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Chromosomal Breakpoints and Transposable-element-insertion Sites in Salivary Gland Chromosomes of *Chironomus riparius* Meigen (Diptera, Chironomidae) from Trace Metal Polluted Stations

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Abstract: We studied the distributions of somatic chromosome rearrangements and copies of three transposable elements (NLRCth1, CTRT1 and TFB1) in the salivary-gland chromosomes of *Chironomus riparius* Mg. larvae. Larvae were collected from four stations (Chaya River and The Farm in Bulgaria, and Pazar fountain and Derincay River in Turkey), whose sediments had different concentrations of several trace metals. The number of cytogenetic aberrations was higher in larvae from those stations whose sediments had higher concentrations of trace metals. Through FISH analysis of the cytogenetic localization of the three transposable elements, we found fixed insertion sites in the centromere regions and many variable insertion sites. NLRCth1, CTRT1, TFB1 insertion sites and locations of somatic-rearrangements occurred significantly more often in the proximal than in the distal regions of the polytene chromosomes. In addition, some common breakpoints of aberrations co-localized with NLRCth1, CTRT1 or TFB1 insertion sites in the populations of *C.riparius* samples: from Pazar (for all studied TEs: from 35.7% to 57.1%); Derincay River (from 38.8% to 69.4%); The Farm (from 34.8% to 50%) and Chaya River (from 23.3% to 50%). We advance the hypothesis that chromosome instabilities induced by stress agents in the environment could be related to the position of the TEs in the genome of *C. riparius*.

Key words: Chironomidae, common breakpoints, FISH, polytene chromosomes, somatic aberrations, TEs-NLRCth1, CTRT1, TFB1

Introduction

Transposable elements (TEs) are a major component of all genomes and represent from 3% to 50% of the total genomic DNA, depending on the species (Capy *et al.* 2000). They can induce different forms of chromosome rearrangements manifested the chromosome instability (Lim and Simmons 1994; Regner *et al.* 1996; Zelentsova *et al.* 1999, Aulard *et al.* 2004, Argueso *et al.* 2008, Michailova *et al.* 2009a), non-disjunction and reduced fertility (Evgen'ev *et al.* 1997, Zelentsova *et al.* 1999, Lerman *et al.* 2003) and be a significant source of genetic disease through their mutagenic action (Hedges and Deininger 2007). The capacity of TEs to move and induce mutations in the genome is considered an important driver in species evolution (Zelentsova *et al.* 1999, Guerreiro

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2012). In some cases TEs could have high transposition rates, which are associated to changes in the environmental conditions. Different factors have been proposed as causative agents of TE mobilization in a wide range of organisms: biotic, abiotic stresses, inter-itraspecific crosses, population factors and etc. (GUERREIRO 2012). For instance, some genotoxic stress agents in the environment may contribute to the mobilization of transposable elements, well seen in cells exposed to gamma radiation, which exhibited increased levels of L1 retrotransposition (FARKASH *et al.* 2006) compared to controls. Several environmental stress factors such as heat shock (GETZ and SCHAIK 1991; LERAMAN *et al.* 2003, BOUVET *et al.* 2008), chemical agents (SORIANO *et al.* 1995, MAUMUS *et al.* 2009), some heavy metals (KALE *et al.* 2005, MICHAILOVA *et al.* 2009a), radiation (FARKASH *et al.* 2006, ARGUESO *et al.* 2008) are supposed to activate the TEs and destabilize the genome structure, since different chromosome rearrangements can appear. In the human genome, KALE *et al.* (2005) demonstrated that DNA damage occurs through the mobilization of the LINE 1 retrotransposon by some heavy metals. In addition some studies show that chromosome rearrangements are TEs-mediated and can occur by ectopic and nonhomologous recombination between TEs copies (LIM and SIMMONS 1994; MIECZKOWSKI *et al.* 2006; DELPART *et al.* 2009). In this aspect many studies have been performed in *Drosophila* (ZELENTSOVA *et al.* 1999, LADEVEZE *et al.* 2001, CASALS *et al.* 2003, AULARD *et al.* 2004; CASALS *et al.* 2005), in *Anopheles* species (MATHIOPOLOUS *et al.* 1998), in mammals (DEININGER *et al.* 2003, HEDGES and DEININGER 2007) and in yeast (MIECZKOWSKI *et al.* 2006).

In some Chironomidae species, several TEs have been found: MEC (BLINOV *et al.* 1991), TEC (WOBUS *et al.* 1990), NLRCth1 (BLINOV *et al.* 1993), NLRCth2 (BLINOV *et al.* 1997), CTRT1 (GRUHL *et al.* 2000), TFB1 (HANKELN and SCHMIDT 1990). Little is known about the environmental factors that can influence TEs activity in this group of insects (BOVERO *et al.* 2002, ILKOVA *et al.* 2007; MICHAILOVA *et al.* 2007; ZAMPICININI *et al.* 2011) but BOVERO *et al.* (2002) and MICHAILOVA *et al.* (2009a) noticed that chromosome breaks occur more frequently in genomic sites containing blocks of repetitive DNA, composed by satellite DNA or/and copies of transposable elements.

The purpose of the present study is threefold: 1) To analyze the distribution and frequency of somatic rearrangements in salivary gland chromosomes of a widely distributed model Chironomid species – *C. riparius,* whose larvae were collected from some trace-metal polluted Bulgarian and Turkish stations; 2) To locate the insertion sites of three transposable elements – NLRCth1, CTRT1 and TFB1- along the polytene chromosomes of *C. riparius* larvae collected from these stations; 3) To test the hypothesis of co-localization between insertion sites of the above mentioned TEs and the breakpoints of aberrations raised under stress agents in the environment.

Material and Methods

Material

We used IV stage (VI-VII phase) larvae of *C. riparius* collected from two polluted water basins in Bulgaria (Chaya River, Asenovgrad, 2009, and The Farm, a water pool near Plovdiv, 2009) and in Turkey (Derincay River, near Corum, 2009, and a fountain in Pazar, 2010).The egg masses, collected from the Pazar fountain were reared under standard laboratory conditions (constant aeration and temperature of 18-20°C, 16h light &8h dark; feeding 2 times a week) and a trace metal polluted sediment (Table 1). All the sediments contained concentrations of trace metals higher than those of reference data for fossil sediment (FORSTNER and SALOMONS 1980) (Table 1). Detailed analysis of trace metal concentrations in the sediment has been performed by MICHAILOVA *et al.* (2009b; 2012).

Methods

Cytogenetic methods

The conventional acetic-orcein method for preparing salivary gland polytene chromosome preparation was applied (MICHAILOVA 1989). Larvae of *C. riparius* were identified by the species-specific cytogenetic markers of the polytene chromosomes (MICHAILOVA 1989; KIKNADZE *et al.* 1991). The standard chromosome maps done by HÄGELE (1970) and KIKNADZE *et al.* (1991) were used for detailed cytogenetic mapping of chromosome aberrations and TE-insertion-site locations. Frequencies of somatic chromosome aberrations were estimated as percentage both over the total number of studied larvae and analyzed cells of a given population.

Table 1. Trace metals concentration (μg/g) in the sediments of the lab. culture from Pazar, the three field stations according to MICHAILOVA *et al*. (2012) and fossil sediment according to FORSTNER and SOLOMONS (1980).

