© Comparative Cytogenetics, 2008 . Vol. 2, No. 1, P. 21-35. ISSN 1993-0771 (Print), ISSN 1993-078X (Online)

Mystery of *Chironomus dorsalis* Meigen karyotype (Diptera: Chironomidae)

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Abstract. The study of the karyotype structure of *Chironomus dorsalis* Meigen, 1818 from the "pseudothummi" cytocomplex was performed. Arms C and D were mapped for the first time according to Keyl-Dévai system (Keyl, 1962; Dévai et al., 1989) in addition to arms A, E, and F, mapped by Keyl (1962). The structure of the centromeric regions of *Ch. dorsalis* has been described in more detail and compared with the reference species, *Ch. piger* Strenzke, 1959, and the other species of the "thummi" cytocomplex. It was discovered that *Ch. dorsalis* karyotype differs clearly from other *Chironomus* species by the banding pattern of centromeric regions on the chromosomes AE and BF. Two thick C-positive bands are located on the centromeric region of each of these chromosomes. The hypothesis was suggested that chromosomes AE and BF of *Ch. dorsalis* are dicentric with one active centromere. The data obtained also support previously postulated hypothesis that the "pseudothummi" cytocomplex should be considered as more primitive and ancestral to other cytocomplexes of the genus *Chironomus* Meigen, 1803. Natural populations of *Ch. dorsalis* from various regions of Russia were studied for the first time as well the chromosomal polymorphism in some of these populations was quantitatively estimated that allowed us to assess the size of banding sequence pool for this species, which consists of 8 banding sequences.

Key words: *Chironomus dorsalis*, "pseudothummi" cytocomplex, karyotype, centromere, dicentric, banding sequence.

Introduction

Comparative study of the karyotypes of the species belonging to the genus *Chironomus* Meigen, 1803 has made it possible to discover a number of chromosomal evolutionary patterns in this genus (Keyl, 1961, 1962; Martin, 1979; Wülker, 1980; Wülker et al., 1989; Kiknadze et al., 1989, 1991, 2007; Petrova, 1989, 1990; Michailova, 1989; Shobanov, 2000; Gunderina et al., 2005). Unlike the majority of other studied animal species, reciprocal translocations of whole arms have played the leading role in the karyotype evolution of *Chironomus* species. These translocations combine with chromosomal rearrangements of other types, such as paracentric inversions, fusion of chromosomal arms, local amplification of centromeric DNA, and change in the number and location of nucleolar organizers. A typical haploid karyotype of the species belonging to the genus *Chironomus* comprises four chromosomes formed by seven chromosomal arms

(A, B, C, D, E, F, and G), which correspond to gene linkage groups. The reciprocal translocations of whole arms in the species evolution have led to the existence of various combinations of chromosomal arms in the karyotypes of various *Chironomus* species. According to different combinations of chromosomal arms, all the *Chironomus* species are now grouped in the following cytocomplexes: AB CD EF G ("thummi"), AE CD BF G ("pseudothummi"), AC ED BF G ("parathummi"), AB ED CF G ("camptochironomus"), AF BE CD G ("matures"), AD CB EF G ("lacunarius") and AG CD BF E ("columbiensis"). In addition to these main cytocomplexes, their modifications connected with the fusion of individual arms also occur (Keyl, 1962; Martin, 1979; Wülker, 1980). As the chromosome breaks during reciprocal translocations of whole arms took place in the centromeric chromosomal regions, it was of interest to study these regions in various cytocomplexes to find out the fate of the centromeres. So far, the morphology of centromeric bands in polytene chromosomes has been sufficiently well studied only for the "thummi" cytocomplex species (Keyl, 1962; Hägele, 1977; Sigareva, 1981; Hankeln et al., 1994). The centromeres in polytene chromosomes are detectable as single dense intensively stained C-positive bands. They are precisely mapped and have their own designations on the chromosomal cytological map of each species. In the mitotic chromosomes of chironomids, the centromeres are detectable as primary chromosome constrictions; however, C-heterochromatin is undetectable there in the majority of species due to a very low content of centromeric DNA in the chironomid genome (Hägele, 1977). Consequently, polytene chromosomes are more appropriate for studying the centromeric regions of *Chironomus* species.

The centromeric regions of other cytocomplexes have not been studied in detail, and only sparse data on the localization of centromeric bands in the "pseudothummi" cytocomplex are available (Keyl, 1962; Kiknadze et al., 1991; Shobanov, Petrova, 1995); however, these data are rather contradictory. In this work, we attempted to describe in more detail the structure of the centromeric region in a member of the "pseudothummi" cytocomplex, *Ch. dorsalis*, as compared with the reference species, *Ch. piger* ("thummi" cytocomplex). Moreover, we were first to map arms C and D of *Ch. dorsalis* in addition to arms A, E, and F, mapped by Keyl (1962). Arms B and G yet remain unmapped due to their complex rearrangements as compared with the standard pattern of *Ch. piger* Strenzke, 1959. Natural populations of *Ch. dorsalis* from various regions of Russia were studied for the first time and the chromosomal polymorphism in some of these populations was quantitatively estimated, which allowed us to assess the size of banding sequence pool for this species.

Material and Methods

Polytene chromosomes of *Ch. dorsalis* salivary glands were studied in the last (fourth) instar larvae. Table 1 lists the sites of larval sampling and the volumes of samples. The larvae were fixed with the Carnoy mixture (96% alcohol and glacial acetic acid at a ratio of 3:1). Salivary glands were excised from the fixed larvae for karyological analysis, and the bodies were used for morphological preparations.

Mitotic chromosomes were studied in the cells of gonads and nerve ganglia. Squash preparations of polytene and mitotic chromosomes were made conventionally using aceto-orcein staining (Kiknadze et al., 1991). Polytene chromosomes were mapped according to Keyl (1962; arms A, E, and F) and Dévai et al. (1989, arms B, C, and D). Banding sequences

Table 1. Collection sites and numbers of *Chironomus dorsalis* larvae analyzed.

