Karyotypes of Chironomus Meigen (Diptera: Chironomidae) species from Africa

Wolfgang F. Wülker¹, I.I. Kiknadze², A.G. Istomina²

¹ Zur Bitzenmatte 9, D79249 Merzhausen, Germany ² Institute of Cytology and Genetics, Russian Academy of Sciences, Siberian Branch, Novosibirsk, 630090, Lavrentiev pr. 10, Russia

Corresponding author: W.F. Wülker (Wolfgang.Wuelker@biologie.uni-freiburg.de)

Abstract
The karyotypes of six African Chironomus species (Ch. alluaudi Kieffer, 1913, Ch. transvaalensis Kieffer, 1923, Ch. sp. Nakuru, Ch. formosipennis Kieffer, 1908, Ch. prope pulcher Wiedemann, 1830, Ch. sp. Kisumu) were investigated; four of these karyotypes were described for the first time (Ch. sp. Nakuru, Ch. formosipennis, Ch. prope pulcher, Ch. sp. Kisumu). Of the six Chironomus karyotypes, three had “pseudothummi” cytocomplex chromosome arms combinations AE CD BF G (Ch. alluaudi, Ch. transvaalensis, Ch. sp. Nakuru), two had “thummi” cytocomplex arms combinations AB CD EF G (Ch. formosipennis, Ch. prope pulcher), and one had “parathummi” arm combinations AC BF DE G (Ch. sp. Kisumu). Thus, three of the ten main cytocomplexes known were detected in Africa. Detailed photomaps of all chromosome arms, with the exception of arms B and G, were prepared for the karyotypes of Ch. alluaudi, Ch. transvaalensis, Ch. sp. Nakuru, Ch. prope pulcher; the karyotypes of Ch. formosipennis, Ch. sp. Kisumu could only be fragmentarily mapped.

Endemic African banding sequences were characteristic for most of the chromosomal arms in all species studied. However, basic sequences, which can be present in different Chironomus species on different continents (Wülker, 1980; Kiknadze et al., 2008), were also detected also in several African species (Ch. alluaudi, Ch. sp. Nakuru, and Ch. formosipennis). The banding sequences of African species studied allow discussion of the derivation of modern banding patterns from hypothetical species, living before separation of cytocomplexes and continents.

Keywords
Chironomus, karyotype, banding sequences, chromosomal polymorphism, chromosomal evolution
Introduction

As shown by cytogenetic analysis of chromosomal evolution, the divergence of animal karyotypes during speciation was mainly mediated by para- and pericentric inversions, altering the gene orders in linkage groups (Dobzhansky 1970, White 1977, King 1993, Zdobnov et al. 2002). The other types of chromosomal rearrangements (translocations, fusions, duplications) play an additional role in rearrangements of the linear structure of genome. Alteration of the gene orders in chromosomes during evolution can be visualized in Diptera, which possess polytene chromosomes with distinct banding sequences. The bands of polytene chromosomes, which form species-specific banding sequences, are considered as genetic markers to analyze divergence patterns of the linear genome structure during evolution. The use of the number of chromosomal breakpoints as a divergence measure provided establishment of phylogenetic relationships between species (Kiknadze et al. 2008). Species of the genus Chironomus have four giant chromosomes with seven chromosome arms (A-G). Based on the different combination of the arms, caused by whole-arm translocations, the Chironomus species are grouped into several cytocomplexes (Keyl 1962, Wülker 1980). Cytocomplex is not a taxonomic term. It includes the species with definite chromosome arms combinations, but not similar morphologically. Comparison of banding sequences between species from different cytocomplexes have shown that karyotypes can include species-specific sequences and so called basic sequences, common to more than one cytocomplex and in more than one continent. Such basic sequences were probably present before the separation of species and cytocomplexes (Keyl 1962, Wülker 1980).

By global analysis of banding sequences in Eurasia, North and South America, Australia, we have traced banding sequence changes during Chironomus species divergence and continent dispersal (Martin et al. 1974, Wülker 1980, Wülker et al., 1989, Kiknadze et al. 2003, 2008). It was shown that in Eurasia, North America, and Australia, banding sequence pools of many species were represented mainly by endemic continent-specific sequences. However, basic sequences, common for different continents were also found in karyotypes of some species in addition to the endemic sequences. Such basic sequences were noted also in two African Chironomus species (Ch. alluaudi, and Ch. sp. Nakuru) (Martin 1979, Wülker 1980). It was of interest to study how often such basic sequences can be found among African species. However, the data on Chironomus karyotypes in Africa are very scanty despite there being much information on the morphology of African chironomids. Wülker (1980) has presented photographs of seven chromosome arms of Ch. alluaudi; Martin (1979) has quoted the arm F banding sequence of Ch. transvaalensis; Wülker et al. (1989) included the banding sequences of Ch. transvaalensis arms A, and F in their list of Chironomus sequences, and have shown the position of Ch. alluaudi and Ch. transvaalensis on the phylogenetic tree.

This paper contains full descriptions of the karyotypes of six African Chironomus species (Ch. alluaudi, Ch. transvaalensis, Ch. sp. Nakuru, Ch. formosipennis, Ch. prope pulcher, Ch. sp. Kisumu). Among them four karyotypes are described for the first time.
(Ch. sp. Nakuru, Ch. formosipennis, Ch. prope pulcher, Ch. sp. Kisumu). Detailed photomaps of arms A, C, D, E, and F are presented for Ch. alluaudi, Ch. transvaalensis, and Ch. sp. Nakuru. The chromosome arms could be mapped only partly for Ch. formosipennis, Ch. prope pulcher and Ch. sp. Kisumu.

