Karyotype divergence between Palearctic and Nearctic populations of the Holarctic species *Chironomus plumosus* L. (Diptera, Chironomidae).


Malcolm G. Butler¹. Department of Zoology, North Dakota State University, Fargo, ND 58105, U.S.A.  mbutler@plains.nodak.edu  tel: 701 231 7398  fax: 701 231 7149

Iya I. Kiknadze, Veronica V. Golygina and Albina G. Istomina. Laboratory of Cell Biology, Institute of Cytology and Genetics, 630090 Novosibirsk, Russia.

Jon Martin. Department of Genetics, University of Melbourne, Parkville, Victoria 3052, Australia.

Wolfgang F. Wülker. Institut für Biologie 1 (Zoologie) Albert-Ludwigs-Universität, Hauptstr. 1, D-79104 Freiburg, Germany.

James E. Sublette and Mary F. Sublette. 3550 Winslow Dr., Tucson, AZ 58715, U.S.A.

¹ Author to whom all correspondence should be sent.
Karyotype divergence between Palearctic and Nearctic populations of the Holarctic species *Chironomus plumosus* L. (Diptera, Chironomidae).


**Abstract**

Macrogeographic patterns of chromosomal banding sequences were studied in natural populations of the Holarctic species *Chironomus plumosus*. Of the 31 inversion sequences now known, 16 are endemic to the Palearctic, 7 are endemic to the Nearctic, and 8 are Holarctic sequences common to both zoogeographic zones. Differences in the sets of inversion sequences found on each continent, plus differing frequencies of Holarctic sequences, result in great overall divergence of karyotypes on the two continents. The karyotype of Nearctic *C. plumosus* differs from that of Palearctic populations primarily by the presence of a homozygous Nearctic sequence in arm A (n'plu A9), along with fixation (h'plu C2, h'plu E2, and h'plu F1), or high frequency (h'plu D2), of Holarctic sequences which are present but less frequent in the Palearctic. Although long continental isolation has led to great divergence of karyotypes on opposite sides of the Atlantic Ocean, all populations of *C. plumosus* show sufficient cytogenetic similarity to constitute a single Holarctic species.

*Key words:* karyotype, inversion polymorphism, cytogenetic distances, *Chironomus.*
Introduction

The study of chromosomal polymorphism in natural populations of animals allows us to evaluate the cytogenetic differentiation of populations, to determine the role of fixed chromosomal rearrangements in speciation, and to reconstruct the cytogenetic history of species and their patterns of migration (Dobzhansky et al. 1977; White 1977; Krimbas and Powell 1992). For these purposes, analysis of chromosomal polymorphism in Holarctic species is most useful. The midge *Chironomus plumosus* L. is a good candidate for such analysis because it is widely distributed in both the Palearctic and Nearctic, and is frequently sampled as a biological indicator of lake eutrophy (Thienemann 1913; Saether 1975, 1979; Wiederholm 1980).

The karyotype and chromosomal polymorphisms in Palearctic *C. plumosus* have been studied extensively (Keyl and Keyl 1959; Keyl 1962; Maximova 1976; Vest Pedersen 1978, 1984; Belyanina et al. 1983; Kiknadze 1987; Kiknadze et al. 1987a, 1991a; Michailova 1989; Michailova and Petrova 1991; Petrova et al. 1992; Shobanov 1994a, 1994b, 1994c). A high level of polymorphism has been found in all Palearctic populations studied, with 20 banding sequences (standard plus inversion sequences) characterized (Shobanov 1994a, 1994b). In addition, several rare inversion sequences have been described (Michailova and Petrova 1991; Ilyinskaya 1995) but were not documented by photographs suitable for mapping, precluding their use for comparative analysis of chromosomal polymorphism in different populations. As different maps of the chromosome banding patterns have been used in some of these descriptions, it is beneficial to have a uniform standard by which the banding patterns can be compared in different populations of *C. plumosus*.

