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Selection against transposable elements in *D. simulans* and *D. melanogaster*

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Summary

The insertion site numbers of the transposable elements (TEs) copia, mdg1, 412 and gypsy were determined in various natural populations of *Drosophila melanogaster* and *D. simulans* by *in situ* hybridization. We showed that, while all elements except gypsy had many insertion sites scattered over the chromosomes in *D. melanogaster*, only the 412 element in *D. simulans* presented a high number of insertions, and this number was lower than in *D. melanogaster*. This low 412 site number per genome in *D. simulans* was associated with a lower proportion of insertions on the X chromosome in comparison with *D. melanogaster*, as determined in diploid genomes (0·090 for *D. simulans* against 0·137 for *D. melanogaster*) and in haploid genomes (0·102 against 0·146), each value being, moreover, lower than the value of 0·20 expected on the hypothesis of no selection against insertional mutations. These results suggest that selection is a major mechanism explaining 412 copy number regulation in *Drosophila*, and is stronger in *D. simulans* than in *D. melanogaster*.

1. Introduction

The idea that *D. simulans* has fewer TE copies than its sibling species *D. melanogaster* comes mainly from the observation that the chromosomes of *D. melanogaster* carry approximately 3 times as much middle repetitive DNA as those of *D. simulans* (Dowsett & Young, 1982), and that no sequence length variation is detected in various *D. simulans* lines (Aquadro *et al.* 1988). Only scattered and sometimes indirect data are, however, available concerning the exact number of copies of TEs in chromosomes from natural populations of *D. simulans* (Dowsett & Young, 1982; Vaury *et al.* 1989; Csink & McDonald, 1990; Martin *et al.* 1983; Leibovitch *et al.* 1992; Nuzhdin, 1995), and although many TE families can be detected simultaneously in both species (Brookfield *et al.* 1984), we have little indication of the partitioning of the copies between heterochromatin and euchromatin. We thus do not know whether the overall low amount of repetitive sequences found in *D. simulans* is really due to fewer euchromatic copies in each family, as suggested by the observation that the copy number per family of various elements on the X chromosome is three times less in *D. simulans* than in *D. melanogaster* (Nuzhdin 1995). This is of importance, since the overall lower amount of middle repetitive sequences in *D. simulans* than in *D. melanogaster* has been suggested as reflecting a higher effective species population size for *D. simulans*, with the underlying hypothesis that selection against TE insertions is similar in the two species (Aquadro *et al.* 1988; Aquadro, 1992). Only a lower number of copies scattered over the chromosomes of *D. simulans* in comparison with *D. melanogaster* should be considered as evidence of a possible influence of effective species population size, although a very low rate of transposition could also account for such distribution. There is, however, no evidence of a low rate of transposition in *D. simulans* (Eeken *et al.* 1987; Inoue & Yamamoto, 1987), and it has been shown theoretically that negative selection leads to fewer copy numbers on the X chromosomes than on the autosomes (Charlesworth & Langley, 1991; Montgomery *et al.* 1987; Langley *et al.* 1988); this is because hemizygosity in *Drosophila* males allows selection to be stronger on the X chromosomes. A lower proportion of TE insertions on the X in comparison with the autosomes is not expected with low transposition rate.

We therefore collected flies from various, geographically distinct, natural populations of *Drosophila melanogaster* and *Drosophila simulans*, and analysed them for the presence and localization of the four retrotransposable elements copia, mdg1, 412 and

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gypsy. We found that the elements behaved differently within species and between them, and that selection acts against 412 insertions more strongly in *D. simulans* than in *D. melanogaster*. Such negative selection accounts for the low 412 copy number in *D. simulans*, without implying a major influence of population size.

2. Materials and methods

(i) *Natural populations collected*

We collected flies from various, geographically distinct, natural populations. Seven populations of *D. melanogaster* (France, 3 populations; Portugal, 2; Congo, 1; Arabia, 1) and 12 populations of *D. simulans* (France, 5 populations; Portugal, 2; Congo, 1; Cordoba, 1; Arabia, 1; Israel, 1; South Russia, 1) were collected simultaneously in the same areas whenever possible; they were then maintained in the laboratory as isofemale lines with around 50 pairs every generation. The populations were analysed for their TE copy number as soon as possible after their arrival in the laboratory.

