A temperature cline in copy number for 412 but not roo/B104 retrotransposons in populations of *Drosophila simulans*

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The copy number of the retrotransposable element 412 of *Drosophila simulans* from populations collected worldwide shows a negative correlation with minimum temperature. No association was detected for the roo/B104 element. The possibility that selective pressures might regulate the 412 copy number in these natural populations is supported by detection of selection against the detrimental effects of 412 insertions (estimated by the proportion of insertions on the X chromosome in comparison with the autosomes) but not roo/B104. These data reveal different spatial patterns for two element families, and strongly suggest that some factors in the environment, such as temperature, may interfere with the control of retrotransposition, thus affecting important aspects of genomic evolution.

Keywords: roo/B104; 412; retrotransposable elements; temperature cline; *Drosophila simulans*

1. INTRODUCTION

Transposable elements (TEs) are of major importance in maintaining and reshaping the genetic variability of populations. The way in which they respond to environmental factors thus determines their impact on the potential of natural populations to adapt to new environments. However, induction of TE transpositions by environmental stresses has been demonstrated only in a few cases. For example, thermal stress amplifies transposition and transcription of TEs in tobacco-cultured cells (Hirochika *et al.* 1996), whereas in *Saccharomyces cerevisiae* and *Candida albicans*, heat shocks stimulate transcription of TEs and other genes (Chen & Fonzi 1992; Kang *et al.* 1992) and increase transposition in *S. cerevisiae* (Paquin & Williamson 1984). In *Drosophila*, heat shocks amplify the amount of transcripts of the copia element (Strand & McDonald 1985), but without any association with copy number (Csink & McDonald 1990). Although some interpretations argue in favour of mobilization of copia-like elements after heat shocks (Junakovic *et al.* 1986; Ratner *et al.* 1992), other results show no effect under similar stresses (Arnault & Dufournel 1994; Arnault *et al.* 1997). The existence of a relationship between environmental factors and mobilization of TEs in *Drosophila* is thus an open question. Direct relationships between environmental conditions and TE copy number have not been reported in natural populations, although the somatic excision rate of the mariner element increases with the latitude of origin of populations of *Drosophila simulans* (Giraud & Capy 1996) and is related to temperature (Garza *et al.* 1991); the 412 element follows a gradient in copy number in natural populations of *D. simulans* between the Southern and Northern Hemispheres (Vieira & Biémont 1996a). In

this paper, we determine the copy number of retrotransposable element roo/B104 in natural populations of *D. simulans* and we analyse previous data on 412 by relating them to environmental conditions. We show that the variation in copy number between geographically distant populations is not associated with environmental factors for roo/B104, in contrast with the retrotransposable element 412 for which a negative correlation with minimum temperature is observed. Moreover, selection against the detrimental effects of transposable element insertions is not involved in the regulation of roo/B104 copy number, in opposition to the 412 element. The different behaviour of the two element families reveals different influences of the environment on retrotransposon regulation and thus on genomic evolution.

2. MATERIAL AND METHODS

(a) Natural populations

We considered 50 samples of *D. simulans* populations collected in Europe, Africa, the Middle East and America previously analysed for their copy number of the 412 element (see Vieira & Biémont (1996a) for the map of sampling localities). Recently caught wild populations of *D. simulans* were maintained in the laboratory as isofemale lines, whereas older populations were maintained in small mass cultures. One female larva from each of two isofemale lines per population, or two female larvae from each mass-mated stock, were analysed for the roo/B104 copy number.

