

# Long-term evolution of the *roo* transposable element copy number in mutation accumulation lines of *Drosophila melanogaster*

JULIA DÍAZ-GONZÁLEZ, J. FERNANDO VÁZQUEZ, JESÚS ALBORNOZ  
AND ANA DOMÍNGUEZ\*

Área de Genética, Departamento de Biología Funcional, Universidad de Oviedo, 33071 Oviedo, Spain

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## Summary

The rate of insertion of transposable elements (TEs) is a fundamental parameter to understand both their dynamics and role in the evolution of the eukaryotic genome. Nonetheless, direct estimates of insertion rates are scarce because transposition is in general a rare phenomenon. A great deal of our previous work on transposition was based on a set of long-term mutation accumulation (MA) lines of *Drosophila melanogaster* started in 1987 (Oviedo lines), where *roo* was found highly active, with a rate of insertion of  $7 \times 10^{-4}$  insertions per element and generation, as compared with other 15 TE families that presented transposition rates around  $10^{-5}$ . Here, we study the evolution of the *roo* transposition rate, by *in situ* hybridization, after 60–75 additional generations of MA in two subsets of the Oviedo lines, O and O', which had achieved average numbers of *roo* insertions of 77 and 84, respectively. In the O lines, insertions accumulated at a rate that remained constant ( $7 \times 10^{-4}$  insertions per element and generation); however, the subset of lines O' showed a lower accumulation rate of  $4 \times 10^{-4}$  insertions per element per generation, suggesting a regulation of transposition that depends on the number of elements. However, one of the O' lines reached a number of 103 insertions, departing from the group mean by 4.6 SD, and showing that it escapes regulation. Hence, 'de novo' mutations affecting the regulation of transposition are relatively common. These results are discussed in relation to the possible mechanisms of containment of TEs.

## 1. Introduction

Transposable elements (TEs) are mobile, repetitive DNA sequences that constitute a structurally dynamic component of genomes. They have been found in nearly all eukaryotic organisms and make up a large part of their genome. In *Drosophila melanogaster*, TEs account for 20% of the genome, 5.3% of the euchromatin and 50% of the heterochromatin (Quesneville *et al.*, 2005). Each TE family has a distinctive number of copies in natural populations (Vieira *et al.*, 1999; Kaminker *et al.*, 2002). The forces that control the abundance and distribution of TEs in genomes have been the subject of a great deal of empirical and theoretical research, although their nature and evolutionary significance is still a matter of debate (Nuzhdin, 1999; Kidwell, 2005; Lee & Langley, 2010). Several non-excluding hypotheses have been

proposed to explain the constraint of TE copy number. The elements could be maintained by a balance between transposition and selection, either against deleterious effects of insertions or opposing deleterious effects of rearrangements due to unequal exchange among insertions (Charlesworth & Langley, 1989; Pasyukova *et al.*, 2004). The rate of transposition could also be self-regulated, i.e. the transposition rate decreases as the copy number increases (Charlesworth & Langley, 1986, 1989; Biémont, 1994). Moreover, transposition can be repressed by the host (Brookfield, 1991). Recent investigations have shown that transposition can be regulated in the *Drosophila* germline through small interference RNAs (see Aravin *et al.*, 2007). Small RNA pathways of gene silencing are ancient mechanisms that, besides gene regulation, are involved in the control of viruses and TEs (Vagin *et al.*, 2006).

A key parameter to understand the dynamics of TEs is their transposition rate. However, there have