(ii) Insertion site number estimations

Diploid genomes. To get a quick estimation of the TE insertion site number of the *D. melanogaster* and *D. simulans* natural populations for copia, mdg1, gypsy and 412, one female larva for two to three isofemale lines was analysed directly per population and per element. We thus obtained the insertion site number of diploid individuals. In addition the sites were precisely localized on the polytene chromosomes of *D. simulans* using the Lefevre (1976) and Sorsa (1988) photographic maps of *D. melanogaster*.

Haploid genomes. The site number estimated directly by analysing larvae from the isofemale lines is sensitive to the degree of homozygosity of the flies in the lines. Indeed, the number of insertion sites detectable in diploids by *in situ* hybridization decreases with increasing homozygosity (because the polytene chromosomes are composed of the two parental homologous chromosomal sets), and since there is only one X in males and two Xs in females, the degree of homozygosity might differ slightly between the X chromosomes and the autosomes. So, for the element 412 which showed a high number of sites in both species (see Section 3), we crossed males from the natural populations with females from inbred lines with well-known profiles of 412. For *D. melanogaster* we used the highly inbred line 16 as in Biémont *et al.* (1994), and for *D. simulans* we used a recent line (MK) maintained by brother–sister matings for seven generations. Although this number of generations of inbreeding was low, the number of insertion sites in this *D. simulans* line MK (five sites for 412) was low enough to obtain flies which were homogeneous for their 412 profiles. The copy number of a haploid genome was thus obtained by subtracting the site number of the inbred line from that of the hybrid larva; because there is no recombination in *Drosophila* males, we reconstructed intact chromosomes.

(iii) *Probes used*

We used the probe cDm5002 containing the copia element (5 kb) (Levis *et al.* 1980; Dunsmuir *et al.* 1980); the A fragment of the mdg1 element inserted at the *Hind* III site of the pBR322 plasmid (Ilyin *et al.* 1980; Tchurikov *et al.* 1981); the clones Dm111 containing the full length gypsy element inserted at the *Bam* H1 site of pBR322 (Bayev *et al.* 1984); the complete 412 element (Finnegan *et al.* 1978) inserted in pBR322.

(iv) In situ *hybridization*

Polytene chromosome spreads from salivary glands of third instar female larvae taken from the isofemale lines were prepared and treated with nick-translated, biotinylated DNA probes (Biémont, 1994). Insertion sites were visualized as brown bands resulting from a dye-coupled reaction with peroxidase substrate and diaminobenzidine.

3. Results

(i) *TE insertion site number in diploids*

As seen in Table 1, the elements copia, gypsy and mdg1 in the natural populations of *D. simulans* were characterized by a very low insertion site number on all chromosome arms (determined on diploid genomes of females taken directly from the isofemale lines). Most of the copia insertions were in the same sites (42B, 42C, 82E) in all the populations, and these three fixed copia sites were the same as those already reported in a sample of various strains (Leibovitch *et al.* 1992). The insertion polymorphism of gypsy was higher than for copia, with no apparent fixed sites. Very few insertion sites were observed for mdg1, and the low intensity of the hybridization signal contrasted with the very strong signal observed in *D. melanogaster*. This suggests that our mdg1 probe from *D. melanogaster* had only weak homology with the *D. simulans* mdg1 element. Note, however, that many bands were detected in *D. simulans* heterochromatin by our mdg1 probe when comparing Southern blots loaded with DNA from adult flies, in which heterochromatic and euchromatic insertions are detected, and salivary glands, in which only euchromatic insertions are revealed due to the polytenization of the chromosomes (unpublished results), suggesting that the conditions of the Southern blots allowed detection of the slightly diverged mdg1 known to exist in heterochromatin (Shevelyov *et al.* 1989). The case of 412 was quite different. This element was well scattered over the chromosome arms in all the *D. simulans* lines

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Table 1. *Mean insertion site numbers of TEs*