The presence of further basic banding sequences in the karyotypes of African Chironomus species was discovered, along with endemic continent-specific (Ethiopian region) sequences.

Evolutionary divergence of “thummi“ and “pseudothummi” cytocomplexes is discussed.

Material and methods

Forth instar larvae of African Chironomus species were used for karyotype study. 35 years ago, one of us (W.W.) had the opportunity to visit Kenya (22.12.1975–16.01.1976). From a base at the house of relatives in Nairobi, he went with family (wife and 3 sons) to collect chironomids to the west to Lake Nakuru and Lake Victoria, to the north to Anderle Mount Ca. 3000 m above N.N., and to the southeast to Tsavo National park, Mombasa and vicinity. Other material was contributed by colleagues: Mount Elgon and Lake Naivasha (Peter N. Cox), Mount Kenya, near 4350 m (scientific excursion of University Erlangen, Germany, under Prof. Dr. Rüppell), Zigi River, Tanzania (Dr. J. Grunewald). The list of collection sites of Chironomus larvae is presented in Table 1. We have not identified species Ch. sp. Nakuru and Ch. sp Kisumu, but the study of the banding sequences of their karyotypes was very important for purpose of our paper.

Larvae were fixed in ethanol-glacial acetic acid (3:1). The technique of chromosome preparation was as usual (Keyl, 1962). The identification of chromosome banding sequences follows by Keyl (1962) for arms A, E, and F, and by Dévai et. al., (1989) for arms C and D.

To trace the relationship of African Chironomus banding sequences with sequences from other continents, we compared them with known basic sequences; if basic sequences for some of species were unknown, we compared them with Chironomus piger standard (ST).

We have pointed to previous literature on morphological characteristics of species studied at the beginning of each species description. Most part of the material (larvae, pupa, adults and karyotype slides) is now deposited in Zoologische Staatssammlungen in Münich (Germany).

Equipment of the Center of Microscopy Analysis of Biological Objects of SB RAS in the Institute of Cytology and Genetics (Novosibirsk) was used in accomplishment of this work: microscope “Axiokop” 2 Plus, CCD camera AxioCam HRc, software package AxioVision 4 (Zeiss, Germany).
Results

**Chironomus alluaudi Kieffer, 1913**
http://species-id.net/wiki/Chironomus_alluaudi

**Previous reports:** Kieffer 1913, imago.
Freeman 1957, imago.
Wülker 1980, photo of arms A-G
Wülker et al. 1989, phylogenetic position
Kiknadze et al. 2004, list of banding sequences of arms A, C, D, E, and F

**Karyotype** (Fig. 1a). Haploid number n=4, arm combination AE CD BF G (“pseudo-otthummi” cytocomplex), centromere bands not heterochromatinized, nucleolus in arm G (terminal), at least 3 Balbiani Rings (BRs) on arm G, inversion polymorphism in arms C and G.

Banding sequences (Fig. 1b-g)

**Arm A** (Fig. 1b) has the sequence all A1 identical with the main sequence of arm A found in many Chironomus species (Ch. holomelas Keyl, 1961, Ch. melanescens Keyl, 1961, etc.) and it is considered a cosmopolitan basic sequence (holA1).

**Table 1.** Collection sites and number of specimens of African Chironomus species.

| Species                  | Collection sites                                                                 | Collection date | Collector                  | Number of specimens |
|--------------------------|----------------------------------------------------------------------------------|-----------------|----------------------------|---------------------|
| *Chironomus alluaudi*    | Kenya: drinking troughs brooks and pools at Endebess/Mt. Elgon, mountain lakes W of Nakuru, Aberdare mountains up to 3300 m, ponds at MtKenya 4350 m, near Limuru (north of Nairobi), Athi-river south of Nairobi, Amboseli-park | 29.12.75, 03.01.76, 12.01.76, 13.01.76, 10.01.76 | P. N. Cox, W. Wülker, G. Rüppell, W. d’Oleire-Oltmanns, H. Koehler, W. Wülker | 120                  |
| *Chironomus transvaalensis* | Kenya: pool east Lake Victoria, north and south Athi-river near Nairobi, west of Mombasa, Tansania: Kikuwi-river | 27.12.75, 13.01.76 | W. Wülker J. Grunewald     | 115                  |
| *Chironomus sp. Nakuru*  | Kenya: brook south east Lake Nakuru                                              | 26.12.75        | W. Wülker                 | 9                   |
| *Chironomus formosipennis* | Kenya: Lake Naivasha, Tansania: Zigi-river, running waters                       |                 | P. N. Cox, J. Grunewald   | 15                  |
| *Chironomus prope pulcher* | Kenya: two pools in short distance, River Athi south of Nairobi                  | 13.01.76        | W. Wülker                 | 6                   |
| *Chironomus sp. Kisu**Mu* | Kenya: flat pools 10 and 22 km east Lake Victoria, together with *Ch. transvaalensis*, brook in Amboseli-park (Kilaguni) | 27.12.75, 10.01.76 | W. Wülker                 | 7                   |
Karyotypes of Chironomus Meigen (Diptera, Chironomidae) species from Africa

allA1 1a-2c 10a-12c 3i-2d 9c-4a 13a-19f

Arm E (Fig. 1, c, 7, b) has the sequence allE1 identical with Chironomus piger ST (cosmopolitan basic sequence).
allE1 1a - 13g

