

Siberian species of the *riihimakiensis*-group in the genus *Chironomus*

2. Inversion polymorphism and cytophylogeny

(Insecta, Diptera, Chironomidae)

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Of the three taxa described in a previous paper (Kiknadze et al. 1992), spec. All is very close to *C. riihimakiensis*, differing only in the amount of centromeric heterochromatin and the banding pattern of chromosome arm F. The banding pattern of spec. Tu1 is 10 inversion steps, that of *C. tuvanicus* 24 inversion steps apart from *C. riihimakiensis*. The most important deviations are present in arms D and F of *C. tuvanicus*. All chromosome arms sometimes show primitive or basic banding patterns, identical with those of other groups of primitive *Chironomus* species (*aberratus*-group, *longistylus-tenuistylus*, *riparius-piger*, *plumosus*-group and species with *salinaris*-type larvae). The evolutionary importance of chromosome fusion, change of centromeric heterochromatin and number of nucleoli are evident in the *riihimakiensis*-group.

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Introduction

Cytotaxonomical studies of the genus *Chironomus* revealed a number of groups of closely related species or pairs of siblings: *staegeri*-group with 3 species (Wülker et al. 1971), *plumosus*-group with at least 11 species (Ryser et al. 1983, Dévai et al. 1983, Beljanina et al. 1983, Michailova & Fischer 1986, Kiknadze 1987, Kerkis et al. 1988, 1989, Shobanov & Demin 1988, Demin & Shobanov 1990, Kiknadze et al. 1991a, b), *aberratus*-group with 5 species (Wülker 1991a). *Camptochironomus tentans* - *C. pallidivittatus* are siblings (Beermann 1955), as well as *C. riparius (thummi)* - *C. piger* (Keyl & Strenzke 1956, Keyl 1957) and *C. longistylus* - *C. tenuistylus* (Wülker 1991b). These findings are of profound importance for understanding the principles that underlie the micro-evolutionary process. We found another group of closely related *Chironomus* species around *C. riihimakiensis* Wülker 1973 and described in a previous paper (Kiknadze et al. 1992) karyotypes and morphology of larvae, pupae and adults for three taxa (*C. spec. All*, *C. Tu1*, *C. tuvanicus*, spec. nov.) from the Altai and Tuva (Siberia). In the present paper, we will give data on the inversion polymorphism in these species and show the cytophylogenetic relationships between each other and to some well-known *Chironomus* species like *C. piger*, *C. plumosus* and the *aberratus*-group.

We are sure that the *riihimakiensis*-group consists of more species. There are unpublished results on chromosomally defined species from Scandinavia (*C. spec. Sk22*), the Irkutsk region in Siberia (*C. spec. V. Provis*), and Yakutia (*C. spec. Le1*) which are closely related to this group.

Moreover, another species which we call *C. spec. Tu3* was discovered in Tuva; its karyotype shares many common sequences with those of the *riihimakiensis*-group. However, we refrained from including *Tu3* in the *riihimakiensis*-group, because its arms C and D lacked nucleoli and arm G showed a different morphology. Despite, we use the *Tu3* banding patterns in the phylogenetic schemes.

Material and methods

Most of the material investigated and the methods applied are the same as in Kiknadze et al. 1992. Additional material: *C. spec. Sk22* Christiansborgvann Bergen Norway, 4/15-24/1980, 10 larvae, crew of Zoological Museum Bergen and W. Wülker; *C. spec. Le1*, mouth of river Lena, 6/28/1987 and 7/15/1987, 22 larvae, T. salova; *C. spec. Tu3*, Kyzyl/Tuva, Jenisei river, old channels, 5/1990, 43 larvae, M. T. Siirin. The methods of chromosome preparation are based on Keyl & Keyl 1959, principal thoughts on the evolution in the genus *Chironomus* can be found in Keyl 1962. References on standardization of banding patterns see Kiknadze et al. 1992.

Results

Inversion polymorphism and cytophylogenetic relations of banding patterns

The comparative study of the chromosomal polymorphism among Siberian species of the *riihimakiensis*-group have shown that all three species were not very polymorphic (Table 1). Rare homo- and heterozygous inversions were identified in *C. spec. All* and *C. spec. Tu1*. Inversion polymorphism was higher in *C. tuvanicus*, but the spectrum of the inversions was not very diverse.