Element	*D. simulans*	*D. melanogaster*	Element	*D. simulans*	*D. melanogaster*
copia	30	14	412	27	16
X	0·1 (0·3)	2·7 (1·7)	X	1·2 (1·1)	4·3 (1·3)
2L	0·03 (0·2)	4·3 (2·0)	2L	2·7 (1·6)	7·4 (2·2)
2R	2·1 (0·3)	5·3 (1·4)	2R	4·5 (2·1)	6·4 (2·0)
3L	0·2 (0·5)	4·0 (1·6)	3L	2·1 (1·3)	5·6 (2·7)
3R	1·0 (0·0)	6·7 (2·5)	3R	3·2 (1·8)	7·4 (1·7)
Total	3·4 (0·6)	23·0 (4·6)	Total	13·2 (3·8)	31·1 (4·1)
mdg1	31	15	gypsy	26	15
X	0·0 (0·0)	3·5 (1·2)	X	0·3 (0·5)	0·2 (0·6)
2L	0·0 (0·0)	4·5 (1·6)	2L	0·1 (0·4)	0·1 (0·4)
2R	0·3 (0·6)	4·7 (2·4)	2R	0·2 (0·4)	0·2 (0·4)
3L	0·04 (0·2)	4·3 (2·0)	3L	0·3 (0·7)	0·2 (0·4)
3R	0·3 (0·5)	7·5 (2·7)	3R	0·6 (0·7)	0·2 (0·4)
Total	0·6 (0·6)	24·5 (3·3)	Total	1·6 (1·3)	0·9 (1·0)

Mean insertion site numbers of the copia, mdg1, 412, and gypsy elements in X, 2L, 2R, 3L, and 3R chromosomal arms for diploid genomes of female larvae from isofemale lines from 12 natural populations of *D. simulans* and 7 natural populations of *D. melanogaster*. Two to three isofemale lines were analysed per population.
Standard deviations are in parentheses; the values above the columns are the sample sizes.

studied, the mean value over the populations being equal to 13·2 sites per diploid genome (Table 1) (see Vieira & Biémont (1996) for a detailed analysis of 412 insertion site number in various natural populations of *D. simulans*). In *D. melanogaster*, copia, mdg1 and 412 all had many copies inserted over the chromosome arms (see Table 1) as well as in the chromocentre, which was always marked by our probes. Gypsy, however, had a low copy number similar to, though slightly smaller than, that observed in *D. simulans*. We eliminated from the analysis the insertions localized in the centromeric regions 20, 40, 41, 80 and 81, because TE site localization in these regions is difficult and not reliable for all chromosomes; moreover, it is well known that these regions accumulate TE copies in β-heterochromatin (Miklos *et al.* 1988; Vaury *et al.* 1989; Charlesworth *et al.* 1994; Carmena & Gonzalez, 1995; Pimpinelli *et al.* 1995).

(ii) *412 insertion site number on the X versus the autosomes*

It has been proposed that, in theory, the transpositional increase in TE copy number in the *Drosophila* genome may be opposed either by the regulation of the rate of transposition with increasing copy number, or by selection against insertional mutations (Charlesworth & Langley, 1991; Montgomery *et al.* 1987; Langley *et al.* 1988). Under the first hypothesis, an equiproportionality between the X and the autosomes is expected in the genome, while the second hypothesis predicts a lower number of insertions on the X chromosome than on the autosomes because a higher proportion of deleterious mutations are eliminated in the hemizygous males. A frequency of elements on the X of 0·20 is thus expected if insertions are neutral. This neutral model considers that in the *D. melanogaster* genome 40% of the X and 22% of the autosomes are heterochromatic, the heterochromatic Y chromosome constitutes 20% of the haploid genome of the male, and that transposition is independent of sex and of location in euchromatin versus heterochromatin (Charlesworth & Langley, 1991; Montgomery *et al.* 1987; Langley *et al.* 1988). The second model of copy number regulation based on selection acting against deleterious insertions (Charlesworth & Langley, 1991; Montgomery *et al.* 1987; Langley *et al.* 1988) considers that the ratio of the mean selection coefficients on hemizygous or homozygous mutations for the autosomes over the X is equal to 0·618, with a dominance coefficient for heterozygotes equal to 0·35. A proportion of TE insertions on the X of 0·13 is thus predicted. Two other models of selection based on ectopic exchanges between the repetitive sequences, thus inducing deleterious chromosome rearrangements, predict a proportion of insertions on the X chromosome to be 0·16, if all inserted elements can recombine with any element located elsewhere in the genome, or to be 0·18 if elements recombine only with other elements located in the same chromosomal region (Langley *et al.* 1988).