\* Corresponding author: Ana Domínguez. Tel: +34-985102719. Fax: +34-985103534. e-mail: sanjurjo@uniovi.es

been few systematic studies on spontaneous transposition rates of TEs, other than those involving hybrid dysgenesis (Eggleston *et al.*, 1988; Harada *et al.*, 1990; Nuzhdin & Mackay, 1995; Domínguez & Albornoz, 1996; Pasyukova *et al.*, 1998; Nuzhdin, 1999; Maside *et al.*, 2000, 2001; Alonso-González *et al.*, 2006; Papaceit *et al.*, 2007; Vázquez *et al.*, 2007). The outcome of these studies, covering 34 families of elements, is that most individual rates are zero (90 estimates out of 117 were zero including *in situ* and Southern experiments or 71 out of 96 when only *in situ* data are considered). Some elements such as *roo* and *copia* were shown to transpose in several studies (6 out of 7 and 5 out of 11, respectively), while element 412 did not transpose in any of the six experiments where it was considered. The mean overall transposition rate in these studies is of the order of  $10^{-4}$  but indirect estimates based on the analysis of nested TEs are one or two orders of magnitude lower (Bergman & Bensasson, 2007).

In order to study TE dynamics, long-term mutation accumulation (MA) lines of *D. melanogaster*, derived from a genetically homogeneous population, have been monitored for activity of several families of TEs (Alonso-González *et al.*, 2006) over generations. The element *roo* was found highly active in this genetic background, with a rate of insertion of  $7 \times 10^{-4}$  insertions per element and generation, as compared with other 15 TE families that presented transposition rates around  $10^{-5}$  or lower (Domínguez & Albornoz, 1996; Maside *et al.*, 2001; Alonso-González *et al.*, 2006; Vázquez *et al.*, 2007). Mutations consisting of indels or reordenations within the element (structural mutation) occur at a rate in the order of  $10^{-5}$  as well (Domínguez & Albornoz, 1996, 1999; Albornoz & Domínguez, 1999). This result is consistent with the reported structural degeneration of the elements in the *D. melanogaster* genome in spite of being extremely young (Kaminker *et al.*, 2002; Bergman & Bensasson, 2007). Structural degeneration is not limited to the TEs in the reference sequence, but is a general phenomenon in populations of *Drosophila* (Alonso-González *et al.*, 2003).

Element *roo*, with 137 copies, is the most abundant TE in the euchromatic portion of the reference genome of *D. melanogaster* (Quesneville *et al.*, 2005). Here, we check the long-term evolution of the number of elements per genome and the transposition rate of *roo* in two different sets of MA lines, both derived from the same isogenic base, which have been accumulating *roo* insertions for more than 350 generations. This provides an excellent material to check the possible mechanisms that constrain TE copy number and limit their proliferation in natural populations.

## 2. Materials and methods

### (i) MA lines

In a previous experiment (Santiago *et al.*, 1992), a set of MA lines was established from a *D. melanogaster* base population, which was isogenic for all chromosomes, and carried the recessive eye-colour mutation *sepia* (*se*) as an indicator of possible contamination. The isogenic stock was classified as Q (weak P) or M' (pseudo-M) for the P–M system of hybrid dysgenesis. Two sets of MA lines derived from that base (Alonso-González *et al.*, 2006) were used in the present experiment (Fig. 1). All the lines were maintained with two pairs of flies per generation.

O set (formerly named Oviedo set): consisting of the eight original lines surviving in 2009. These lines were maintained at  $24 \pm 1$  °C during the experiment. The insertion pattern of *roo* was determined in 2009 (generations G371–G376) and compared with the data obtained in 2005 (G297–G324).

O' set (formerly named Oviedo-28 set): lines derived from one of the O lines (C66) at generation 115 (G115 of the O set corresponds to the generation zero, G'0, of the O' set). Sixty lines (201–260) were maintained at  $28 \pm 1$  °C for 75 generations, while 20 lines (261–280) were maintained at  $24 \pm 1$  °C as controls. From generation G'76 onwards, all the lines of the O' set were maintained at  $24 \pm 1$  °C. The insertion pattern of *roo* was determined for the 22 surviving lines in 2009 (G'268–G'275) and compared with the data obtained in 2005 (G'213).

All lines were maintained with one or two pairs of parents per generation, reared in the standard medium of this laboratory (brewer's yeast–agar–sucrose) in glass vials (20 mm diameter and 100 mm height).

