

Copy number of *P* elements, *KP*/full-sized *P* element ratio and their relationships with environmental factors in Brazilian *Drosophila melanogaster* populations

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The *P* transposable element copy numbers and the *KP*/full-sized *P* element ratios were determined in eight Brazilian strains of *Drosophila melanogaster*. Strains from tropical regions showed lower overall *P* element copy numbers than did strains from temperate regions. Variable numbers of full-sized and defective elements were detected, but the full-sized *P* and *KP* elements were the predominant classes of elements in all strains. The full-sized *P* and *KP* element ratios were calculated and compared with latitude. The northernmost and southernmost Brazilian strains showed fewer full-sized elements than *KP* elements per genome,

and the strains from less extreme latitudes had many more full-sized *P* than *KP* elements. However, no clinal variation was observed. Strains from different localities, previously classified as having *P* cytotype, displayed a higher or a lower proportion of *KP* elements than of full-sized *P* elements, as well as an equal number of the two element types, showing that the same phenotype may be produced by different underlying genomic components of the *P*–*M* system.

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Introduction

After entering the *Drosophila melanogaster* genome by horizontal transfer from *D. willistoni* (Anxolabéhère *et al.*, 1988; Daniels *et al.*, 1990), *P* elements have spread vertically worldwide and evolved into a heterogeneous family including the ancestral sequence and a collection of internally deleted elements. *P* elements have been classified into two structural types: type I, which includes the full-sized 2.9 kb *P* element and elements with small deletions at the 3' end, and type II, which have much larger deletions. While the full-sized *P* element is able to encode a single 87 kDa polypeptide that is the functional transposase (Rio *et al.*, 1986), the defective ones cannot produce their own transposase, but they can be mobilized if this enzyme is supplied in *trans*.

P element transposition occurs in germline cells of the progeny of a cross between males of a strain that contains *P* elements (*P*) and females devoid of *P* elements (*M*). The progeny of this cross displays the *P*–*M* hybrid dysgenesis syndrome (Kidwell *et al.*, 1977). Since dysgenesis does not occur in the reciprocal cross, the repression of *P* element mobilization is thought to be maternally inherited through a cytoplasmic condition not permissive for *P* element transposition, called the *P* cytotype. *M* cytotype, which is found in strains lacking *P*

elements, allows *P* element transposition (Engels, 1983). According to the ability to induce (*P* activity potential) or to repress (*P* susceptibility) hybrid dysgenesis, strains of *D. melanogaster* are classified into *P*, *Q*, *M'* and *M* phenotypes. *P* strains exhibit low to high levels of *P* activity potential and low levels of *P* susceptibility, while *Q* strains show extremely low levels of both *P* activity and *P* susceptibility. Both types have been shown to possess 30–50 copies of *P* elements per genome, of which about a third are the 2.9 kb elements and the rest are deleted derivatives (Bingham *et al.*, 1982; Black *et al.*, 1987; O'Hare *et al.*, 1992). *M* and *M'* strains do not exhibit any significant level of *P* activity. *M* strains are devoid of any *P* elements and show extremely high *P* susceptibility (Bingham *et al.*, 1982). On the other hand, *M'* strains vary from extremely high to moderately low *P* susceptibility (Anxolabéhère *et al.*, 1985) and possess from a few to 50 copies per genome (Anxolabéhère *et al.*, 1984; Black *et al.*, 1987), most of which, if not all, are defective sequences (Bingham *et al.*, 1982; Black *et al.*, 1987). Among them, the most frequently deleted derivative is *KP*, a type II element (deleted at positions 808–2560) that encodes a 207 aa protein that has been considered as an important repressor of transposition (Lee *et al.*, 1996; Simmons *et al.*, 2002).

