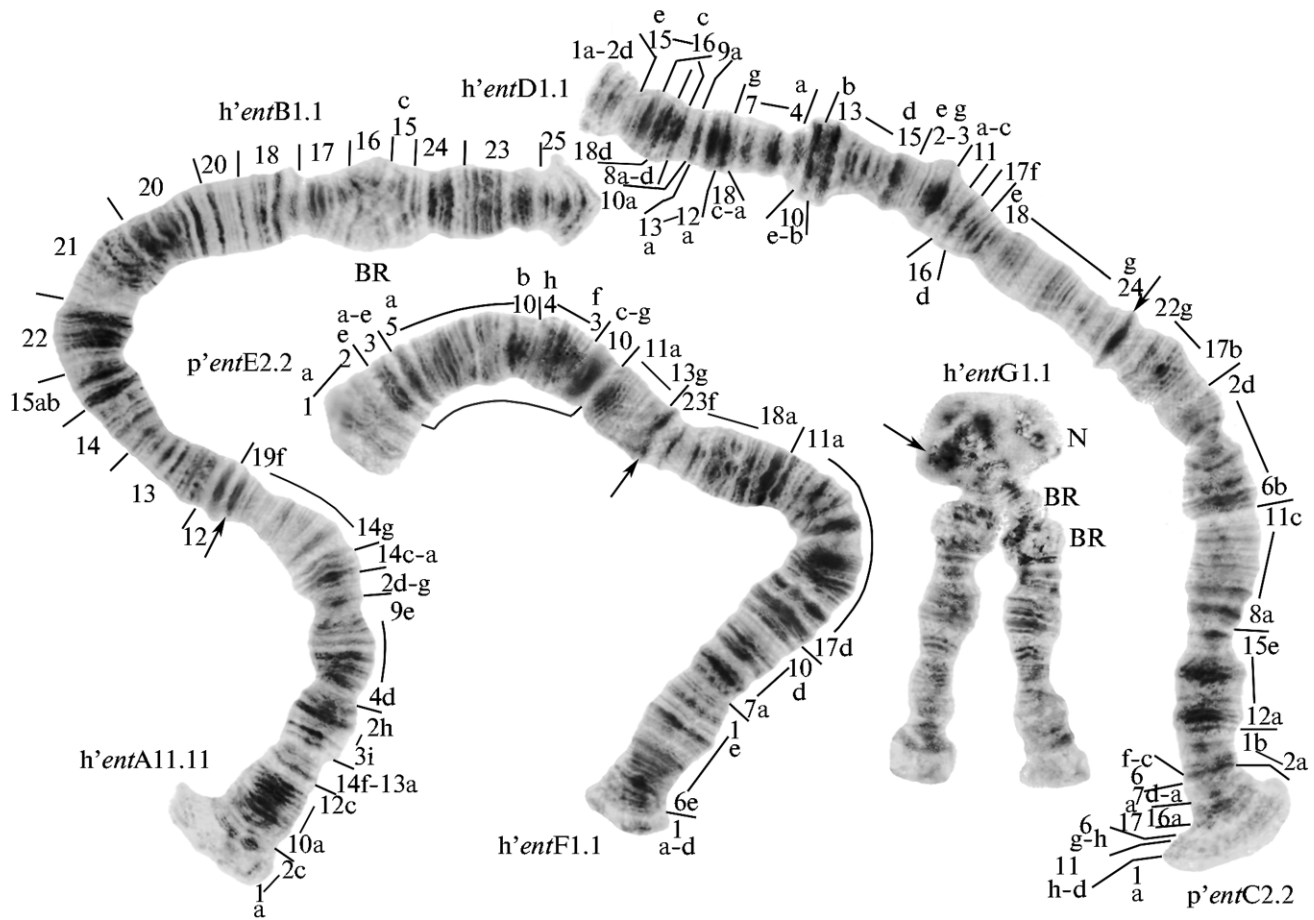
drastic changes in the lake ecosystem, and also describes their karyological specificity in the Baikal region in the 1980s and at present.

## MATERIALS AND METHODS

Our material included IV instar larvae of *C. entis* (1981, 2009) and *C. borokensis* (2009) collected in Lake Kotokel from depths of 4–5 m: 47 larvae of *C. entis* in 1981, 24 larvae of *C. entis* and 26 larvae of *C. borokensis* in 2009. For comparison we also used the data of 1983 on 30 larvae of *C. borokensis* collected in Lake Dukhovoe from the depths 2–3 m (Proviz, 1989). The larvae were fixed in the mixture of 96% ethanol and glacial acetic acid (3 : 1). The preparations were made using the ethyl-orcein technique (Demin and Il'inskaya, 1988; Demin and Shobanov, 1990). The polytene chromosomes were documented at 90× lens magnification using the MKU-1 microfilm attachment (in 1981–1983) or at 100× lens magnification using the Carl Zeiss Axiostar Plus microscope (The Electron Microscopy Equipment Center, the Limnological Institute of the Siberian Branch of the Russian Academy of Sciences) and AxioVision Rel. 4.7.1 software. Chromosome arms A, C, D, E, and F of *C. entis* were mapped following the *piger* standard (Keyl, 1962; Devai et al., 1989), and arm B, after Maksimova (1976). The banding sequences are designated as follows: the first symbol refers to the geographic distribution (p' for the Palaearctic, n' for the Nearctic, h' for the Holarctic) (Golygina, 1999); it is followed by the abbreviated species name (*ent*), the arm (A), and the sequence number, for example, p'entA1 (in the homozygous state, p'entA1.1). Chromosomes of *C. borokensis* were mapped according to the standard of Maksimova (1976); since the range of *C. borokensis* is limited to the Palaearctic, the geographic symbols are not needed. The level of chromosome polymorphism of the populations was estimated by the fraction of heterozygous individuals in the sample (%) and the number of heterozygous inversions per individual.