	Cr	Cu	Mn	Pb	Cd
Fossil sediment (lake)	59	25	406	16	0.2
Pazar fountain	0.111±0.001	49.63±0.67	44.51±0.72	57.2±0.41	0.21±0.01
Derincay River	191±1.4	56.1±2.1	725.6±6.2	16.5±0.11	0.19±0.01
The Farm, Plovdiv	1302±15.6	86.42±1.7	73.76±1.05	339±1.51	4.73±0.08
Chaya River, Asenovgrad	70.4±0.97	314.3±2.2	434.7±3.2	585.9±1.6	7.30±0.09

Chromosome aberrations are considered somatic when they affected one cell or a clonal cell lineage of the salivary gland of a larva (SELLA *et al*. 2004). A breakpoint occurring in more than one individual was considered a chromosomal locus more prone to breakage than others and defined as a "common" breakpoint (BOVERO *et al*. 2002). In this study "common" breakpoints have been recorded after comparison of the breakpoints of somatic rearrangements found here and those previously described for *C. riparius* from other polluted regions (PETROVA *et al*. 2004; SELLA *et al*. 2004; ILKOVA *et al*. 2007; MICHAILOVA *et al*. 2009a, 2012). Following BOVERO *et al*. (2002), we divided the chromosomes AB, CD and EF in two regions: proximal and distal to the centromere. Chromosome G is very short and could not be divided into a proximal and distal part. The number of individuals and cells analyzed cytogenetically is shown in Table 2.

In situ hybridization (FISH)

We used the probes of three transposable elements: NLRCth1, CTRT1 and TFB1. NLRCth1 belongs to the LINE (Long Interspersed Nuclear Elements) family of retroelements. It contains two open reading frames, ORF1 and ORF2. The NLRCth1 probe is a 502 bp long amplicon obtained using the following primers: NLR3790-F (5'- AGCAGCTACAGGCCAATAAGTCTAC-3') and NLR4291-R (5'-GATACAGTGCTGTATCATCTGCGAA-3'). CTRT1 is a SINE transposable element (Short Interspersed Nuclear Element). We obtained the CTRT1 310 bp long probe by using the following primers: CTRT18-F (5'-ATCCCAGGCCATGTCTAATTTTC-3') and CTRT327-R (5'-GAATCTATTGTACCACACCCTTTAGACC-3'). TFB1 is a fold-back transposable element. The TFB1 326 bp probe was obtained by using the following primers: TFB1311-F (5'-GCAACGACTATTCCTACCTTGCC-3') and TFB1636-R (5'-TCACACCGTTTTCACGTGTGAATCT-3').

Amplicons corresponding to the three probes were first obtained by amplifying approximately 50 ng of genomic DNA with the above listed primer pairs according to the following conditions: 1x Taq buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP; 0.5 μM each primer; and 1 u Taq DNA polymerase (Fisher) in a final volume of 30 μL. The amplification profile was: 3' at 95°C followed by 30 cycles of 30 sec at 94°, of 30 sec at 52° and of 45 sec at 72°. A 1/1000 aliquot was re-amplified and labeled as above, except that 0.15 mM TTP plus 0.05 mM Digoxigenin-11-dUTP were used instead of TTP.

The locations of the copies of the TEs along the salivary gland chromosomes were determined by FISH, following the methods of SCHMIDT (1992) and HANKELN *et al*. (1993). This included several steps: rehydratation of chromosome preparations in ethanol, heat stabilization at 70°C, and denaturation in SSC solutions. The hybridization was carried out at 54°C overnight in a wet chamber. The hybridization signals were detected by the anti-digoxigenin antibody (Roche).

The numbers of individuals and cells analyzed by FISH are shown in Table 2. The hybridization signals were considered fixed when they were found in all the salivary gland cells of all the studied individuals of a sample. The signals were considered variable when they were found in all or nearly all the salivary gland cells (either in the homozygous or the heterozygous state) of one or more than one individual but not in all sampled individuals (MICHAILOVA *et al*. 2009a).

Statistical analysis

By means of the G-test (SOKAL and ROHLF 1995), we checked whether the somatic breakpoints and TE in-

Table 2. Number of studied individuals and cells of *C. riparius* by different methods

	Pazar fountain, Turkey		Derincay River, Turkey		The Farm, Bulgaria		Chaya River, Bulgaria	
	Individuals	cells	Individuals	cells	Individuals	cells	Individuals	cells
Cytogenetic method	14	288	18	378	15	439	20	526
FISH with NLRCth1 probe	2	19	2	28	1	12	4	80
FISH with CTRT1 probe	2	9	2	17	2	23	3	43
FISH with TFB1 probe	2	24	3	67	2	25	3	66

sertion-sites along the chromosomes were randomly distributed or occurred significantly more often in the proximal chromosomal regions than in the distal ones. Correlations between frequencies of common breakpoints and frequencies of the sites of transposable elements in proximal and distal regions were estimated using the Spearman correlation coefficient, rs (Sokal and Rohlf 1995).

Results

Cytogenetic characteristics of the larvae of the *C. riparius* populations

The karyotype of salivary gland cells of all the larvae studied did not differ from the standard karyotype of the IV instar larvae of *C. riparius*. This species belongs to the "thummi" cytocomplex (Keyl 1962). The chromosome set is 2n=8 with chromosome arm combinations: AB, CD, EF and G. In chromosome G 3 Balbiani Rings (BRa, BRb and BRc) and one Nucleolar organizer (NOR) are located. Chromosomes AB and CD are metacentric, chromosome EF is submetacentric, and chromosome G is acrocentric (Figs. 1 a, b, c, d).

1. Somatic chromosome rearrangements

(a) Pazar fountain (Turkey)

The types and frequencies of the observed somatic chromosome rearrangements can be seen in Table 3. Heterozygous pericentric inversions, six hetero-

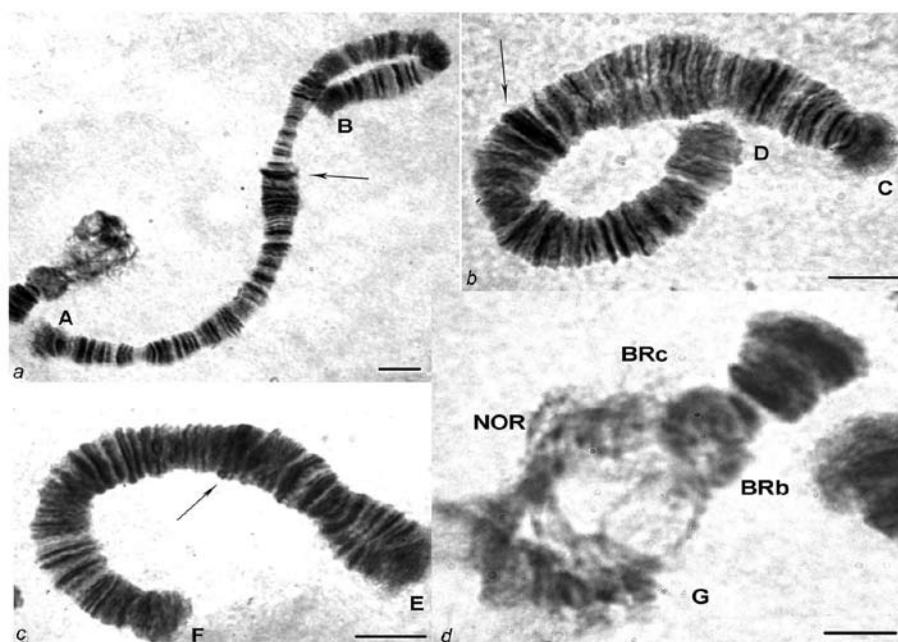

Fig. 1. Polytene chromosomes of *Chironomus riparius*: standard karyotype
(a) chromosome AB, (b) chromosome CD, (c) chromosome EF, (d) chromosomes G with Balbiani rings (BRb, BRc) and Nucleolar organizer (NOR). → centromere region. Bar: 100 µm

zygous paracentric and one deletion were found. Most (70%) of the chromosome inversions were concentrated in the CD chromosome.