To analyze the cytogenetic relationships between the species of the *riihimakiensis*-group, a comparison of the band sequences was made.

Tab. 1. Chromosomal polymorphisms in the *riihimakiensis*-group.

Species collection sites and dates	Arm band sequences and frequencies (in brackets)						Inversion heterozy- gotes frequen- cies	Average number of hetero- zygous inversions per larva	Chromo- somes frequen- cies	Number of larva
	A	B	C	D	E	F				
<i>C. sp. All</i>										
Chikhachev ridge, small water body										
July, 1988	A11(1.0)	B11(1.0)	C11(1.0)	D11(1.0)	E11(1.0)	F11(1.0)	0	0	0	20
July, 1990	A11(1.0)	B11(1.0)	C11(1.0)	D11(1.0)	E11(1.0)	F11(1.0)	0	0	0	55
Yenisei, Kyzyl										
May, 1990	A22(1.0)	B22(0.5) B12(0.5)	C11(1.0)	D11(1.0)	E11(1.0)	F11(0.4) F12(0.6)	0.6	1.1	0.2	10
<i>C. sp. Tu1</i>										
Kyzyl, pond										
Sept, 1988	A22(1.0)	B22(1.0)	C11(1.0)	D11(1.0)	E11(1.0)	F11(1.0)	0	0	0	11
May, 1990	A22(1.0)	B22(1.0)	C11(1.0)	D11(0.97) D12(0.03)	E11(1.0)	F11(1.0)	0.03	0.03	0.07	32
<i>C. tuvanicus</i>										
Yenisei, Kyzyl										
May, 1990	A11(1.0)	B11(0.92) B12(0.08)	C11(0.44) C22(0.09) C12(0.47)	D11(1.0)	E11(1.0)	F11(0.63) F22(0.01) F12(0.36)	0.65	0.88	0	100

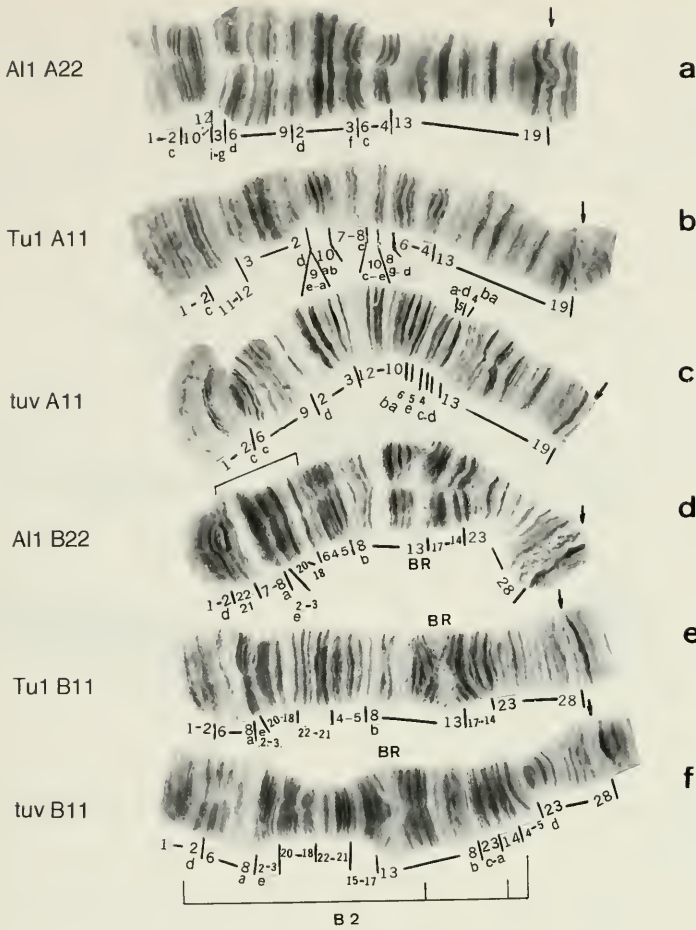


Fig. 1. a-c: Banding pattern of arms A. a. *C. spec.* A11 - A11A22. b. *C. spec.* Tu1-Tu1A11. c. *C. tuvanicus-tuv*A11. d-e: Banding pattern of arms B. d. *C. spec.* A11-A11B22. e. *C. spec.* Tu1-Tu1B11. f. *C. tuvanicus-tuv*B11. The break points of inversions are indicated by brackets. Arrows indicate centromeric bands. BR = Balbiani rings.