Location	Population	Collection date	Number of larvae
Yaroslavl Prov. Latka river, Borok	YAR-LA	29.05.1986	10
Novosibirsk Prov. The basin of Eltsovka river, gard. com. «Kristall» The basin of Ziryanka river, gard. com. «Genetic» Puddle near the Berdsk pond	 NSK-SK NSK-GE NSK-BL	 27.07.1990 30.07.1999 25.06.2007	 7 12 50
Republic of Altai Aiya Lake	RAL-AY	09.1989	14
Primorsky Terr. Brook near Shamora bay	PRI-SH	14.11.2000	23

in each arm were designated with the symbol of species, symbol of arm, and number of sequence, for example, dorA1, dorG1, dorG2.

C-banding (Hägele, 1977; Sigareva, 1981) was used for detection of the centromeric bands.

A comparative study of the banding sequences from the banding sequence pools of *Ch. dorsalis* and other *Chironomus* species allowed us to detect the number of breakpoints in *Ch. dorsalis* chromosomes that determined the divergence of banding sequences and to construct an NJ (neighbour-joining) phylogenetic tree for the chromosomal evolution of the genus in question. The MEGA2 (©Kumar et al., 2001, Arizona State University, USA) software package was used for constructing the tree.

Equipment of the Center of Microscopy analysis of biological objects SB RAS in the Institute of Cytology and Genetics was used in accomplishment of this work: microscope "Axioskop" 2 Plus, CCD-camera AxioCam HRc, software package AxioVision 4 (Zeiss, Germany).

Results

Karyotype

The karyotype of *Ch. dorsalis* was for the first time described by H.-G. Keyl and I. Keyl (1959). The photomaps of arms A, E, and F were also first reported by Keyl (1962). Brief information about the karyotype was provided by Kiknadze et al. (1988, 1991) and Michailova (1989). Characteristic of *Ch. dorsalis* karyotype is the arm combination AE CD BF G ("pseudothummi" cytocomplex, Fig. 1). Chromosome AE is a submetacentric; chromosomes CD and BF – metacentrics; and chromosome G – a telocentric. The only nucleolus is localized to the short chromosome G near the centromeric–telomeric region. Three Balbiani rings are morphologically distinguishable: two rings are localized to arm G and the third, to arm B (Fig. 1).

A peculiar specific feature of the *Ch. dorsalis* karyotype is the presence of two intensively stained bands similar to centromeres in the centromeric region of chromosomes AE and BF (Fig. 1). Both bands in each chromosome are C-positive (Fig. 2, a). One or two thin C-positive bands are located between these thick bands (Fig. 2, c, e). To the understand-

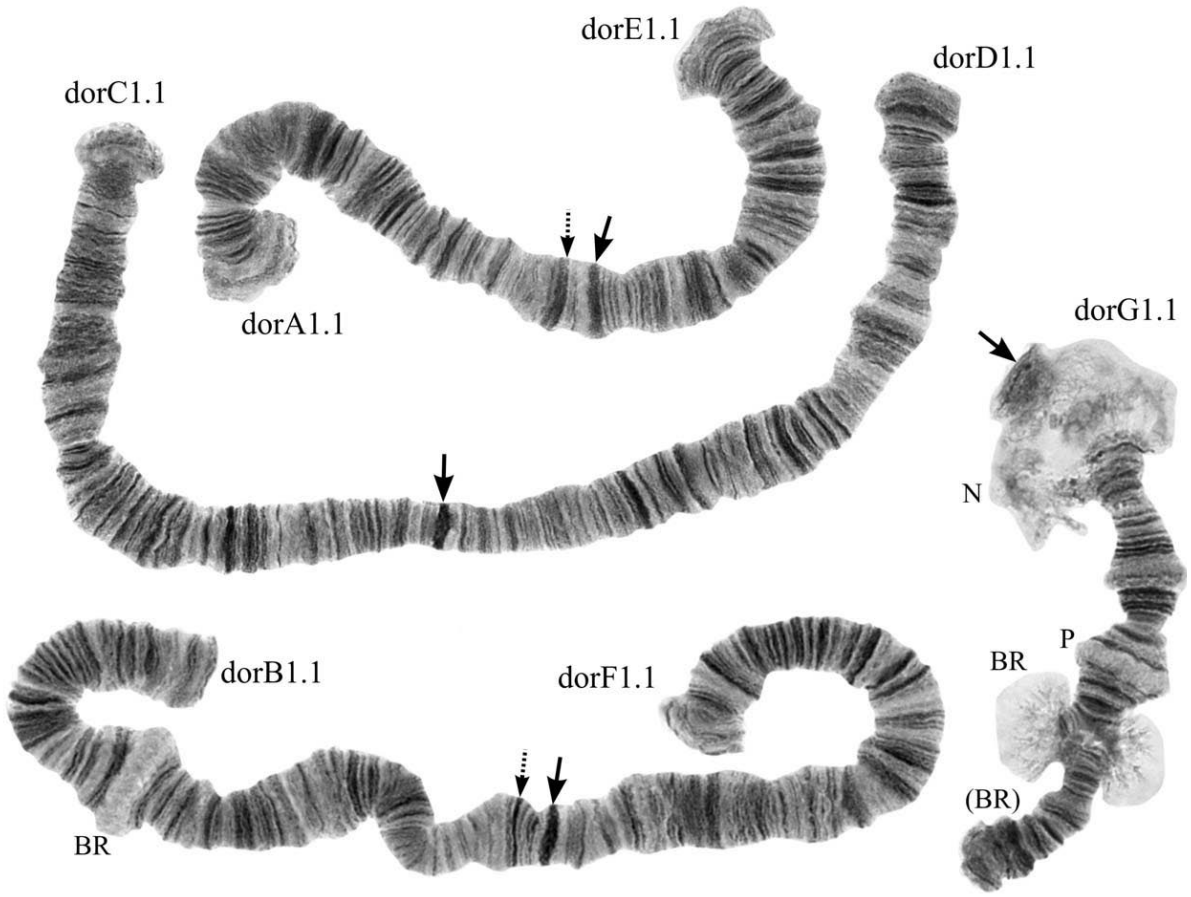


Fig. 1. Karyotype of *Chironomus dorsalis*. dorA1.1, dorB1.1, etc., are the designations of genotypic combinations of banding sequences in chromosome arms; N, nucleolus; BR, Balbiani ring; P, puff. Solid arrows show the centromeric bands and dashed arrows indicate additional C-positive bands in the centromeric regions of chromosomes AE and BF.

ing of the origin of these bands, we thoroughly compared the banding sequences of *Ch. dorsalis* and some of the "thummi" cytocomplex species.