Altogether, 14 “common” breakpoints (58.33% of all breakpoints) of aberrations were found. They were significantly more frequent in the proximal part of the CD chromosome than in the distal part (G=7.01 df=1; P<0.01). Only one aberration was found in chromosome G, no aberrations were found in arm B and EF chromosome (Table 3).

(b) Derincay River (Turkey)

The types and the frequencies of the observed somatic chromosome rearrangements found in the salivary gland chromosomes of *C. riparius* larvae from this station can be seen in Table 3. Altogether, 42 different types of heterozygous paracentric inversions were found as well as few types of heterozygous pericentric inversions. They were located in chromosomes AB, CD and EF (Fig. 2a). Two deletions were detected in chromosome G only and they affected BRc or both BRc and BRb (Fig. 2b).

The total number of common breakpoints of somatic aberrations was 49 (66.2% of all breakpoints). Common breakpoints were localized significantly more often in proximal than in distal parts of chromosome AB (G=14.59 df=1; P<0.001), while in chromosomes CD (G=3.54 df=1; P<0.1) and EF (G= 3.67 df=1; P<0.1) they were randomly distributed.

(c) The Farm (Bulgaria)

The type and frequencies of the observed somatic chromosome alterations are given in Table 3. Almost 95% of the aberrations were heterozygous inversions (33) localized in chromosome arms A, B, C, D, F and G. Heterozygous pericentric inversions affected chromosomes AB, CD and EF. Two deletions of chromosome G were detected in BRc or in both BRc and BRb.

Forty-six (70.8%) out of 65 breakpoints were common breakpoints. They were significantly localized in the proximal parts of chromosome AB (G=11.78 df=1; P<0,001) and CD (G=23.16 df=1; P<0.001) rather than in the distal parts. In chromosome EF they were randomly distributed.

(d) Chaya River (Bulgaria)

The frequency and location of the somatic chromosome rearrangements established in this locality are presented in Table 3. The majority of them (78%) was heterozygous paracentric inversions and affected all chromosomes (total number is 39), while the heterozygous pericentric inversions were detected in chromosome AB, CD and EF. Three deletions and three deficiencies were established in chromosome G with low frequency (Table 3). Two amplifications were found in arm F.

The common breakpoints estimated were 60, i.e. 70.6% of all the observed breakpoints. They were concentrated significantly more often in the proximal part of the chromosomes AB (G=4.87 df=1; P<0.05) and CD (G=12.06 df=1; P<0.001) than in the distal parts, while in the chromosome EF they were randomly distributed.

2. Distribution of the transposable elements

a) Pazar fountain (Turkey)

The total number of the TE clusters in all the chromosomes as well as the number of fixed and variable signals can be seen in Table 4. For the three TEs, the number of all insertion sites in the whole genome was significantly higher in the proximal than in the

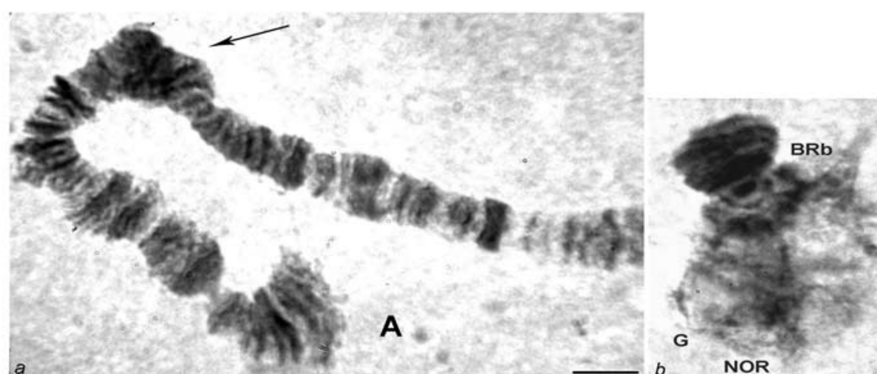

Fig. 2. Somatic chromosome aberrations in *C. riparius* (Derincay River)
(a) pericentric heterozygous inversion in chromosome AB →; (b) deletion of BRc in chromosome G →
Bar: 100 µm

Table 3. Somatic chromosome rearrangements in *Chironomus riparius* (Het.inv. – Heterozygous paracentric inversion; Pericentric inv. – Heterozygous pericentric inversion)

stations	Pazar fountain			Derincay River			The Farm (Plovdiv)			Chaya River (Asenovgrad)		
	Type and localization of aberrations	% indiv	% cells	Type and localization of aberrations	% indiv	% cells	Type and localization of aberrations	% indiv	% cells	Type and localization of aberrations	% indiv	% cells
Chromosome AB				Pericentric inv.	61.11	11.11	Pericentric inv.	40.00	2.05	Pericentric inv.	45.00	4.75
	Het.inv. B2b-B2h	7.14	0.35	Het. inv C3d-D1d	5.56	0.26	Het.inv. B1e-B2h	6.67	0.23	Het.inv. B2d-B2g	7.14	0.86
	Het.inv. B4b-B4i	7.14	0.35	Het. inv C4a-C4e	5.56	0.26	Het. inv. B4b-B4h	6.67	0.23	Het.inv. B4b-B4h	7.14	0.58
				Het. inv. D3h-E2l	5.56	0.26	Het. inv. B4h-C2h	6.67	0.23	Het.inv. C2a-C2g	7.14	0.29
				Het.inv. E1b-E2n	5.56	0.53	Het. Inv. B4h-C2c	6.67	0.23	Het. inv. C3a-C3f	16.67	0.56
				Het. inv. E2l-E3o	5.56	0.26	Het. inv. E2a-E3g	6.67	0.23	Het. inv. E1a-E1b	7.14	0.58
				Het. inv. E3a-F1c	5.56	0.26	Het. inv. E2d-E3e	6.67	0.23	Het. inv. E3f-E3n	7.14	0.86
				Het. inv. F1g-F2k	5.56	0.26	Het. inv. E2d-E3n	6.67	0.23	Het. inv. F1d-F1g	7.14	0.58
				Het. inv. F2a-F3l	5.56	0.26	Het. inv. E2g-E3e	6.67	0.23	Het inv. E2a-E2h	16.67	0.56
							Het. inv. E2n-E3n	6.67	0.23	Het inv. F4a-F4d	16.67	0.56
										Het inv. F4d-F4g	16.67	0.56
										Het inv. F4d-F4k	16.67	0.56
										Het inv. G2c-G2k	16.67	0.56
Chromosome CD	Pericentric inv.	35.71	3.47	Pericentric inv.	27.78	2.65	Pericentric inv.	20.00	0.68	Pericentric inv.	30.00	1.71
	Het.inv. B4i-B4l	7.14	0.35	Het.inv. A3b-B3a	5.56	0.26	Het. inv. B4j-B5p	6.67	0.23	Het. inv. A1c-A1e	7.14	0.29
	Het.inv. C6i-D3e	7.14	0.35	Het.inv. A4b-B1d	5.56	0.26	Het. inv. B4j-B5a	6.67	0.23	Het inv. A2e-A2g	16.67	0.56
	Het.inv. C5a-D1b	7.14	0.35	Het.inv. B4a-B5a	5.56	0.26	Het. inv. B4q-B5p	6.67	0.23	Het inv. A3b-A3e	16.67	0.56
	Het.inv. C5b-C6e	7.14	0.35	Het.inv.B4e-B5m	5.56	0.26	Het. inv. B4q-B5i	6.67	0.23	Het. inv. A3c-A3f	7.14	0.58
				Het. inv. B4j-B5a	11.11	0.79	Het. inv. B5a-C1a	6.67	0.23	Het. inv.B3b-B3h	7.14	0.29
				Het. inv. B4j-B5p	5.56	0.26	Het. inv. B5a-C1j	6.67	0.23	Het inv. B3b-B3g	16.67	0.56
				Het. inv. B4j-C2f	11.11	0.53	Het. inv. B5i-C2f	6.67	0.23	Het. inv. B5f-B5m	10.00	0.85
				Het. inv. B4l-B5a	11.11	0.53	Het. inv. B5i-C1j	6.67	0.23	Het.inv. B5m-B5o	33.33	1.12
				Het.inv. B4l-B5p	5.56	0.26	Het. inv. B5m-C1j	6.67	0.23	Het inv. C1e-C1h	16.67	0.56
				Het. inv. B4l-C1f	5.56	0.26	Het. inv. B5p-C2l	6.67	0.23	Het inv. C2e-C2h	33.33	1.12
				Het.inv.B4m-B5p	5.56	0.26	Het. inv. C1c-C2f	6.67	0.23	Het.inv. C2l-C2m	16.67	0.56
				Het.inv. B4n-C1j	5.56	0.26	Het. inv. C1h-C2h	6.67	0.23			
				Het. inv.B4n-B5p	5.56	0.26						
				Het.inv. B5a-C1b	5.56	0.26	Het. inv. C4c-C6d	6.67	0.23	Het inv. C5a-C5g	16.67	0.56
				Het. inv. B5a-C1f	5.56	1.32	Het. inv. C5a-C6e	6.67	0.23	Het. inv. C5b-C5d	7.14	0.29