No longitudinal, latitudinal, or altitudinal clines of inversion polymorphism are known in *C. plumosus*, in contrast to dipteran species such as *Drosophila melanogaster* and
D. subobscura, which have clear-cut clines of several inversion sequences (Krimbas and Loukas 1980; Knibb 1982; Lemeunier et al. 1986; Krimbas and Powell 1992). In addition to inversion polymorphism, genomic polymorphism occurs in Palearctic C. plumosus populations. This is associated with the presence of additional B-chromosomes (Keyl and HŠgele 1971; Ilyinskaya and Petrova 1985) and with the appearance of triploids (Belyanina et al. 1983; Dyomin and Ilyinskaya 1986; Ilyinskaya 1995).

In contrast to this knowledge of Palearctic C. plumosus, the karyotype and chromosomal polymorphism of Nearctic C. plumosus have not been studied extensively. Only a few short publications have reported the presence of cytologically identified C. plumosus in some North American lakes, including the discovery of an uncommon banding sequence in arm A of American C. plumosus (Wülker et al. 1968; Martin, unpublished list of North American Chironomus; Butler et al. 1995). It thus remains unknown whether or not the very long continental isolation of Palearctic and Nearctic C. plumosus populations has been accompanied by a strong divergence of karyotypes on the different continents. Data from other Holarctic chironomids indicate that the magnitude of such cytogenetic differentiation may vary considerably. In some species there is dramatic karyotype divergence between Old World and New World populations. For example in the subgenus Camptochironomus, the karyotype of Nearctic C. tentans differs from that of Palearctic C. tentans by the presence of unique "Nearctic" inversion sequences in all seven chromosomal arms (Acton 1958, 1962; Acton and Scudder 1971; Kiknadze et al. 1996a; Gunderina et al. 1996). Much less karyotype divergence was found in Palearctic and Nearctic C. pallidivittatus, a sibling species of C. tentans (Kiknadze et al. 1998), where only two of seven chromosomal arms have fixed Nearctic sequences. However, in addition to these Nearctic sequences, the karyotype of Nearctic C. pallidivittatus is characterized by fixed Holarctic sequences in arms C and D, which are polymorphic in Palearctic C. pallidivittatus. Similarly, only two fixed Nearctic sequences were found in the Holarctic midge Glyptotendipes barbipes (Martin and Porter
Thus there is insufficient data on patterns of karyotype divergence among Holarctic species during continental isolation to draw general conclusions.


It is still unknown which sibling species from the *plumosus* group inhabit the Nearctic region. Prior to 1995, only *C. plumosus* and *C. vancouveri* had been reported as cytologically identified species of the *plumosus* group in North America (Wülker et al. 1968; Michailova and Fisher 1986). Recently the presence of *C. entis* in North American chironomid communities was described (Butler et al. 1995) and the presence of *C. balatonicus* and *C. muratensis* has been claimed (Dinsmore & Prepas 1997). The karyotype of *C. entis* will be considered in a companion paper to this one (Kiknadze et al., in prep.), but we have not encountered *C. balatonicus* nor *C. muratensis*, even in small samples from the reported locations.

This paper is devoted to the study of karyotype structure and chromosomal polymorphism in Holarctic *C. plumosus* from the Nearctic and Palearctic Regions. Patterns of karyotype divergence during continental isolation of populations are discussed.

**Materials and methods**
Final instar larvae of *C. plumosus* L. were collected for morphological and cytogenetic analysis from Palearctic (Russian) and Nearctic (North American) waterbodies. Eighteen Palearctic and forty Nearctic natural populations of *C. plumosus* have been studied. The locations of the wild populations and the collection dates are given in Table 1. Larvae were identified by morphological and karyological characters (Keyl and Strenzke 1956, Keyl 1962, Kiknadze et al. 1991c). Larvae were fixed in a 3:1 mixture of 100% ethanol and glacial acetic acid, and each larva was used for both karyological and morphological study. Isolated salivary glands were squashed for polytene chromosome preparations, and the head capsule and larval body were mounted on permanent slides for morphological analysis. The results of the morphological study will be presented elsewhere.