D. melanogaster populations show different ratios of full-sized and defective *P* elements, and their frequencies and *P*–*M* phenotypes vary worldwide. According to Anxolabéhère *et al.* (1989), North American populations are virtually all *P* and *Q*, and most of them possess many full-sized *P* elements and a mix of deleted elements. On the other hand, in North Africa, Europe and Asia,

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populations are mostly *M'*. *P* and *Q* populations are found only at the Eurasian coastline and are also found in Central Africa. European populations possess fewer full-sized *P* elements and their frequencies gradually decrease from France to Central Asia where populations have many copies of *KP* elements (Black *et al*, 1987). In eastern China, Japan, Australia and Africa, both full-sized and *KP* elements are the predominant classes (Todo *et al*, 1984; Anxolabéhère *et al*, 1985, 1988, 1990; Sakoyama *et al*, 1985; Black *et al*, 1987; Boussy *et al*, 1998; Itoh *et al*, 1999, 2001; Itoh and Boussy, 2002). These data show that full-sized *P* and *KP* elements are the predominant classes in all populations, excepting those from the Americas, which seem to possess many other defective elements. O'Hare *et al* (1992), for example, found seven full-sized *P* and two *KP* elements, and 19 other defective elements in a single strain. Since the available information about the South American populations seems to be restricted to just one population from Peru (Black *et al*, 1987), in this study we determined the overall copy number and the ratio of full-sized *P* and *KP* elements, aiming for a better understanding of the geographical differentiation of *D. melanogaster* populations in Brazil. In addition, we addressed the question whether the environmental factors, namely latitude or temperature, exert any influence on *P* element copy numbers and *KP*/full-sized *P* element ratios.

Material and methods

Strains

D. melanogaster isofemale lines from eight distant Brazilian regions were set up from collections made in 1996–2000, and were maintained in laboratory as highly inbred small cultures since then. The strains had already been classified according to the *P*–*M* phenotype by Vetorazzi *et al* (1999) as moderate *P* (MA, from São Luis-MA and PI, from Teresina-PI; MG, from Santana do Riacho-MG and SP, from São José do Rio Preto-SP), *Q* (PR, from Maringá-PR and RS₂, from Porto Alegre-RS) and *M'* (SC, from Joinville-SC). The strain RS₁ (from Santa Maria-RS) had not been classified according to the *P*–*M* system. The strain Harwich-w (strong *P*) was used as a control. Figure 1 indicates the localities from which the strains were collected.

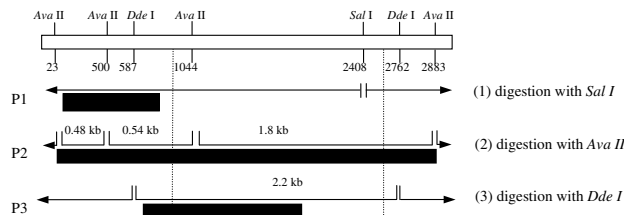
Southern blot analysis

Genomic DNA was isolated from pools of about 50 individuals. Approximately 5 µg of genomic DNA of each strain was digested with the *SalI*, *AvaII* and *DdeI* restriction endonucleases, aiming to investigate the overall number of *P* element copies, and the proportion of full-sized *P* and *KP* elements. The blots were hybridized with the ECL hybridization buffer (Amersham Pharmacia Biotech do Brasil™) and the appropriate probes. DNA digested with *St*all was probed with a 764 bp 5'-end sequence of *D. melanogaster P* element amplified from the pπ25.1 plasmid (Houck *et al*, 1991). DNA digested with *AvaII* was probed with a 2.9 kb sequence amplified from pπ25.1 (Haring *et al*, 1995), and DNA digested with *DdeI* was probed with a 896 bp fragment obtained by *P* element digestion with *PvuII* (Figure 2). The DNA fragments were then electrophoresed, transferred to nylon membrane and fixed. The membranes



Figure 1 Map of Brazil with the localities on which the flies were collected and the respective latitudes. MA: São Luis, 2.31°Slat; PI: Teresina, PI, 5.09°Slat; MG: Santana do Riacho, MG, 18.20°Slat; SP: São José do Rio Preto, SP, 20.49°Slat; PR: Maringá, PR, 23.25°Slat; SC: Joinville, SC, 26.18°Slat; RS₁: Santa Maria, RS, 29.41°Slat and RS₂: Porto Alegre, RS, 30.02°Slat.