Arm C (Fig. 1, d) has two sequences, allC1 and allC2, differing by a simple inversion. The sequence allC1 differs greatly from the basic sequence in arm C; therefore we have compared it with Chironomus piger ST: differing by seven inversion steps from pigST:

allC1 1a-2g 11c-10a 16a-17a 6h-2h 11d-15e 9f-7a 17b-22g
allC2 1a-2g 11c-10a 16a-17a 15e-11d 2h-6h 9f-7a 17b-22g
hyp 5 1a-2g 11d-15e 17a-16a 10a-11c 7a-9f 6h-2h 17b-22g
hyp 2+3+4 1a-2g 11d-15e 16a-17a 10a-11c 7a-9f 6h-2h 17b-22g
hyp 1 1a-2g 17a-16a 15e-11d 11c-10a 9f-7a 6h-2h 17b-22g
pigST 1a-2g 2h-6h 7a-9f 10a-11c 11d-15e 16a-17a 17b-22g

Arm D (Fig. 1, e) has single sequence allD1 differing by one inversion step from pigST:
allD1 1a-9e 19h-10a 20a-24g
pigST 1a-9e 10a-19h 20a-24g

Arm B (Fig. 1, a) not mapped, monomorphic. The common BR is not developed.
Figure 1b-g. Homozygous banding sequences of *Chironomus alluaudi* in arms A, E, C, D, F and G. The designations are the same as in Fig. 1.

**Arm F** (Fig. 1, f) has the sequence allF1, identical with pigST (cosmopolitan basic sequence).

allF1 1a–23f

**Arm G** (Figs 1, a, g) not mapped, has two sequences allG1 and allG2 differing by one simple inversion in the central part of arm G, including two of the Balbiani rings.
In total, the banding sequence pool of *Ch. alluaudi* contains 9 sequences. Six of them endemic for Africa (Ethiopian sequences), three of them (allA1, allE1, allF1) belong to the category of cosmopolitan basic sequences. *Ch. alluaudi* can be considered as a *Chironomus* species with a primitive karyotype (Wülker, 1980, 2010).

**Larva:** “thummi-type” (no tubuli laterales) on abdominal segment VII). Mentum with high lateral tooth, median tooth as in other *Chironomus* species, pectin epipharyngis about 11 teeth, antenna black with 4 segments, paralabial plates about 40 striae.

**Distribution:** different places in Africa (Freeman, 1957), Kenya (leg. Wülker, Jan. 1976). Dunking troughs brooks and pools at Endebess/Mt Elgon (N. Cox leg.); mountain lakes W of Nakuru, Aberdare mountains up to 3300m, ponds Mt. Kenya 4350m (Oltmanns leg.) near Limuru (north of Nairobi), Athi river south of Nairobi, Amboseli-park (Wülker leg.)

*Chironomus transvaalensis* Kieffer, 1923
http://species-id.net/wiki/Chironomus_transvaalensis

**Previous reports:** Kieffer 1923, imago.
Mc Lachlan 1969, 1971: larva and pupa.
Freeman 1957, imago.
Martin 1979, banding sequence of chromosome arm F.
Wülker, Dévai and Dévai 1989, banding sequences of arms A, E, and F, phylogenetic position of species.

**Karyotype** (Fig. 2, a). Haploid number n=4, arm combination AE CD BF G (“pseudothummi” cytocomplex), centromeric bands not heterochromatinized, nucleolus in arm C, inversion polymorphism in arms C and G.

**Banding sequences** (Fig. 2, b-f).

**Arm A** (Fig. 2, b) has the sequence trvA1, differing by only one inversion step from the basic sequence holA1.

| trvA1 | holA1 |
|-------|-------|
| 1a-2c 10a-12c 3i-c 5a-9e 2d-3b 4d-a 13a-19f | 1a-2c 10a-12c 3i-c 2d-3b 9e-5a 4d-a 13a-19f |

**Arm E** (Fig. 2, c) has the banding sequence trvE1, differing only by one step from basic sequence aciE1 (*Ch. acidophilus* Keyl, 1960 etc.)

| trvE1 | aciE1 |
|-------|-------|
| 1a-2b 5a-10b 3e-2c 4h-3f 10c-13g | 1a-3c 10b-5a 4h-3f 10c-13g |

**Arm C** (Fig. 2, d, j) has two banding sequences, trvC1 and trvC2, differing by one simple inversion (Fig. 2, j). The sequence trvC1 is formed by four inversion steps from a basic sequence, (lonC1), found in several *Chironomus* species (*Ch. longisty- lus* Goetghhebuer, 1921, *Ch. anthracinus* Zetterstedt, 1860 etc.).
trvC1 1a-2e 11d-12d 2f-6b 13a-15e 8a-11c 6gh 17a-16a 7d-a 6f-c 17b-22g
hyp 3 1a-2e 11d-12d 6b-2f 13a-15e 8a-11c 6gh 17a-16a 7d-a 6f-c 17b-22g
hyp 2 1a-2e 2f-6b 12d-11d 13a-15e 8a-11c 6gh 17a-16a 7d-a 6f-c 17b-22g
hyp 1 1a-2e 2f-6b 11d-12d 13a-15e 8a-11c 6gh 17a-16a 7d-a 6f-c 17b-22g
lonC1 1a-2e 2f-6b 11c-8a 15e-13a 12d-11d 6gh 17a-16a 7d-a 6f-c 17b-22g

Arm D (Fig. 2, e) has the sequence trvD1 differing from pigST by four inversion steps.
trvD1 1a-2h 4b-7e 12d-7f 19h-13a 4a-3a 20a-24g
hyp 2 1a-2h 4b-7e 7f-12d 13a-19h 4a-3a 20a-24g
hyp 1 1a-2h 19h-13a 12d-7f 7e-4b 4a-3a 20a-24g
pigST 1a-2h 3a-4a 4b-7e 7f-12d 13a-19h 20a-24g

Arm B (Fig. 2, a) not mapped, monomorphic. BR is well developed.