Table 3. Continued.

stations	Pazar fountain			Derincay River			The Farm (Plovdiv)			Chaya River (Asenovgrad)		
	Type and localization of aberrations	% indiv	% cells	Type and localization of aberrations	% indiv	% cells	Type and localization of aberrations	% indiv	% cells	Type and localization of aberrations	% indiv	% cells
Chromosome CD				Het.inv. B5a-C1a	5.56	0.26	Het. inv. C5d-C6g	6.67	0.23	Het inv. C5b-C5g	16.67	1.12
				Het. inv. B5a-C1j	11.11	0.53	Het. inv. C6a-D3c	6.67	0.23	Het inv. C6a-C6g	16.67	0.56
				Het.inv. C1a-C2a	11.11	0.53	Het. inv. D1d-D3d	13.33	0.46	Het. inv. C6c-C6f	7.14	0.29
				Het. inv. C1j-C2l	5.56	0.26	Het. inv. D3a-D4i	6.67	0.23	Het inv. D2b-D2f	16.67	0.56
				Het. inv. C4e-D4i	5.56	0.26	Het. inv. D3a-D4f	6.67	0.23			
				Het.inv. C4e-E2a	5.56	0.26	Het. inv. E3a-E3i	6.67	0.23			
				Het.inv. C6a-D4f	5.56	0.26						
				Het.inv. D3a-E2g	5.56	0.79						
				Het. inv. D3a-E1i	5.56	0.26						
				Het. inv. E1i-E2g	5.56	13.23						
				Het. inv. E2b-E3i	5.56	0.26						
Chromosome EF				Pericentric inv.	11.11	1.06	Pericentric inv.	13.33	0.91	Pericentric inv.	50.00	2.79
				Het.inv. A1c-A2a	5.56	0.26	Het. inv. B4b-C2d	6.67	0.23	Het.inv. A2d-A2g	16.67	0.56
				Het.inv. A3e-A5e	5.56	0.26	Het. inv. C3e-C4h	6.67	0.23	Het. inv. B1d-B1p	7.14	0.29
				Het.inv. A4a-B1r	5.56	0.26	Het. inv. C4h-D1d	6.67	0.23	Amplification B2p	7.14	0.29
				Het.inv. A5e-B1p	5.56	0.26				Amplification B3h	16.67	1.68
				Het. inv. B1c-B1r	11.11	0.79				Het inv.B2m-B2n	16.67	0.56
				Het.inv. B2a-B2h	5.56	0.26				Het inv.B3k-B3m	16.67	0.56
				Het.inv.C2b-C2f	5.56	0.26				Het inv. C1a-C1e	16.67	1.68
										Het inv. C2b-C2e	16.67	0.56
										Het. inv. C4e-C4l	7.14	0.29
										Het inv. D2b-D2f	16.67	0.56
Chromosome G										Het inv. A1c-A1e	50.00	2.23
				Het.inv. (sec. A)	11.11	13.23	Het. inv. Bc-Ce	6.67	1.37	Het.inv. A1de-A2a	28.57	3.46
										deletion BRc	45.00	2.47
	deletion BRc	7.14	1.74	deletion BRc	38.89	5.56	deletion BRc	60.00	7.29	deletion BRb	28.57	2.01
				deletionBRc/BRb	16.67	0.79	deletion BRc/BRb	40.00	1.59	deletion BRc/BRb	40.00	2.28
										Deficiency E2de	14.29	0.58
										Deficiency (sec. E)	16.67	0.56
										Deficiency (sec.A)	16.67	1.12

Table 4. Number (N) of TEs signals in *C. riparius*

Stations	Pazar fountain			Derincay River			The Farm (Plovdiv)			Chaya River (Asenovgrad)		
TE	Total N of signals	N of variable signals	N of fixed signals	Total N of signals	N of variable signals	N of fixed signals	Total N of signals	N of variable signals	N of fixed signals	Total N of signals	N of variable signals	N of fixed signals
NLRCth1	70	53	17	105	88	17	58	41	17	191	174	17
CTRT1	62	45	17	101	84	17	148	131	17	84	67	17
TFB1	88	74	14	210	196	14	152	135	17	107	94	13

distal regions of the chromosomes (for NLRCth1, G=9.79; df=1; $P < 0.01$; for CTRT1, G=21.9; df=1; $P < 0.001$; for TFB1, G=6.74; df=1; $P < 0.01$).

The fixed locations of hybridization signals of the three TEs appeared only in the proximal part of the chromosomes AB, CD and EF (Table 5). No fixed signals were found in chromosome G. Variable insertion sites were TFB1 seen in both the proximal and the distal parts of the chromosomes but never in the centromere regions. NLRCth1 variable signals were 75.7% of all detected signals; CTRT1 were 75.6% and 83% for TFB1.

b) Derincay River (Turkey)

Table 4 shows the total number of insertion sites of the three TEs as well as the number of the fixed and variable signals. NLRCth1 signals were randomly distributed in the proximal and distal regions of chromosomes (G=2.81; df=1; $P<0.1$) while the CTRT1 and TFB1 signals occurred with a significantly higher frequency in the proximal than the distal part of all the chromosomes (CTRT1 G=15.38; df=1; $P<0.001$; TFB1 G=8.11; df=1; $P<0.01$).