Banding sequences

Arm A is monomorphic (Fig. 3, a; Tables 2, 3). The only banding sequence dorA1 differs from the standard sequence of *Ch. piger* arm A (pigA1) by three overlapping inversions:

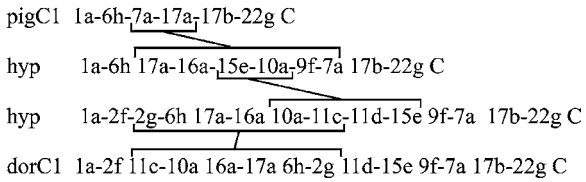
pigA1 1a-2c-2d-12c-13a-19f C
hyp 1a-2c 12c-10a-9e-4a-3i-2d 13a-19f C
hyp 1a-2c 4a-9e-10a-12c 3i-2d 13a-19f C
dorA1 1a-2c 4a-9e 2d-3i 12c-10a 13a-19f C

Of the species from the "pseudothummi" cytocomplex, dorA1 is most close to the sequences of *Ch. melanescens* Keyl, 1961 and *Ch. holomelas* Keyl, 1961, differing from them by only one simple inversion.

A specific feature of dorA1 is a very intensive staining of bands 19ef in chromosome AE (Fig. 2, b). It should be noted that only 19f of these two bands gives a C-staining (Fig. 2, c). This band is similar to the centromeric band typical of the genus *Chironomus* in its thickness and staining intensity. The adjacent thin C-positive band has no analogs in the reference species *Ch. piger* (Figs. 2, c; 4, a).

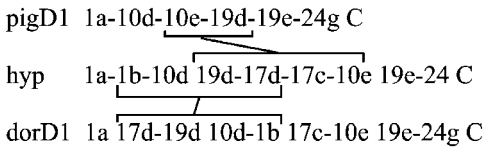
Arm B is monomorphic (Fig. 3, e; Tables 2 and 3). We succeeded in mapping only the most conserved regions of arm B (Fig. 3, e) due to complex rearrangements distinguishing dorB1 and pigB1. A specific feature of arm B is an unusually intensive staining of band 28b, which is almost as intensive as that of the common centromeric bands (Figs. 2, d; 3, e; 4, b). This band is C-positive (Fig. 2, e). BR is developed on region 7 (Figs. 1; 3, e).

Arm C is monomorphic (Fig. 3, c; Tables 2, 3). The sequence dorC1 differs from the standard pigC1 by three included inversions:



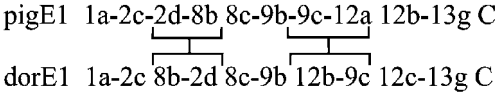
No closely related sequence was found in the species of "pseudothummi" cytocomplex.

Arm D is monomorphic (Fig. 3, d; Tables 2, 3). The sequence dorD1 differs from the standard pigD1 by two overlapping inversions:



No closely related sequence was found in the species of "pseudothummi" cytocomplex.

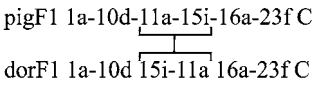
Arm E is monomorphic (Fig. 3, b; Tables 2, 3). The sequence dorE1 differs from the standard pigE1 by two nonoverlapping inversions:



All the bands corresponding to the standard *Ch. piger* are identifiable in arm E. An intensively stained band, C-positive and similar in its morphology to the centromeric band, is detected to the left of band 13g, the last in this arm (Figs. 2, b, c; 4, a).

No closely related sequence is detectable in the arm E of other "pseudothummi" species.

Arm F is monomorphic (Fig. 3, f; Tables 2, 3). The sequence dorF1 differs from the standard by one simple inversion:



All the bands corresponding to the standard *Ch. piger* are distinctly identifiable in arm F, including the typical C-positive centromeric band to the left of region 23 (Figs. 2, d, e; 4, b).

The sequence dorF1 is identical to the sequence holF1 of *Ch. holomelas* from the "pseudothummi" cytocomplex.

Arm G is low polymorphic and has two banding sequences, dorG1 and dorG2 (Fig. 5; Tables 2, 3). The sequence dorG1, observed as a rule in a homozygous state (Fig. 5, a, b), is predominant; dorG2 has been found only once in a heterozygous state and differs from dorG1 by a simple inversion (Fig. 5, c). Only one BRc is very intensively developed in the cells of the main and lateral lobes of *Ch. dorsalis* salivary gland (Fig. 5, a), whereas the additional BRa is activated only in the special lobe (Fig. 5, b). These rings are similar to the BRc and BRa of *Ch. piger*. The BRb of *Ch. dorsalis* is usually a puff (Fig. 5, a, b).