Polytene chromosome squashes were prepared by a routine aceto-orcein method (Keyl and Keyl 1959; Kiknadze et al. 1991c). Mapping of the polytene chromosomes was made according to Keyl (1962) for arms A, E, and F and according to Devai et al. (1989) for arms B, C, and D. Unfortunately, there has not been one common system for mapping *C. plumosus* polytene chromosomes. Some authors use the generally accepted mapping system for the genus *Chironomus*, with the *C. piger* banding pattern as a standard (Keyl 1962; Devai et al. 1989), while others employ the mapping system of Maximova (1976), in which the *C. plumosus* banding pattern serves as a standard (Belyanina et al. 1983; Michailova 1989; Petrova et al. 1992; Shobanov 1994a, 1994b, 1994c), and Vest Pedersen (1978, 1984) uses his own system. We prefer the mapping system of Keyl (1962) and Devai et al. (1989) because it allows the direct comparison of banding patterns in *C. plumosus* and its siblings with the banding patterns in the other species of the genus *Chironomus* (Keyl 1962; Martin 1979; Wülker et al. 1989).
Each arm sequence is prefixed by an abbreviation of the species name as established by Kiknadze et al. (1996a), eg., for *C. plumosus*: plu A1, plu A2, etc. for arm A, and plu B1, plu B2, etc. for arm B. Because some banding sequences are known only in Nearctic *C. plumosus*, and others only from the Palearctic, special symbols are used to indicate continental distribution: p'plu A1, p'plu B3, etc. for Palearctic sequences and n'plu A9, n'plu B4, etc. for sequences in the Nearctic. Sequences occurring in populations on both continents are denoted as Holarctic (h’plu A2, h’plu B1, etc.). After the initial designation of a sequence in this paper, the prefix may be dropped where it is not essential to an understanding of the meaning. A comparative listing of the notations used by several authors for banding sequences of the seven chromosome arms of *C. plumosus* is given in Table 2.

Quantitative analysis of chromosomal polymorphism was carried out for 15 Palearctic and 12 Nearctic populations of *C. plumosus*, most represented by samples sizes exceeding 20 larvae (Tables 3, 4) but some as small as 8 larvae. The set of inversion sequences present was recorded for additional populations represented by smaller samples listed in Table 1. Karyotypes from ten populations in Wisconsin and five in Indiana, sampled in 1964-67, were scored approximately 30 years ago, prior to recognition of rare Nearctic sequences in arms B and D. Quantitative data from these populations are thus not used in our analyses, although some collections were large.

Heterozygote frequencies within each population were tested for conformity to expectation under Hardy - Weinberg equilibrium using the $P^2$ test. The significance of the differences among populations in the frequencies of alternative sequences was tested with Fischer's test (Snedecor and Cochran 1967). Genetic distances ($D_N$) between populations analyzed quantitatively were calculated with Nei's method (Nei 1972), and a dendrogram was constructed by UPGMA cluster analysis (Sneath and Sokal 1973). Slides of karyotypes plus larval head capsules and abdominal tubuli from all specimens examined are held at the
Results

Karyotype and chromosomal polymorphism in Palearctic C. plumosus

The karyotype of Palearctic C. plumosus is presented in Fig. 1. As detailed above, this karyotype has been described by numerous authors, but with great differences in the system used to map the polytene chromosomes. For consistency with mapping of other Chironomus species, we use the C. piger standard of Keyl (1962) and Devai et al. (1989). The karyotype of C. plumosus is very polymorphic in all Palearctic populations studied, with all chromosomal arms except arm G having inversion sequences. The basic homozygous and heterozygous inversions are shown in Fig. 2.

Arm A has eight sequences in the populations studied (Tables 3, 5; Figs. 1, 2a, 2d-2h). Sequences p'plu A1 and h'plu A2 predominated. Other sequences were found only as heterozygotes, and they appeared regularly but at low frequency in many of the populations. Sequences p'plu A1 and h'plu A2 differ by a simple inversion (Figs. 2a, 2d; Table 5), for which the exact determination of the break points in regions 12 and 13 was very difficult because of a series of very thin bands located in these regions (Figs. 1, 2a, 2d). The morphology of these thin bands can change near the break points, resulting in different descriptions of banding sequences by different investigators. Our description corresponds to that of Keyl (1962).