## RESULTS AND DISCUSSION

**Karyotype of *C. entis*** (Kerkis et al., 1988; Demin and Shobanov, 1990; Kiknadze et al., 1991, 2000; Golygina, 1999).  $2n = 8$  (Fig. 1), chromosome arm combination AB, CD, EF, G (*thummi* cytocomplex). Chromosomes I (AB) and II (CD) are metacentric, chromosome III (EF) is submetacentric, and the short chromosome IV (G) is acrocentric. The nucleolus is



**Fig. 1.** Karyotype of *Chironomus entis* from Lake Kotokel. A, B, C, D, E, F, and G are chromosome arms; 1–25 are chromosome segments; a, b, c, d ... are bands; 1.1, 2.2, 11.11 are banding sequences; N is the nucleolar organizer, BR is the Balbiani ring; arrows mark centromeres, bracket marks the homozygous inversion.

located in arm G near the centromere; there are two Balbiani rings in arm G and one in arm B. The banding sequences in arms A, C, D, E, and F are designated according to Kiknadze and co-authors (2000, 2004), those in arm B, according to Kerkis and co-authors (1988).

Arm A is represented by two sequences in the population examined: h'entA11 and p'entA16, the latter being found for the first time in this species. Sequence A11 was found both in the homozygous state (Fig. 1) and in heterozygotes with A16 (Fig. 2a), while A16 was found only in heterozygotes with A11. Sequence A16, as well as A11, differs from h'entA1 in a simple inversion:

h'entA1 1a-2c 10a-12c 14f-13a 3i-2h 4a-4c 2g-2d 9e-4d 14g-16d 17a-19f

h'entA11 1a-2c 10a-12c 14f-13a 3i-2h 4d-9e 2d-2g 4c-4a 14g-16d 17a-19f

p'entA16 1a-2c 10a-12c 14f-13a 16d-14g 4a-4c 2g-2d 9e-4d 2h-3i 17a-19f

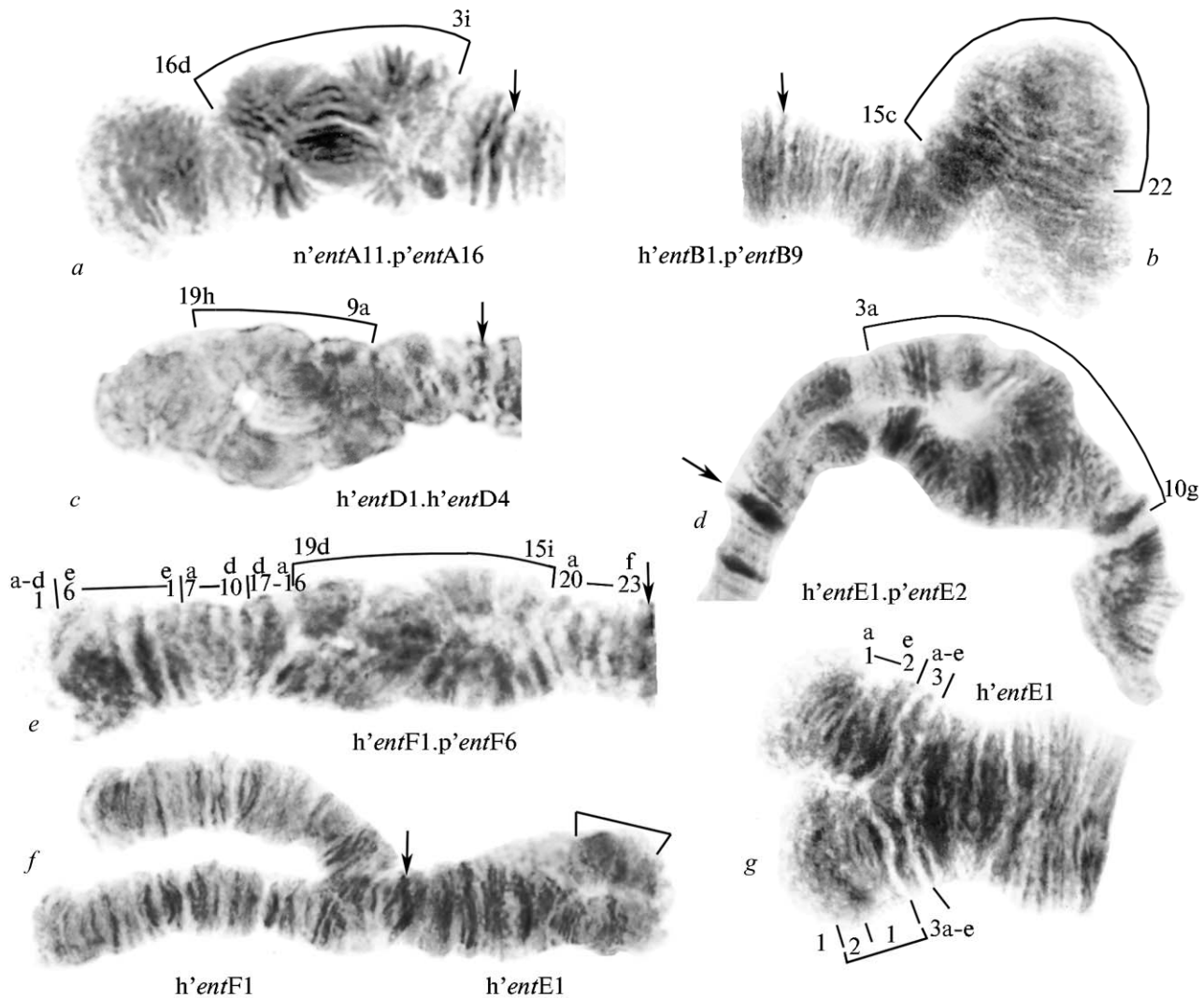
Sequence A11 was originally described from the Nearctic populations (Golygina, 1999) and later discovered in Finland (Michailova, 2001) and in two lakes in Chita Province where it existed only in the homozygous state (Petrova and Klishko, 2005).