The fixed signals of the TEs occurred in proximal regions of chromosomes AB, CD, EF and never in distal regions (Table 5). Variable insertion sites were seen both in the proximal and the distal parts of the chromosomes but never in the centromere regions. 83% of NLRCth1 and CTRT1 insertions and 93% of TFB1 insertions were variable. Some of the variable signals of TFB1 occurred either in the homozygous or in the heterozygous state in cells of the same individual (i.e. signals in arm A at the sites A1a, A4a, C2h, C4a; in arm B at the sites F4i, G1a; in arm D at the sites C6i, F2o; in arm E: A1a; in arm F at the site B3b).

c) The Farm (Bulgaria)

The number of the fixed and variable signals of the TEs can be seen in Table 4. The NLRCth1 copies appeared to be significantly more abundant in the proximal parts of all three chromosomes than in the distal parts (G=15.9; df=1; $P<0.001$). CTRT1 and TFB1 copies were localized randomly in all chromosomes (CTRT1, G=5.42; df=1; $P<0.02$; TFB1, G=3.7; df=1; $P<0.1$).

The fixed insertion sites of the three TEs were concentrated in the proximal part of the chromosomes AB, CD and EF (Table 5). No fixed signals were found in chromosome G. Variable insertion sites were seen both in the proximal and the distal parts of the chromosomes but never in the centromere regions. The variable insertions of NLRCth1 were 70.7% of all the insertions; of CTRT1 88.5% and of TFB1 89 %.

d) Chaya River (Bulgaria)

The fixed, variable and total numbers of insertion sites of the three TEs are shown in Table 4. No significant differences were found in the distribution of NLRCth1 insertion sites in proximal and distal parts of the chromosomes (G=0.81; df=1; $P>0.1$). In contrast CTRT1 and TFB1 signals were concentrated significantly more often in the proximal parts of the chromosomes than in the distal parts (CTRT1 G=5.02; df=1; $P<0.05$ and TFB1 G=10.93; df=1; $P<0.001$).

The three TEs had fixed insertion sites concentrated in the proximal part of the chromosomes AB, CD and EF (Table 5, Figs. 3 and 4). No fixed signals were established in chromosome G. The variable insertions were distributed along the chromosome arms but never in the centromere regions (Table 5, Fig. 4). For NLRCth1 the variable insertions were 91%, for CTRT1 79.7% and for TFB1 87.8%.

Some of the variable insertion sites of NLRCth1 appeared both in the homozygous and the heterozygous state in the cells of one and the same individ-

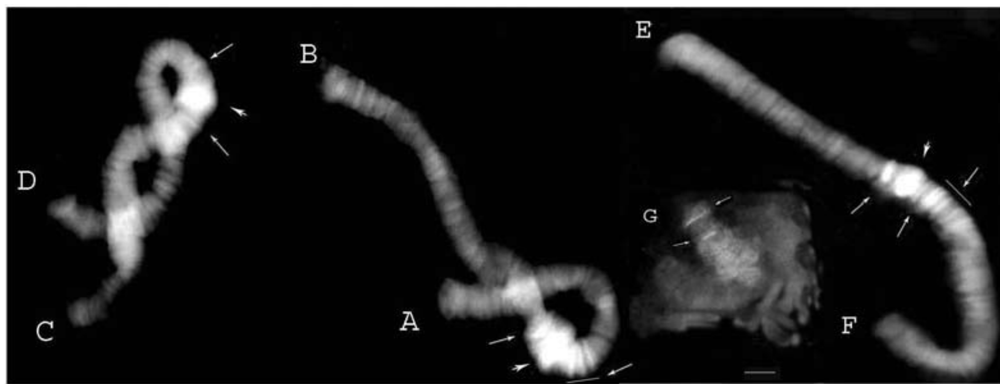

Fig. 3. Signals of CTRT1 in the polytene chromosomes AB, CD, EF and G of *C. riparius* from Chaya River (Asenovgrad); variable signals ⟶
fixed signals →
Bar: 10 µm

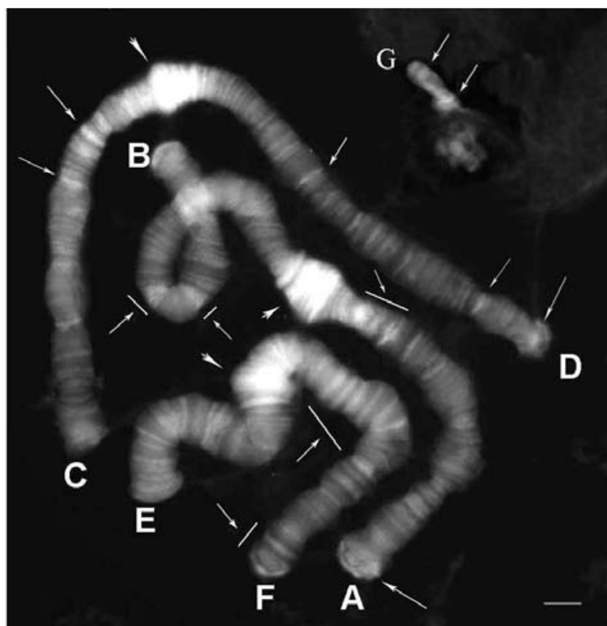

Fig. 4. Signals of TFB1 in the polytene chromosomes AB, CD, EF and G of *C. riparius* from Chaya River (Asenovgrad); variable signals ⟶
fixed signals →
Bar 10 µm

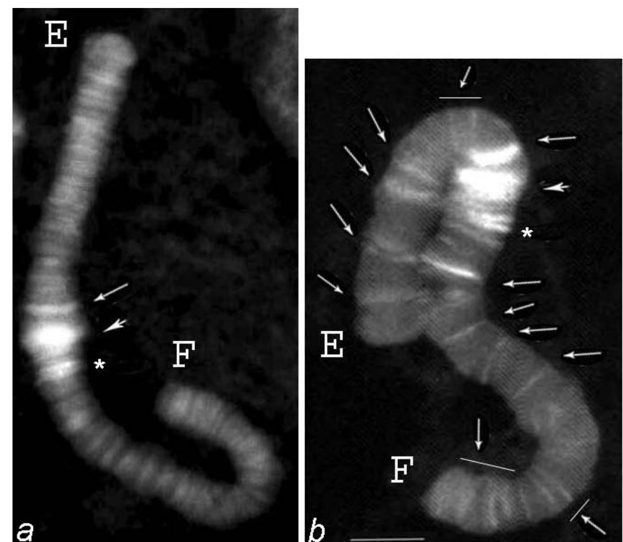

Fig. 5. Signals of NLRCth1 in chromosome EF of *C. riparius*; Bar: 10 µm
(a) strong fixed signals and a strong variable signal in proximal part of arm E (B1r) and a variable homozygous signal in arm F (B2q) – * ;
(b) strong fixed signals in proximal part of chromosome EF, a strong variable signal in proximal part of arm E (B1r); variable signals along arms E and F;
a variable heterozygous signal in arm F (B2q) – *
variable signals ⟶
fixed signals →

uals (in arm A at the site D1d and G2a; in arm D at the site C4e; in arm F at the site B2q) (Figs. 5a, b).

