

Chromosomal distribution of the 412 retrotransposon in natural populations of *Drosophila simulans*

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The insertion site localization of the 412 retrotransposable element was analysed by *in situ* hybridization to the polytene chromosomes of 57 individual genomes from 25 natural populations of *Drosophila simulans*. The 412 insertion sites along the chromosomes show a tendency to aggregate in the distal and proximal ends of the 2R arm, and in several local regions along the 3R arm. The distribution of the 412 insertion sites, weighted by DNA content, along the chromosome arms reveals an overall tendency for the site number to increase from the middle of the arm to the base and tip, with a decrease at the tips, especially pronounced for the X chromosome. Such a distribution differs slightly from that of *D. melanogaster*, which globally shows an increase of the 412 site number from base to tip of the chromosome arms, indicating differing behaviour of the 412 element in the two species. These results are discussed in connection with the recombination rate along the chromosome arms.

Keywords: chromosomal distribution, *Drosophila simulans*, natural populations, retrotransposon.

Introduction

The presence of many transposable elements (TEs) in the genomes of eukaryotes is well established. Their role and the ways in which they are regulated in natural populations, are, however, matters of speculation. From data from *Drosophila melanogaster*, it is clear that the increase in TE copy number within a genome is mainly counterbalanced by selection acting against either TE insertion mutations or deleterious chromosome rearrangements arising from ectopic exchanges between insertion sites (Charlesworth & Charlesworth, 1983; Langley *et al.*, 1988; Charlesworth & Langley, 1989; Stephan & Langley, 1992). Although some interpretations of the data favour the ectopic exchange model (Charlesworth *et al.*, 1994a,b; Sniegowski & Charlesworth, 1994; Nuzhdin, 1995), recent results on both *D. melanogaster* and *D. simulans* are more in agreement with selection against insertional mutations (Biémont, 1992; Biémont *et al.*, 1994; Hoogland & Biémont, 1996; Vieira & Biémont, 1996a,b). The

main bulk of the data comes from *D. melanogaster*, but it is becoming evident that this species, with its high copy number for most TEs, is not typical of its species group. More knowledge of TE distributions over the chromosome arms in other species is thus necessary, but, so far, only partial data are available, such as the positions of TEs in laboratory stocks, information only of their presence/absence or of their copy numbers, and localizations restricted to the X chromosomes. Even *D. simulans*, the closest species to *D. melanogaster*, is not yet well known, although data are now accumulating (Leibovitch *et al.*, 1992; Caggese *et al.*, 1995; Nuzhdin, 1995; Vieira & Biémont, 1996a,b). *Drosophila simulans* has a total number of TEs that is about one-third of that in *D. melanogaster* (Dowsett & Young, 1982; Csink & McDonald, 1990; Nuzhdin, 1995; Vieira & Biémont, 1996a,b), as a result, presumably, of stronger selection against insertions in the *D. simulans* genome than is found in *D. melanogaster* (Nuzhdin, 1995; Vieira & Biémont, 1996b). For example, the retrotransposable element 412 has a high copy number in both species, but has only half as many copies in *D. simulans* as in *D. melanogaster*.

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Here, we present an analysis of the distribution of this element in the chromosomes in 25 geographically distinct natural populations of *D. simulans*.

Materials and methods

Drosophila populations

Flies from 25 geographically distinct, natural population samples of *D. simulans* were used: France (5 populations), Portugal (2), Russia (1), Spain (1), Arabia (1), Israel (1), Congo (1), USA (2), Brazil (1), Uruguay (1), Australia (2), New Caledonia (4), Tahiti (1), Madagascar (1) and Réunion (1). They were maintained in the laboratory as isofemale lines with about 50 pairs every generation (see Vieira & Biéumont, 1996a for details). The populations were analysed for their 412 insertion localization as soon as possible after their arrival in the laboratory.

In situ hybridization

Polytene chromosomes from salivary glands of third instar female larvae were prepared and treated with nick-translated, biotinylated DNA probes, as previously described (Biéumont, 1994). Insertion sites were visualized as brown bands resulting from a dye-coupled reaction with peroxidase substrate and diaminobenzidine. We used as probe the 412 element (Finnegan *et al.*, 1978) inserted in pBR322, provided by N. Junakovic. A genomic DNA sequence associated with the probe hybridized to the 21D region and thus acted as a positive control for hybridization.

