

# The relationship between chromosome rearrangements and repetitive DNA clusters in *Chironomus riparius* Meigen (Diptera: Chironomidae) from anthropogenically polluted Palaeartic regions

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**Abstract.** We compared chromosomal localization of aberration breakpoints and repetitive DNA clusters (Alu, Hinf repeats and NLRcTh1 retro-element copies) in polytene chromosomes among several Palaeartic population samples of *Chironomus riparius* Mg. from sites polluted with heavy metals. Both breakpoints and repetitive DNA clusters were significantly more frequent in proximal than in distal chromosomal regions, and they were found to be significantly associated.

**Key words:** *Chironomus riparius*, polytene chromosomes, aberration breakpoints, repetitive DNA clusters, polluted sediments

## INTRODUCTION

The midge *Chironomus riparius* Meigen, 1804 is distributed all over the Palaeartic region. A standard karyotype of the species is well characterized at the cytogenetic level and generally found to be monomorphic (Michailova, 1989; Kiknadze et al., 1991). A high level of polymorphism due to somatic aberrations has recently been observed in populations of *Ch. riparius* larvae living in sediments polluted with heavy metals (Michailova et al., 1996, 1998, 2000; Bovero et al., 2002; Sella et al., 2004). Insertional polymorphism for a non-LTR mobile element (NLRcTh1) was also recently analyzed in the same populations using the transposon display method (TID) (Zampicinini et al., 2004). Additionally, localization of Hinf and Alu satellite DNA clusters

was analyzed using FISH (Hankeln et al., 1989; Bovero et al., 2002). NLRcTh1 copies were found to have polymorphic insertions in all populations, while Alu and Hinf repeats have fixed positions.

The purpose of the present study is to compare the chromosomal localization of aberration breakpoints and repetitive DNA clusters (Alu or Hinf repeats or NLRcTh1 copies) in different populations of *Ch. riparius*, to analyze their distribution along the chromosomes and to see whether they are significantly associated.

## MATERIAL AND METHODS

Data on *Ch. riparius* breakpoint locations were obtained from 13 Palaeartic populations (6 from Italy, 3 from Bulgaria, 3 from Russia, 1 from Armenia) already described by Sella et al. (2004)





Table (completion).

Arm G			
Alu	Hinf	NLRCTh1	br. p.
	A1a		
	A1d		
	A2b	<b>A2b</b>	A2b
	Bc		Bc
	Dc		Dc, Ca
	NORb		Da

and Petrova et al. (2004). All these samples, with the exception of Corio, Italy (Sella et al., 2004), were collected in heavy metal polluted sediments.

Data on Alu and Hinf locations were obtained from one Italian and two Bulgarian populations (Bovero et al., 2002; Michailova et al., 2007, in press). Data on NLRCTh1 location were taken from Michailova et al. (2007, in press).

Cytogenetic, FISH and statistical methods are described in Bovero et al. (2002). Repetitive DNA clusters and NLRCTh1 insertions were mapped into the standard *Ch. riparius* chromosome map (Hägele, 1970; Kiknadze et al., 1991).

Common breakpoints are defined as those found in more than one individual; fixed insertions are those found in all sampled individuals and cells; variable insertions are those which appear only in one or more than one but not in all cells of an individual. To check the distribution randomness of breakpoints and repetitive DNA along the chromosomes, each arm was divided into two arbitrary proximal and distal sections, relative to the centromere, whose boundaries are indicated in Bovero et al. (2002).

## RESULTS

Within the studied *Ch. riparius* populations, larvae had a standard karyotype of  $2n = 8$ , with chromosome arm combinations AB, CD, EF, G, three Balbiani rings and a Nucleolar Organiser in chromosome G, providing reason to ascribe them to the “*thummi*” cytochrome (Keyl, 1962).

Localization of chromosome rearrangements, DNA clusters (Alu and Hinf) and NLRCTh1 retro-

element in polytene chromosomes of *Ch. riparius* is shown in Table. We found altogether 27 common breakpoints, 22 Alu, 34 Hinf and 76 NLRCTh1 insertions.

In whole genome we established 9 sites of coincidence of DNA clusters and breakpoints of aberrations (in arms A, C and D – 1 site; in arms F and G – 3 sites). Also in all arms we observed sites of coincidence of repetitive DNA clusters and transposable element (in arm A – 1 site; in arms B, C, E – 2 sites; in arm D – 1 site, in arm F – 5 sites).