Sequence p'plu A3 (pluIa of Keyl 1962; Tables 2, 5) was found in one population (V-VU) in far eastern Europe, but in none of the Siberian populations. However, a similar sequence,
p'plu A7, was observed in five Siberian populations (Table 3; Fig. 2g). Earlier, Kiknadze (1987) and Kiknadze et al. (1987a) mistakenly described p'plu A7 as p'plu A3. Sequence p'plu A7 actually differs from p'plu A3 by a complex rearrangement in the proximal part of that sequence (Table 5; Fig. 2g).

Sequence p'plu A4 is formed from A1 by several complex paracentric inversions (Table 5; Fig. 2e). It is typical of European populations of *C. plumosus* (Kiknadze et al. 1991a; Shobanov 1994a, 1994b) and was found in only one Siberian population. The Siberian sequence p'plu A5 is close to the European p'plu A4, but differs from it by a simple paracentric inversion on the distal part of the arm A (Table 5; Fig. 2f). Sequence p'plu A6 differs from A1 by a very short paracentric inversion in region 14 - 17 of arm A (Table 5; Fig. 2h). Sequence p'plu A8 was found only once, in the heterozygous state. It results from a simple inversion of A2 spanning most of that arm (Table 5).

The banding sequence of *C. plumosus* arm B can be derived from the standard *C. piger* arm B sequence only by a series of very complex rearrangements. Thus we have been unable to map this arm, but can distinguish three sequences (Tables 3, 5; Figs. 1, 2b, 2i). Sequences h'plu B1 and h'plu B2 are predominant. The rare sequence, p'plu B3, is formed from B1 by a small simple inversion (Fig. 2i) and was found only once, in the heterozygous state.

Arm C has only two sequences, p'plu C1 and h'plu C2, which differ by a simple inversion (Tables 3, 5; Figs. 1, 2j). In arm D, five sequences are known for Palearctic *C. plumosus* and three were found in the populations studied (Tables 2, 3, 5; Figs. 1, 2k). Sequences p'plu D1 and h'plu D2 differ by a simple inversion (Fig. 2k). Sequence p'plu D3 is formed by complex paracentric inversion of D1 (Table 5). Sequences p'plu D4 and p'plu D5 were described by Shobanov (1994b).
Arm E has two sequences, p'plu E1 and h'plu E2, differing by a simple inversion (Tables 2, 3, 5; Figs. 1, 2l). We have used the mapping of these two sequences given by Keyl (1962), but repeated analysis of the banding sequences of the arm E suggests an alternative version (Fig. 4e; Table 5). In situ hybridization experiments with cloned DNA would be necessary to prove which of these versions is correct.

Arm F has three sequences (Tables 2, 3, 5), and the predominant sequences h'plu F1 and p'plu F2 differ by a simple inversion in regions 11 - 17 (Figs. 1, 2m). Sequence p'plu F3 differs from F1 by a simple inversion near the distal end of the arm (Fig. 2n). Although rare, this sequence occurs in a number of other Palearctic Chironomus species (Keyl 1962; Martin 1979) and is phylogenetically basal to sequences h'plu F1 and p'plu F2. Arm G, which bears the nucleolus and two Balbiani rings, is monomorphic. A third Balbiani ring is located very near the nucleolus and has only been identified by electron microscopy (Istomina et al. 1989). The homologues of G are usually not paired (Fig. 1).

A total of 24 banding sequences is now known in Palearctic C. plumosus, with 22 of these observed in the Palearctic populations studied here (Tables 3, 6). The number of sequences we found in a population ranged between 9 and 16, with 9 to 19 genotypic combinations of these sequences found in different populations (Table 4). All populations studied had a high level of chromosomal polymorphism, with 37% to 93% of the larvae in each population heterozygous for at least one arm. The average number of heterozygous inversions per larva ranged from 0.47 to 1.71 (Table 4). Genomic polymorphism in the form of additional B-chromosomes also was found in all but three populations (Table 4), and heterozygosity of the centromeric bands was observed in some populations (Fig. 2m). Of the 24 sequences known in the Palearctic, 16 have been found only in the Palearctic and 8 are Holarctic (Table 6). Cytogenetic differentiation between Palearctic populations ranges from $D_N = 0.002$ to $D_N = 0.317$, with an average genetic distance of $D_N = 0.071 \pm 0.008$. 
Karyotype and chromosomal polymorphism in Nearctic C. plumosus

The karyotype of Nearctic C. plumosus differs from that in the Palearctic by the presence of unique Nearctic sequences and the fixation of some sequences polymorphic in the Palearctic. Banding patterns of Nearctic C. plumosus are illustrated in Figs. 3 and 4 and described in Table 5.