Localization of 412 insertion sites

The localization of the 412 insertion sites was determined on diploid individuals (one larva for two to three isofemale lines analysed directly per population). The sites were precisely localized on the polytene chromosomes following the Lefevre (1976) and Sorsa (1988) photographic maps established for *D. melanogaster*, which are very close to *D. simulans* except for the major inversion 85A–93F in the 3R chromosome arm.

Estimation of DNA content

The DNA content per division of the polytene chromosomes was estimated following Bolshakov *et al.* (1985) and Sorsa (1988) using data from *D. melanogaster*. The estimate of Bolshakov measures the number of silver grains in a region after ³H-thymidine incorporation. The estimate of Sorsa is based

on the number of bands seen in a division, according to the equation: DNA content per chromatid = LC × NB × 21.6 kb, where LC is a coefficient corresponding to the relative length of bands in the division, NB is the number of bands in the division, and 21.6 kb is the average amount of DNA per band plus adjacent interband.

Statistical tests

The statistical analyses were based on the number of insertion sites (a site was represented only once per population, even if an insertion at that site was detected in various individuals) rather than on the number of copies; this eliminates any bias caused by high site frequencies resulting from relatedness between individuals or populations, or preferential insertions into specific regions. In all the analyses we excluded the centromeric regions 20, 40–41 and 80–81 because: (i) it is well known that these regions accumulate TE copies in β -heterochromatin (Miklos *et al.*, 1988), which is a trap for many nonmobile ancient copies of TEs (Vauray *et al.*, 1989); (ii) although such an accumulation of insertions in the centromeric region is easily seen for the 412 element by *in situ* hybridization, precise site localization and DNA content estimation in these regions are difficult to obtain and are not reliable.

To analyse the distribution of the 412 insertion sites along the chromosome arms we divided each arm into 120 parts following Bridges's lettered subdivisions. The presence of an insertion site was denoted by 1 and its absence by 0. The distribution of the 1s and 0s along the chromosomes was analysed by four nonparametric statistics in which the sites coded 1 were characterized by their ranks in the ordered sequence (see Aulard *et al.*, 1995 for detailed formulae of these statistics). We thus calculated:

- 1 the sum of ranks of 1 (*SR*), which tests the existence of an accumulation of sites at one of the extremities of the chromosome arm;
 - 2 the number of runs (blocks of 0 or 1) (*NR*), which tests the autocorrelation between sites; a high value of this test detects a regular distribution, a low value suggests partition by blocks;
 - 3 the longest run (*LR*), which detects a local accumulation of sites;
 - 4 the variance of ranks (*VR*), which detects aggregation of 1, either in the middle of the arm (low variance value) or at its two ends (high variance value).
- The null hypothesis for all these statistics was the random distribution of insertion sites over the chromosomes.

To analyse the relationship between TE insertion site number and DNA content, the nonparametric Spearman's rank correlation (ρ), with correction in case of ties (Lehmann, 1975), was used because of the non-normality of the data.

Results

Figure 1 shows the cumulative distributions of the 412 insertion sites along the chromosome arms for the 57 genomes. As revealed by the statistics presented in Table 1, these distributions did not show any significant tendency for the X, 2L and 3L chromosome arms. However, groupings of the 412 insertion sites were detected along the 3R arm ($NR' = -3.21$, $P < 0.01$), and an accumulation at both extremities was detected for the 2R arm ($NR' = -2.39$, $P < 0.05$; $VR' = 2.63$, $P < 0.01$). For the statistics that showed the same tendency over the five chromosome arms (Table 1), we calculated:

$$U = (\Sigma S')/\sqrt{5}$$

over all arms, which is normally distributed according to the central limit theorem. We thus detected a global aggregation of insertion sites at the two ends of the chromosomal arms ($U_{VR'} = 2.96$; $P < 0.01$),