### (ii) Analysis of transposition rate

Initially, all lines in each set were expected to share the same pattern of *in situ* hybridization sites. This was confirmed by the constancy of the insertion pattern among the lines in each set. Line differences will thus reflect new insertions and excisions that have been fixed in the lines. Under the neutral model, the number of mutations occurring per generation and locus is  $\mu \times 2N_e$  (where  $\mu$  is the mutation rate and  $N_e$  is the effective size) and their probability of fixation is  $1/(2N_e)$ . Thus, the fixation rate under neutrality is equal to the mutation rate. In small populations, detrimental mutations with a selective disadvantage smaller than  $1/(2N_e)$  will behave as effectively neutral (Hedrick, 2010). Comparisons between the number of insertions and transposition rates determined in 2009 with the respective parameters determined in 2005 for the same lines were made by paired Student *t*-test. The dependence of the transposition rate per element per generation in 2009 on the number of elements in

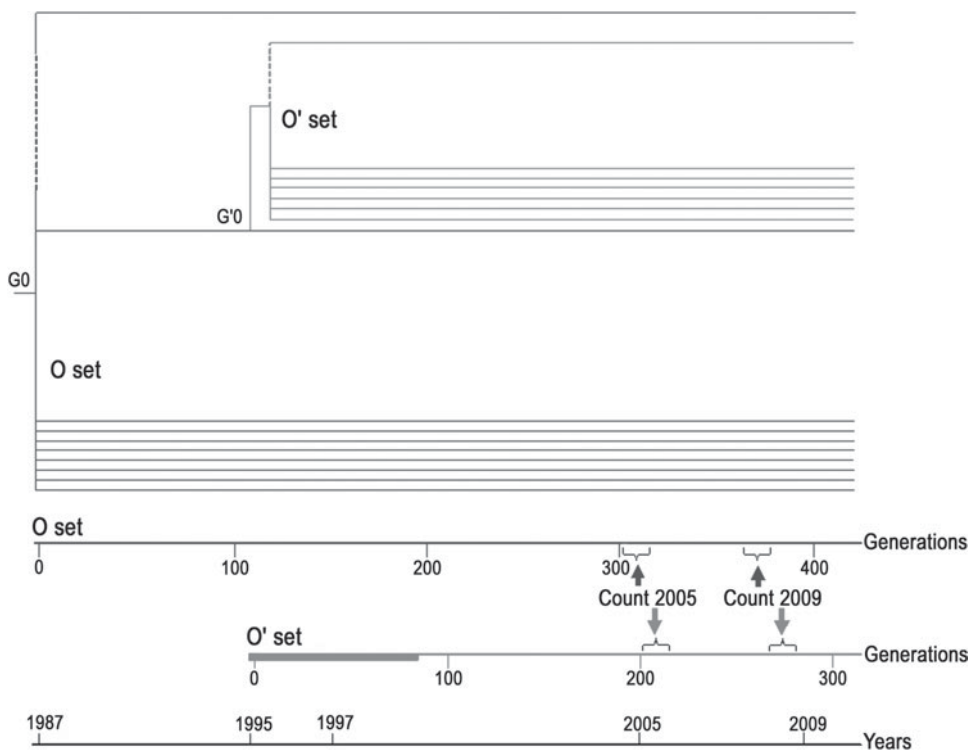


Fig. 1. Diagrammatic representation of the two sets of MA lines and test that were made over time.

2005 was studied by regression. The homogeneity of regression slopes in the two sets of lines was tested by an analysis of covariance using SPSS, following Sokal and Rohlf (1995).

(iii) *Fluorescent in situ hybridization (FISH)*

*In situ* hybridization to polytene chromosomes was carried out following the protocol of Gatti *et al.* (1994). The probe used was the internal 2.3 kb *SalI-HindIII* fragment labelled with digoxigenin-11-dUTP using Nick Translation Mix (Roche). Signals were detected with anti-digoxigenin-rhodamine Fab fragments (Roche). Probe was hybridized to polytene chromosome squashes of salivary glands from third-instar larvae. After hybridization, slides were air-dried and stained with 4',6-diamidino-2-phenylindole. Slides were examined under a fluorescence microscope (Olympus BX61). Chromosomal locations of *roo* elements were determined at the level of cytological subsections on standard Bridges map of *D. melanogaster* (Lefevre, 1976). In general, two nuclei were analysed per slide.