Arm B is usually monomorphic; only one larva collected in 1981 had a previously unknown heterozygous inversion with a sequence resembling h'pluB1; here it is designated as p'entB9 (Fig. 2b). Using the mapping system of Maksimova (1976), this sequence can be described as follows:

h'pluB1 12-25

h'entB1 12-15b 22-15c 24 23 25

p'entB9 12-22 24 23 25



**Fig. 2.** Chromosome polymorphism in the population of *Chironomus entis* from Lake Kotokel: heterozygotes *h'entA11.p'entA16* (a), *h'pluB1.p'entB9* (b), *h'entD1.h'entD4* (c), *h'entE1.p'entE1* (d), *h'entF1.p'entF6* (e) and disrupted conjugation of homologs in arms F and E (f, g). Brackets mark the segments with inversions; other designations as in Fig. 1.

Arm C is represented by only one sequence, *p'entC2*, which is common in the Palaearctic populations but has not been found in the Nearctic ones (Golygina, 1999; Kiknadze et al., 2000):

*p'entC2* 1a 11h-11d 6g-6h 17a-16a 7d-7a 6f-6c 2c-1b 12a-15e 8a-11c 6b-2d 17b-22g

Arm D revealed two sequences: *h'entD1* and *h'entD4*; the latter was found only in heterozygotes with D1 (Fig. 2c)<sup>1</sup> and differs from D1 in a simple inversion of the greater part of the arm:

*h'entD1* 1a-2d 15e-16c 18d 8a-8d 9a-10a 13a-12a 18c-18a 7g-4a 10e-10b 13b-15d 2e-3g 11a-11c 16d-17f 18e-18g 19a-19h 20a-24g

*h'entD4* 1a-2d 15e-16c 18d 8a-8d 19h-19a 18g-18e 17f-16d 11c-11a 3g-2e 15d-13b 10b-10e 4a-7g 18a-18c 12a-13a 10a-9a 20a-24g

Sequence D4 was previously known only from the Nearctic and designated as “*n'entD4*” (Kiknadze et al., 2000). The finding of this sequence in East Siberia suggests a Holarctic distribution: *h'entD4*.

Arm E revealed two sequences: *h'entE1* and *p'entE2*. Of these, E1 was found only in heterozygotes E1.2 (Fig. 2d), whereas E2 was found both in hetero- and homozygotes E2.2 (Fig. 1).

<sup>1</sup> Sequence *h'entD4* was found only in one larva characterized by compact chromosomes, hence the indistinct banding pattern of arm D fragment containing the inversion in Fig. 2c.

**Table 1.** Parameters of chromosome polymorphism in the populations of *C. entis* and *C. borokensis* from Kotokel and Dukhovoe lakes

Species, locality, and collection date	n	Heterozygosity, %	Inversions per ind.	Number of heterozygous inversions in chromosome arms, %						
				A	B	C	D	E	F	G
<i>C. entis</i> , Lake Kotokel, 25.V.1981	47	83.3	1.27	60.0	3.3	–	–	63.3	–	–
<i>C. entis</i> , Lake Kotokel, 15.VI.2009	24	54.2	0.67	25.0	–	–	4.2	20.8	4.2	–
<i>C. borokensis</i> , Lake Dukhovoe, 26.V.1983	30	43.3	0.47	16.7	20.0	–	10.0	–	–	–
<i>C. borokensis</i> , Lake Kotokel, 15.VI.2009	26	53.8	0.73	19.2	46.2	–	7.7	–	–	–

**Table 2.** Genomic combinations of banding sequences in the populations of *C. entis* and *C. borokensis* from Kotokel and Dukhovoe lakes

Genomic combinations							Frequency of genomic combinations in different samples, %			
A	B	C	D	E	F	G	1e	2e	1b	2b
11.11	1.1	1.1	1.1	1.2	1.1	1.1	23.3	8.3		
11.11	1.1	1.1	1.1	2.2	1.1	1.1	16.7	45.8		
11.11	1.1	1.1	1.4	2.2	1.1	1.1		4.2		
11.11	1.1	1.1	1.1	2.2	1.6	1.1		4.2		
11.16	1.1	1.1	1.1	1.2	1.1	1.1	40.0			
11.16	1.1	1.1	1.1	2.2	1.1	1.1		25.0		
11.16	1.1	1.1	1.1	1.2	1.1	1.1		12.5		
11.16	1.1	1.1	1.1	2.2	1.1	1.1	16.7			
11.16	1.4	1.1	1.1	2.2	1.1	1.1	3.3			
1.1	1.1	1.1	1.1	1.1	1.1	1.1			56.6	46.2
1.1	1.1	1.1	1.2	1.1	1.1	1.1			6.7	
1.1	1.2	1.1	1.1	1.1	1.1	1.1			16.7	34.6
1.1	1.2	1.1	1.2	1.1	1.1	1.1			3.3	
1.2	1.1	1.1	1.1	1.1	1.1	1.1			10.0	
1.4	1.1	1.1	1.1	1.1	1.1	1.1			6.7	
1.4	1.1	1.1	1.2	1.1	1.1	1.1				7.7
1.4	1.2	1.1	1.1	1.1	1.1	1.1				11.5