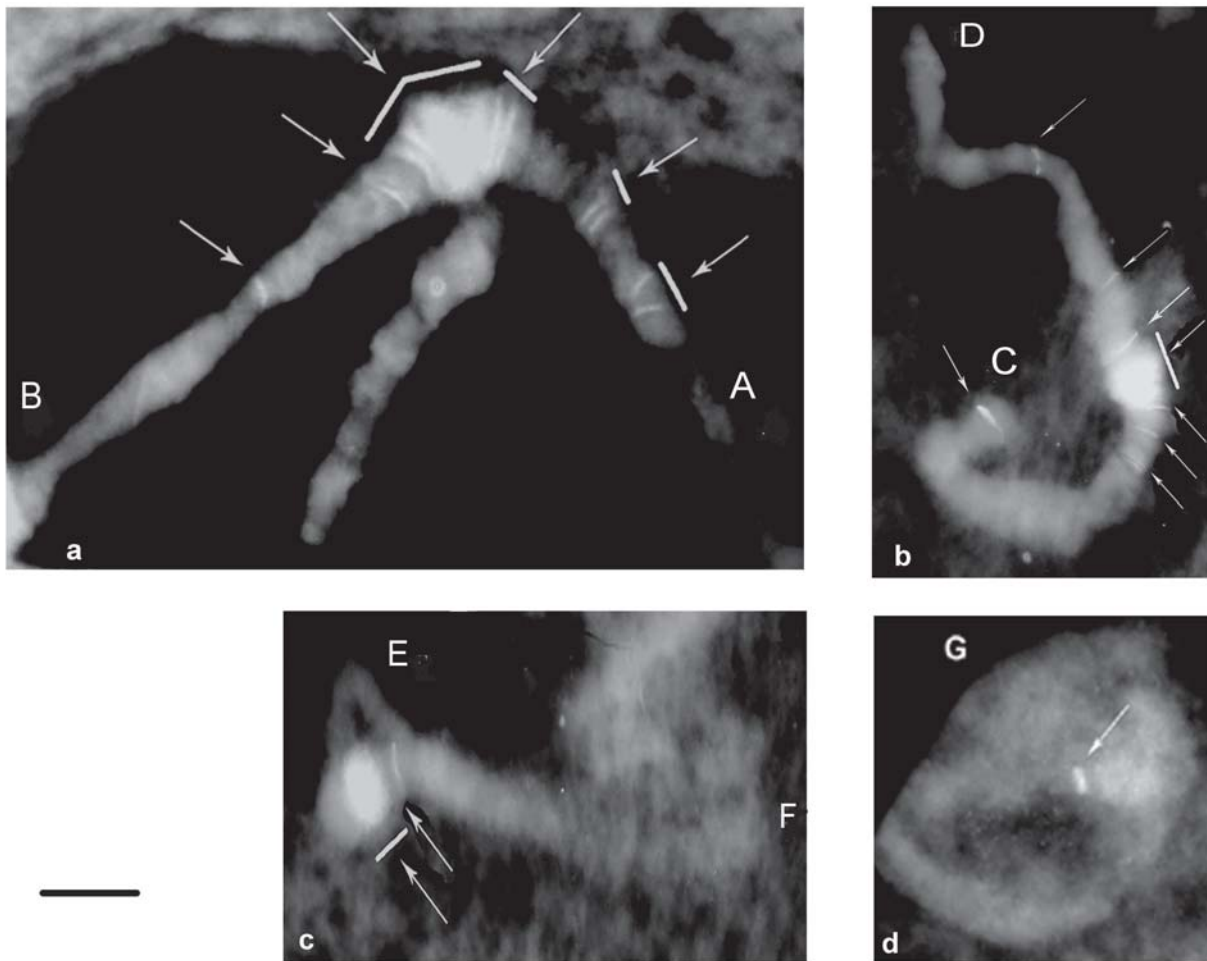
In all Palaeartic populations examined in the present study, locations of Alu and Hinf DNA clusters were found to be fixed.

Of the 76 NLRCTh1 retro-element insertions, 14 were fixed and 62 variable; of 62 variable signals, 36 occurred only once (Fig. 1). Variable signals are dispersed over the chromosome arms. In contrast, the fixed signals are located exclusively in the centromeres of chromosomes AB, CD, EF. Also, some signals are occurred close to the centromeres (for instance, D1d in arm A, D3d, D3h and E3e in arm B, C2l in arm C, C4e in arm D, B2h in arm E, B2m in arm F). In arm G the signal appeared in A2b.

Frequencies of Alu and Hinf DNA clusters, and of most copies of the NLRCTh1 retrotransposon are significantly concentrated in the proximal parts of chromosomes AB, CD and EF (G test, d.f. = 1; for AB,  $G = 17.14$ , for CD,  $G = 9.62$ , for EF,  $G = 24.99$ ,  $P < 0.001$ ). The frequency of rearrangement breakpoints was significantly higher in proximal than in distal parts of all chromosomes ( $G = 12.68$ , d.f. = 1,  $P < 0.001$ ), where the constitutive heterochromatin is localized.

Correlation between the frequency of repetitive DNA locations and common break points in proximal and distal regions is significant (Spearman  $r = 0.69$ ,  $P < 0.05$ ).

Ten out of 27 (37%) common breakpoints coincided either with Alu, Hinf, or NLRCTh1 retro-element location.



**Fig. 1, a-d.** Localization of retrotransposon NLRCh1 in the polytene chromosomes of *Chironomus riparius*. Arrows indicate NLRCh1 insertions. **a** - chromosome AB. **b** - chromosome CD. **c** - chromosome EF. **d** - chromosome G. Bar = 10  $\mu$ m.

## DISCUSSION

Alu and Hinf clusters have been studied in Italian (Bovero et al., 2002), German (Hankeln et al. 1989) and Bulgarian (Bovero et al., 2002; Michailova et al., 2007, in press) populations. In all studied populations the locations of these repetitive DNA clusters were found to be fixed. They can therefore be considered cytogenetic species-specific markers and can be used to differentiate *C. riparius* from the homosequential species *C. piger*. These clusters, as well as NLRCh1 copies, are located mainly in constitu-

tive heterochromatin sites, previously identified by Hägele (1977) and Michailova et al. (1997). Since 37% of common breakpoints occur in such heterochromatic regions, our data support the view that these regions are hot spots for ectopic recombination and rearrangement breakpoints. This is in agreement with Bovero et al. (2002), who found that somatic breaks in populations subjected to heavy metal pollution, occurred significantly more often in proximal repetitive DNA-rich regions than in distal ones. The present data also suggest that heterochromatin structure

is a key factor in inducing chromosomal instability following exposure to heavy metals, and confirm the topical role of repetitive DNA clusters in chromosome rearrangements.

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