Arm A has two sequences in the Nearctic, the rarer of these being the Holarctic sequence h'plu A2 (Tables 3, 5; Figs. 3a, 3b). In most of the North American populations studied, the uniquely Nearctic sequence n'plu A9 predominated (Table 3). These two arm A sequences differ by a large simple inversion (Figs. 3a, 3b; Table 5).

Four sequences in arm B have been found in North American populations (Tables 3, 5; Figs. 3c-3e). The dominant sequence is h'plu B1, and a second Holarctic sequence, h'plu B2, was found only in the heterozygous state. Two Nearctic sequences, n'plu B4 and n'plu B5, were also rare and found only as heterozygotes (Tables 3, 5; Figs. 3d, 3e). Sequence n'plu B4 results from an inversion located in region 5 - 10 (Fig. 3e), whereas n'plu B5 is formed by a complex inversion of B1 and has not been mapped (Fig. 3d).

Arm C is monomorphic for the Holarctic sequence h'plu C2 (Table 3; Fig. 3f).

Arm D is polymorphic, with five sequences recorded (Tables 3, 5). The most frequent sequence is h'plu D2 (Fig. 4a), and the other four sequences are Nearctic: n'plu D6, n'plu D7, n'plu D8, and n'plu D9 (Figs. 4b-4d). All of these sequences are formed from D2 by simple inversions (Table 5). These uniquely Nearctic sequences are rare (Table 3) and are found mainly in the heterozygous state.
Arms E and F are both monomorphic in the Nearctic (Table 3), with fixation of Holarctic sequences h'plu E2 (Fig. 4e) and h'plu F1 (Fig. 4f), respectively. Arm G is monomorphic for h'plu G1, as in Palearctic populations.

Thus, the karyotype of Nearctic *C. plumosus* differs from that in the Palearctic by the following features: high frequency of n'plu A9 found in both homozygous and heterozygous states, fixation of Holarctic sequences h'plu C2, h'plu E2, and h'plu F1, and a high frequency of h'plu D2 (Figs. 5a, 5b). In addition, six Nearctic sequences (n'plu B4, n'plu B5, n'plu D6, n'plu D7, n'plu D8, and n'plu D9) are unique to Nearctic *C. plumosus*, but are rare and found mainly as heterozygotes.

In total, fifteen banding sequences were recorded from Nearctic populations (Tables 3, 6) with 7-13 sequences and 7-14 genotypic combinations per population (Table 4). The number of inversion heterozygotes ranged from none to 52% in Nearctic populations, and the average number of the heterozygous inversions per larva varied from 0.0 to 0.57 (Table 4). These Nearctic populations are therefore somewhat less polymorphic than those of the Palearctic.

Of the 15 banding sequences found in the Nearctic, 8 were Holarctic, and 7 were Nearctic (Table 6). Palearctic and Nearctic populations have a total of 31 sequences - 16 Palearctic, 7 Nearctic, and 8 Holarctic (Table 6). The presence of uniquely Nearctic sequences contributes substantially to overall karyotypic divergence between Palearctic and Nearctic *C. plumosus*. Cytogenetic distances between Nearctic populations of *C. plumosus* vary from $D_N = 0.001$ to $D_N = 0.105$, with the average $D_N = 0.020\pm0.003$. On the other hand, the average cytogenetic differentiation between Palearctic and Nearctic *C. plumosus* populations is $D_N = 0.699\pm0.013$. However this level of differentiation is considerably less than that observed between Nearctic and Palearctic *C. tentans* (Kiknadze et al. 1996a, Gunderina et al.)
Therefore we conclude that *C. plumosus* is a single species with highly differentiated populations on either side of the Atlantic Ocean (Fig. 6).