(b) In situ hybridization

Polytene chromosomes from salivary glands of third-instar female larvae were prepared and treated with a nick-translated, biotinylated roo/B104 DNA probe (Scherer *et al.* 1982), as previously described (Biémont 1994). Insertion sites were visualized as brown bands resulting from a dye-coupled reaction

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with peroxidase substrate and diaminobenzidine. The insertion site numbers of the 412 and roo/B104 elements were determined on all chromosome arms (X, 2L, 2R, 3L, 3R) and were summed to give the total number of labelled sites per diploid genomes. We did not take into account the insertions localized in the centromeric regions 20, 40, 41, 80 and 81, because TE site number estimations in these regions are difficult and not reliable for all chromosomes. Because the *in situ* technique does not allow distinction of homozygous from heterozygous sites, any increase in homozygosity level in the populations sampled, resulting from inbreeding during laboratory culture, may lead to an underestimation of total insertion sites in the diploid genomes. However, it has been shown previously for the 412 element that the use of diploids gives reliable data (Vieira & Biémont 1996a,b).

(c) *Geographical and climatic records*

As environmental variables, we considered latitude, longitude, minimum, maximum and average temperatures, total duration of sunshine in a year, vapour pressure (in millibars), minimum, maximum and average relative humidity, amount of precipitation (in millimetres), and number of days with amount of precipitation equal to or greater than 1.0 mm.

(d) *Statistical analysis*

Because we are dealing with spatially autocorrelated data (i.e. values of close localities are more similar than values from more distant localities), the assumption that the variables are independently and identically distributed is not met. Thus, classical parametric statistical tests cannot be used, as for example the ANOVA associated with the multiple regression model. This ANOVA can be performed but the *p*-values classically associated with it are not reliable. Mantel tests of association between similarity matrices (Mantel 1967), which take spatial autocorrelation into account, were thus applied to establish the relationships between the copy numbers of 412 and roo/B104 and latitude, longitude and environmental characteristics, between pairs of populations. Because two larvae were analysed per population for both 412 and roo/B104, we considered all 100 larvae in the calculation. For each variable, a 100 × 100 matrix of pairwise similarities (the measure of resemblance between two values of this variable) was calculated. For each pair of variables, *x* and *y*, the matrices were multiplied element by element and the sum of the product, $\sum_{xy}$, was tested by comparison with the null distribution. This reference distribution was determined by a randomization procedure in which rows and columns of one matrix were permuted, while keeping the other matrix constant, and the $\sum_{xy}$ value calculated for each permutation. Fifty-thousand permutations were done to obtain precise *p*-values (Jackson & Somers 1989). The observed $\sum_{xy}$ values were then converted into a correlation coefficient, r_{xy} , between matrices (Smouse *et al.* 1986). Note that this r_{xy} value is quite different from the classical Pearson correlation coefficient, *r*, in that r_{xy} is in practice much lower than *r* and presents a different null distribution which, at least with our data, is very skewed. Hence only the *p*-values associated with the r_{xy} coefficient are directly interpretable. To determine the influence of latitude in the association between 412 copy number and climatic variables, we calculated partial Mantel tests (Smouse *et al.* 1986) in which the rows and columns of the similarity matrix of the 412 copy number (the dependent variable) were permuted while the similarity matrices of the explicative variables, latitude and the climatic variables, were held constant. This last test took into account the spatial autocorrelation of climatic variables according to latitude.