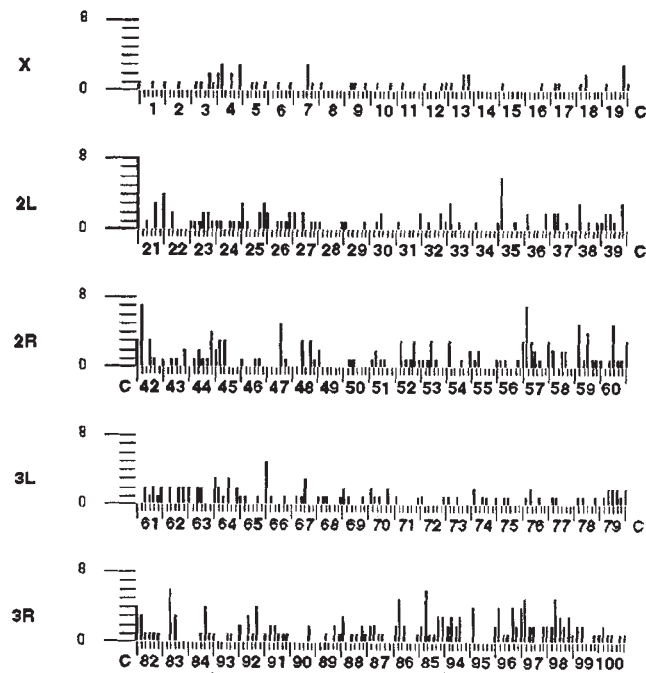


Fig. 1 Localization on the chromosome arms of the 412 retrotransposable element insertion sites found in 57 genomes from 25 populations of *Drosophila simulans*. C: chromocentre. The inversion 85A–93F is marked by arrows.

with a more pronounced accumulation at the distal part of the arms ($U_{SR'} = -2.63$; $P < 0.01$).

We have looked for a possible relationship between 412 insertion site number and DNA content along the chromosomes of *D. simulans*, as found previously in *D. melanogaster* for various TEs (Hoogland & Biéumont, 1996). As seen in Table 2,

Table 1 Nonparametric statistics for detecting structure in the distribution of the 412 element insertion sites over the chromosome arms of *Drosophila simulans*

Statistics	Chromosome arms				
	X	2L	2R	3L	3R
	(43)	(62)	(70)	(62)	(79)
SR'	-1.46	-0.84	-1.05	-0.77	-1.76
NR'	0.09	0.46	-2.39*	0.08	-3.21**
LR	0.74	0.28	0.36	0.90	0.40
VR'	0.31	1.53	2.63**	1.52	0.62

SR : sum of ranks; NR : number of runs; LR : longest run; VR : variance of ranks (see text for details).

For all statistics, but LR , we calculated the normal approximation because the observed number of insertion sites was greater than or equal to 10. The statistic with a prime:

$$S' = \frac{S_{\text{obs}} - E(S)}{\sqrt{\text{Var}(S)}}$$

is normally distributed. S_{obs} is the observed value, $E(S)$ and $\text{Var}(S)$, respectively, the mean and variance of the statistic S . For the LR statistic, the exact probability is given.

The numbers of insertion sites observed are in parentheses.

* $P < 0.05$; ** $P < 0.01$.

Table 2 Spearman's rank correlation coefficients for 412 insertion site number versus DNA content (Sorsa and Bolshakov estimations) in *Drosophila simulans*

Chromosome arms	DNA content estimation	
	Sorsa	Bolshakov
X	0.50*	0.51*
2L	0.18	0.27
2R	0.18	0.17
3L	0.47*	0.57**
3R	0.02	0.24

*One-tailed $P < 0.05$; **one-tailed $P < 0.01$.

the two variables are significantly correlated for the X and 3L arms using both the Bolshakov and the Sorsa estimates of DNA content. We thus divided the number of 412 insertion sites in each of Bridges's divisions by the DNA content in the division. Figure 2 thus represents the distribution in each chromosome arm of the number of 412 insertion sites weighted by DNA content. In agreement with the above results on the insertion site distribution along the chromosomes, there was a global tendency for 412 insertion site number to decrease from the tip and base to the middle of the chromosomal arms, with a decrease at the extreme tip of chromosomes, especially for the X (Fig. 2).

To see whether the above result was specific to *D. simulans* or whether it is also seen in *D. melanogaster*, we used the insertion sites of 412 in *D. melanogaster* available in the literature. We pooled the data from a French population, Valence (185 insertion sites: Aulard *et al.*, 1995), and a Maryland population (165 insertion sites: Charlesworth & Lapid, 1989; Charlesworth *et al.*, 1992a). Figure 3 represents the distribution of 412 insertion site number weighted by DNA content along an average chromosome (the five arms are superimposed) for

each species. It appears that *D. melanogaster* differs from *D. simulans* by a less pronounced accumulation of 412 insertions in specific regions, and with the number of 412 insertions clearly increasing from the base to the tip of the average chromosome. In contrast, on average, the 412 insertion site number in *D. simulans* decreases from the tip and the base to the middle of the chromosome arms, with a sharp decrease at the tip especially caused by the X chromosome. The chi-squared values calculated on each chromosome arm from data on insertion site numbers weighted by Bolshakov DNA content estimate per division have been summed to test the difference between the distributions observed in the two species. There is no significant difference in the two average distributions ($\chi^2_{90} = 100.5$; $P = 0.21$), but a significant difference between the two distributions on the X chromosome is found ($\chi^2_{18} = 32.96$; $P < 0.05$). However, the two sets of data were not entirely comparable because with *D. simulans* we were dealing with few individuals from many various populations, whereas the *D. melanogaster* data concerned many individuals in only two populations. More data on various *D. melanogaster* populations are thus needed to confirm these latter results.