To be sure that the two ways of analysing genomes gave similar estimates of the proportion of insertion sites on the X chromosomes, we compared the values obtained in haploids and diploids (female genomes from the isofemale lines). The distributions of the proportion of insertion sites on the X chromosomes presented in Fig. 1 for both species were compared by analyses of variance on arcsin-square root transformed data, so as to take into account both intra- and inter-population variabilities. No statistically significant difference was detected between the two values ($F = 0·15$ for *D. melanogaster*, $F = 0·77$ for *D.*

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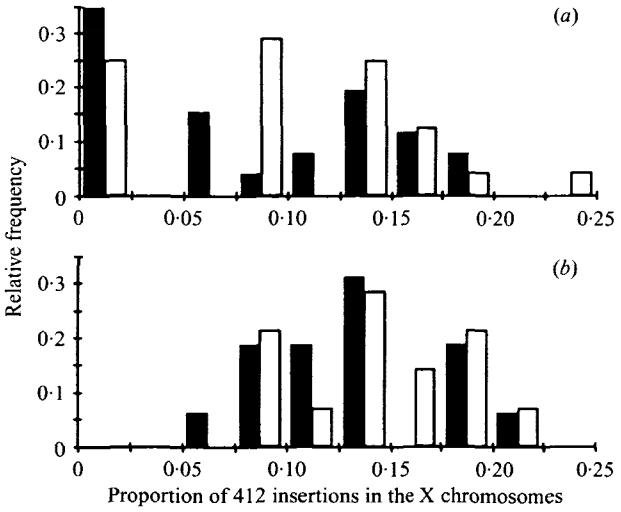

Fig. 1. Distributions of the proportion of 412 insertion sites on the X chromosomes in female larvae from the isofemale lines (black columns) and crosses with the inbred lines (white columns), for *D. simulans* (*a*) and *D. melanogaster* (*b*).

simulans), implying that both haploids and diploids gave reliable estimations of the proportion of 412 insertion sites on the X chromosomes.

Since the element 412 presented high insertion site numbers in both *D. melanogaster* and *D. simulans*, making the above theoretical assumptions testable in both species, we compared the proportions of insertions on the X chromosomes with the 0·20, 0·18, 0·16 and 0·13 theoretical values. As seen in Table 2, the proportions of 412 on the X for *D. melanogaster* were statistically significantly lower than the expected theoretical value of 0·20 but fitted the 0·13 value well, as tested by χ^2, and this was true whatever the technique used for estimating the insertion site numbers. The comparison was more ambiguous for the ectopic exchange model because the theoretical (0·16 and 0·18) and observed (0·137 and 0·146) values were intermediate between 0·13 and 0·20, making the statistical test less discriminating.

The fact that haploids and diploids gave similar results allowed us to compare the theoretical expectations with the proportions on X chromosomes of the insertion site numbers of the elements copia and mdg1 of *D. melanogaster*, for which only data on diploids were available. As seen in Table 2, the deviation from randomness and the agreement with the selected model were also observed for the proportion of insertions on the X chromosome for these two elements, with in addition a clear rejection of the ectopic exchange model for copia but not for mdg1 (the number of gypsy insertions was too small for statistical analysis of insertions on the X chromosomes).

For *D. simulans* the proportion of 412 on the X chromosomes was also statistically significantly lower than the expected theoretical value of 0·20, but fitted the 0·13 value only for data on haploid genomes. For diploids even the selected model was rejected as a result of both low values of the proportions tested and higher sample sizes, which made the χ^2 tests more efficient. Both models of ectopic exchange were statistically rejected. Actually the proportions of 412 insertions on the X in the *D. simulans* populations appeared always significantly lower than the values observed in *D. melanogaster* (*D. simulans* 0·102, *D. melanogaster* 0·146 for the haploid genomes; analysis of variance: $F = 4{\cdot}75$, $P < 0{\cdot}05$; *D. simulans* 0·090, *D. melanogaster* 0·137 for the diploid isofemale line genomes; analysis of variance: $F = 8{\cdot}79$, $P < 0{\cdot}01$).