Peculiarities of the centromeric region banding patterns in chromosomes AE and BF

As is mentioned above, the presence of two large intensively stained bands in the centromeric regions of chromosomes AE and BF (Fig. 4) is a specific feature of *Ch. dorsalis* karyotype as compared with the other *Chironomus* species studied so far. This leads to difficulties

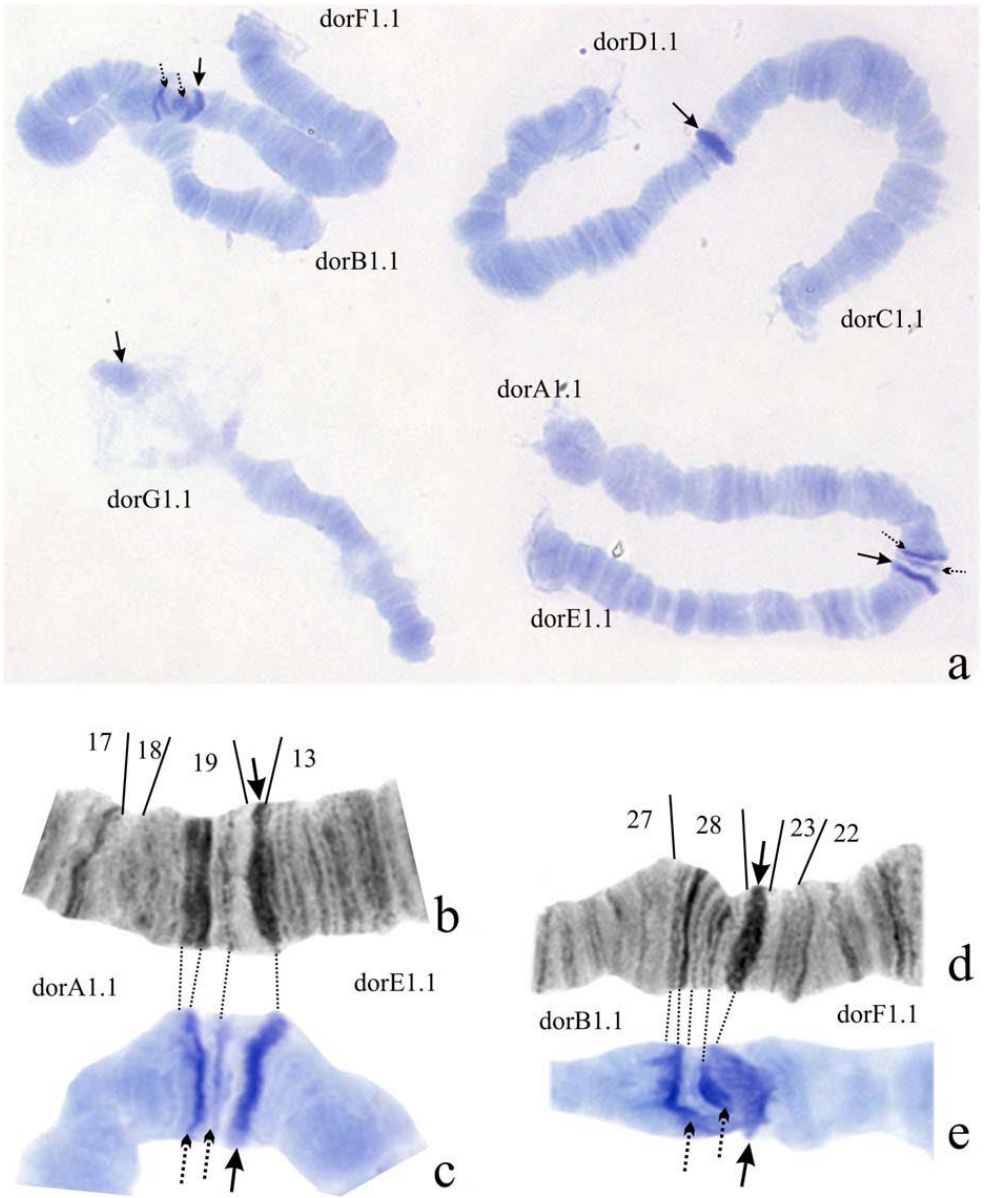


Fig. 2. C-staining of *Chironomus dorsalis* polytene chromosomes. **a -** karyotype. **b, c -** comparison of the chromosome AE centromeric region stained with aceto-orcein (**b**) and C-banding (**c**). **d, e -** comparison of the chromosome BF centromeric region stained with aceto-orcein (**d**) and C-banding (**e**). The designations are the same as in Fig. 1.

in detection of the proper centromeric band. As a rule, only the centromeric bands in polytene chromosomes give a distinct C-positive staining in the karyotypes of the standard, *Ch. piger*, and other previously studied *Chironomus* species (except for *Ch. riparius* Meigen, 1804 with local amplification of centromeric DNA) (Hägele, 1977; Sigareva, 1981; Hankeln et al., 1994). Unfortunately, the C-staining of mitotic chromosomes of the majority

Table 2. Frequencies of banding sequences in natural populations of *Chironomus dorsalis*.

Banding sequence	Population				
	YAR-LA n=10	NSK-GE n=12	NSK-BL n=50	RAL-AY n=14	PRI-SH n=23
dorA1	1	1	1	1	1
dorB1	1	1	1	1	1
dorC1	1	1	1	1	1
dorD1	1	1	1	1	1
dorE1	1	1	1	1	1
dorF1	1	1	1	1	1
dorG1	1	1	1	1	0.978
dorG2	0	0	0	0	0.022
Total number of banding sequences	7	7	7	7	8

of *Chironomus* species is unsuccessful due to small amounts of centromeric heterochromatin (Hägele, 1977). As has been shown, both thick bands in the *Ch. dorsalis* chromosomes AE and BF are C-positive (Fig. 2c, e). This suggested that the *Ch. dorsalis* chromosomes AE and BF could be dicentric. On the other hand, the chromosomes CD and G each carry only one typical C-positive centromeric band (Fig. 2, a). It should be noted also that one or two very thin additional C-positive bands are observable between the two large C-positive bands in the chromosomes AE and BF in the majority of cases (Fig. 2, c, e). Study of the morphology of C-positive bands allows us to suggest that the C-positive band adjacent to 13g region on arm E was the centromeric band of chromosome AE, and the C-positive band located near band 23g on arm F was the centromeric band of chromosome BF, because both bands displayed a dense vacuolized structure characteristic of the centromeric regions in the genus *Chironomus* (Fig. 2, b, d).