Notes: 1e, *C. entis* from Lake Kotokel, 25.V.1981; 2e, *C. entis* from Lake Kotokel, 15.VI.2009; 1b, *C. borokensis* from Lake Dukhovoe, 26.V.1983; 2b, *C. borokensis* from Lake Kotokel, 15.VI.2009.

h'entE1 1a-2e 10g-10c 3f-4h 10b-5a 3e-3a 11a-13g

p'entE2 1a-2e 3a-3e 5a-10b 4h-3f 10c-10g 11a-13g

It should be noted that sequence p'entE2, occurring in many regions of the Palaearctic, was found only in the heterozygous state in Chita Province; this sequence was not found in the Nearctic (Belyanina et al., 1992; Kiknadze et al., 2000; Petrova and Klishko, 2005).

Besides the heterozygous inversions E1.E2, many larvae (one-third of the samples in 2009) revealed disrupted conjugation of homologs at the terminal

segments (Fig. 2g), probably due to an inversion whose exact position remains unknown.

Arm F revealed sequence p'entF6 which was new for the species in question. This sequence, found in heterozygotes F1.F6 (Fig. 2e), differs from F1 in a simple inversion:

h'entF1 1a-1d 6e-1e 7a-10d 17d-16a 15i-11a 18a-19d 20a-23f

p'entF6 1a-1d 6e-1e 7a-10d 17d-16a 19d-18a 11a-15i 20a-23f

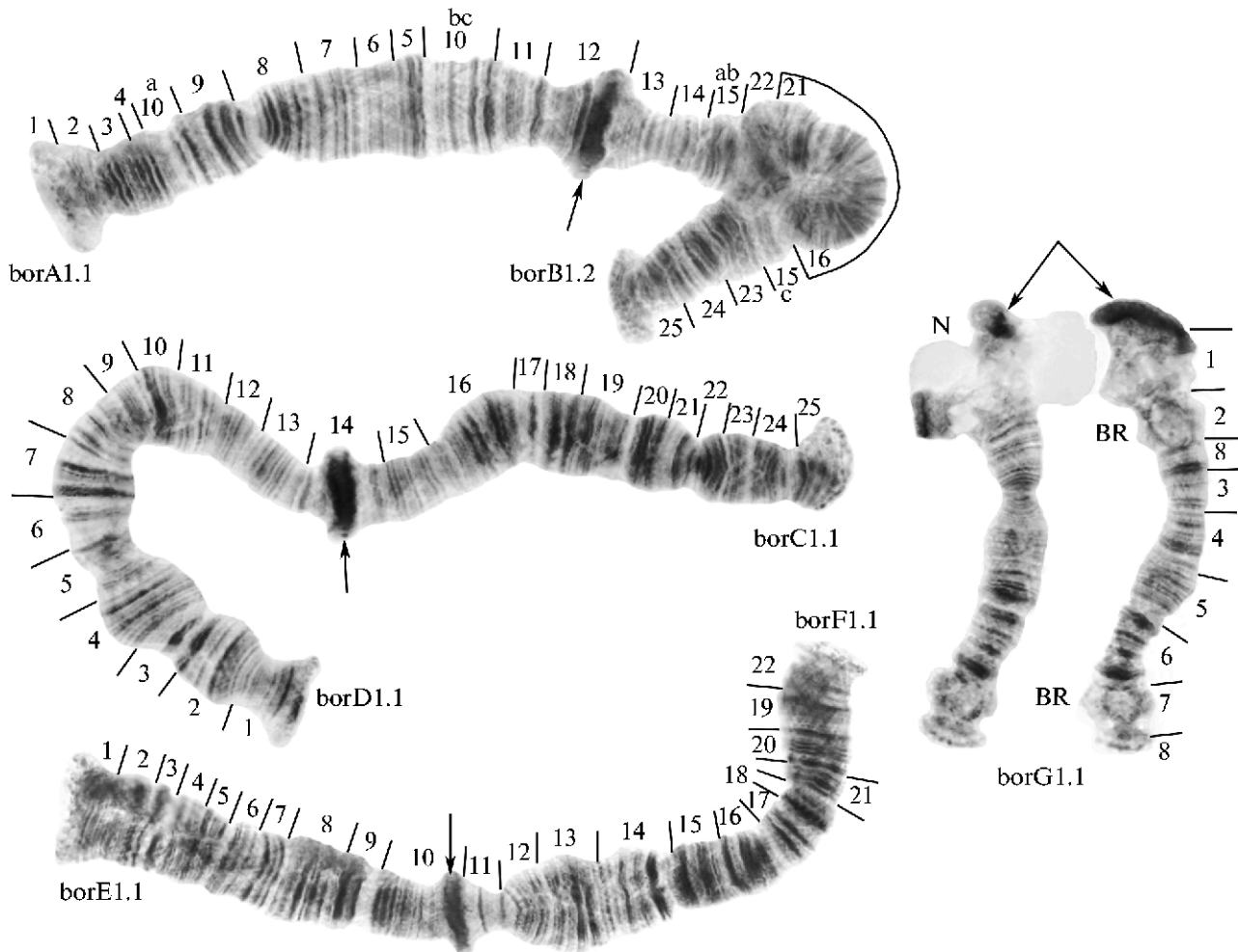


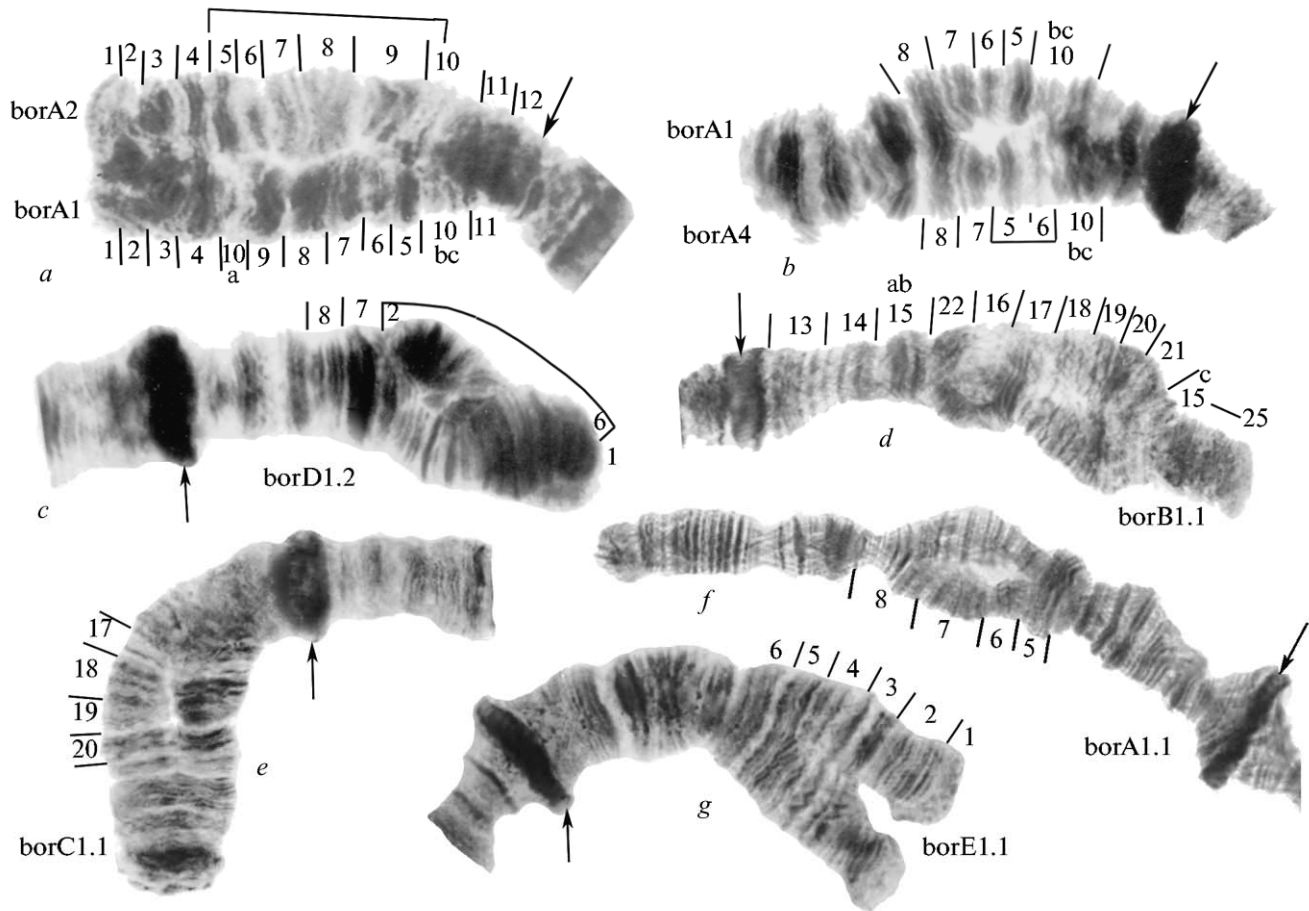
Fig. 3. Karyotype of *Chironomus borokensis* from Lake Kotokel: 1.1, 1.2 are banding sequences of chromosome arms; other designations as in Fig. 1.

In the material of 2009, a single case of mosaicism with respect to conjugation of arm F homologs was observed: in one cell of the salivary gland the homologs were not conjugated along nearly the entire arm (Fig. 2f), whereas in the other nuclei conjugation was normal.

The results of studies of chromosome polymorphism are shown in Tables 1 and 2. Comparative analysis of chromosome polymorphism parameters of *C. entis* from Lake Kotokel reveals both qualitative and quantitative changes in the genetic structure of the population that have occurred since the first karyological examination in 1981. The mean number of inversions per individual has decreased considerably by 2009: from 1.27 to 0.67 (Table 1), due to a smaller number of heterozygous inversions in arms A and E. The general index of heterozygosity has also decreased from 83.3 to 54.2%. The frequency of se-

quence p'entA16, which was found in Lake Kotokel only in the heterozygous state in 1981 and 2009, has decreased nearly twofold: from 60.0 to 25.0%. The frequency of heterozygotes with sequence p'entE2, widespread in the Palearctic, has decreased from 63.3 to 20.8% due to stabilization of this inversion sequence in the homozygous state (Table 2). The increasing frequency of homozygotes entE2.2 indicates that this sequence may have a higher adaptive value during the period in question.