(iii) *Test of transposition rate hypothesis*

A very low rate of transposition could account for the low number of TE insertion sites in *D. simulans*. We

Table 2. *Tests of the proportions of TE insertions on the X chromosomes compared with the autosomes in* D. melanogaster *and* D. simulans *genomes*

Element	No. on X	No. on autosomes	Proportion on X	Neutral model† 0·20	Negative selection models† 0·18	0·16	0·13
D. melanogaster							
copia[a] (14)	38	284	0·118	13·5***	8·4***	4·2*	0·4
mdg1[a] (15)	53	314	0·144	7·1**	3·2	0·7	0·7
412[a] (16)	68	430	0·137	12·5***	6·4*	2·0	0·2
412[b] (14)	37	217	0·146	4·7*	2·0	0·4	0·6
D. simulans							
412[a] (26)	32	338	0·090	29·8***	18·8***	12·5***	4·8*
412[b] (24)	25	219	0·102	14·5***	9·9**	6·0*	1·6

Tests of the proportions of transposable elements on the X chromosomes compared with the autosomes in *D. melanogaster* and *D. simulans* genomes, in larvae from: [a] the isofemale lines (diploid genomes), [b] the crosses with the inbred lines (haploid genomes).

† Chi-squared tests of neutral and negatively selective containment models of copy number (see text).

The numbers of female larvae analysed per element are in parentheses.

Significance levels: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

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tested this hypothesis by estimating the level of 412 insertion site heterozygosity in theoretical flies resulting from crossing isofemale lines of *D. simulans* (in which the 412 insertions were precisely localized on the chromosome arms) from different populations. This degree is in fact a measure of the heterogeneity in insertion profiles of the various populations, and a low value is thus expected to reveal high occupancy frequency among populations resulting from either a low transposition rate or a recent invasion so that populations had not enough time to differentiate. We preferred to calculate this heterozygosity level instead of element frequency per site because the mixing of various different population samples made an average frequency meaningless. With a computer we analysed the expected insertion profiles of the individuals obtained from theoretically crossing the isofemale lines from the various populations. We then calculated the number of labelling sites (NS) and the number of heterozygous sites (NH) in these flies (Biémont & Gautier, 1988; Biémont, 1995). The proportion of heterozygous sites (NH/NS) is thus an estimate of the degree of heterogeneity in insertion profiles between the populations. We found a value of degree of heterogeneity equal to 0·95, which is very close to values already reported in *D. melanogaster* for various elements (Biémont & Gautier, 1988; Biémont *et al.* 1994), clearly indicating that our *D. simulans* populations bore different insertions. Low transposition rate is thus not the main factor determining the low copy number of the 412 element in *D. simulans*, in agreement with the lower proportion of insertions on the X, which was not expected under the low transposition rate hypothesis.

4. Discussion

The very low copy number of mdg1 and the fixed sites of copia globally contributed to the low amount of transposable elements seen in *D. simulans* (Dowsett & Young, 1982; Vaury *et al.* 1989; Csink & McDonald, 1990; Martin *et al.* 1983; Leibovitch *et al.* 1992; Nuzhdin, 1995). The polymorphism of insertions observed for 412, as well as gypsy, furnishes a strong argument against any explanation of this overall low element copy number in *D. simulans* based on global inactivity of TEs, which was suggested by the presence of fixed sites of copia and the known absence of copia-homologous transcripts within the *D. simulans* genome (Csink & McDonald, 1990). An overall lower transposition rate in *D. simulans*, although there is no direct support of such a hypothesis in the literature (Eeken *et al.* 1987; Inoue & Yamamoto, 1987), could explain a lower copy number of TEs; it cannot, however, account for the lower ratio of insertions on the X over the autosomes, and the high value of heterozygosity level at site location between populations (a measure of degree of heterogeneity) is not in favour of this hypothesis either. Moreover, *D. melanogaster* and *D. simulans* exhibited similar gypsy mean insertion site numbers, suggesting that the regulation in site number depends also on the TE considered and not only on a global characteristic of the genome.