As C-staining of mitotic chromosomes was unsuccessful, we studied mitosis to find the bridges and the fragments that had to be generated in mitosis in the presence of dicentric chromosomes. No abnormalities were found in mitosis. This suggests that once chromosomes AE and BF are dicentric, one of the centromeres must be inactivated.

Chromosomal polymorphism

Before this work, the chromosomal polymorphism of *Ch. dorsalis* has not been actually studied, except for the data on the absence of polymorphism in German populations (Keyl, 1962) and the presence of single heterozygous inversion in arm G in Bulgarian populations (Michailova, 1989). The majority of populations that we have studied were also monomorphic with the exception of one Far Eastern population, which displayed a single heterozygote in arm G (Fig. 5, c; Tables 2, 3). Correspondingly, the banding sequence pool of this species is small, comprising only eight banding sequences.

Discussion

The data obtained in this work demonstrate that *Ch. dorsalis* is widely distributed not only in Western Europe (Fauna Europaea, 2007; www.faunaeur.org, on August, 2007), but also in Russia. The karyotype of *Ch. dorsalis*

Table 3. Frequencies of genotypic combination of banding sequences in natural populations of *Chironomus dorsalis*.

Cenotypic combination of banding sequences	Population				
	YAR-LA n=10	NSK-GE n=12	NSK-BL n=50	RAL-AY n=14	PRI-SH n=23
dorA1.1	1	1	1	1	1
dorB1.1	1	1	1	1	1
dorC1.1	1	1	1	1	1
dorD1.1	1	1	1	1	1
dorE1.1	1	1	1	1	1
dorF1.1	1	1	1	1	1
dorG1.1	1	1	1	1	0.967
dorG1.2	0	0	0	0	0.043
Total number of genotypic combination of banding sequences	7	7	7	7	8
% of larvae with heterozygotic inversions	0	0	0	0	4.3
Average number of heterozygotic inversions per larvae	0	0	0	0	0.04

in all the populations studied is identical and displays a low polymorphism. The published data on a high polymorphism of this species in English and Scottish populations (Acton, 1956, 1957) are erroneous, as the author studied *Ch. luridus* Strenzke, 1959, which is actually a highly polymorphic species, rather than *Ch. dorsalis*. Thus, *Ch. dorsalis* can be considered a low polymorphic species. The size of its banding sequence pool is small and comprises only eight banding sequences, which is five–sevenfold lower than those of highly polymorphic *Chironomus* species (Kiknadze et al., 2004b).

According to the banding pattern of *Ch. dorsalis* polytene chromosomes, its karyotype is comparatively close to the standard karyotype of *Ch. piger*. In particular, arm F differs by only one inversion; arms D and E, by two inversions; and arms A and C, by three inversions. In the NJ phylogenetic tree (Fig. 6), *Ch. dorsalis* is located in the cluster neighbouring *Ch. piger*; as for the pseudothummi cytocomplex species, *Ch. dorsalis* is most closely related to *Ch. luridus*.

A comprehensive study of the banding patterns of *Ch. dorsalis* polytene chromosomes has detected peculiar specific features of the centromeric regions in two chromosomes, AE and BF. These chromosomes of *Ch. dorsalis* differ by the reciprocal translocations of whole arms from the chromosomes AB and EF of the species forming the "thummi" cytocomplex, including the standard, *Ch. piger*. The difference between the centromeric regions of *Ch. dorsalis* and *Ch. piger* (as well as of other members of the "thummi" cytocomplex) consists in that all the *Ch. piger* chromosomes have only one distinct C-positive centromeric band, whereas two *Ch. dorsalis* chromosomes carry two large C-positive bands each. As all the bands in *Chironomus* species, centromeric bands included, are personified and have their own precise designations (Keyl, 1962; Dévai et al., 1989), we could compare the banding sequences in the centromeric regions of *Ch.*

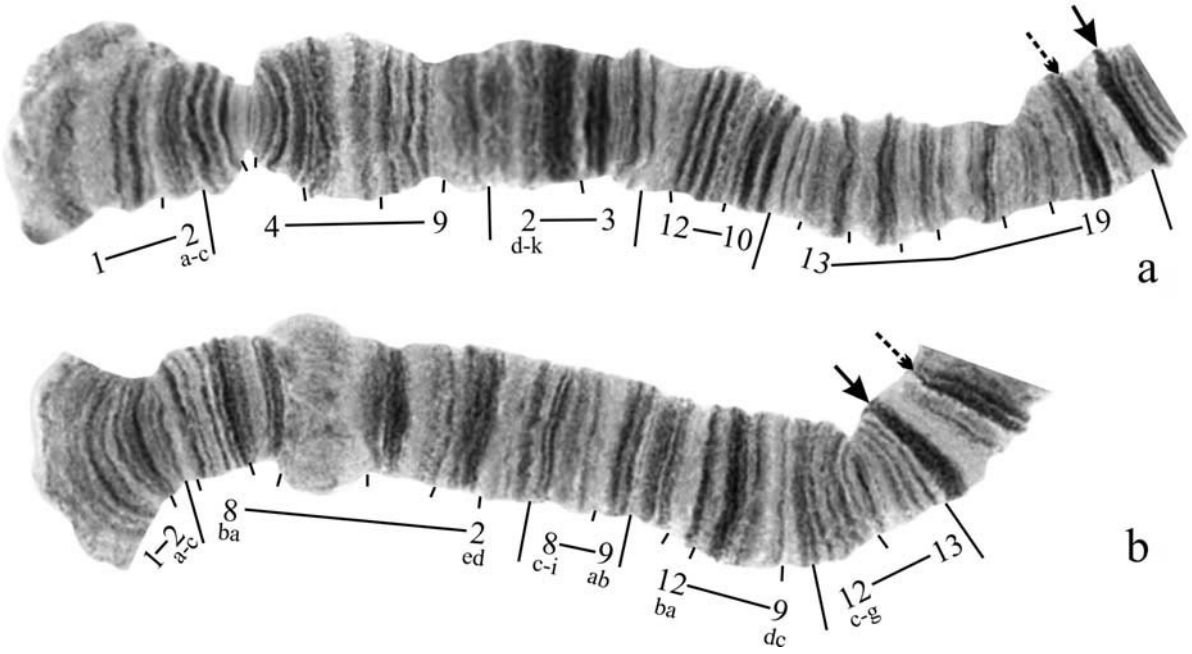


Fig. 3, a, b. Mapping of *Chironomus dorsalis* chromosome arms in chromosome I. **a** - dorA1.1. **b** - dorE1.1. The designations are the same as in Fig. 1.