The inversion spectrum observed in 2009 lacked the rare sequence p'entB9 but included two new ones, h'entD4 and p'entF6. The sample of 1981 included 5 genotypic combinations of banding sequences, of which the combination of h'entA11.p'entA16 and p'entE1.2 was the most common (40.0%; see Table 2). The material of 2009 included 6 genotypic combinations, the prevalent ones being h'entA11 and p'entE2.2



**Fig. 4.** Chromosome polymorphism in the populations of *Chironomus borokensis* from Kotokel and Dukhovoe lakes: heterozygotes *borA1.2* (a), *borA1.4* (b), *borD1.2* (c) and disrupted conjugation of homologs in arms B, C, A, and E (d–g). Brackets mark the segments with inversions; other designations as in Fig. 1.

(45.8%), and also *h'entA11.p'entA16* and *p'entE2.2* (25.0%).

In general, the present level of chromosome polymorphism of *C. entis* in the Baikal region resembles that of most Palaearctic and Nearctic populations but differs from that in Lake Shaksha (Chita Province). The latter population is characterized by a high heterozygosity level (100%) and a large mean number of inversions per individual (1.6) (Belyanina et al., 1992; Golygina, 1999; Kiknadze et al., 2000; Petrova and Klishko, 2005).

**Karyotype of *C. borokensis*** (Kerkis et al., 1988; Kiknadze et al., 1991, 1996).  $2n = 8$  (Fig. 3), chromosome arm combination AB, CD, EF, G (thummi cyto-complex). The long chromosomes I (AB) and II (CD) are metacentric, chromosome III (EF) is submetacentric, and the short chromosome IV (G) is telocentric. The centromeres are large and heterochromatic, this

feature differentiating *C. borokensis* from the rest of *Chironomus* species of the group *plumosus*. The nucleolar organizer (N) and two Balbiani rings (BR) are located in arm G, one more BR is located at segment 16 of arm B. The banding sequences are designated according to Kerkis and co-authors (1988).

The population of Lake Kotokel revealed two sequences in arm A: *borA1* in the homozygote A1.1 (Fig. 3) and in the heterozygote A1.4 (Fig. 4b). Three sequences were found in the larvae from Lake Dukhovoe: *borA1* in the homozygote A1.1 and in the heterozygotes A1.2 (Fig. 4a) and A1.4, and also *borA2* and *borA4* which were found only in the heterozygous state.

Sequence *borA2* was found in all the previously studied populations (Kerkis et al., 1988; Kiknadze et al., 1991, 1996; Golygina et al., 2003) except for those from Chita Province (Petrova and Klishko,

2005); sequence *borA4*, involving segments 6, 5, and partly 10, was recorded for the first time. In Lake Ivan (Chita Province), one more sequence, *borA3*, was found in the same arm (Petrova and Klishko, 2005); it differs from *borA1* in a simple inversion:

*borA1* 1-4 10a-5 10b-12

*borA2* 1-12

*borA3* 1-4 10a-7b 10bc 5-7c 11 12

*borA4* 1-4 10a-7 5 6 10bc 11 12

Arm B is represented by sequences *borB1* (Fig. 4d) and *borB2* (Fig. 3), differing in an inversion in the median part of the arm:

*borB1* 25-23 15c 21-16 22 15b-13

*borB2* 25-23 15c 16-21 22 15b-13

The former sequence was recorded in the homozygote B1.1 and in the heterozygote B1.2 in the material from both lakes, the latter, only in the above heterozygote.

Arm D also revealed two sequences *borD1* and *borD2* that occurred in the heterozygote D1.1 and in the heterozygote D1.2 (Fig. 4c).

*borD1* 1-13

*borD2* 1 6-2 7-13

Sequences *borB2* and *borD2* are characterized by the same distribution as *borA2*.

Arms C, E, F, and G in the examined populations were represented by the standard sequences only:

*borC1* 25-14

*borE1* 1-10

*borF1* 22 19-21 18-11

*borG1* 1 2 8 3-8

A similar structure of these arms was found only in the population from Chita Province (Petrova and Klishko, 2005). In addition to the standard homozygotes, hetero- and homozygotes *borF1.2* and *borF2.2* were found in Yaroslavl Province (Kerkis et al., 1988), *borG1.2* and *borG2.2* in Yakutia (Kiknadze et al., 1996), *borF1.2*, *borF2.2*, and *borG1.2* in Primorye Territory (Golygina et al., 2003).

Besides large chromosome transformations, lack of conjugation in arms A, B, C, and E was observed in several cases in the populations examined, without

any apparent changes in the banding patterns (Figs. 4d–4g).