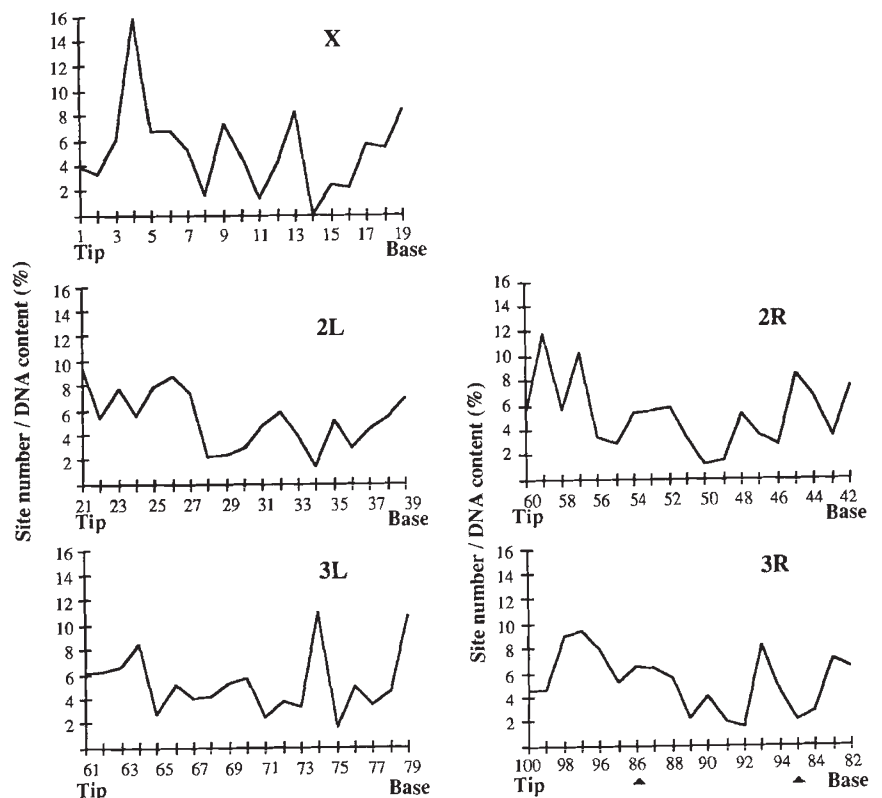


Fig. 2 Distribution per Bridges's division of the 412 insertion site number weighted by DNA content from Bolshakov along the five chromosome arms of *Drosophila simulans*. The inversion 85A-93F is marked by arrows.

Because the Bolshakov and Sorsa estimators of DNA content give similar distributions of DNA content along the chromosome arms (Hoogland and Biémont, 1996), only data from the Bolshakov estimate were used.

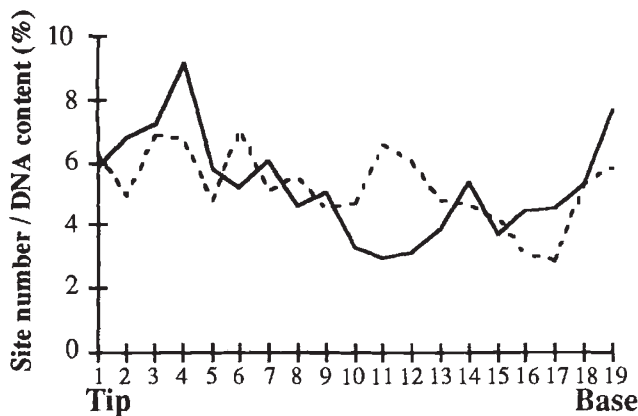


Fig. 3 Distribution along the 19 divisions of an average chromosome arm (the five arms are superimposed) of the relative (in %) 412 insertion site number weighted by DNA content (Bolshakov estimation: see Fig. 2) in *Drosophila simulans* (solid line) and in *Drosophila melanogaster* (dashed line).