dorsalis, *Ch. piger*, and *Ch. muratensis* Ryser, Scholl et Wuelker, 1983 and find out from the morphology which particular *Ch. dorsalis* large band was the true candidate centromeric band (Figs. 2, 4). This morphological analysis suggested that the chromosome AE (pseudothummi cytocomplex) and chromosome AB ("thummi" cytocomplex) had an identical centromeric band, which was retained with arm A during the translocation, whereas the translocation break occurred to the right of this band. The second thick C-positive band in chromosome AE could correspond to the heterochromatized band 19f of arm A.

According to our opinion, the centromeric band in *Ch. dorsalis* chromosome BF was carried with the arm F, while the second C-positive band can correspond to the heterochromatized band 28b of arm B. We believe that the presence of two C-positive bands in the centromeric regions of chromosomes AE and BF can indicate their dicentric nature.

How could such dicentric chromosomes have originated during evolution? It is known that the karyotypes of primitive chironomids (subfamily Tanypodinae) have 14 one-armed chromosomes, which formed two-armed chromosomes (subfamilies Chironominae and Orthocladiinae) during evolution (Petrova, 1989, 1990). Two variants of the fate of centromeric bands are possible after the fusion of rod-shaped chromosomes: first, when two true telocentrics, lacking the second arm, are fused, their centromeres are united into a large centromere, functioning as a whole, and, second, if the rod-shaped chromosomes still had very short second arms, then the centromeres in two-armed chromosomes will be separated by short regions and a dicentric chromosome will be formed (Holmquist, Dancis, 1980). Thin interstitial bands observed between two centromeric bands can be regarded as relic

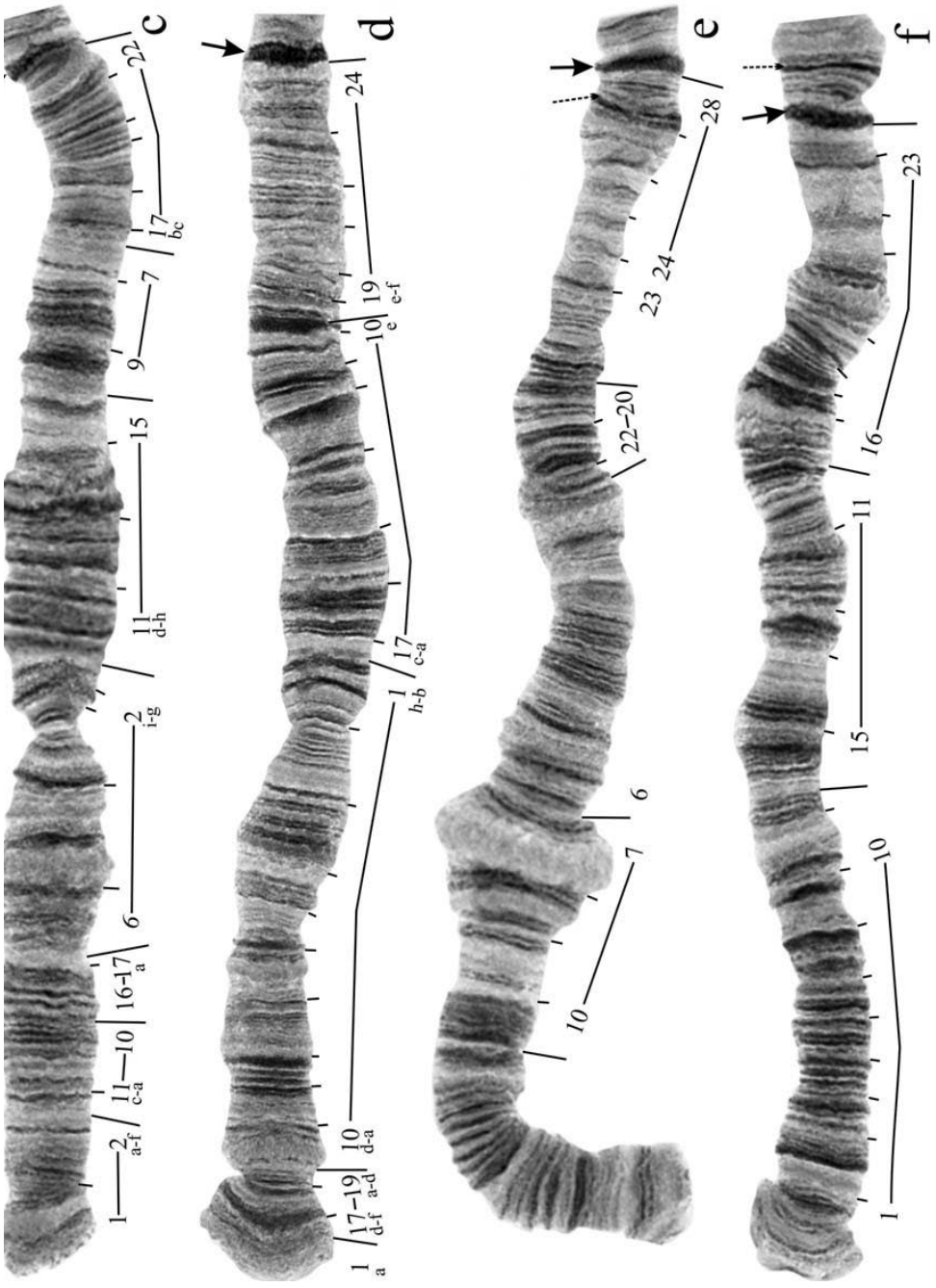


Fig. 3 c-f. Mapping of *Chironomus dorsalis* chromosome arms in chromosome II (**c**, **d**), and chromosome III (**e**, **f**): **c** - dorC1.1; **d** - dorD1.1; **e** - dorB1.1; **f** - dorF1.1. The designations are the same as in Fig. 1.