C. spec. A11

Arm A occurs in two sequences (Fig. 1a): A11A1 as in *riihimakiensis*, *holomelas* and many other species (basic pattern, Wülker 1980). A11A2 is different by one simple inversion:

A11A1	1-2c	10-12	3-2d	9	-	4	13-19
A11A2	1-2c	10-12	3i-g 6d-9 2d-3f			6c-4	13-19

A11A11 was dominating in the Altai population, A11A22 in the Tuvinian population. No larvae heterozygous for inversions have been found in both populations (Table 1).

Arm B occurs in two sequences, which differ in one simple inversion (Figs 1d, 5d, Table 1). Both patterns are also present in *C. riihimakiensis*:

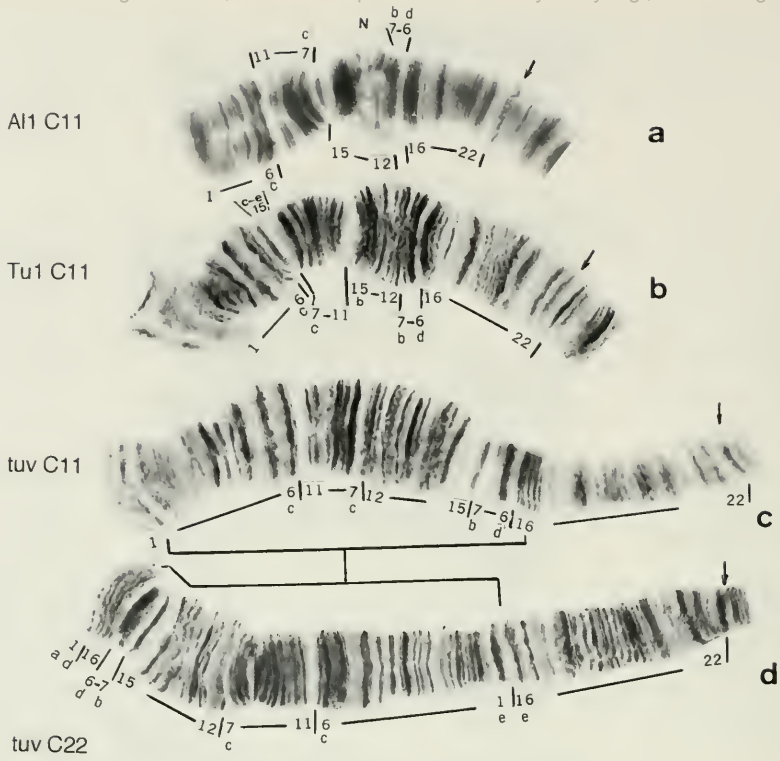


Fig. 2. Banding patterns of arms C. a. *C. spec.* Al1-Al1C11. b. *C. spec.* Tu1-Tu1C11. c-d. *C. tuvanicus* (c-*tuv*C11, d-*tuv*C22). Designations as in Fig. 1, N = nucleolus.

Al1B1	1-2d	22-21	18-20	3-2e	8a-6	4-5	8b-13	17-14	23-28	
Al1B2	1-2d	22-21	7-8a	2e-3	20-18	6f-a	4-5	8b-13	17-14	23-28

Arms C, D and E are monomorphic (Figs 2a, 3a, 4a, Table 1). Band sequences are as in *riihimakiensis* and *aberratus*. Arms C and D have one large nucleolus each (Figs 2a, 3a, 4a).

Arm F occurs in two sequences (Fig. 4d, Table 1). Al1F1 is 3, Al1F2 4 inversion steps from *riihimakiensis* 2, a pattern which was not known at the time of the species description by Wülker 1973, but was detected by him later (unpublished) in material from northern Sweden (Veittijärvi near Luleå, pool near Arnemark, pool in swamp near Abisko Tourist station). *riih* 2 is a very common pattern in the genus *Chironomus*, present in at least 12 species all over the holarctis (Wülker 1991a):

<i>riih</i> F2 = <i>aberr</i> F1	1	-	10	17	-	11	18 - 23	
inter	1	8b - 2	8c-10	17	-	11	18 - 23	
Al1F1	1	9b - 8c	2 - 8b	12 - 17	10 - 9c	11i-a	18-23	
Al1F2	1	9b	8b-2	8c - 9a	12 - 17	10 - 9c	11i-a	18-23

Arm G carries one well developed nucleolus near the centromere region. Two large puffs (possibly Balbiani rings) are developed in this arm. The banding pattern is ill-defined which hinders mapping (Fig. 5a).