a Full-sized *P* element = 2.9 kb



b *KP* element = 1.15 kb

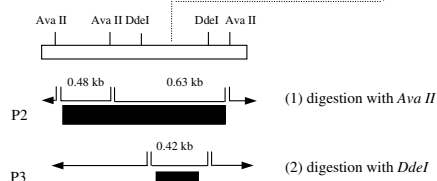


Figure 2 Maps of *P* elements indicating the restriction sites of the endonucleases used in the analyses, the restriction fragments produced by each one and the location where the used probes anneal: (a) full-sized *P* element and (b) *KP* element. Restriction sites (vertical lines), restriction fragments (broken horizontal lines) and probes (black boxes) are indicated. (a1) *SalI* restriction fragment and probe (P1: 764 bp 5'-end sequence of *D. melanogaster P* element) used to estimate *P* element copy number analysis. *AvaII* restriction fragments and probe (P2: 2.9 kb *P* element sequence contained pπ25.1 plasmid) used to identify both full-sized *P* element (a2) and *KP* element (b1). *DdeI* restriction fragments and probe (P3: *PvuII* restriction fragment of *P* element contained in pπ25.1 plasmid) used to identify *KP* element (a3 and b2).

were washed for 40 min in primary buffer containing urea (6 M urea, 0.4 SDS and 0.5 M × SSC), at 42°C, and for 10 min in secondary buffer (2 × SSC) at room temperature.

Relative proportion of full-sized *P* and *KP* elements

Scanning densitometry was performed by rendering the Southern blot autoradiographs as TIFF files, and the images were analysed with the public domain image analysis software NIH image ver. 1.60 (<http://rsb.info.nih.gov/ni-image>). The relative numbers of full-sized *P* and *KP* elements in each strain were estimated by calculating the ratio of the observed band densities for *KP* and full-sized *P* elements. For the statistical analysis, the *KP/P* element ratios were \log_{10} -transformed.

Statistical analysis

Linear regression analysis was performed to evaluate the influence of latitude on copy number and the *KP/P* element ratios of each strain, as well as the influence of the overall copy number on the *KP/P* element ratios. The influence of mean annual temperatures of the localities where the collections were made on copy number was also tested by Student's *t*-tests. For this analysis, the strains were grouped into two classes. The first class included the strains MA and PI, from populations of equatorial regions, with elevated mean temperature all year long (above 22°C and up to 40°C in the summer), and SP, from a population of a tropical region (mean annual temperatures above 22°C with lows rarely below 15°C in the winter). The second class included the southeastern strain MG and the southern strains PR, SC, RS₁ and RS₂. MG was from a mountainous region in southeastern Brazil, with moderate temperatures in the summer and an average temperature below 12°C in winter and autumn. The southern strains were from a subtropical region characterized by well-defined seasons with temperatures ranging from 2°C to 15°C in the winter and 25°C to 35°C in the summer.

Results

Figure 3a and Table 1 show that the total number of *P* elements varied among strains from nine (MA) to 19 (RS₁). Figure 3b shows genomic DNA digested with *Ava*II and probed with 2.9 kb *P* element sequence amplified from $p\pi 25.1$. The 1.8 and 0.48 kb bands present in all lines correspond to the expected sizes of an *Ava*II-digested 2.9 kb *P* element. The 0.54 kb band runs with the 0.48 kb band in agarose gels and cannot be easily distinguished. Since the intensity of each fragment corresponds to its amount in the genome, the variation in intensity observed in the 1.8 kb band can be used as an indication of variation in the number of full-sized *P* elements (smaller in MA, PI and RS₁ lines). The signal intensity of the 0.63 kb band, which represents the *KP* element, is very strong and, although visible in all strains, is stronger in MA, PI, SC, RS₁ and RS₂. The 0.48 kb band can be generated by the full-sized *P* element, the *KP* element and other defective elements with no deletion in the 22–500 nt region.