**3. Results**

The distribution of *roo* insertions in the two sets of MA lines was determined and compared with the ancestral pattern inferred in a previous experiment (Vázquez *et al.*, 2007) to identify new insertions (see Fig. 2). The evolution of the number of *roo*

insertions along generations of MA is shown in Fig. 3. Several new insertions that have been originally identified in the lines in 2005 were not fixed (18.97% in the set Oviedo and 21.43% in the set Oviedo-28), as they were not mapped in the present experiment. No excisions of the ancestral patterns (63 elements for the O lines and 72 for the O' lines) were found throughout the experiment. The number of elements of the O lines in 2009 ranged between 70 and 89 with a mean of  $81.38 \pm 2.02$ , significantly exceeding that of the previous account, in 2005, that was  $77.50 \pm 2.02$  ( $P=0.0049$ , paired Student *t*-test). The number of elements in the O' set of lines in 2009 ranged between 77 and 103 with a mean of  $86.27 \pm 1.12$  elements per individual, to be compared with the mean of  $84.09 \pm 0.91$  in the previous determination in 2005 ( $P=0.0058$ , paired Student *t*-test). Line 203 departed 3.01 SD from the mean in the count of 2005 and 4.11 SD in the last determination and can therefore be considered an outlier differing from the rest of the lines.

The rates of insertion were calculated both per generation and per element per generation (Table 1). Rates for the O lines remained constant across generations, while the O' lines that started from a higher number of elements show a decrease that is significant when expressed as insertion rate per element per generation. The overall regression coefficient of insertion rate per element per generation on copy number was non-significant (Table 2). The regression coefficient of number of copies predicting transposition rate per element and generation can be compared across