General morphology mostly as in *C. riihimakiensis*. G-homologs do not pair as a rule. 20 % of the larvae had B-chromosomes.

C. spec. Tu1

Arm A is monomorphic. The derivation from *riihimakiensis* is possible on the way of Sk22 (see Introduction) and two hypothetical forms. Note the complex arrangement in region 7 - 10 which is characteristic for this species (Fig. 1b):

<i>riihA1</i>	1-2c	10	-	12	3	-	2d	9	-	4	13-19
		┌──────────────────────────┐									
Sk22A1	1-2c	9a-e		2d-3		12-10a		8-4		13-19	
		┌──────────────────────────┐									
<i>hyp</i>	1-2c	11-12	3-2d	9e-a	10a-e		8-4		13-19		
		┌──────────────────────────┐									
<i>hyp</i>	1-2c	11-12	3-2d	9e-a	10ab	7-8	10e-c		6-4	13-19	
		┌──────────────────────────┐									
Tu1A1	1-2c	11-12	3-2d	9e-a	10ab	7-8c	10c-e		8g-d	6-4	13-19
		┌──────────────────────────┐									

Arm B is monomorphic. The TuB1 sequence differs from A11B1 and *riihB1* by complex changes in the distal part of the arm which seem to involve 2 inversion steps:

A11B1 = <i>riihB1</i>	1-2d	22-21	18-20	3-2e	8a-6	4-5	8b-13	17-14	23-28
		┌──────────┐							
<i>hyp</i>	1-2d	21-22	18-20	3-2e	8a-6	4-5	8b-13	17-14	23-28
		┌──────────────────────────┐							
Tu1B1	1-2d	6-8a	2e-3	20-18	22-21	4-5	8b-13	17-14	23-28

Arm C is monomorphic and can be derived from *riihimakiensis* and A11 through one simple inversion (Fig. 2b):

<i>riihC1</i> , A11C1	1-6c	11-	7c	15	-	12	7b-6d	16-22
		┌──────────────────┐						
Tu1C1	1-6c	15c-e	7c	-11	15b-	12	7b-6d	16-22

Arm D occurs in two sequences, but only 1 larva was a heterozygote (Tu1D12), Tu1D1 is as *riihD1* and A11D1; Tu1D2 carries one simple short inversion (Figs 3b, 5e, Table 1):

Tu1D1	1-3	11-	18f	7d-4	10-7e	18g-22	
		┌──────────┐					
Tu1D2	1-3	17-	11	18a-f	7d-4	10-7e	18g-22

One large nucleolus is developed in arm D as a good marker.

Arm E is noted for a specific sequence following a large inversion (Fig. 4b):

<i>riihE1</i> , A11E1	1	-	3e	5-10b	4-3f	10c-13
		┌──────────────────┐				
Tu1E1	1	3f-4	10b-5	3e-2	10c-13	

Arm F as in *C. piger* (basic pattern Wülker 1980), shared by *C. alluaudi*, *longistylus*, spec. Apple Valley, *hyperboreus* and *islandicus*, Wülker 1991) and therefore differs from *riihF2* by inversion 17-11.

Arm G carries one nucleolus and two BRs, one more BR is possibly developed at the very end of the distal part of the arm (Fig. 5b). G-homologs are paired as a rule, but they do not conjugate tightly. 7 % of the larvae had B-chromosomes.