The observation of a lower insertion site number of 412 in *D. simulans* in comparison with the value observed in *D. melanogaster* thus apparently agrees with the hypothesis of a larger species effective size of *D. simulans* as proposed by Aquadro *et al.* (1988) and Aquadro (1992). This difference in mean insertion site numbers could indeed theoretically result from a loss of those insertion sites with low selection coefficient that at larger population size were effectively selected against and thus dramatically reduced in frequency (Charlesworth & Langley, 1991). Moreover, if selection acted against the mutational effects of insertions, then the number of 412 insertions on the X chromosome is theoretically expected to be less than the number of insertions on the autosomes (the expected proportion on the X is 0·13) (Montgomery *et al.* 1987; Langley *et al.* 1988). The proportions of 412 insertions on the X chromosomes in *D. simulans* and *D. melanogaster* are compatible with this hypothesis, and reinforce the conclusion of Aquadro *et al.* (1992) that insertions are selected against in the *Drosophila* genome. However, the lower proportion of insertions in the X chromosome of *D. simulans* in comparison with *D. melanogaster* may in fact reveal some specific characteristics of the *D. simulans* genome. This may concern either the intensity of selection acting against insertion sites, the proportion of heterochromatin versus euchromatin, or the average value of dominance effect (h).

A smaller h value in *D. simulans*, and therefore a stronger recessivity of mutations or a longer persistence of recessive alleles in populations (Charlesworth *et al.* 1992*b*) in this species, could indeed explain the low proportion of TE copies in the X chromosome. A global increase in TE copy number is, however, expected in the *D. simulans* genome if h is small, which is not observed, the number of TE copies being about 3 times less in *D. simulans* than in *D. melanogaster* (Dowsett & Young, 1982; Leibovitch *et al.* 1992; Nuzhdin, 1995). There is, however, no need to involve a population size effect, in agreement with the suggestion from a theoretical study which indicates that an effective population size of less than 100 is required for purifying selection against TE insertions to be significantly weaker (Charlesworth & Charlesworth, 1983). Since populations of *D. melanogaster* do not reveal such a small effective size, other mechanisms such as stronger selection should account for the overall lower TE insertion site number of *D. simulans*. The proportions of 412, copia and mdg1 element insertions on X chromosomes of *D. melanogaster* populations, which do not contradict the theoretical value of 0·13, strongly reinforce the validity of the negative selection hypothesis. The discrepancies

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in proportions of TE insertion sites on X chromosomes sometimes reported when different populations or TEs were compared (Langley *et al.* 1988; Charlesworth *et al.* 1992*a*, *b*; Biémont, 1993; Biémont *et al.* 1994; Aulard *et al.* 1995) might thus only reflect specific characteristics of the population under study (Vieira & Biémont, 1996). Indeed, for an effect of selection to be detected on the X chromosome, the host population might have been submitted to selection for a long time, especially if selection coefficients were low. The lack of a difference in TE distribution between the X chromosome and the autosomes should thus be not considered to reflect only absence of selection but instead recent movements in the genome of the element under study, whatever their causes, with equal probability of partition of the insertions over the chromosome arms (Vieira & Biémont, 1996). As reported here, negative selection shows up unambiguously when the proportions of TE insertions on X chromosomes are averaged over various populations. This is an important point, because the fact that a higher relative abundance of elements on autosomes than on X chromosomes is not always observed (Montgomery *et al.* 1987; Charlesworth *et al.* 1992*a*, *b*) is generally used as an argument opposing the model of selection against insertional mutations (see Charlesworth 1991 for a discussion), thus favouring the alternative model of a dominant deleterious effect of chromosomal rearrangements due to recombinational events between TE insertions (Langley *et al.* 1988; Charlesworth, 1991). Another argument against the negative selection hypothesis comes from theoretical assumptions which postulate that the value of the selection coefficient against insertions should be close to the transposition rate. The selection coefficient is, however, estimated as 0·01–0·02 (Charlesworth, 1991) and the transposition rate as 10^{-4} (Charlesworth & Lapid, 1989; Charlesworth *et al.* 1992*b*; Suh *et al.* 1995), suggesting that selection is too strong to adequately maintain TE copy number. Note, however, that the estimated selection coefficient comes from data on lethal and deleterious viability genes in natural populations (see Charlesworth, 1991) and not from those genes with a TE inserted, and that we do not yet have a direct estimation of transposition rate in natural populations.

Our results favour the idea that selection against the detrimental effect of TE insertions is a major force explaining copia, mdg1 and 412 copy number containment in the *Drosophila* genome of natural populations, and that this purifying selection is stronger in *D. simulans* than in *D. melanogaster*, at least for the 412 element. The reasons for this selective difference remain to be understood to explain why *D. melanogaster* has been invaded by transposable elements while the *D. simulans* genome maintains them mostly in heterochromatin or fixed euchromatic sites.

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