3. RESULTS AND DISCUSSION

Like the 412 element (Vieira & Biémont 1996a), the roo/B104 element appeared to be represented by a relatively high mean copy number in *D. simulans*. However, while 412 varied in copy number from 1 to 23, following a gradient from the Southern to the Northern Hemisphere, the copy number of roo/B104 varied from 22 (in a sample from the Seychelles) to 55 (in a sample from the Canary Islands). A population from Seattle (USA) showed an extreme value of roo/B104 copy number (94 insertions), which was much higher than the other populations and so was excluded from the analysis. Although two individuals per population was a small sample size, an analysis of more larvae in some populations (C. Vieira, unpublished results) shows that low and high values of insertion site numbers did actually characterize the populations and were not the result of chance only, with the values coming from the left or right tail of a large distribution. We thus investigated whether the variation in 412 and roo/B104 copy numbers was associated with latitude or longitude, using the Mantel test of matrix association (Mantel 1967). The 412 copy number was found to be significantly associated with latitude (50 populations, $p=0.00002$, figure 1; and see Vieira & Biémont (1996a) for the map of the sampling localities), whereas no significant relationships were found with the number of copies of the roo/B104 element (50 populations, $p=0.035$ for latitude, figure 2). The latitudinal cline for 412 copy number strongly suggests an association of specific environmental conditions with copy number regulation. From data gathered by the World Meteorological Organisation (World Meteorological Organisation 1982), we extracted various climatological variables, averaged over 30 years, for the regions where our populations live. From these variables, the minimum temperature and vapour pressure were found to be significantly associated with the 412 copy number ($p=0.00002$ for temperature, figure 1; $p=0.005$ for vapour pressure). To test a possible influence of latitude on the correlation we used a partial Mantel test (Smouse *et al.* 1986). This test showed that the 412 copy number was significantly associated with minimal temperature independently of latitude ($p=0.002$), and this association remained even when three populations with extremely low temperature values (see figure 1) were removed from the calculation ($p=0.02$). Such data removal is justified by the fact that the Mantel correlation is very sensitive to extreme values. The association between 412 copy number and vapour pressure was no longer significant when the effect of latitude was taken into account ($p=0.13$), suggesting that this association was only due to the correlation of vapour pressure with latitude. This last result shows the power of the partial Mantel test in taking into account the effect of indirect correlations, thus increasing our confidence in the above significant association found between the 412 copy number and minimum temperature.

Because the 412 element is sensitive to environmental factors, either directly or indirectly, it could be argued that selective pressures might regulate its copy number in natural populations. One source of information concerning the effect of selection on the containment of TE copy number is the relative abundance of elements on the X chromosome and on the major autosomes. The

Figure 1. Plots of copy number per diploid genome of the retrotransposable element 412 versus latitude and minimum temperature ($^{\circ}\text{C}$). Each locality was represented by two values.

Figure 2. Plots of copy number per diploid genome of the retrotransposable element roo/B104 versus latitude. Each locality was represented by two values.

selection hypothesis indeed 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. Under a model of selection acting against deleterious insertions, a proportion of TE insertions on the X chromosome of 0.13 is predicted (Charlesworth & Langley 1991; Montgomery *et al.* 1987; Langley *et al.* 1988). Under two other models of selection based on deleterious chromosome rearrangements due to ectopic exchanges between the repetitive sequences, a proportion of insertions on the X chromosome of 0.16 is predicted if all inserted elements can recombine with any element located elsewhere in the genome, or 0.18 if elements recombine only with other elements located in the same chromosomal region

Figure 3. Distributions of the proportion of insertion sites on the X chromosomes in female larvae for the 412 (black bars) and roo/B104 (white bars) retrotransposable elements in natural populations of *D. simulans*.

(Langley *et al.* 1988). A frequency of elements on the X chromosome of 0.20 is expected if insertions are neutral (Montgomery *et al.* 1987; Langley *et al.* 1988). The observation of this 0.20 value does not exclude other explanations based on TE copy number regulation not affecting the proportion of insertions on the X chromosome. It has been shown previously that the 412 element follows a model of selection, with an average proportion of insertions on the X chromosome being not different from 0.13 (Vieira & Biémont 1996a,b). For roo/B104, the proportion of insertions on the X chromosome determined in this study was found to be 0.20 ± 0.01 (see figure 3), a value that implies either absence of selection against the detrimental effects of the roo/B104 insertions or a recent invasion of roo/B104 in *D. simulans*. The two distributions of the proportion of insertions on the X chromosomes for both elements are presented in figure 3. Note that the variance of the proportion of insertions on the X chromosome is in great part due to the existence of populations with a very low insertion site number, and this effect is particularly evident for the 412 element. These results are compatible with natural selection acting against insertional mutations of the 412 element, but not of roo/B104. This may be related to the action of temperature as the environmental factor, and thus suggests a differential effect of the environment on the regulation of TEs. Selection against 412 insertions, but not roo/B104, has been reported in other experiments carried out with *Drosophila melanogaster* (Montgomery *et al.* 1987; Langley *et al.* 1988; Charlesworth *et al.* 1992a,b; Aulard *et al.* 1995) and *D. simulans* (Vieira & Biémont 1996b). From our data, the number of 412 insertions was too small in many populations for us to analyse statistical variation with minimal temperature in the proportion of insertions on the X chromosomes.