Arm F (Fig. 2, f) has the banding sequence trvF1 differing from cosmopolitan basic pigST by three inversion steps.
trvF1 1a-2a 10d-3f 14f-16g 14e-11a 2b-3e 17a-23f
hyp 2 1a-2a 10d-3f 16g-14f 14e-11a 2b-3e 17a-23f
hyp 1 1a-2a 10d-3f 3e-2b 11a-14e 14f-16g 17a-23f
pigST 1a-2a 2b-3e 3f-10d 11a-14e 14f-16g 17a-23f

Figure 2a. Karyotype of *Chironomus transvaalensis*. p – puff and the designations are the same as in Fig. 1
Figure 2b-f. Homozygous banding sequences of *Chironomus transvaalensis* in arms A, E, C, D and F.

**Arm G** (Fig. 2, g-i) has three banding sequences, trvG1, trvG2, and trvG3. The sequence trvG2 differs from trvG1 by a short inversion in the region BR1 (Fig. 2, h); the sequence trvG3 – by long inversion of central part of arm G (Fig. 2, i). Both last sequences were found as heterozygotes. There are four Balbiani rings.

In total, the banding sequence pool of *Ch. transvaalensis* contains 10 sequences, all of them are Ethiopian endemic sequences.

**Larva:** tubuli laterales at abdominal segment VII. Other characters - Mc Lachlan, 1969.  
**Distribution:** various places in Africa, Freeman (1957); Blantyre Malawi (Mc Lachlan), Wülker, 1957: pool east Lake Victoria, Kikuwi-river, Tanzania (J. Grunewald), Pretoria South Africa, Israel (Martin, personal communication).
Figure 2g-j. Homozygous and heterozygous banding sequences of *Chironomus transvaalensis* in arm G (g-i) and heterozygous inversion in arm C (j). Brackets above arms indicate the localization of inversions. The designations are the same as in Fig. 1.
Chironomus sp. Nakuru

Previous report: Wülker, 1980, banding pattern of arms A, E, and F. This species was not identified as well as Ch. sp. Kisumu because there was no additional possibility to collect larvae for rearing. However, the study of Ch. sp. Nakuru karyotype was very important for comparative analysis of Ethiopian Chironomus banding sequences with Chironomus sequences of the other continents.

Karyotype (Fig. 3, a). Haploid number n=4, arm combination AE CD BF G ("pseudothummi" cytocomplex), centromeric bands not heterochromatinized, nucleoli on arms F and G, Balbiani rings on arms G, B, and A. Chromosomal polymorphism was not recorded.

Banding sequences (Fig 3, b-f).

Arm A (Fig. 3, b) has the banding sequence nakA1 identical with cosmopolitan basic sequence found in many species (Ch. holomelas, Ch. melanescens, etc.)

nakA1 1a-2c 10a-12c 3i-2d 9c-4a 13a-19f

Arm E (Fig. 3, c) has banding sequence nakE1 differing by two inversion steps from the cosmopolitan basic sequence lonE1 (Ch. longistylus, Ch. anthracinus etc.).

nakE1 1a-3e 12g-a 5a-10b 4h-3f 10c-11d 13a-g
hyp1 1a-3e 12g-a 11d-10c 3f-4h 10b-5a 13a-g
lonE1 1a-3e 5a-10b 4h-3f 10c-11d 12a-g 13a-g

Figure 3a. Karyotype of Chironomus sp. Nakuru. The designations are the same as in Fig. 2a.
Figure 3b-f. Homozygous banding sequences of *Chironomus* sp. Nakuru in arms A, E, F, C and D. The designations are the same as in Fig. 1.
Arm C (Fig. 3, e) has the sequence nakC1 differing by four inversion steps from basic pattern lonC1 (Ch. longistylus, Ch. anthracinus, etc.) and by seven inversion steps from Chironomus piger ST (Fig. 7, b).

\[
\text{nakC1}\quad 1a-2e\ 4i-2f\ 5a-6b\ 13f-11d\ 11c-8a\ 15e-14a\ 6gh\ 17a-16a\ 7d-a\ 6f-c\ 17b-22g
\]

\[
\text{hyp1+2}\quad 1a-2e\ 4i-2f\ 5a-6b\ 11d-13f\ 14a-15e\ 8a-11c\ 6gh\ 17a-16a\ 7d-a\ 6f-c\ 17b-22g
\]

\[
\text{lonC1}\quad 1a-2e\ 2f-4i\ 5a-6b\ 11c-8a\ 15e-14a\ 13f-11d\ 6gh\ 17a-16a\ 7d-a\ 6f-c\ 17b-22g
\]

Arm D (Fig. 3, f) has the banding sequence nakD1 differing from pigST by three inversion steps.

\[
\text{nakD1}\quad 1a-2h\ 8c-13c\ 8b-3a\ 13d-15e\ 19h-16a\ 20a-24g
\]

\[
\text{hyp}\ 1\quad 1a-2h\ 13c-8c\ 8b-3a\ 13d-15c\ 16a-19h\ 20a-24g
\]

\[
\text{pigST}\quad 1a-2h\ 3a-8b\ 8c-13c\ 13d-15c\ 16a-19h\ 20a-24g
\]

Arm B (Fig. 3, a) not mapped, monomorphic. The common BR is not developed.