3. Association between TEs insertion sites and chromosome aberration breakpoints

a) Pazar fountain (Turkey)

Ten out of the 14 common breakpoints (71.4%) co-localized with NLRCth1, CTRT1 or TFB1 insertions. Five out of the 14 common breakpoints (35.7%) were found to have NLRCth1 insertions (they were localized in CD chromosome). However, no significant correlation between cytological locations of common breakpoints and of NLRCth1insertion sites was established (rs=0.45; df=10; P>0.05). Similarly eight out of the 14 common breakpoints (57.1%) coincided with CTRT1 insertion sites, but the correlation was not significant (rs=0.34; df=10; P>0.05). In contrast, six out of 14 (42.8%) common breakpoints coincided

Table 5. Cytogenetic localization of the insertion sites of NLRCth1, CTRT1 and TFB1 in *C. riparius* polytene chromosomes

Stations	Chromosomes	NLRCth1	CTRT1	TFB1
Pazar fountain	AB	A1a, A3b, B4d, C2e, C2h, C4a, C4c, D1d, *centr D1klD2abcdef;* D3d, D3h, E1b, E1d, E2f, E2l, E3n, E3o, F1a, F1b, F4a, G1a, G1l	A2h, B2d, **B4b**, B4d, B4f, C2h, C4a, D1d, *centr D1klD2abcdef* ; E1d, E3a, E3e, F1a, F1d, F2a, F2e, F3b, F3f, G1g,	A1a, A1g, A3e, A4a, A4c, A4h, B1g, B2d, **B2h**, C1a, C1c, C1d, C1e, C2e, C2h, C3d, C4a, C4c, D1b, D1d, *centr D1kl D2def*; D3d, D3h, E1b, E3a, E3e, F1d, F2a, F2i, F3f, F3h, G1a, G1c, G3p,
	CD	A2e, A2f, A2g, B1c, B2a, B4a, B4b, B4c, B4d, B4e, B4j, B5a, B5i, C1j, **C2l**, *centr* ***C3cdefghi***; **C4e**, F1b, F1e, F2j, F2a-e	A2g, A3d, B2a, B4a, **B4i**, 5a, B5i, C1i, C2f, **C2l**, *centr* ***C3cdefghi***;	A1a, A1b, A2e, A2g, A2i, B1c, B2a, B2g, B4a, B5a, B5p, C1j, **C2l**, *centr* ***C3cdefghi***; C4d, **C4e**, C6a, C6c, D3a, D3c, D4i, D4k, E2b, E3e, E4e, F2o,
	EF	B1r, *centr B2jk*; B2q, B2m, B3b, B3k, B3o, D3d	A1d, A2i, A3c, A5e, A5f, A5g, *centr B2jk*; B3o, B4d, B4f, C1e, C2b	A1a, A3e, B1r, *centr B2jk*, B2q , B3o, B4f, C1a, C1e, C2b, C4a, C4e, D1e, D2o, D3g,
	G	A1b, A2b, Ba, Bb, E2e	A1b, A2b, sec. B, Cb, **Ca-d**, E2e,	A1b, Ba
Derincay River	AB	A1a, A1b, A1j, A2h, A4a, A4c, A4e, B2d, B2h, B3d, B4b, B4d, B4f, B4h, C1c, **C4a**, C4c, **D1d**, *centr D1klD2abcde****D2f***; **D3d**, **D3h**, E1d, **E1f**, E2b, **E3a**, E3e, F1f, F3b, F3h, F3i, F4e, F4g, G1a, G1c, G1g, G1l, G2r, G3b, G3n, G3p	A1a, sec. A1a-j, A1g, A2d, A3a, A3e, A4c, A4e, B1g, B3j, sec. B4, B4b, B4d, B4f, C1c, C1e, **C3d**, **C4a**, C4c, **D1d,** *centr D1klD2abcde****D2f***; **D3d**, E1d, E2l, **E3a**, E3e, E3n, F1d, F2a, F3a, G1a, G1g, G3b	A1a, A1g, A2h, A3e, A4a, A4c, A4e, A4h, B1a, B1c, B1d, B1e, B1g, B1l, B2d, B2h, B3d, B3j, B4d, B4f, B4h, C1c, C1e, C2a, **C2e**, C2h, C2j, C3a, **C3d**, **C4a,** C4c, **C4e**, D1c, **D1d**, *centr D1kD2ab****D2e f***; **D3d**, **D3h**, E1b, E1d, **E1f**, E2d, E2l, **E3a**, E3e, E3h, E3l, E3n, F1a, F1d, F2a, F2e, F2g, F2i, F3a, F3b, F3f, F3h, F4a, F4c, F4d, F4e, F4g, F4i, G1a, G1c, G1g, G1l, G2a, G2p, G2l, G3b, G3p
	CD	A1a, A1b, A2i, A3d, A3f, A4c, A4d, B4j, B4l, B4n, B5a, B5i, B5m, C2l, *centr C3cdefghi,* C4e, D1e, D1h, D3a, D3e, D4d, E2b, E2d, E3a, E3e, F1b, F1e, F2c, F2j,	A3d, A3f, B1c, B2a, B2g, B4e, B4j, B4n, B5a, B5p, C2l, *centr C3cdefghi*; C4a, C4e, C6i, D1d, D1e, D3a, D4i, D4k, E2b,	A1a, A1h, A2b, A2g, A2i, A3c, A3d, A3f, A4a, A4b, A4d, B1a, B1b, B1c, B2a, B3e, B3h, B4a, B4g, B4j, B4n, B5a, B5i, B5m, B5p, C1a, C1d, C1e, C1g, C1j, C2a, C2b, C2f, C2j, C2l, C2m, C3a, C3b, *centr C3cdefghi*; C4a, C4b, C4c, C4d, **C4e**, C5c, C5d, **C6a**, C6i, D1a, D1d, D1e, **D3a**, D3e, D4b, D4d, **D4f**, **D4i**, D4k, E1e, E1i, E4e, F1b, F2a, F2b, F2e, F2j, F2o
	EF	A1a, A1b, A3c, A4f, A5e, **B1c**, **B1r**, *centr B2jk;* B2q, B3n, B4d, B4f, C1a, C1e, C2d, C4h, D1d, D2h, D3d, D3g,	A1d, **A2a**, A2e, A2f, A2i, A3c, A5e, A5f, A5g, **B1c**, **B1r**, *centr B2jk*; B2q, B3b, B3c, B3d, B3h, B3o, B4d, B4f, C1e, C2b, C2f, C4a, D1d, D1g, D2e, D2o,	A1a, A1c, **A2a**, A2c, A2g, A2i, A2k, A3c, A4a, A4f, A5e, A5g, **B1c**, **B1r**, *centr B2jk*; B2l, B2m, B2n, **B2q**, B3b, B3d, B3h, B3g, B3k, B3o, B4d, B4f, B4g, C1a, C1e, **C2b**, C2d, **C2f**, C3b, C3d, C3f, C4a, C4c, C4e, C4h, D1d, D1g, D2e, D2h, D2o, D3b, D3f, D3g,
	G	A1b, **Ba**	A1b, **A2b**, **Ba**, sec. B, Bc,	A1b, A1e, sec. **A**, **A2b**, **Ba**, Bc, Cb, **Cd**, Ce, Db, E1c, E2e,
The Farm (Plovdiv)	AB	C4a, C4c, D1d, *centr* ***D1klD2abcdeD2f***; **D3d**, **D3h**, E1d, E1f, G1g,	A1j, A2h, A4a, A4e, B1g, B2b, B2d, B3h, B3j, **B4b**, B4d, **B4h**, C1c, C2e, C2h, C3d, C4a, C4c, D1d, *centr* ***D1klD2abcdeD2f***; **D3d**, E1d, E1f, E2i, E2l, E3a, **E3e**, **E3n**, F1d, F2a, F2e, F2i, F3a, F3f, F3h, F4a, F4g, G1a, G1b, G1g, G2l, G3b	A1a, A4a, A4c, A4e, A4h, B1c, B1g, B2b, B2d, B3d, **B4h**, B4g, C1c, C2e*, C4a, C4c, D1d, *centr* ***D1klD2abcdef***; **D3d**, **D3h**, E1b, E1d, E1f, E2l, E2n, E3a, **E3e**, E3l, **E3n**, F1d, F2a, F2e, F3b, F3f, F4a, F4e, G1a, G1c, G1g, G1l, G2a, G2l, G2p, G3b, G3p,

Table 5. Continued.