**Discussion**

We have presented the first global analysis of banding patterns and chromosomal polymorphism in *Chironomus plumosus*, based on material from both Palearctic and Nearctic populations. A clear difference is seen between Palearctic and Nearctic *C. plumosus* karyotypes. This divergence is characterized by the presence, in North American populations, of endemic Nearctic sequences found in both homozygous and heterozygous states. In addition, a single Holarctic sequence is fixed in each of arms C (h'plu C2), E (h'plu E2), and F (h'plu F1), whereas these arms are polymorphic in the Palearctic. In arm D, the Holarctic sequence h'plu D2 can be said to be fixed in the Nearctic; although this sequence is not homozygous in all populations, the other Nearctic arm D sequences are derived from h'plu D2 by inversions. Sequence p'plu D1, which is common in the Palearctic, was not found in Nearctic populations.

Fifteen inversion sequences were recorded in Nearctic *C. plumosus*, and 24 inversion sequences are now known from the Palearctic. In total, Holarctic *C. plumosus* has 31 sequences; 16 of them are Palearctic, 7 are Nearctic, and 8 are Holarctic (Table 6). However, karyotype divergence between Palearctic and Nearctic *C. plumosus* is not as pronounced as between Palearctic and Nearctic *C. tentans* (Kiknadze et al. 1996a). Homozygous Nearctic sequences are present in all chromosomal arms of *C. tentans*. In contrast, only one chromosomal arm in *C. plumosus* (arm A) commonly has homozygous Nearctic sequences (Fig. 5), with other Nearctic sequences appearing in the heterozygous state. However, it is important to emphasize that three sequences - h'plu C2, h'plu D2 and h'plu E2 - are fixed in the Nearctic, whereas these occur at lower frequencies in the Palearctic. Thus, four out of seven chromosomal arms differ substantially between Nearctic and Palearctic *C. plumosus*. 
Cytogenetic differentiation among populations within each zoogeographic zone is much less significant than that seen between Palearctic and Nearctic populations. A dendrogram of cytogenetic distances among 15 Palearctic and 12 Nearctic populations shows a clear-cut separation of populations on the two continents (Fig. 6). The overall differentiation between Palearctic and Nearctic populations in *C. plumosus* \( (D_N = 0.699 \pm 0.013) \) is considerably lower than that seen between Palearctic and Nearctic populations of *C. tentans* \( (D_N = 1.650 \pm 0.021) \), which should in fact be viewed as different species (Kiknadze et al. 1996a). We consider Palearctic and Nearctic *C. plumosus* to be a single Holarctic species with highly differentiated populations on each continent. The level of karyotype divergence within *C. plumosus* is closer to that seen in Holarctic *C. pallidivittatus* (Kiknadze et al. 1998) and Holarctic *Glyptotendipes barbipes* (Martin and Porter 1973).

As was mentioned above, *C. plumosus* has a large number of sibling species in the Palearctic. A Nearctic sibling species, *C. vancouveri*, was described from Canada by Michailova and Fischer (1986). Whether or not Nearctic *C. plumosus* and *C. vancouveri* are different species remains an open question. We have found the karyotypes of Nearctic *C. plumosus* and *C. vancouveri* to be very similar. For both, the most common sequences (h’plu A2, h’plu B1, h’plu B2, h’plu C2, h’plu D2, h’plu E2, h’plu F1, and h’plu G1) are identical. The most important aspect of cytogenetic similarity is the fixation in the homozygous state of identical Holarctic sequences h’plu C2, h’plu D2, and h’plu E2. The only difference is the high level of chromosomal polymorphism in Nearctic *C. plumosus*, while *C. vancouveri* is monomorphic. However, monomorphism in *C. vancouveri* could be due to the limited number of egg masses used to found the *C. vancouveri* laboratory stock studied by Michailova and Fisher (1986). Further comparative investigation is needed, including analysis of C-banding (Michailova and Fischer 1984), to resolve the question as to the potential identity of Nearctic *C. plumosus* and *C. vancouveri*. 
Finally, we note that most of the banding sequences known in Holarctic *C. plumosus* (52%) are restricted to the Palearctic, with only 23% endemic to the Nearctic. This suggests that the species originated in the Palearctic. The appearance of unique Nearctic sequences demonstrates the potentially important role of chromosomal rearrangements in the adaptation of a species to the environmental conditions of another continent.
Acknowledgements
We thank J.K. Cooper, K.M. Giovannielli, A.M. Johnson, and R.L. Rezanka for collecting much of the Nearctic *C. plumosus* material, and T. Tank for help with manuscript preparation. This research was supported by grants from the Russian Fund for Fundamental Research, the North Dakota Water Resources Research Institute, Region VIII of the U.S. Environmental Protection Agency, and the Research and Consulting Committee at North Dakota State University.
References