Arm F (Fig. 3, d) has the banding sequence nakF1 formed by four inversion steps from pigST.

\[
\text{nakF1}\quad 1a-i\ 10a-14e\ 6e-2a\ 7a-9f\ 14f-23f
\]

\[
\text{hyp}\ 3\quad 1a-i\ 10a-14e\ 2a-6e\ 7a-9f\ 14f-23f
\]

\[
\text{hyp}\ 2\quad 1a-i\ 10a-14e\ 9f-2a\ 14f-23f
\]

\[
\text{hyp}\ 1\quad 1a-i\ 14e-10a\ 9f-2a\ 14f-23f
\]

\[
\text{pigST}\quad 1a-i\ 2a-9f\ 10a-14e\ 14f-23f
\]

The arm F of Chironomus sp. Nakuru has a nucleolus in region 17–19.

Arm G (Fig. 3, a) has the banding sequence nakG1. It differs from the most of Chironomus species arm G by numerous Balbiani rings. It is possible to suggest that some of them can be nucleoli. But it is often impossible to differentiate nucleoli and Balbiani rings without electron microscopy or in situ hybridization.

In total, seven banding sequences are found in sequence pool of Ch. sp. Nakuru, six chromosomal arms have Ethiopian endemic sequences, and one arm (A) a cosmopolitan basic sequence.

**Larva:** long tubuli laterales at abdominal segment VII, extremely long antenna, gula light, no dark stripe on clypeus.

**Distribution:** brook to SE of Lake Nakuru, Kenya

Chironomus formosipennis Kieffer, 1908

http://species-id.net/wiki/Chironomus_formosipennis

**Previous reports:** Kieffer 1908, imago.
Freeman 1957, imago.
Dejoux 1970, imago.
Dejoux 1970, pupa.
Dejoux 1970, larva.

**Karyotype** (Fig. 4). Haploid number n=4, arm combinations AB CD EF G (“thumi” cytocomplex), centromeric bands not heterochromatinized, nucleoli in arms A and G, Balbiani ring in arm G. Chromosomal polymorphism was not recorded.

Banding sequence was determined only in arm E. The sequence frmE1 was identical with the cosmopolitan basic pattern, aprE1 (as in *C. aprilinus* Meigen, 1818)

frmE1  1a-3c 10b-3f 10c-13g

**Larva:** long tubuli laterales at abdominal segment VII. Other characters - Dejoux, 1970.

**Distribution:** Lake Naivasha, Kenya, Zigi-river, Tanzania, running waters.

**Chironomus prope pulscher** Wiedemann, 1830

**Previous reports:** Wiedemann 1830, imago.
Freeman 1957, imago.
Dejoux 1968, imago, pupa, larva.

![Figure 4](image-url)  
**Figure 4.** Karyotype of *Chironomus formosipennis*. The designations are the same as in Fig. 1.
The association to this species is based on one male adult from the collecting sites of the larvae.

**Karyotype** (Fig. 5, a). Haploid number n=3, arm combination AB CD FEG (modified “thummi” cytocomplex), centromeric bands not heterochromatinized, nucleolus in arm F (at the very telomeric end) and nucleolus-like bodies at the ends of arms A, B, E; Balbiani rings are in arms G and B. Chromosomal polymorphism in arm C (Fig. 5, a).

Banding sequences (Fig. 5, a, b-e).

**Arm A** (Fig. 5, b) has the banding sequence pulA1, formed by four inversions from pigST

pulA1  1a-3i 8g-6a 16d-17h 11e-9a 4ab 5c-4c 16c-12a 18a-19f
hyp 3+4 1a-3i 8g-6a 16d-17h 11e-9a 4abcd-5e 16c-12a 18a-19f
hyp 1+2 1a-3i 8g-6a 5c-4a 9a-11e 17h-16d 16c-12a 18a-19f
pigST  1a-3i 4a-5e 6a-8g 9a-11e 12a-17h 18a-19f

**Arm B** (Fig. 5, a) not mapped, monomorphic. It has a sequence pulB1. The common BR is well developed.

**Arm C** (Fig. 5, a) not mapped. It has two banding sequences pulC1 and pulC2 differing by a simple inversion, which involved practically the whole central part of arm C.

**Arm D** (Fig. 5, c) has the sequence pulD1, formed by five inversion steps from pigST

A. Karyotype of *Chironomus prope pulcher*. The designations are the same as in Fig. 1.
Figure 5b-e. Homozygous banding sequences of *Chironomus* prope *pulcher* in arms A, D, E, F, and G.
Arm E (Fig. 5, e) has the banding sequence pulE1, formed by three inversion steps from pigST.

| Banding Sequence | Description |
|------------------|-------------|
| pulE1            | 1a-2e 6e-4a 13d-12a 3f-a 6f-11d 13e-g |
| hyp 2            | 1a-2e 6e-4a 13d-12a 11d-6f 3a-f 13e-g |
| hyp 1            | 1a-2e 6e-4a 3f-a 6f-11d 12a-13d 13e-g |
| pigST            | 1a-2e 3a-f 4a-6e 6f-11d 12a13d 13e-g |

Arm F (Fig. 5, d) has the sequence pulF1, formed by one simple inversion from pigST.

| Banding Sequence | Description |
|------------------|-------------|
| pulF1            | 1a-10d 19d-11a 20a-23f |
| pigST            | 1a-10d 11a-19d 20a-23f |

The characteristic of arm F in *Ch. prope pulcher* is the presence of the nucleolus at the telomeric end, which is a rare event among *Chironomus* species.