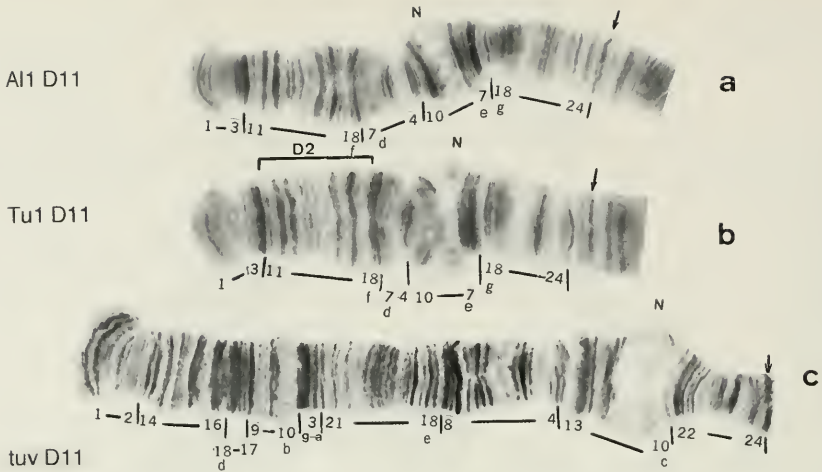


Fig. 3. Banding patterns of arms D. a. *C. spec.* All1. b. *C. spec.* Tu1. c. *C. tuvanicus*. Designations as in Figs 1 and 2.

C. tuvanicus

Arm A is monomorphic (Table 1). The *tuvA1*, species specifically, results from two non-overlapping inversions from the *holomelas* sequence (Fig. 1c):

<i>hol, rihiA1, AllA1</i>	1-2c	10	-	12	3-2d	9	-	4	13-19
<i>tuvA1</i>	1-2c	6c-9 2d-3 12-10 6ba 5e				4cd 5a-d		4ba	13-19

Arm B occurs in two sequences that differ in one large included inversion (Fig. 1f), *tuvB1* can be connected to Tu1B1 by two inversions, *tuvB2* presupposes two more:

Tu1B1	1-2d	6-8a	2e-3	20-18	22-21	4-5	8b-13	17-14	23-28				
<i>hyp</i>	1-2d	6-8a	2e-3	20-18	22-21	15-17	13-8b	5-4	14	23-28			
<i>tuvB1</i>	1-2d	6-8a	2e-3	20-18	22-21	15-17	13-8b	23c-a	14	4-5	23d-28		
<i>tuvB2</i>	1	14	10e-8b	23c-a	10f-13	17-15	21-22	18-20	3-2e	8a-6	2d-a	4-5	23d-28

8 % heterozygotes B12 have been found.

Arm C1 can be connected to arms C of *riihimakiensis* and spec. All1 by a short inversion. The arm occurs in two sequences (Fig. 2c, Table 1) that differ in one simple inversion:

<i>riihC1, AllC1</i>	1-6c	11-7c	15-12	7b-6d	16-22		
<i>tuvC1</i>	1-6c	11-7c	12-15	7b-6d	16-22		
<i>tuvC2</i>	1a-d	16d-a	6d-7b	15-12	7c-11	6c-1e	16e-22

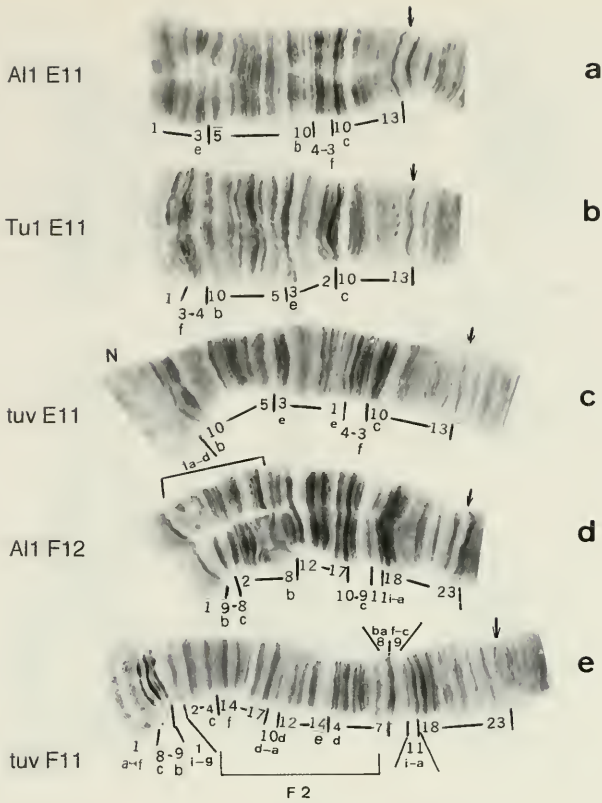


Fig. 4. a-c: Banding patterns of arms E. a. *C. spec.* Al1-Al1D11. b. *C. spec.* Tu1-TuD11. c. *C. tuvanicus-tuvD11*. d-e: Banding patterns of arms F. d. *C. spec.* Al1-Al1F12. e. *C. tuvanicus-tuvF11*. Designations as in Fig. 1.