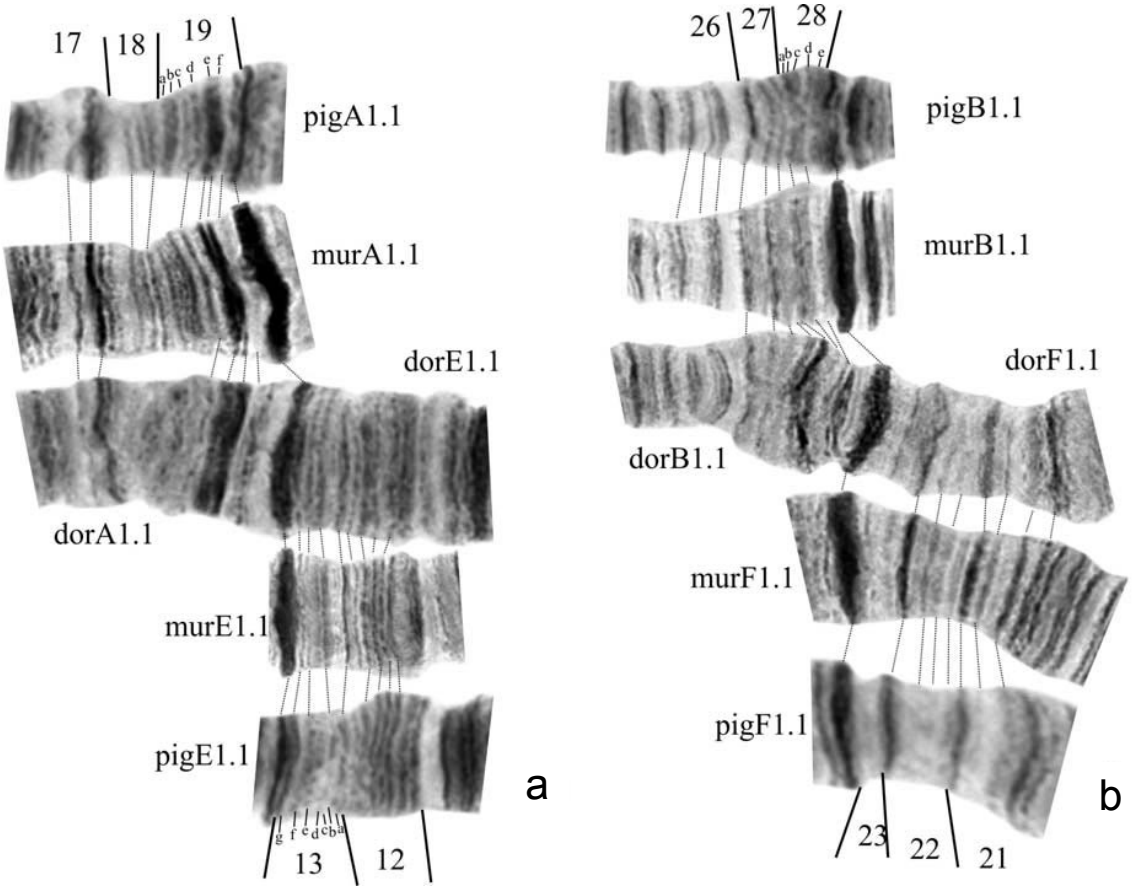


Fig. 4. Mapping of the centromeric regions in *Chironomus dorsalis*, *Ch. piger*, and *Ch. muratensis* chromosomes AE (**a**) and BF (**b**).

structures of the translocation process or the result of other chromosomal rearrangements, deletions or duplications (Lentzios et al., 1980). According to Hsu et al. (1975), one of the centromeres in a dicentric chromosome is, as a rule, inactivated, and its presence does not cause any chromosomal rearrangements during the evolution. Presumably, both variants were realized in the karyotype of *Ch. dorsalis*, namely, formation of the united centromere in chromosome CD and retention of two centromeres in chromosomes AE and BF with inactivation of one of the centromeres.

When the combinations AB CD EF G and AE CD BF G were formed, the inactivated centromere also lost its morphological properties (heterochromatization and C-staining). Consequently, only one centromeric band is distinctly detectable in each chromosome in the "thummi" cytocomplex.

It should be noted that until recently the "thummi" cytocomplex has been considered as ancestral for the other cytocomplexes (Wülker, 1980; Shobanov, 2000), and appearance of additional large bands in the centromeric region of certain pseudothummi species has been regarded as the origination of a neocentromere after the loss of the centromeric band during translocation (Shobanov, Petrova, 1995).