Arm G (Fig. 5, e) is joined with arm E. There is large Balbiani ring near the site of fusion, and a small Balbiani ring or puff in the center of arm G. A small nucleolus is possibly developed at the telomeric end of arm G.

In total, eight banding sequences were recorded in the *Ch. prope pulcher* banding sequence pool. All of them are endemic for Ethiopia. There are no basic sequences.

- **Larva:** long tubuli laterales on abdominal segment VII. Other characters - Dejoux, 1968.
- **Distribution:** two pools within a short distance, River Athi south of Nairobi, Kenya.

**Chironomus sp. Kisumu**

Karyotype (Fig. 6, a). Haploid number n=4, arm combination AC BF DE G (“parathummi” cytocomplex), centromeric bands not heterochromatinized, nucleoli in arms E and G, Balbiani rings in arms B and G. Chromosomal polymorphism was not recorded.

Banding sequences (Fig. 6, b-f)

Arm A (Fig. 6, b) has the sequence kisA1, formed by 3 inversion steps from pigST.

| Banding Sequence | Description |
|------------------|-------------|
| kisA1            | 1a-k 19d-16b 10a-16a 2a-9e 19ef |
| hyp 1            | 1a-k 19d-16b 16a-10a 9e-2a 19ef |
| pigST            | 1a-k 2a-9e 10a-16a 16b-19d 19ef |

Arm C (Fig. 6, c) has the sequence kisC1, formed by 8 inversion steps from pigST.

| Banding Sequence | Description |
|------------------|-------------|
| kisC1            | 1a-2e 5d-6f 22e-17a 2f-5c 16h-14a 11f-13f 6g-11e 22fg |
| hyp 4+7          | 1a-2e 5d-6f 22e-17a 2f-5c 6g-11e 11f-13f 14a-16h 22fg |
| hyp 3            | 1a-2e 5d-6f 22e-17a 16h-14a 13f-11f 11e-6g 5e-2f 22fg |
| hyp 2            | 1a-2e 5d-6f 6g-11e 11f-13f 14a-16h 17a-22e 5e-2f 22fg |
hyp 1 1a-2e 22e-17a 16h-14a 13f-11f 11e-6g 6f-5d 5e-2f 22fg
pigST 1a-2e 2f-5c 5d-6f 6g-11e 11f-13f 14a-16h 17a-22e 22fg

Arm D (Fig. 6, d) has the sequence kisD1, formed by 6 inversion steps from Chironomus piger ST:
kisD1 1a-e 3g-1f 18c-16a 14c-15e 23d-18d 8d-4a 14b-9a 23c-24g
hyp 4+5 1a-e 3g-1f 18c-16a 15c-14e 23d-18d 8d-4a 14b-9a 23c-24g
hyp 3 1a-e 3g-1f 14c-15c 16a-18c 18d-23d 8d-4a 14b-9a 23c-24g
hyp 1+2 1a-e 3g-1f 4a-8d 23d-18d 18c-16a 15e-14c 14b-9a 23c-24g
pigST 1a-e 1f-3g 4a-8d 9a-14b 14c-15e 16a-18c 18d-23d 23-24g

Arm E (Fig. 6, e) has the sequence kisE1, formed by two inversion steps from Chironomus piger ST

Figure 6a. Karyotype of Chironomus sp. Kisumu.
Figure 6b-f. Homozygous banding sequences of Chironomus sp. Kisumu in arms A, C, D, E and F.

kisE1 1a-3f 6g-4a 11d-7a 12a-13g
pigST 1a-3f 4a-6g 7a-11d 12a-13g

Presence of a nucleolus in region 13 in arm E is a great characteristic of the Ch. sp. Kisumu karyotype.

Arm B (Fig. 6, a) not mapped. It has one sequence – kisB1. The common BR is well developed.

Arm F (Fig. 6, f) has the sequence kisF1. It was mapped only fragmentarily because of complex inversions in comparison with Chironomus piger ST. The presence of a large Balbiani ring situated just near the centromeric band is a characteristic of arm F in the Ch. sp. Kisumu karyotype. There is pericentric inversion in the chromosome BF (Fig. 6, a, f).
Arm G (Fig. 6, a) is longer than usual in *Chironomus* species. There is a nucleolus and four Balbiani Rings on arm G. One of Balbiani Rings, noted by the black dot in Fig. 6, a, was developed only in some cells of the salivary gland cells.

In total, seven Ethiopian endemic banding sequences are found in the sequence pool of *Ch.* sp. Kisumu. All these sequences differ from *Ch. parathummi* Keyl, 1961 sequences.

**Larva:** long tubuli laterales on abdominal segment VII.
**Distribution:** near Victoria lake, Kenya.

**Discussion**

Karyotypes of six African *Chironomus* species were studied. Four of these karyotypes were described for the first time (*Ch.* sp. Nakuru, *Ch. formosipennis*, *Ch. prope pulcher*, *Ch.* sp. Kisumu). Detailed photomaps of arms A, C, D, E, and F were presented, also for the first time, for *Ch. alluaudi*, *Ch. transvaalensis*, and *Ch.* sp. Nakuru.