The *Dde*I digests, probed with a *Pvu*II fragment, are shown in Figure 3c. All strains showed the 2.2 and 0.42 kb bands expected from full-sized *P* and *KP* elements, respectively, with variable signal intensities. Besides the full-sized *P* and *KP* elements, other internally deleted elements occur, with a maximum of six in the PI strain. The proportion of full-sized elements per genome seems to increase from the South to about 5.09°S lat, but

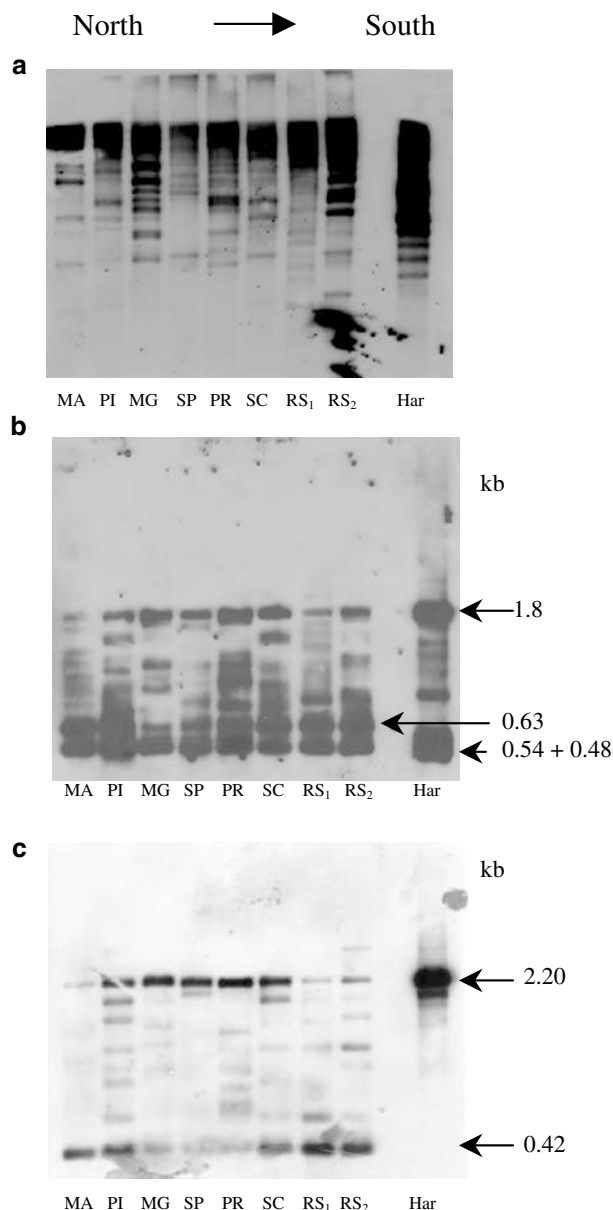


Figure 3 Southern blot analysis of genomic DNA of eight strains of *D. melanogaster* digested with (a) *Sall* and probed with a 764 bp 5'-end sequence of *D. melanogaster* *P* element; (b) *Ava*II and probed with a 2.9 kb sequence from complete *P* element; (c) *Dde*I and probed with a 896 bp fragment obtained from digestion of *P* element with the restriction endonuclease *Pvu*II. *Ava*II restriction fragments are indicated: 0.48 kb (produced by the full-sized *P* and *KP* elements), 0.54 kb (produced only by the full-sized *P* element), 0.63 kb (produced only by *KP* elements) and 1.8 kb (produced only by full-length *P* elements). *Dde*I restriction fragments are indicated: 2.2 kb (produced only by full-sized *P* elements), 0.42 kb (produced only by *KP* elements). Lines are arranged in each blot from left to right by latitude. Har: *D. melanogaster* Harwich.

this qualitative pattern does not extend to MA, the northernmost population. The highest proportion of *KP* elements per genome appears among both northernmost (2.32–5.09°S lat) and southernmost (29.41–30.02°S lat) populations.

The *KP*/full-sized *P* element ratios in the genomic DNA of a particular strain were evaluated by the ratio of

Table 1 P element copy number and KP/P element ratios in eight strains of *D. melanogaster* from Brazil

Strains	P element overall copy number	KP/full-size P ratios
MA	9	2.30
PI	12	1.04
MG	19	0.79
SP	11	0.56
PR	15	0.64
SC	13	1.00
RS ₁	16	2.15
RS ₂	15	1.14

the observed signal intensities of the expected fragment from KP (0.42 kb) and full-sized P (2.2 kb) elements. The values shown in Table 1, which reflect the signal intensities observed in Figure 3c, indicate that the strain SP possesses the highest proportion of full-sized elements (KP/P ratio = 0.56) and the strain MA the lowest (KP/P ratio = 2.30).