47 % heterozygotes *tuwC12* and 9 % homozygotes *tuwC22* have been found. Arm D is monomorphic (Table 1). The *tuwD1* sequence is species specific (Fig. 3c) and can be reached from the D of *riih* over the D of *piger* by 9 inversion steps:

<i>riihinakiensis</i>	1 - 3	11 - 18f	7d - 4	10 - 7e	18g-24
<i>hyp</i>	1 -	7d	18f	7e	18g-24
<i>piger</i>	1	-	-	-	-24
<i>hyp</i>	1-2	21	-	3	22-24
<i>hyp</i>	1-2	14	- 21	3 - 13	22-24
<i>hyp</i>	1-2	14	- 21	3a-g 10b - 4	10c - 13 22-24
<i>tuwD1</i>	1-2	14-16	18d-17	9-10b 3g-a 21-18e	8-4 13 - 10c 22-24

The large nucleolus is a good marker for arm D.

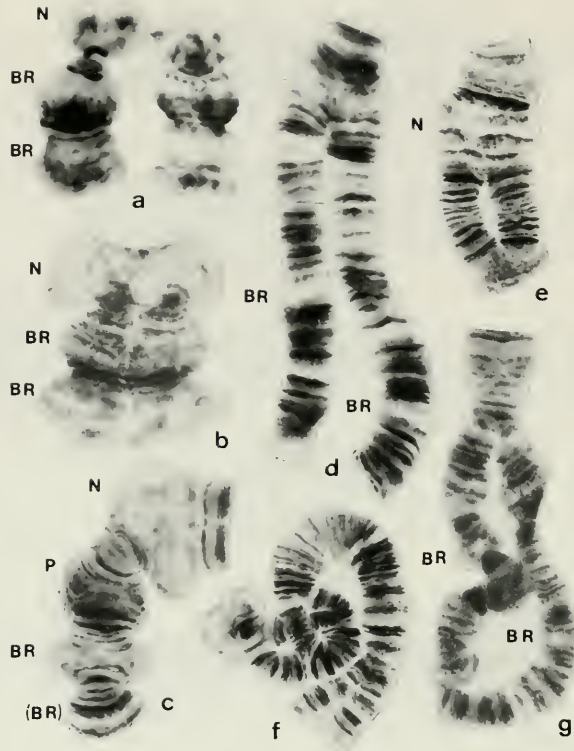
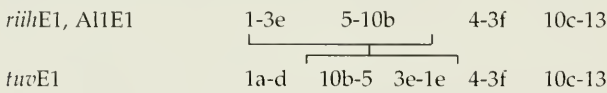
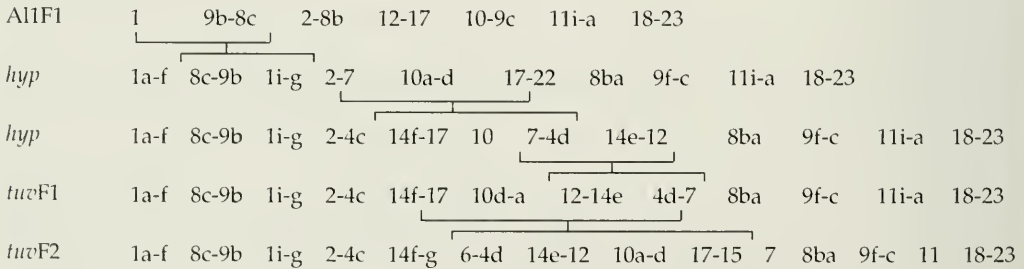


Fig. 5. Morphology of arm G and inversion heterozygotes in the *riihimakiensis*-group. a. A11G11. b. Tu1G11. c. *tuv*G11. d. A11B12. e. Tu1D12. f. *tuv*F12. g. *tuv*B12. N = nucleolus, BR = Balbiani ring, P = puff.