Among the species studied, three species (*Ch. transvaalensis*, *Ch. prope pulcher*, *Ch.* sp. Kisumu) have only endemic Ethiopian banding sequences in their karyotypes, while cosmopolitan basic banding sequences were discovered in the karyotypes of the other species, along with endemic sequences (*Ch. alluaudi*, *Ch.* sp. Nakuru, *Ch. formosipennis*). The presence of these basic sequences indicates a relationship of African *Chironomus* species to *Chironomus* species from other continents before their separation (Kiknadze et al. 2008).

The results on African species are relevant the problem whether or not the chromosome arm combination of the “thummi” cytocomplex is rare in Southern continents. At the moment, one species in South America (*Chironomus* sp. Las Brisas, Wülker, Morath, 1989), one species in India (*Ch. javanus* Kieffer, 1924), and two species in Australia (*Ch. javanus*, *Ch. queenslandicus* Martin, 2005) are known to have this “thummi” cytocomplex chromosome arm combination (Martin 2010, Martin, pers. comm. and this paper).

Earlier it was demonstrated (Wülker 1980), that the presence of basic sequences in arms A, E, F of some *Chironomus* species of the “thummi” and “pseudothummi” cytocomplexes supports an idea that the basic sequences existed in hypothetical stem species before the separation of the complexes. The results of this paper contribute to the understanding of chromosome arms C and D in phylogeny in both cytocomplexes, in addition to data on arms A, E and F published earlier (Wülker 1980, 2010, Kiknadze et al. 2008).

Keyl (1962) established the hypothesis, that “the hypothetical species, which crossed the border between “thummi” and “pseudothummi” cytocomplexes” had most probably three banding patterns in arm E (in Keyl’s terms): standard as *Ch. piger* Strenzke, 1959, pattern as *Ch. aprilinus* Meigen, 1838 and others, pattern as *Ch. aberratus* Keyl, 1961 and others. We can ask, whether these three patterns are known today in both cytocomplexes. This is indeed so (Fig. 7, a) with the exception of the fact that
Figure 7a-c. Relations of recent species and hypothetical “basic” species before separation of the cytocomplexes in arm E (a), arm C (b), and arm D (c). The data of Keyl (1962) and Kiknadze (unpublished) were also used. Dots — inversion steps between banding sequences; ST — piger standard after Keyl (1962) and Dévai et al. (1989). all — alluaudi, atr — arella, apr — aprilinus, abe — aberratus, cra — crassicaudatus, fro — frommeri, hol — holomelas, hpi — heteropilicornis, kii — kiiensis, pil — pilicornis, plu — plumosus, pst — pseudothummi, sax — sataxilis, trv — transvaalensis.
the pattern of *Ch. aberratus* itself is not known in the “pseudothummi” cytocomplex, but there are the sequences trvE1 and nakE1 which differ only by 1–2 inversions from abeE (Fig. 7, a).

In arms C and D, an accumulation of species with the identical sequences was previously observed only in the “thummi” cytocomplex (Wülker, 2010). With the data of this paper we can propose that chromosome arms C and D had also two patterns before separation of the cytocomplexes (*Chironomus piger* ST sequence sensu Keyl, 1962 and basic pattern sensu Wülker, 1980, 2010). Fig. 7, b shows that pattern ST and basic themselves are not found in “pseudothummi” cytocomplex (question marks in Fig. 7, b), but there are several species, which have banding patterns differing only by a few inversions from ST and basic. The African species (*Ch. alluaudi, Ch. transvaalensis, Ch. sp. Nakuru, Ch. formosipennis*) play an important role in the development of the arm C and D phylogeny. Fig. 7, c demonstrates that there are ST and basic patterns in the ‘thummi’ cytocomplex, but only patterns close to ST were found in the “pseudothummi” cytocomplex: allD1 only by one, yosD1 by two, nakD1 by three, and trvD1 by four inversions from ST.

A great peculiarity of some African *Chironomus* karyotypes is the presence of large numbers of functionally active chromosome sites, especially Balbiani rings. For example 5 BRs were found in *Ch. transvaalensis* (Figs 2, a, 2, g-i), 6 BRs in *Ch. sp. Nakuru* (Fig. 3, a). Most *Chironomus* species have two or three visible BRs since e.g. many species have the gene for BR4 but do not express it, and the number seen may also vary with developmental stage.

**Acknowledgements**

Travel of W.W. was sponsored by Deutsche Forschungsgemeinschaft. In Nairobi, family B. and R. Seelmann-Eggebert offered great hospitality and cooperation, Prof. Schneider and Prof. Kaib gave important informations. Prof. Dr. Vareschi, Drs. Reyer, Pater N. Cox, Oltmanns, d’Oliveira and J. Grunewald helped in collection of the larvae. Wülker’s family contributed encouragement, car driving and food. Many thanks to all of them.

The work was supported by the Presidium of the Russian Academy of Sciences under the program “The Origin and Evolution of Biosphere”, subprogram 2 project “The Role of Chromosome Rearrangements in Speciation and Evolution”; the program “Biodiversity and Gene Pool Dynamics”, subprogram “The Dynamics of Plant, Animal, and Human Gene Pools”, project “The Mechanisms of Genetic Variation of Diptera Populations and Species” and by the grant of RFBR No 09-04-01440.

**References**

Dejoux C (1968) Contribution à l’étude des premiers états des chironomides du Tchad (Insectes, Diptéres) (1e note). Hydrobiologia 31: 449–464. doi:10.1007/BF00134446
Karyotypes of Chironomus Meigen (Diptera, Chironomidae) species from Africa

Dejoux C (1970) Contribution à l’étude des premiers états des chironomides du Tchad (Insectes, Diptères) (4e note). Cahiers ORSTOM, série Hydrologie. 4(2): 39–51.