Figure 4 shows the scatter plot of the overall copy number against the log-transformed KP/P ratios (Figure 4a), and the scatter plots of the KP/P ratios and overall copy numbers plotted against latitude (Figure 4b,c). All the fitted regression lines have slopes that are not different from zero (log KP/P ratio = 0.283–0.0186 copies, $F_{1,6} = 0.44$, $P = 0.531$; log KP/P ratio = 0.112–0.00437 latitude, $F_{1,6} = 0.26$, $P = 0.625$; copy number = 10.4 + 0.172 latitude, $F_{1,6} = 2.95$, $P = 0.137$). Since the value of the copy number of the MG strain was above the 95% confidence interval, we re-evaluated the influence of latitude on the copy number of each strain excluding the value of MG (Figure 4d). In this new analysis, the fitted regression line had a significant slope (copy number = 9.45 + 0.182 latitude, $F_{1,5} = 10.11$, $P = 0.025$). In order to test the hypothesis that temperature was the underlying factor responsible for the above dependence, we grouped the strains into two classes: those from equatorial and tropical climates (Maranhão, Piauí, and São Paulo) and those from temperate climates (Minas Gerais mountain region, Santa Catarina and Rio Grande do Sul), as described in Material and methods. In agreement with the second regression analysis, the strains from the warmer climates exhibited a significantly lower number of P elements ($t_7 = 3.74$; $P = 0.013$).

Discussion

Copy number and environmental factors

P element copy numbers in *D. melanogaster* populations vary worldwide. The values reach 50 copies in North America (Ronsseray *et al.*, 1989), 30 in Western Europe (Anxolabéhère *et al.*, 1985) and only 12 copies in Russia

(Biémont *et al.*, 1990), suggesting a western–eastern gradual reduction of copy numbers. The overall copy numbers of P elements in Brazilian *D. melanogaster*