Stations	Chromosomes	NLRCth1	CTRT1	TFB1
The Farm (Plovdiv	CD	B4a, B4b, B4c, B4d, B4e, B4f, B4g, B4h, B4i, **B4j**, **B5m**, **B5p**, **C1a, C2f**, **C2l**, *centr* ***C3****cdef-ghi*; C4c, **C4e**, **C5a**, D3e, F2j	A1a, A1h, A2b, A2g, A2i, A3b, A3d, A3f, A4b, B1c, B2a, B3e, B3h, B4a, B4e, **B4j**, B4n, **B5a**, **B5p**, **C2f**, **C2l**, *centr* ***C3****cdef-ghi*; **C4e**, **C5d**, C5e, **C6e**, **C6g**, C6i, D1d, D1e, **D3a**, D4d, D4f, **D4i**, D4k, E1e, E1k, E2a, E2b, E2d, E3a, E3c, E3e, F1b, F1c, F1e, F2b, F2c	A1a, A2b, A2f, A2g, A4a, B1c, B2a, B2g, B3e, B4a, **B4j**, **B5a**, **B5m**, **B5p**, C1b, C1g, **C1j, C2f**, **C2l**, *centr* ***C3****cdefghii*; **C4e**, **C6a**, C6b, C6c, **C6d**, D1e, **D3a**, D4d, D4f, **D4i**, D4k, E1e, E1i, E2b, F1b, F2a, F2j, F2m, F2o
	EF	A1a, A1b, B1h, B1m, B1r, *centr* ***B2jk***; B2m, B2o, **B2q**, D2h, D3d	A1b, A1c, A1d, A2a, A2b, A2i, A3e, A4f, A5e, A5f, A5g, B1h, B1l, B1m, B1r, *centr B2jk*; **B2q**, B3b, B3h, B3o, B4f, C1a, C1e, C2b, C2f, C3b, C3f, D2e, D2h, D2o, D3f	A1a, A1b, A1c, A1d, A1e, A2e, A2i, A3c, A3e, A4a, A4d, A4e, A4f, A5e, A5g, B1c, B1h, B1r, *centr* ***B2jk***; **B2q**, B3b, B3h, B3k, B3o, **B4b**, B4d, B4f, B4g, C1a, C1e, C2b, C2f, C3b, C4a, C4c, C4e, C4h, D1g, D2e, D2h, D2o, D3b, D3f, D3g,
	G	A2b, **Ba**, E2e,	A1a, A1b, A2b, A2c, sec. A, sec B**, C**, D, E, **Ba**, **Bc**, Db, E2e,	A1b, A1e, A2b, **Bc**, Cb, **Cd**, Ce, E1c, E2e, sec. B,
Chaya River (Asenovgrad)	AB	A1a , A1e, A1g , A1j, A2h, A3b, A3d, A4a, A4c, A4e, A4h, B1d, B1e, B1g, B1k, B1l, B2b, **B2d**, B2h, B3h, B3j, **B4b**, B4d, **B4h**, B4f, C1c, C1e, C2e, C2h, C2l, C3d, C4a, C4c, D1d, D1e, *centr* ***D1kl****D2abcde****D2f***; D3d, D3e, **E1a**, **E1b**, E1d, E1f, E2b, E2l, E3a, E3e, **E3n**, F1b, F1d, F2a, F2e, F3b, F3f, F4a, **F4g**, G1a, G1e, G1g, G2a, **G2k**e, G3b, G3f, G3n, G3o, G3p	A1a, A2g, A4a, A4e, B1g, B3dfhj, **B4b**df**h**, C2e, C4a, C4c, D1d, D2d, *centr* ***D1kl****D2abcde****D2f***; D2l, D3d, E1f, **F4eg**, G1e, G1g, G2r, G3d, G3j, G3p	A1ab, A3e, A4h, **B4h**, C2f, C2i, C2j, C3d, C4a, C4c, D1d, *centr D2ab****D2def***; D3b, D3h, **E1b,** E1d, E1f, E2d, E2l, E3a, E3e, E3m, **E3n**, F3b, F3h, **F4g**, G1a, G1c, G1g, G1l, **G2k**, G2l, G3f, G3mn, G3op
	CD	A1a, A1g, A1h, A2bc, A2g, A3c, A3d, A3e, A3f, A4ab, A4c, B1c, B2a, B2g, B3e, B4a, B4d, B4e, B4g, B4j, B4n, B5a, B5i, **B5m**, B5n, B5p, C1c, C1j, C2f, **C2l**, C3a**,** *centr* ***C3****cdefghi*; C4b, C4e, **C5a**, C5c, **C5d**, C5e, **C6a**, C6i, D1a, D1d, D1e, **D2b**, D3a, D4f, D4i, E1e, E1i, E3d, E3e, E4e, F2e, F2j, F2m	A1h, A3df, A4b, B4i, B4l, B4n, B5i, C2f, C2h-i, C2l, *centr* C3c*C3efgh*i, C4e, C5cde, C6i, D1c, D2bc, D3a, D4i, E2b, E3ae, E4e, F2de, F2m, F2o	A1ab, A1h, A2f, B2a, B4a, B5a, B5i, B5p, C1j, C2fg, C2l, *centr* C3c*C3efgh*i; C4a, C4bc, C4e, C5e, C6i, D1d-c, D3a, D3e, D4f, D4i, D4k, E1e, E2b, F1b, F2no
	EF	A1a, A1c, A1d, A1e, A2a, A2i, A3c, A3e, A4d, A4f, A5e, A5g, B1c, B1e, B1m, B1r, B2d, B2i, *centr* ***B2jk***; **B2m**, B2q, B3b, B3d, **B3h**, B3o, B4d, B4f, **C1a**, **C1e**, **C2b**, C2f, C3b, C3d, C3f, C4a, **C4e**, C4h, D1d, D1g, D2e, D2h, D2o, D3b, D3d,	A1a, A2a, A2i, A3e, A5e, A5g, B1r, *centr* ***B2jk***; B2q, B3bdfk, B3o, **C1e**, C1h, C2f, C4h, D1g, D2e, D2o, D3f	A1a, A2ab, A2h, A4f, A4g, A5g, B1c, B1h, B1r, **B1p**, B2fgh, *centr* ***B2jk***; **B2m**, B2q, B3b, B3d, B3k, B3o, B4df, **C2b**, C2f, C4a, C4h, D1d, D1g, D2e, D2o, D3g, D3fg
	G	**A1a**, A1b, A1d, **A1e**, A2b, A2c, **Ba**, Bb, **Bc**, Cd, Da, E1c,E2e,	A1b, A2b, Ce, E1c,	A1b, **A1e**, A2b, **Ba**, **Cd,**Ce, E1c,

Fixed TE location (italic type); Variable TE location (normal type). Coincidence between common breakpoints locations and TEs locations (bold type.

with TFB1 insertion sites and the correlation was significant (rs=0.79; d f=10; P<0.01).