Data on the chromosome polymorphism and genotypic combinations of banding sequences are presented in Tables 1 and 2. The heterozygosity level in *C. borokensis* populations from the Baikal region is 53.8% (Lake Kotokel) and 43.3% (Lake Dukhovoe), which is lower than in populations from Yakutia (100%) and Yaroslavl Province (68.0%) (Kerkis et al., 1988; Kiknadze et al., 1996) but much higher than in those from Chita Province (20.0%) (Petrova and Klishko, 2005). The mean number of inversions per individual is greater in Lake Kotokel (0.73) than in Lake Dukhovoe (0.47) but smaller than in Yaroslavl Province (0.90). The differences in these parameters between the two populations from the Baikal region are determined by the high frequency (46.2%) of heterozygotes *borB1.2* in Lake Kotokel (Table 2). Such high frequency of this inversion was not recorded in *C. borokensis* populations from other regions, either. The greatest number of genotypic combinations of banding sequences (six), observed in the population from Lake Dukhovoe, is determined by the combination of the standard sequences and four inversion ones. As a rule, only one inversion sequence was present in an individual karyotype; two sequences, namely *borB2* and *borD2*, were observed in only one case. Four genotypic combinations are present in Lake Kotokel, two of them including two inversion sequences: *borA4* and *borB2*, *borA4* and *borD2*. Individuals with standard sequences prevail in both lakes: 56.6% in Lake Dukhovoe and 46.2% in Lake Kotokel.

Thus, the results obtained have allowed us to characterize the karyotypic specificity of populations of two dominant chironomid species in Lake Kotokel. Changes in the genetic structure of *C. entis* population that have occurred since our first research, may be related to transformations in the lake ecosystem. The fraction of heterozygous individuals, reflecting the adaptive capacities of the population (Dobzhansky, 1970; White, 1977), has decreased by 1.5 times, and the number of inversions per individual, by almost 2 times. The appearance of *C. borokensis*, which has now colonized the medium depth zone (4 m) and has become dominant in the southern hollow of the lake, also appears to be related to the changes in the environment. The level of heterozygosity and the number of inversions per individual in the population of *C. borokensis* are now almost the same as in that of *C. entis*, and exceed the values previously observed in Lake Dukhovoe by 1.2 and 1.5 times, respectively.



The demonstration of intraspecific microevolutionary processes in the populations of *C. entis* and *C. borokensis* shows that cytogenetic monitoring of the ecological condition of Lake Kotokel holds much promise and should be continued. The new banding sequences found in the Baikal region enrich our knowledge of the karyotypes of these species.

#### ACKNOWLEDGMENTS

The authors are sincerely grateful to N.A. Shobanov (Institute for Biology of Inland Waters, RAS) for valuable advice and discussion, and to D.V. Matafonov, N.M. Pronin (Institute of General and Experimental Biology, SB RAS), and N.G. Sheveleva (Limnological Institute, SB RAS) for help with material collection.

This work was carried out within the framework of National Research Project VII 62-1-4 "A Complex Study of the Supralittoral Zone as an Essential Component of the Littoral Zone in Lake Baikal" and partly supported by the Siberian Branch of RAS (Integration Project M-49).

#### REFERENCES

1. Bazova, N.V., Matafonov, D.V., and Pronin, N.M., "On Structural Changes in the Bottom Invertebrate Communities of Lake Kotokel (Lake Baikal Basin)," *Vestnik Buryat. Gos. Sel'skhoz. Akad.* **2** (19), 101–106 (2010).
2. Belyanina, S.I., Loginova, N.V., and Sigareva, L.E., "A Morphological Characteristic of *Chironomus entis* (Diptera, Chironomidae) and Its Karyotypic Variation in Different Geographic Zones," *Zool. Zh.* **71** (8), 32–38 (1992).
3. Belykh, O.I., Tikhonova, I.V., Sorokovikova, E.G., et al., "On Finding of Toxic *Microcystis* in Lake Kotokel (Buryatia)," *Vestnik TGU Biol.*, No. 330, 172–175 (2010).
4. Belykh, O.I., Sorokovikova, E.G., Fedorova, G.A., et al., "Presence and Genetic Diversity of Microcystin-Producing Cyanobacteria (*Anabaena* and *Microcystis*) in Lake Kotokel (Russia, Lake Baikal Region)," *Hydrobiol.* 2011. DOI 10.1007/s10750-011-0724-2.
5. Bukhteeva, N.M., "Karyotype and Inversion Polymorphism in *Chironomus plumosus* L. from East Siberia," *Tsitologiya* **16** (3), 358–361 (1974).
6. Demin, S.Yu. and Il'inskaya, N.B., "Changes in Compactness of Polytene Chromosomes from Various Organs of *Chironomus plumosus* Larvae," *Tsitologiya* **30** (4), 407–415 (1988).
7. Demin, S.Yu. and Shobanov, N.A., "Karyotype of the Midge *Chironomus entis* of the *plumosus* Group from the European Part of the USSR," *Tsitologiya* **30** (11), 1364–1372 (1990).
8. Devai, G.Y., Miskolczi, M., and Wülker, W., "Standardization of Chromosome Arms B, C, and D in *Chironomus* (Diptera: Chironomidae)," *Acta Biol. Debr. Oecol. Hung.* **2**, 79–92 (1989).
9. Dobzhansky, T.H., *Genetics of the Evolutionary Process* (Columbia Univ. Press, N.Y., 1970).
10. Golygina, V.V., *Karyotypic Divergence of Holarctic Chironomus Species of the Group plumosus in the Palaearctic and the Nearctic* (Diptera, Chironomidae). Candidate's Dissertation in Biology (Novosibirsk, 1999).
11. Golygina, V.V., Martin, I., Kiknadze, I.I., et al., "*Chironomus suwai*, a New Species of the *plumosus* Group (Diptera, Chironomidae) from Japan," *Aquatic Insects* **25** (3), 177–189 (2003).
12. Golygina, V.V., Kiknadze, I.I., Istomina, A.G., et al., "Cytogenetic Divergence of Genomes in *Chironomus plumosus* Group (Diptera: Chironomidae)," *Comp. Cytogen.* **1** (1), 17–32 (2007).
13. Kerkis, I.E., Filippova, M.A., Shobanov, N.A., et al., "Karyological and Genetic-Biochemical Characteristics of *Chironomus borokensis* sp. n. of the *plumosus* Group," *Tsitologiya* **32** (10), 1046–1054 (1988).
14. Keyl, H.-G., "Chromosomenevolution bei *Chironomus*. II Chromosomenumbauten und phylogenetische Beziehungen der Arten," *Chromosoma* **13**, 464–514 (1962).
15. Kiknadze, I.I. and Istomina, A.G., "Karyotypes and Chromosome Polymorphism in Siberian Species of Chironomidae (Diptera)," *Sibir. Ekol. Zh.* **7** (4), 445–460 (2000).
16. Kiknadze, I.I., Shilova, A.I., Kerkis, I.E., et al., *Karyotypes and Morphology of Larvae of the Tribe Chironomini: an Atlas* (Nauka, Novosibirsk, 1991) [in Russian].
17. Kiknadze, I.I., Istomina, A.G., Gunderina, L.I., et al., *Karyotypes of Chironomid Midges in the Yakutian Cryolithic Zone. The Tribe Chironomini* (Nauka, Novosibirsk, 1996) [in Russian].
18. Kiknadze, I.I., Butler, M.G., Golygina, V.V., et al., "Intercontinental Karyotypic Differentiation of *Chironomus entis* Shobanov, a Holarctic Member of the *C. plumosus* Group (Diptera, Chironomidae)," *Genome* **43** (5), 857–873 (2000).
19. Kiknadze, I.I., Golygina, V.V., Istomina, A.G., and Gunderina, L.I., "Chromosome Polymorphism during Population and Species Divergence in Non-Biting Midges (Diptera, Chironomidae)," *Sibir. Ekol. Zh.* **11** (5), 635–652 (2004).
20. Linevich, A.A., *Chironomidae of Lake Baikal and the Baikal Region* (Nauka, Novosibirsk, 1981) [in Russian].
21. Linevich, A.A. and Proviz, V.I., "*Chironomus plumosus* L. under the Conditions of Baikal and the Baikal Region," in *Diptera and Their Significance for Rural Economy* (Zool. Inst., Leningrad, 1987), pp. 129–134 [in Russian].