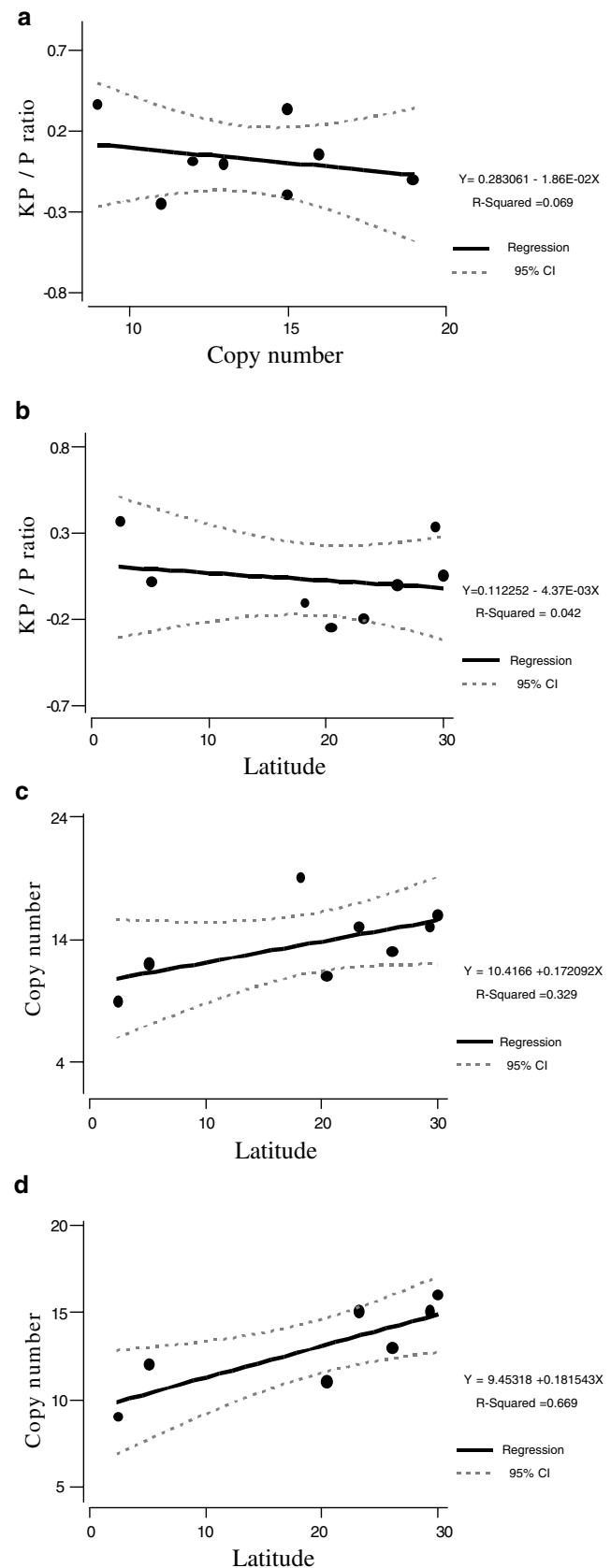


Figure 4 Regression analyses of the overall P element copy number against the ratio of KP/full-sized P elements per genome and both values against the latitudes of the localities where the *D. melanogaster* flies were collected. (a) KP/full-sized P log-transformed ratio against overall copy number; (b) KP/full-sized P against latitude; (c) overall P element copy number against latitude; (d) overall P element copy number against latitude, excluding the MG copy number and latitude values. The fitted linear regression lines are shown (solid lines) as well as the 95% confidence intervals (dotted lines).

populations varied from 9 to 19 (13.75 ± 3.15 copies), resembling more the Eurasian populations than the North American ones. The variation observed between the strains was lower than that expected, since the geographic variation in mean copy number as well as the Poisson variation between individuals within populations would contribute to a higher variation among the eight populations. However, since the strains have been maintained in laboratory in small mass cultures, their small population sizes could have produced high frequencies of transposable element sites even though the expected variance is Poisson, as predicted by Brookfield and Badge (1997).

Taken together, the regression analysis of the overall *P* element copy number on latitude, and the comparison of the *P* element copy number of strains from equatorial/tropical regions and subtropical regions, allow us to suggest that variation on the *P* element copy number is under the influence of the temperature of the original location of each population. Although indirectly evaluated, by grouping the strains according to the mean annual temperature of their original locations, the strains derived from populations of equatorial and tropical regions showed *P* element copy numbers significantly lower (nine in MA to 11 in SP) than those from temperate regions (15 in RS₂ to 19 in MG). The approach of grouping the strains was chosen because temperature is variable and well defined with highs and lows in the seasons of the southern regions, very different from the warmer temperatures almost all year long found in the southeastern or northern regions. Direct relationships between environmental conditions and transposable element copy number have not been reported in natural populations (Boussy *et al.*, 1988). However, indirect evidence suggesting a negative correlation between minimal environmental temperature and the 412 element copy number was found in *D. simulans* (Vieira and Biéumont, 1996; Vieira *et al.*, 1998). It could be argued that temperature can impose a selective pressure and might regulate the overall *P* element copy number in natural populations from regions with high temperatures throughout the year, since the frequency of genetic abnormalities brought about by the *P* element transposition rises dramatically in dysgenic flies reared above 25°C (Engels and Preston, 1979; Schaefer *et al.*, 1979). However, it cannot be ignored that copy number might also be regulated by host genes whose frequencies vary with environmental conditions (Corces and Geyer, 1991; Costa *et al.*, 1992; Oakeshott *et al.*, 1992; Bhadra *et al.*, 1997). Even the observed correlation might arise by chance if the nearby populations had similar copy numbers and the climate were also geographically clustered.