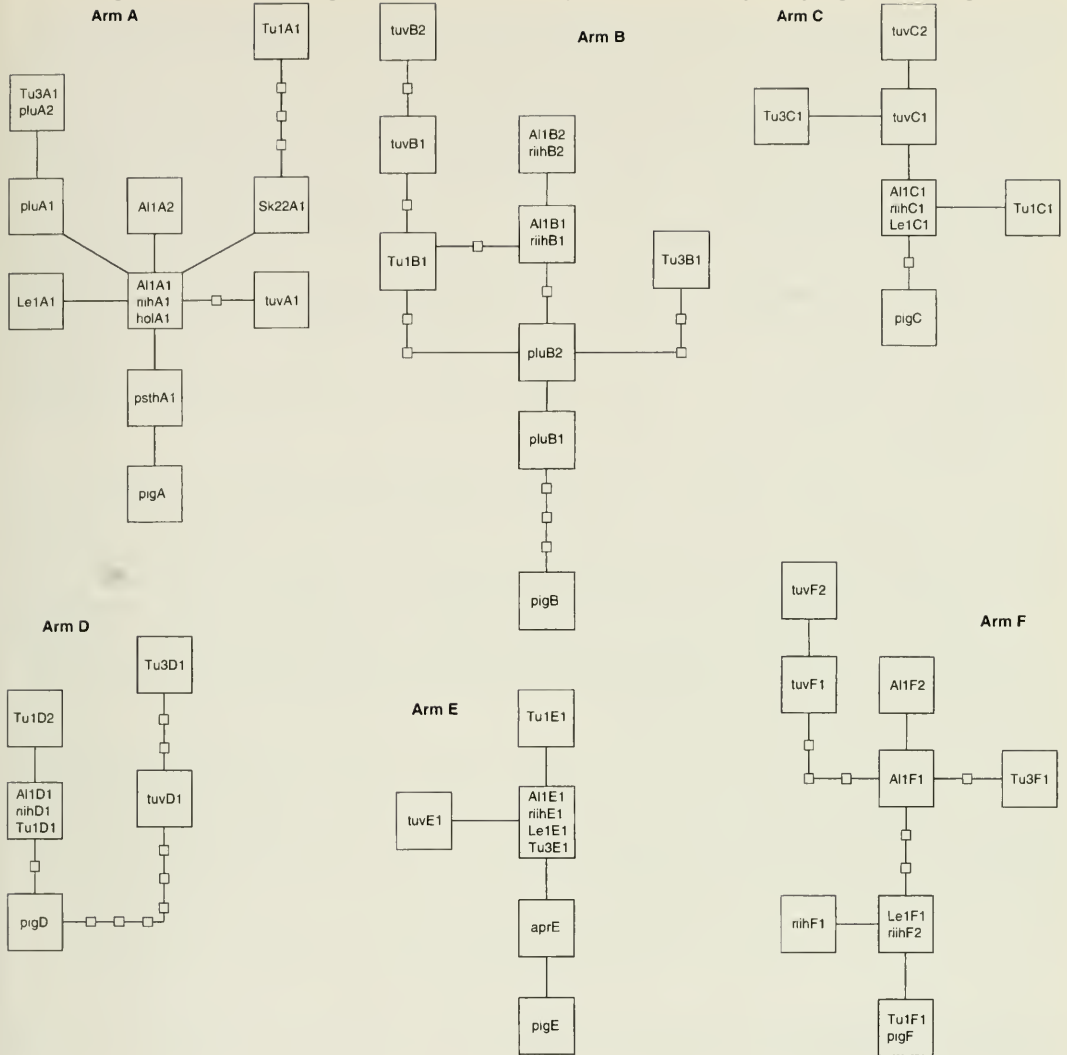
Arm E is fused to arm G. The *tuv*E1 band sequence is one large fixed inversion from *riih*E1 and A11E1 and is species specific (Fig. 4c):



Arm F occurs in two sequences (Figs 4e, 5f, table 1), *tuv*F1 can be derived from A11F1 by 4, from *riih*F2 by 7 inversions:



36 % heterozygotes *tuv*F12 and 1 % homozygotes *tuv*F22 have been found.