22. Maksimova, F.L., "On the Karyotype of *Chironomus plumosus* L. of the Ust-Izhora Natural Population, Leningrad Province," *Tsitologiya* **18** (10), 1264–1269 (1976).
23. Michailova, P., "Cytogenetic Characteristics of Species of the *Chironomus plumosus* Group (Chironomidae, Diptera) in Finland," *Cytobios* **106**, 99–114 (2001).
24. Petrova, N.A. and Klishko, O.K., "Cytodiagnosis, Inversion Polymorphism, and B-Chromosomes of Three Sibling *Chironomus* Species of the *plumosus* Group (Diptera, Chironomidae) from Eastern Siberia," *Zool. Zh.* **84** (8), 838–849 (2005) [*Entomol. Rev.* **85** (7), 729–740 (2005)].
25. Proviz, V.I., *Eco-Morphological and Karyotypic Characteristics of Chironomus plumosus L. (Diptera, Chironomidae) from the Shallow Near-Shore Zone of Baikal*. Candidate's Dissertation in Biology (Irkutsk, 1988).
26. Proviz, V.I., "Karyotypes of *Chironomus plumosus* L. (Diptera, Chironomidae) from Baikal, Kotokel, and Dukhovoe Lakes," in *Worms, Mollusks, and Arthropods* (Nauka, Novosibirsk, 1991), pp. 124–135 [in Russian].
27. Proviz, V.I., "A Study of Chromosome Polymorphism in Three Sibling Species of the Genus *Chironomus*, Group *plumosus* (Diptera, Chironomidae) from the Baikal Region," in *Karyosystematics of Invertebrates* (Zool. Inst., St. Petersburg, 1993), pp. 77–79 [in Russian].
28. Sheveleva, N.G. and Krivenkova, I.F., "Composition and Structure of Zooplankton of Lake Kotokel," *J. Siber. Fed. Univ. Biol.* **3**, 278–291 (2010).
29. Shobanov, N.A., "Morphological Differentiation of *Chironomus* Species of the Group *plumosus* (Diptera, Chironomidae). Larvae," in *Biology, Taxonomy, and Functional Morphology of Freshwater Animals* (Nauka, Leningrad, 1989), pp. 250–279 [in Russian].
30. Shobanov, N.A., *The Genus Chironomus Meigen (Diptera, Chironomidae). Taxonomy, Biology, and Evolution*. Doctoral Dissertation in Biology (St. Petersburg, 2000).
31. Siirin, M.T., Rubtzov, N.B., Karamysheva, T.V., et al., "A Molecular Cytogenetic Characteristic of B-Chromosomes of Chironomidae (Diptera)," *Tsitologiya* **45** (6), 582–589 (2003).
32. Sokolova, V.F., "Bottom Successions in Kotokel and Bol'shoe Eravnoe Lakes, the Transbaikalia," in *Abstracts of Papers, VIII Congr. of the Hydrobiological Society, Russian Academy of Sciences (Kaliningrad, September 16–23, 2001), Vol. 1* (Kaliningrad, 2001), pp. 305–306.
33. White, M.J.D., *Animal Cytology and Evolution. 3rd Ed.* (Cambridge Univ., 1973).