Copy number, ratios of full-sized *P* and *KP* elements and P–M phenotypes

Although the numbers of full-sized *P* and *KP* elements were not directly evaluated, their relative amounts were deduced from the variation in the band intensity of the 2.2 (full-sized *P*) and 0.42 kb (*KP*) fragments generated by *Dde*I. The full-sized *P* and the defective *KP* elements were the predominant types in all the Brazilian *D. melanogaster* strains. These two classes of elements occur with different proportions in different strains: three strains exhibited proportionally more full-sized *P* than

KP elements (MG, SP, PR), three others showed approximately the same proportion (PI, SC, RS₂) and the final two possess more *KP* than full-sized *P* elements (MA and RS₁, the northernmost and southernmost Brazilian strains, respectively). Other internally deleted elements also occur, but in lower numbers, as deduced from the signal intensity of the bands between those of 2.2 and 0.42 kb. The predominance of full-sized *P* plus *KP* elements in the genomes of most populations around the world has been pointed out by Itoh and collaborators (Itoh and Boussy, 2002; Itoh *et al.*, 2001), based on their own surveys or on other authors (Boussy *et al.*, 1988; Nitasaka and Yamazaki, 1994; Bonnivard and Higuier, 1999; French *et al.*, 1999; Itoh *et al.*, 2001; Itoh and Boussy, 2002). Itoh and Boussy (2002) wondered whether this could be the final equilibrium state of the *D. melanogaster* genomes in nature, and suggested that further surveys of wild populations are required. These authors recommended these surveys especially because such predominance was not predicted by any of the theoretical models that have been carried out to evaluate *P* element population dynamics (Brookfield, 1991, 1996; Quesenville and Anxolabéhère, 1997, 1998). Moreover, our study shows that the Brazilian populations seem to be more similar to the Eurasian than the North American ones. No matter what the relative proportions are, the full-sized *P* and *KP* elements seem to be predominant classes of elements in populations of *D. melanogaster* from very distant regions of Brazil (2.31–30.02°S lat).

The observation of hybrid dysgenesis in crosses between strains of *D. melanogaster* could mean that they have functional *P* elements. The ability of a strain to induce (*P* activity potential) or to repress (*P* susceptibility) transposition has often been assayed by gonadal dysgenesis (GD) tests, which are done by crossing unknown strains to the standard M (cross A) and P (cross A*) strains (Kidwell *et al.*, 1977; Engels and Preston, 1980). The molecular basis of the P–M system has been shown to be quite complex. Our data showed a lack of influence of the overall *P* element copy number or the relative proportion of the full-sized *P* and the *KP* elements (*KP/P* ratios) on the *P* activity potential or the *P* susceptibility (GD A and A* indices from Vitorazzi *et al.* (1999), respectively) of each strain (copy number = $23.9 - 15.8 \arcsin A - 22.7 \arcsin A^*$, $F_{2,4} = 1.68$, $P = 0.296$; $\log KP/P = -0.512 + 0.596 \arcsin A + 1.32 \arcsin A^*$, $F_{2,4} = 1.13$, $P = 0.409$, respectively). Disagreements among amounts and types of *P* element and P–M phenotypes have been found in literature. While Anxolabéhère *et al.* (1985) reported a correlation between P–M phenotypes and the overall *P* element numbers in Eurasian strains, Engels (1984), Todo *et al.* (1984) and Boussy *et al.* (1988) showed, in the American and the Australian lines, a positive correlation between the *P* activity and the number of full-sized *P* elements, but not with the overall number of *P* elements. The *P* activity was negatively correlated with the number of *KP* elements. However, such correlation was not observed in *D. melanogaster* samples from Japan and Taiwan (Itoh *et al.*, 2001). This absence of a clear relationship between the P–M phenotypes and the genomic *P* element composition seems to be common. The dependence of the repressing ability of a particular deleted derivative on its insertion site, such as the 1A on the X chromosome, might explain the inconsistencies in results aiming to demonstrate that

certain elements (such as *KP*) act as repressors (Black *et al*, 1987; Jackson *et al*, 1988; Rasmusson *et al*, 1993; Emery *et al*, 1999; Marin *et al*, 2000).

There are probably many modes of repression in natural populations and the results of mixing different repression modes, if different populations get in contact, are presumably complex. Additionally, the finding that full-sized *P* and *KP* elements predominate in wild populations around the world makes this problem even more complex, once it raises the question whether this could be a final equilibrium state in *D. melanogaster* genomes. Further spatial, but also temporal monitoring of wild populations, especially the North American and South American ones, might contribute to clarification of this scenario.

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