Figs 6. Cytophylogeny of the *riihimakiensis*-group. a. arm A; b. arm B; c. arm C; d. arm D; e. arm E; f. arm F. Additional abbreviations: apr = *aprilinus*, hol = *holomelas*, pig = *piger*, plum = *plumosus*, psth = *pseudothummi*.

Arm G is fused to arm E and carries one large nucleolus near the fusion sites, one large puff and one well-developed BR (Fig. 5c). The special lobe was identified in the *C. tuvanicus* salivary gland. An additional BR is developed in the special lobe cells. General morphology of arm G is similar to that in *C. spec.* Al1 and *C. spec.* Tu1, although its homologs are conjugated tightly. B-chromosomes were not found in *C. tuvanicus*.

Discussion

Comparison of the banding patterns of polytene chromosomes in the three species and with *C. riihimakiensis* revealed a high level of similarity in their karyotypes. The presence of a nucleolar organizer in homologue parts of arm D (also in arm C of *C. riihimakiensis* and *C. spec.* Al1), in addition to a typical one in arm G is an important feature of their karyotypes. Arm G is noted for characteristic morphology: In all

species arm G has a large nucleolus at the centromeric end, a massive group of many heavy bands in the centre, and two or three large puffs (BRs). It is difficult to map the arms G in these species because of their ill-defined banding pattern, especially in the distal part, which is greatly decondensed in most of the larvae.

The general morphology of arms G in the *riihimakiensis*-group is similar to those in the *aberratus*-group and in *C. anthracinus*. By this morphology all these species differ clearly from other *Chironomus* species. Moreover, it is necessary to note that some of the sequences in the *riihimakiensis*-group are basic pattern in the sense of Wülker (1980), not only in arms A, E and F, but also in arms C and D. From these basic pattern, we elaborated the cytophylogenetic position of the species via sequential inversion steps, and also showed the connections to well-know karyotypes like those of *C. piger*, *C. plumosus* or *C. aberratus* (Fig. 6).

The data gathered provide evidence that *C. riihimakiensis* and *C. spec.* All are the most closely related species. What makes the difference is the size of centromeric heterochromatin and one fixed inversion (explainable by 3 inversion steps) in arm F (Fig. 6). As was shown by Keyl (1965), the change in the amount of centromeric heterochromatin is an important factor for the speciation in the genus *Chironomus*. So, the karyotypes of the siblings *C. riparius (thummi)* - *C. piger*, *C. plumosus* - *C. borokiensis*, *C. agilis* 1 - *C. agilis* 2, *C. longistylus* - *C. tenuistylus* differ mainly in the DNA content in centromeric heterochromatin (Keyl 1957, 1965, Kerkis et al. 1989, Kiknadze et al. 1991b, Wülker 1991b). The pair *C. riihimakiensis* - *C. spec.* A1 is another example for this kind of speciation.

C. spec. Tu1 and *C. spec.* Sk22 show very similar banding patterns. They are probably different populations of one and the same species. There are 10 steps of inversion rearrangements between *C. riihimakiensis* and *spec.* Tu1 and 24 between *C. riihimakiensis* and *C. tuvanicus* (Fig. 6). So, *C. tuvanicus* may be regarded as the most divergent species of the group. However, the spectrum of common band sequences favors it being considered as a member of the *riihimakiensis*-group. Also in other groups of closely related species and even the groups of sibling species, there are members separated by many inversion steps. For instance, 18 inversion steps were counted in the *plumosus*-group between *C. plumosus* and *C. agilis*, 14 between *C. plumosus* and *C. muratensis* etc. However, as to the spectrum of common band sequences, larval morphology and the level of genetic differentiation, they may legally pertain to the *plumosus*-group (Kiknadze 1987, Kerkis et al. 1989, Filippova et al. 1989).

Beside the fact that the differences between the karyotypes in the *riihimakiensis*-group are due to fixed simple or included paracentric inversions, *C. tuvanicus* is noted for a telomeric fusion of arms G and E. Also the number of nucleoli is different: 3 in *C. riihimakiensis* and *spec.* A11, 2 in *C. spec.* Tu1 and *C. tuvanicus*. In arms C, D and E, the respective banding patterns of the *riihimakiensis*-group have many plesiomorphies in common with other primitive *Chironomus* species (*aberratus*-group, *longistylus-tenuistylus*, *riparius-piger*, *plumosus*-group and species with *salinarius*-type larvae).

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