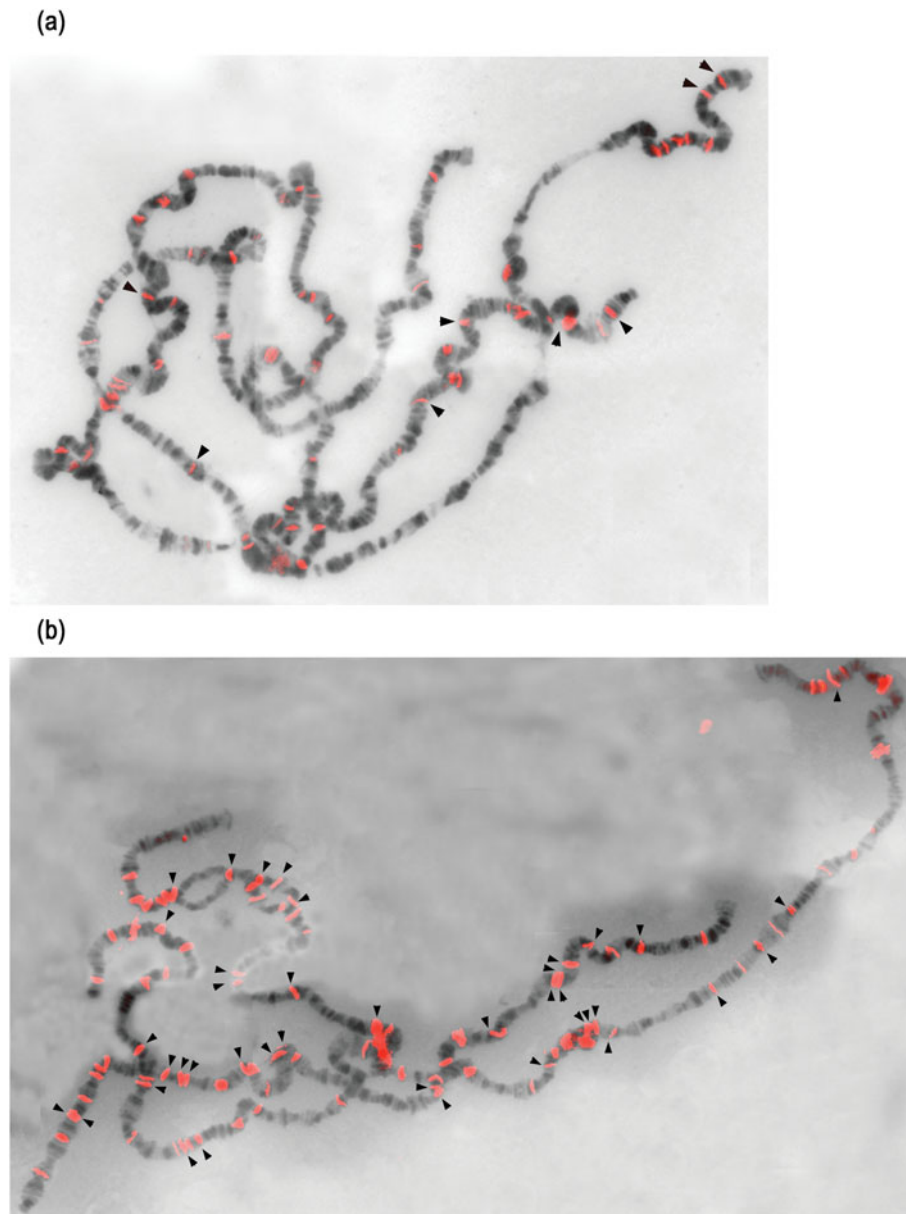


Fig. 2. Cytological detection of *roo* by *in situ* hybridization to polytene chromosomes. Dark arrowheads point to new insertions, the remaining hybridization signals correspond to the original pattern. a) Line O-C20 with 71 insertions, b) Line O'-203 with 103 insertions.

the two sets. Although the difference is not statistically significant ( $P=0.08$ , see Table 2), the regression coefficient for the O set is higher ( $0.606 \pm 0.325$ ,  $P=0.11$ ) than for the O' set ( $-0.118 \pm 0.222$ ,  $P=0.60$ ).

#### 4. Discussion

During 370 generations, the Oviedo MA lines have been accumulating *roo* insertions, starting from 63 elements per genome and reaching a number that varied between 70 and 103. During the second period of MA (2005–2009), the lines O continued to accumulate *roo* insertions at the same rate as before (1987–2005), but the rate of increase in *roo* copy

number has decreased in the lines O' with a larger initial copy number. On the other hand, the regression of transposition rate on *roo* copy number in the O lines, with a lower starting number of copies (77.5), was positive and close to the significance ( $b=0.6$ ,  $P=0.11$ ), while the regression coefficient in the O' lines, with a larger number of copies per haploid genome (84.1) was equal to  $-0.1$ . In a similar experiment with the TEs  *copia* and  *Doc*, Pasyukova *et al.* (1998) observed a positive correlation between transposition rate and copy number in the early stages of the experiment that disappeared later on. This suggests either the existence of a mechanism of transposition regulation dependent on the number of copies

Table 1. Roo *transposition rate*

Year	Rate per generation ( $\times 10^{-2}$ )		Rate per element and generation ( $\times 10^{-4}$ )	
	Lines O ( $n=8$ )	Lines O' ( $n=22$ )	Lines O ( $n=8$ )	Lines O' ( $n=22$ )
2005	4.6 ± 0.8	5.7 ± 0.4	7.6 ± 1.2	8.9 ± 0.6
2009	5.4 ± 1.4	3.8 ± 1.1	7.1 ± 1.9	4.5 ± 1.2
<i>P</i> paired Student <i>t</i> -test	0.71	0.12	0.84	0.03

Table 2. Regression of insertion rate per element and generation on number of elements