Acton, A.B. 1958. A cytological comparison of Nearctic and Palearctic representatives of

Acton, A.B. 1962. Incipient taxonomic divergence in Chironomus (Diptera). Evolution, 16:
330-337.

Acton, A.B., and Scudder G.E. 1971. The zoogeography and races of Chironomus
(=Tendipes) tentans Fab. Limnologica, 8: 83-92.

Belyanina, S.I., Maximova, F.L., Buchteeva, N.M., Ilyinskaya, N.B., Petrova, N.A., and

Chironomus species from lakes in North Dakota and Minnesota, USA. In:
Chironomids, from genes to ecosystems: Proceedings of the 12th International
Symposium on Chironomidae, Canberra, January 23-26, 1994. Edited by P.C.
Cranston, CSIRO, Canberra. pp. 31-37.

and D in Chironomus (Diptera, Chironomidae). Advances in Chironomidology. Part I.


macroinvertebrates in a eutrophic lake in central Alberta. II. Changes in Chironomus


Kiknadze, I.I., and Kerkis, I.E. 1984. Karyotypical characteristics of *Chironomus* f. l. reductus with 2n=6 from the Ob reservoir. [In Russian.] Tsitologia, **26**: 735-739.


Kiknadze, I.I., Siirin, M.T., Filippova, M.A., Gunderina, L.I., and Kalatchikov, S.M. 1991b. The change of the pericentromeric heterochromatin mass is one of important way of chironomid evolution. [In Russian.] Tsitologia, **33**: 90-98.


Maximova, F.L. 1976. The karyotype of *Chironomus plumosus* from the Ust'-Izhora wild population of Leningrad region. [In Russian.] Tsitologia, 18: 1264-1269.


Figure Legends

Fig. 1. Karyotype of Palearctic *Chironomus plumosus*. h'plu A2.2, h'plu B1.1 etc. - genotypic combinations of banding sequences, BR - Balbiani ring, N - nucleolus. Mapping of arms A, E and F is according to Keyl (1962), arms C and D according to Devai et al. (1989). Our version of mapping of regions 3 and 4 on arm E is shown above that arm.

Fig. 2. Inversion polymorphism in Palearctic *C. plumosus*. Homozygous inversion sequences in arms A, B, and D (a-c), heterozygous inversions in arm A (d-f).

Fig. 2 (concluded). Heterozygous inversions in arm A (g-h), arm B (i), arm C (j), arm D (k), arm E (l) and arm F (m-n). Notation is as in Fig. 1.

Fig. 3. Nearctic and Holarctic banding sequences of arms A, B, and C in Nearctic *C. plumosus*. Homozygous sequences in arms A, B, and C (a, c, f); proximal region of heterozygous inversion h'plu A2.n'plu A9 illustrating break point 2cb-18 on the n'A9 homolog (b); heterozygous inversions in arm B (d, e). Notation is as in Fig. 1.

Fig. 4. Nearctic and Holarctic banding sequences of arms D, E, and F in Nearctic *C. plumosus*. Homozygous sequences in arms D, E, and F (a, e, f), heterozygous sequences in arm D (b, c, d). Keyl's mapping of arm E is shown below, and our version above, that arm (e). Notation is as in Fig. 1.

Fig. 5. Comparison of *C. plumosus* karyotypes in (a) the Palearctic and (b) the Nearctic. Notation is as in Fig. 1.

Fig. 6. Dendrogram of genetic distances between populations of *Chironomus plumosus* in the Palearctic and the Nearctic.