The co-localizations between the insertions of copies of all three transposable elements and the breakpoints of aberrations were present mainly in arm C and in arm D (Table 5).

(b) Derincay River (Turkey)

Nineteen out of 49 common breakpoints (38.8%) coincided with NLRCth1 insertion sites. Twenty-two (44.9%) of the common breakpoints coincided with CTRT1 insertion sites and thirty-four (69.39%) coincided with TFB1 insertion sites. The correlation between cytological locations of common breakpoints and those of the CTRT1 and TFB1 were significant (CTRT1, rs=0.59; df=10; P<0.05); TFB1, rs=0.64; df=10; P<0.05) while there was no significant correlation (rs=0.29; df=10; P>0.1) between the location of common breakpoints and the location of NLRCth1 copies.

Co-localizations between the insertions of copies of all three transposable elements and the breakpoints of aberrations were detected in all the chromosomes (Table 5).

c) The Farm (Bulgaria)

Sixteen (34.8%) of the common breakpoints coincided with NLRCth1 insertion sites; twenty-three (50%) co-localized with CTRT1 sites and twenty-two (47.8%) with TFB1 sites (Table 5). Correlations between frequency of common breakpoints and frequency of insertion sites of the three TEs were significant (NLRCth1 rs=0.52; df=10; P<0.05; CTRT1 rs=0.54; df=10; P<0.05 and TFB1 rs=0.53; df=10; P<0.05).

The co-localization between some common breakpoints and insertion sites of all three TEs were detected in chromosomes AB, CD and EF (Table 5).

d) Chaya River (Bulgaria)

Thirty (50%) common breakpoints coincided with NLRCth1 locations. The correlation between the frequency of common breakpoints and the frequency of NLRCth1 insertion sites was not significant (rs=0.43, df =10; P <0.1). In contrast, fourteen (23.3%) common breakpoints significantly co-localized with CTRT1 insertion sites (rs=0.55, df=10; P <0.05) and eighteen (30% of them) common breakpoints coincided significantly with TFB1 insertion sites (rs=0.85, df=10; P <0.01). The co-localizations between common break points and copies of all three TEs were detected in chromosome AB and CD (Table 5).

Discussion

In all the salivary gland chromosomes of the *C. riparius* larvae collected from heavy metal polluted sediments of Derincay River, The Farm (Plovdiv) and Chaya River (Asenovgrad), there was a large range of somatic chromosome rearrangements (heterozygous paracentric and pericentric inversions, deficiency, deletions). In contrast, the number of somatic rearrangements in the sample from the Pazar fountain was 4-6 times lower than that in the samples from the other stations. In addition, the sediment on which Pazar larvae were reared contained trace metals in concentrations lower than those of the other stations. These data support the hypothesis of an overall positive relationship between the frequencies of the different types of somatic aberrations and values of the various concentrations of the trace metals we analyzed (Sella *et al*. 2004, Michailova *et al*. 2011).

Our results showed also that this species has a number of sites of the salivary gland chromosomes that are particularly sensitive to chromosome breakages and are associated with sites of TEs. We advanced the hypothesis that these sites are “hot spots” for chromosome breakages and that mobile elements located in such sites are directly related to the formation of inversion break points. The same relationship has been observed in some *Drosophila* species (Zelentsova *et al*. 1999). Support to this hypothesis is provided by the frequently observed significant associations between the sites of breakpoints and the insertion sites of TEs.

In all the studied localities a significant accumulation of chromosome breaks and TE copies in the proximal parts of the chromosomes was found where the constitutive heterochromatin is localized. In fact heterochromatic regions contain multiple families of transposable elements (Dimitri 1977; Pimpinelli *et al*. 1995; Dimitri and Junakovic 1999; Bovero *et al*. 2002; Dimitri *et al*. 2003; 2009) and in *C. riparius* they are occupied also by clusters of repetitive DNA elements *Alu* and *Hinf* (Ilkova *et al*. 2007). On other hand, some studies in *Drosophila* demonstrated that the recombination rate in the heterochromatic regions is greatly suppressed or reduced (Casals *et*

al. 2006). Also, TEs are expected to be more abundant in regions of low recombination as inversions or inversion breakpoints (Guerreiro *et al.* 2008). All these data may explain the accumulation of TEs in the proximal heterochromatic regions of the chromosomes. So, the drastic reduction of recombination rate in these regions might be the major factor for the fixed localization of the studied TEs in the polytene chromosomes of *C. riparius.* The fixed insertions of the three TEs were found only in the proximal part of the large chromosomes of *C. riparius* from all four localities. Also in a formerly studied population of *C. riparius* (Ilkova *et al.* 2007, Michailova *et al.* 2007) fixed signals of NLRCth1 were observed only in the centromere and pericentromeric regions of AB, CD and EF chromosomes.

Together with the fixed copies of NLRCth1, CTRT1 and TFB1 a high number of variable signals were observed along the chromosome arms of *C. riparius.* Similar polymorphisms have been reported for NLRCth1 (Ilkova *et al.* 2007, Michailova *et al.* 2007) and TEC (Wobus *et al.* 1990) in other *C. riparius* populations and for NLRCth1 and CTRT1 in the *C. piger* genome (Michailova *et al.* 2009a). Our results showed a significant correlation between common breakpoints of aberrations and sites of localization of NLRCth1 (the Farm), CTRT (Derincay River, the Farm, Chaya River) or TFB1 (Pazar, the Farm, Chaya River). In the polytene chromosomes of individuals from Derincay River 77,55% of common breakpoints occurred in sites where repetitive DNA elements are located (NLRCth1, CTRT, TFB1, and/or Alu/Hinf clusters); in the Pazar sample 71,43% common breakpoints coincided with repetitive DNA elements; in the Farm sample 78,26% and in Chaya River sample 61,67%. Most of these breakpoints occur at or very close to the sites of TEs. Similar data of co-localization has been observed in other populations of *C. riparius* from Italy and Bulgaria (Bovero *et al.* 2002; Ilkova *et al.* 2007; Michailova *et al.* 2007), in *Anopheles* (Mathiopolous *et al.* 1998) and *Drosophila* (Regner *et al.* 1996, Zelentsova *et al.* 1999; Casals *et al.* 2005, Delpart *et al.* 2009, Prada *et al.* 2011). 91% of breakpoints of aberrations were localized in Ty sequences or repeated DNA elements in *Saccharomyces* species (Argueso *et al.* 2008).

According to Kale *et al.* (2005), heavy metals and other compounds can cause chromosome damages through the stimulation of mobile elements activity. These authors showed that heavy metals can induce the movement of the human LINE 1 retrotransposons. The cells have a complex homeostatic system which maintains a balance between the external aggressions and the internal regulation of the organisms' metabolic pathways. Mobile elements activity is one of the factors that can influence this balance. Methylation of TEs keeps TEs expression in a repressed state under the cellular control. However, external factors, such as some heavy metals, can affect the regulation of TEs genes, allowing for an increase in aberrant cells and that could be a mechanism for adapting chromosome function to stress (Lönning and Saedler 2002). The ability of some external stress agents to change the TEs activity either directly or indirectly may represent a major factor of genetic instability in somatic cells. Since Chironomids are insects with a significant role for assessing pollutants in aquatic ecosystems, it is necessary to provide a further molecular genome studies to understand the mechanisms of TEs activation in the Chironomids genome.

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