Dévai Gy, Miscołczi M, Wülker W (1989) Standardization of chromosome arms B, C, and D in Chironomus (Diptera: Chironomidae). Acta biologica debrecina, supplement oecologica Hungarica 2(1): 79–92.

Dobzhansky Th (1970) Genetics of the evolutionary process. New-York. 505 p.

Freeman P (1957) A study of Chironomidae (Diptera) of Africa south of the Sahara, part III. Bulletin of the British Museum (Natural History) Entomology 5(9): 321–426.

Keyl H-G (1962) Chromosomenevolution bei Chironomus. II. Chromosomenumbauten und phylogenetische Beziehungen der Arten. Chromosoma 13: 464–514. doi:10.1007/BF00327342

Kieffer J (1908) Chironomidae. Denkschrift Medizinisch-Naturwissenschaftliche Gesellschaft. Jena. 13: 155–162.

Kieffer J (1913) Chironomidae et Cecidomyiidae Voyage de Ch. alluaudi. Et R. Jeanell en Afrique Orientale (1911–1912). Résultats scientifique Insectes Diptéres (Paris) 1–43.

Kieffer J (1923) Chironomidae de l’Afrique de sud. Annales de la Société scientifique de Bruxelles 42 (1): 382–388.

Kiknadze II, Gunderina LI, Istomina AG, Gusev VD, Nemytikova LA (2003) Similarity analysis of inversion banding sequences in chromosomes of Chironomus species (breakpoint phylogeny). In: Kolchanov N, Hofestaedt R (Eds) Bioinformatics of Genome Regulation and Structure. Boston, Dordrecht, London, 245–253.

Kiknadze II, Golygina VV, Istomina AG, Gunderina LI (2004) Pattern of chromosomal polymorphism during population and species divergence in Chironomus (Diptera, Chironomidae). Siberian Oekological Journal 11(5): 635–652 (In Russian).

Kiknadze II, Gunderina LI, Butler MG, Wülker W, Martin J (2008) Chromosomes and continents. In: Dobretsov N, Rozanov A, Kolchanov N, Zavarzin G (Eds) Biosphere Origin and Evolution. Springer-Verlag, 349–369.

King M (1993) Species Evolution: the Role of Chromosomal Change. New-York, 336 pp.

Martin J (1979) Chromosomes as tools in taxonomy and phylogeny of Chironomidae (Diptera). Entomologica Scandinavica. Supplement 10: 67–74.

Martin J (2010) Australian Chironomus species. Available from http://www.genetics.unimelb.edu.au/Martin/AustChironfile/AustChiron.htm [accessed 4 October 2010]

Martin J, Wülker W, Sublette JE (1974) Evolutionary cytology in the genus Chironomus Meigen. Studies in Natural Sciences. (Portales, New Mexico) 1(12): 1–12.

McLachlan AJ (1969) Notes on some larval and pupal chironomids (Diptera) from Lake Kariba, Rhodesia. Journal of Natural History 3: 261–293. doi:10.1080/0022293690770211

McLachlan AJ (1971) Some immature stages of the subgenus Chironomus (Meigen) (Diptera: Chironomidae) from Malawi, Central Africa. Journal of Entomology. Series B, Taxonomy 40(2): 173–178.

Wiedemann CRW (1830) Aussereuropäische Zweißfüllige Insekten. 2: 613.

White MJD (1977) Animal cytology and evolution. Cambridge, 961 pp.

Wülker W (1980) Basic patterns in the chromosome evolution of the genus Chironomus (Diptera). Zeitschrift für Zoologische Systematik und Evolutionsforschung 18(2): 112–123.
Wülker W (2010) The role of chromosomes in chironomid systematics, ecology and phylogeny pp. 1–13. In: Ferrington LC Jr (Ed) Proceedings of the XV International Symposium on Chironomidae. Chironomidae Research Group, University of Minnesota, Saint Paul, Minnesota, 385 pp +VIII. insects.ummz.lsa.umich.edu/~ethanbr/chiro/Docs/Wuelker/polytene.html [accessed on December 01, 2007]

Wülker W, Dévai Gy, Dévai I (1989) Computer-assisted studies of chromosome evolution in the genus Chironomus (Dipt.). Comparative and integrated analysis of chromosome arms A, E and F. Acta biologica debrecina, supplement oecologica Hungarica 2(1): 373–387.

Wülker W, Morath E (1989) South American Chironomus (Dipt.) – karyotypes and their relations to North America. Acta biologica debrecina, supplement oecologica Hungarica 2 (1): 389–397.

Zdobnov EM, von Mering C, Letunic I, Torrents D, Suyama M, Copley RR, Christophides GK, Thomasova D, Holt RA, Subramanian GM, Mueller H-M, Dimopoulos G, Law JH, Wells MA, Birney E, Charlab R, Halpern AL, Kokoza E, Kraft CL, Lai Z, Lewis S, Louis C, Barillas-Mury C, Nusskern D, Rubin GM, Salzberg SL, Sutton GG, Topalis P, Wides R, Wincker P, Yandell M, Collins FH, Ribeiro J, Gelbart WM, Kafatos FC, Bork P (2002) Comparative genome and proteome analysis of Anopheles gambiae and Drosophila melanogaster. Science 298:149–159. doi:10.1126/science.1077061