Discussion

The distribution of TE insertion sites in the chromosomes is of interest in testing models of TE population dynamics. These models postulate that TE copy number is maintained in populations as a result of a balance between transposition, which increases the number of copies, and opposing forces, which tend to reduce it. Such opposing forces include regulation of transposition rate with increasing copy number (Charlesworth & Charlesworth, 1983; Kaplan & Brookfield, 1983; Langley *et al.*, 1983), selection against insertional mutations (Charlesworth & Charlesworth, 1983; Kaplan & Brookfield, 1983; Langley *et al.*, 1983; Kaplan *et al.*, 1985; Montgomery *et al.*, 1987), and selection against deleterious chromosome arrangements induced by ectopic exchanges between TEs (Langley *et al.*, 1988; Stephan & Langley, 1992). Ectopic exchanges are sometimes presented as the main factor explaining the dynamics of TEs (Langley *et al.*, 1988; Charlesworth *et al.*, 1992b, 1994b; Sniegowski & Charlesworth, 1994; Nuzhdin, 1995). In this model, TE site number is negatively correlated with recombination rate, assuming that the frequency of ectopic exchanges in a region is proportional to meiotic exchanges in that region (Langley *et al.*, 1988). To test this hypothesis, authors usually check whether TE insertions accumulate in regions where crossing-over is strongly reduced, such as the bases and tips of the *D. melanogaster* polytene chromosomes (Lindsley & Sandler, 1977). In this species, experimental data available for a survey of 10 TE families (including 412) failed to show an accumulation of TE insertion

sites at the tips of the chromosome (Langley *et al.*, 1988; Charlesworth & Lapid, 1989; Charlesworth *et al.*, 1992b), and there is no consensus for accumulation at the bases, because this depends on whether the centromeric and pericentromeric regions of the chromosomes are included (these are known to be traps for nonmobile ancient copies of TEs; Miklos *et al.*, 1988; Vaury *et al.*, 1989). Indeed when TEs inserted in centromeric and pericentromeric regions are excluded from the data, no simple relationship between insertion site number and recombination rate can be detected in *D. melanogaster* (Biémont *et al.*, 1994; Hoogland & Biémont, 1996). The fact that the 412 element in *D. melanogaster* has never been shown to accumulate in the bases of the autosomes (Charlesworth *et al.*, 1992b; Aulard *et al.*, 1995) may thus rely on the absence of fixed sites of 412 in these proximal regions.

Our data on the distribution of 412 insertions in *D. simulans* reveal an overall tendency for site number to decrease from the tip and the base to the middle of the chromosomal arms, with a sharp decrease at the extreme tips, especially for the X chromosome. One way to test the ectopic exchange model of TE site number containment in *D. simulans* consists of correlating the distribution of 412 insertion site number with the recombination rate along the chromosomes. Unfortunately, we have not yet a complete view of the frequency and distribution of crossovers in *D. simulans*, whose total genetic map length is 1.3 times longer than the *D. melanogaster* map (Ashburner, 1989; True *et al.*, 1996). The X chromosome seems to have the same distribution of recombination rate as *D. melanogaster*, which increases from base to tip with a sharp decrease at the extreme tip (Lindsley & Sandler, 1977; True *et al.*, 1996), suggesting an absence of association between recombination rate and 412 insertion site number in *D. simulans* for this chromosome. The coefficient of exchange value in the third chromosome, which is higher than in *D. melanogaster*, increases from base and tip to the middle of each arm (True *et al.*, 1996). It is thus tempting to conclude that the 412 insertion site number, which decreases from tip and base to the middle of each arm of this third chromosome, was negatively correlated with the recombination rate, in agreement with the ectopic exchange model (Langley *et al.*, 1988; Charlesworth *et al.*, 1992b, 1994b; Stephan & Langley, 1992; Sniegowski & Charlesworth, 1994). We have no information on the coefficient of exchange for the 2L and 2R arms in which the 412 insertion site number evidently increased toward the bases and tips. More information on recombination

rates in *D. simulans* is clearly needed to address properly the question of a relationship between recombination rate and TE chromosomal distribution in this species.

There is little evidence, however, that the 412 insertion site distribution in *D. simulans* differs from that of *D. melanogaster*, at least for the X chromosome. If these two sibling species are submitted to the same evolutionary forces, it is not obvious why the 412 element behaves differently. These data on the 412 element, conjointly with other studies in *D. simulans* (Vaury *et al.*, 1989; Leibovitch *et al.*, 1992; Kimura & Kidwell, 1994; Caggese *et al.*, 1995; Nuzhdin, 1995; Vieira & Biémont, 1996b), reinforce the hypothesis of specific TE behaviours, depending on the TE family and the host species.

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