Our hypothesis postulated above seems

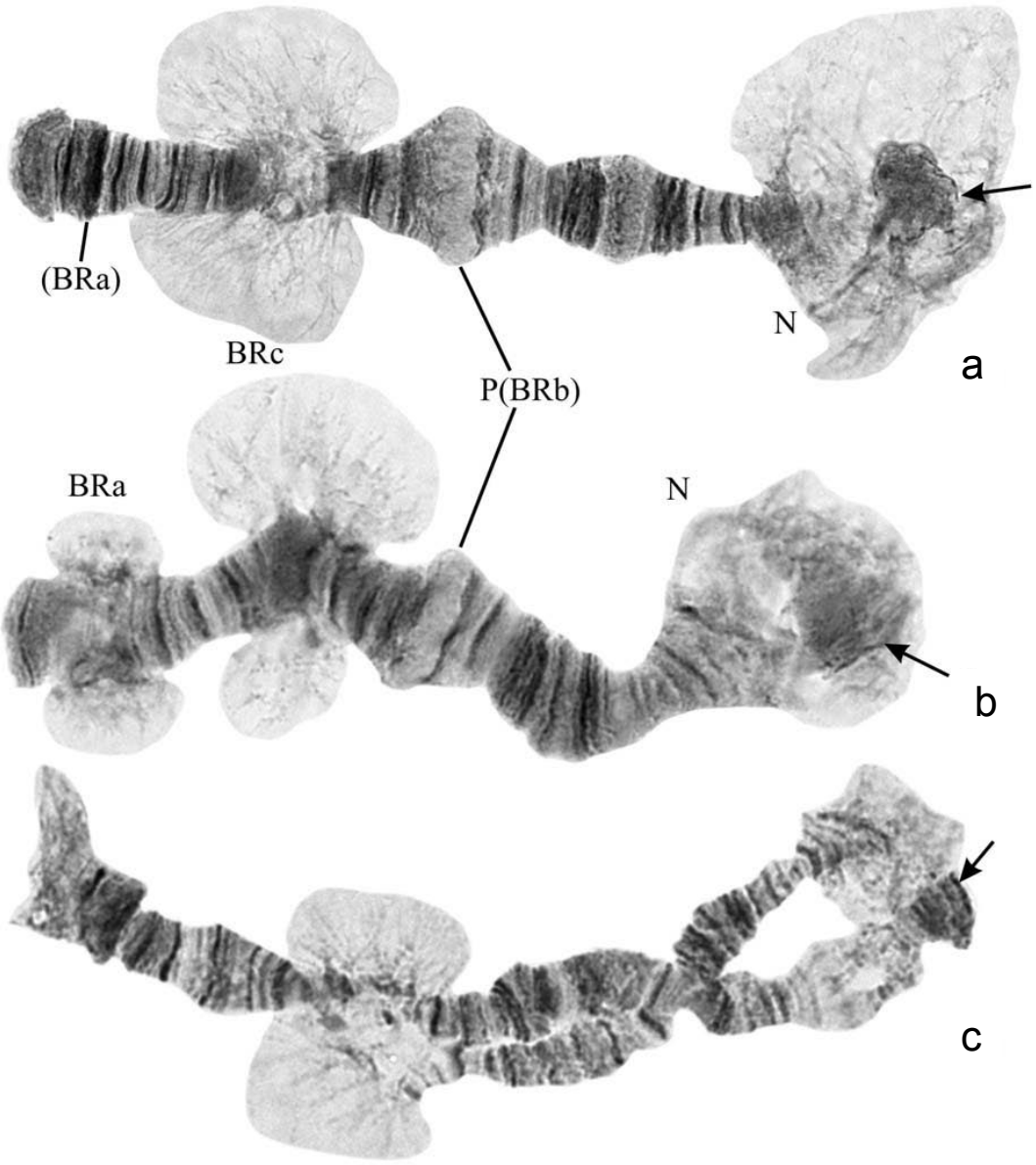


Fig. 5. Chromosome polymorphism in *Chironomus dorsalis* arm G. **a -** dorG1.1 from the cells of salivary gland main lobe. **b -** dorG1.1 from the cells of salivary gland special lobe. **c -** heterozygous inversion dorG1.2. BRa, BRb, and BRc are Balbiani rings a, b, and c. The rest designations are the same as in Fig. 1.

now more preferable, as the data indicating that the pseudothummi cytocomplex is more primitive than the "thummi" cytocomplex (Guriev et al., 2001; Kiknadze et al., 2003, 2004a; Gunderina et al., 2005) are being accumulated, thereby suggesting that the combination AE CD BF G (pseudothummi) was initial for the combination AB CD EF G ("thummi").

Turning back to the hypothesis on the presence of dicentric chromosomes in *Ch. dorsalis* karyotype, it should be noted that the relevant literature reports several cases, although rare, of dicentrics in the normal karyotypes. For example, dicentrics have been found in

Fig. 6. Neighbour-joining phylogenetic tree for the genus *Chironomus* constructed based on the data on the number of chromosome breaks in the main banding sequences of the corresponding species. The "pseudothummi" cytocomplex is shaded in grey.

mammals *Muntiacus muntjak* (Zimmermann, 1780), beetles *Phosphuga atrata* (Linnaeus, 1758), and black flies *Cnephia lapponica* Enderlein, 1921 (Hsu et al., 1975; Lyapunova et al., 1983; Petrova, 1972). In all these cases, it is assumed that one of the centromeres in dicentric chromosomes is inactivated, because the individuals of these species develop normally. Dicentrics are much more frequent in the karyotypes of the cells in various diseases; in these cases, both centromeres remain active, that frequently leads to generation of bridges and fragments during cell division (Choo, 1997; Amor, Choo, 2002; Karpen, Allshire, 1997).

C-staining failed to solve the problem of detecting the true centromeric band, yet allowed us to postulate a possible origin of the *Ch. dorsalis* dicentric chromosomes. Our hypothesis on the existence of dicentric chromosomes in the karyotype of *Ch. dorsalis* can be verified using molecular cytological methods, such as FISH of the centromeric DNA or use of antibodies to the centromere-specific histone H3, which could demonstrate which DNA repeats are contained in the large bands located in the centromeric regions of dicentric chromosomes and in what state is the histone influencing the centromere activity.

Acknowledgements

We are greatly appreciated to A. V. Katokhin for the help in collecting of the material from the Far East of Russia. Financial support was provided by grants from the Presidium of the Russian Academy of Sciences ("Biodiversity and dynamics of gene pools" and "Origin and evolution of biosphere").

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Received March 17, 2008.
Accepted by I.A. Gavrilov, April 24, 2008.
Published June 30, 2008.