Because the TEs located in heterochromatin may contribute by transposition to the pool of elements in euchromatin (Montgomery *et al.* 1987), an alternative hypothesis to explain the different behaviours of the two retrotransposons is to suppose the existence of either a high concentration of 412 in the heterochromatin and/or a high rate of transposition of heterochromatic 412 elements relative to euchromatic ones (Montgomery *et al.* 1987). The first hypothesis is rejected by the observation that the roo/B104 element is proportionally much more abundant in heterochromatin than the 412 element (Charlesworth *et al.* 1994) and so should be less frequent

in the X chromosome than 412 under this hypothesis, which was not observed. Regarding the second hypothesis, we have little information concerning the transposition rate of heterochromatic elements. However, because heterochromatin is largely transcriptionally silent and because transcription is a limiting step for retrotransposition, it seems unlikely that the heterochromatic elements are transposing at a higher rate than the already high value of 10^{-3} observed for euchromatic copies of 412 in natural populations of *D. simulans* (Vieira & Biémont 1997). Hence, the difference in behaviour between 412 and roo/BI04 must depend on specific characteristics of these elements.

It is known that 412 and roo/BI04 present very different expression patterns during development in *D. melanogaster*. Indeed, the 412 class of elements, which includes mdgl and 17.6, are mainly transcribed in early larval and pupal stages (Parkhurst & Corces 1987), and in the visceral mesoderm and the gonads of the embryos (Brookman *et al.* 1992; Ding & Lipshitz 1994). Roo/BI04 is almost only transcribed in embryos (Parkhurst & Corces 1987), especially in the somatic mesoderm and in yolk nuclei of early embryos (Ding & Lipshitz 1994). Moreover, the expression of 412 is proposed to be a direct consequence of transcriptional activation by the UBX transcription factor (Ding & Lipshitz 1994), and this expression can be decreased by 20-OH ecdysone applied to cultured *D. melanogaster* Kc cells (Micard *et al.* 1988; Becker *et al.* 1991). The 412 and roo/BI04 elements thus seem to have very different regulation mechanisms.

A possible action of the environment in the regulation of TEs in natural populations can be supported by several observations: a significant intraspecific variation in copia element transcript level among natural populations of *D. melanogaster* (Csink & McDonald 1990), and an effect of temperature on germinal or somatic excision rates of the mariner element in *D. melanogaster* (Garza *et al.* 1991; Giraud & Capy 1996). This variability has been attributed to trans-acting regulatory factors that could depend on environmental conditions. Hence, trans-acting regulatory factors depending on temperature may regulate 412 copy number, but should have no effect on roo/BI04. Of course, we cannot exclude an indirect effect due to differing regulation of TE copy number by host genes (Corces & Geyer 1991; Bhadra *et al.* 1997) whose frequencies vary with environmental conditions (Costa *et al.* 1992; Oakeshott *et al.* 1992).

The regulation of TE copy number, which differs among natural populations, could contribute to future geographical differentiation of the populations of *D. simulans* following the worldwide invasion of this species, and may have strong evolutionary implications (Arkhipova & Ilyin 1992). The exact influence of the environment on such phenomena, and the impact of new genetic variability due to TEs on the adaptive characteristics of the flies in natural populations are, however, still unknown.

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