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High copy numbers of multiple transposable element families in an Australian population of *Drosophila simulans*

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Summary

Sudden mobilization of transposable elements in *Drosophila* is a well-reported phenomenon but one that usually affects no more than a few elements (one to four). We report here the existence of a *D. simulans* natural population (Canberra) from Australia, which had high copy numbers for various transposable elements (transposons, LTR retrotransposons and non-LTR retrotransposons). The impact of transposable elements on the host genome and populations is discussed.

It is increasingly evident that transposable elements (TEs), which are major components of the genome of many organisms, have played an important role in evolution and can promote new genetic variation. Their rate of movement is thus of great importance for population adaptation. Sudden mobilizations of TEs have been reported in *Drosophila* but they concern either one or a few elements (one to four), or involve specific crosses such as those leading to hybrid dysgenesis (for a review see Biémont; Pasyukova & Nuzdhin, 1993; Petrov *et al.*, 1995; Desset *et al.*, 1999). Few data are available for natural populations. A previous analysis of natural populations of *D. simulans* (Vieira & Biémont, 1996) reported a population from Canberra (Australia) with a very high copy number for the retrotransposable element *412*. We have recently reported data on 36 TEs analysed in various natural populations of *D. simulans* and *D. melanogaster* (Vieira *et al.*, 1999). We worked on fly samples collected from several, geographically distinct, natural populations from Australia (Canberra, Cann River, Eden), France (Valence), Kenya (Makindu), Madagascar, New Caledonia (Amieu), Polynesia (Noumea, Papeete), Portugal (Madeira), Réunion Island, Russia (Moscow) and Zimbabwe. TE copy numbers were determined by *in situ* hybridization to polytene chromosomes from salivary glands of larvae from two isofemale lines per population. From these data, to which the *nomade* and *mariner* element copy numbers were added, we show here that the Canberra population had in fact the highest copy numbers for six TEs out of 26 that showed polymorphism.

As is seen in Table 1, the Canberra population appeared clearly distinct from the other populations. It showed not only a high copy number for TEs such as *412*, *opus* and *I*, but also the presence of many copies of other TEs such as *F*, *nomade* and *mariner*, which were present in zero or one copies in the other populations. The Canberra population also had high copy numbers for *roo*/*B104* and *doc*, although the population's values for these elements were not the highest of all populations.

To test the statistical significance of the high TE copy numbers in the Canberra population we used a randomization procedure. For each TE, the populations were rank-ordered. The populations were then permuted for each TE in each simulation, and the sum of the ranks of all TEs was calculated for each population. We thus compared the observed rank value of the Canberra population with the distribution of the highest rank obtained from 10 000 permutations. We found that the rank value of the Canberra population or higher had a probability of 0·04. Thus, the Canberra population showed high copy numbers for various TEs (transposons, LTR retrotransposons and non-LTR retrotransposons).

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Table 1. *Euchromatic insertion site number per genome of 26 transposable elements in Canberra (averaged over two isofemale lines) compared with the other* D. simulans *populations*

	Canberra	Other populations	
Elements	Site no.	Average site no.	Standard error
LTR retrotransposons			
1731	1·5	0·96	0·98
297	1·0	1·00	0·88
412	59·5	10·10	6·4
BEL	0	0·63	0·7
blood	1·0	2·63	2·8
burdock	3·5	5·42	3·4
copia	4·0	3·90	1·2
coral	3·0	1·79	1·7
flea	1·5	3·58	3·6
gypsy	3·0	1·42	1·3
HMS beagle	2·5	2·79	3·2
mdg3	4·5	3·25	1·8
nomade	7·5	1·43	1·8
opus	8·5	4·50	2·0
prygun	0·5	0·83	1·9
roo/B104	47·5	37·7	6·9
stalker	0	0·42	0·7
tirant	1·0	1·67	1·2
Non-LTR retrotransposons			
doc	19·0	13·4	5·3
F	10·5	1·0	2·8
helena	10·0	10·3	2·2
I	18·5	12·1	3·4
jockey	1·0	3·46	1·6
Transposons			
bari-1	3·5	5·0	2·8
hobo	63·5	66·5	14·6
mariner	7·5	1·0	1·1

Since the *in situ* technique did not allow us to distinguish homozygous from heterozygous sites, the site number estimated directly by analysing larvae from isofemale lines was sensitive to the degree of homozygosity of the flies. Indeed, because the polytene chromosomes are composed of the two parental homologous chromosomal sets, the number of insertion sites detectable in diploids by *in situ* hybridization decreases with increasing homozygosity. Hence a higher level of inbreeding, and therefore of genome homozygosity, in most of the isofemale lines, but not in Canberra, could have led to an overestimation of the copy numbers in this line. To test this hypothesis, we crossed five males and five females from the Canberra populations with females and males, respectively, from an inbred line (Madagascar), which had five homozygous insertion sites for the *412* element. The copy number of the haploid genome was thus obtained by subtracting the site number of the control inbred line from that of the hybrid larvae. We found an average of 53 insertion sites in the haploid genome and 59 in the diploid genome of flies from the Canberra population, which suggests that the Canberra sample was highly homozygous. Thus the high TE values observed for the Canberra population could not result from a higher degree of heterozygosity in this population in comparison with the other population samples. A higher heterozygosity could in any case not have explained the high copy number observed for the elements *F*, *nomade* and *mariner* in the Canberra population, in contrast to only zero or one copies of these elements in the other populations. Since we do not know whether a mobilization of TEs in the Canberra population happened in the wild or in the laboratory, we cannot exclude the possibility that drift, associated with weak purifying selection against TE insertions in a population of dramatically reduced size (Charlesworth & Charlesworth, 1983), or inbreeding were responsible for the accumulation of copies, as suggested in *D. melanogaster* (Nuzhdin *et al*., 1997). Drift may thus explain the fact that not all TE families responded in the same way. Higher transposition rate or the relaxed force of natural selection opposing TE multiplication in inbred lines could be responsible for such a TE accumulation, although it has been shown that the transposition rate of the *412* element is similar in the Canberra population to that in other populations (Vieira & Biémont, 1997), and that inbreeding has no effect on transposition (Labrador *et al*., 1999). However, it is not clear why only the Canberra population, and only some of its TEs, should have been submitted to such inbreeding-related mobilization, but not the other populations nor all the TEs.

These results prompt fascinating questions on the dynamics of genomes, which may become full of TEs. The impact of such a process could be major, in that crossing between natural populations could lead to an overall invasion by many TEs into the genome of the entire species, as was observed for the *P*, *I* and *hobo* TEs (Bucheton *et al*., 1984; Anxolabéhère *et al*., 1988; Periquet *et al*., 1994). We now possess a very useful line for the analysis of the conditions leading to genomic TE invasion.

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