Regression				
Set	<i>R</i>	<i>R</i> <sup>2</sup>	Adjusted <i>R</i> <sup>2</sup>	Std. error of the estimate
Lines O	0.666	0.368	0.262	0.325
Lines O'	-0.118	0.014	-0.035	0.222

ANOVA				
Source	df	Mean square	F	<i>P</i>
Overall regression	1	2.28E-09	0.01	0.927
Difference between regression coefficients	1	8.86E-07	3.29	0.081
Set of lines	1	5.67E-07	2.10	0.159
Error	26	2.70E-07		

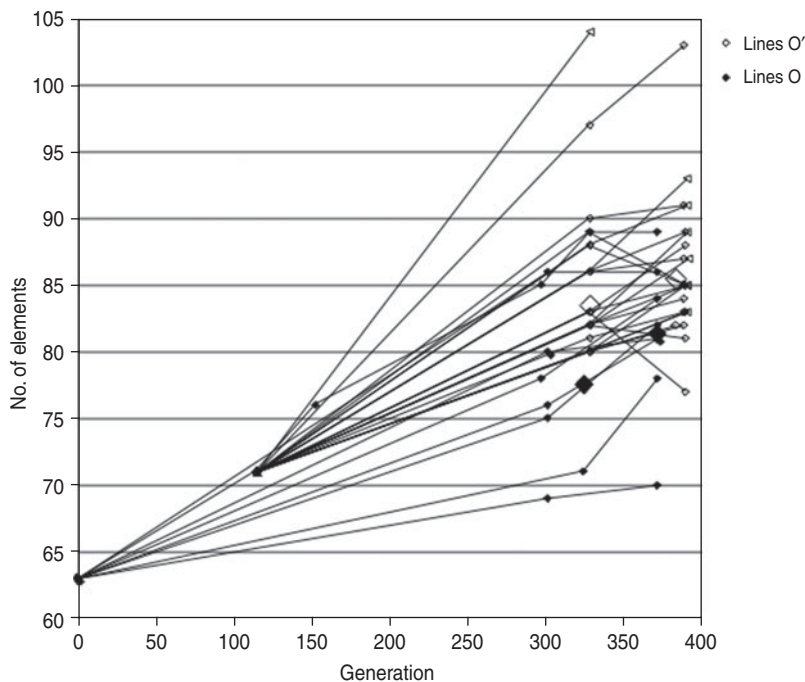


Fig. 3. Number of *roo* insertions against generations.

or, alternatively, the increase of the deleterious effects of insertions with the number of copies (Lee & Langley, 2010). A synergistic effect of insertions on fitness is to be expected from the increased rate of deleterious rearrangements that could be associated with an increase in copy number (Langley *et al.*, 1988; Charlesworth & Langley, 1989).

*D. melanogaster* is the species with more *roo* elements of the genus (de la Chaux & Wagner, 2009; Díaz-González *et al.*, 2010) and the majority of them are very young (Bowen & McDonald, 2001; de la Chaux & Wagner, 2009). The median age of insertion of *roo* is below the detection limit of 0.05 million years obtained from long terminal repeat (LTR) divergence (de la Chaux & Wagner, 2009) indicating that most *roo* elements were recently inserted. This can be related to the reported activity of the element in most laboratory lines studied (Eggleston *et al.*, 1988; Nuzhdin & Mackay, 1995; Pasyukova *et al.*, 1998; Maside *et al.*, 2000, 2001; Vázquez *et al.*, 2007). The number of *roo* elements in the Oviedo lines has surpassed the characteristic number of elements estimated from *in situ* hybridization studies in *D. melanogaster* natural populations ( $67.60 \pm 13.87$ , Vieira *et al.*, 1999;  $63.0$ , Kaminker *et al.*, 2002). In the Oviedo lines, the rate of accumulation of *roo* drops as the number of elements increase, pointing out to regulation as the possible mechanism for the containment of *roo*, and consistent with the proposed density-dependent mechanism of defence by iRNAs (Abrusán & Krambeck, 2006; Lu & Clark, 2010).

The effect of the genetic background on transposition rates was shown in several reports of instability for one or more TEs (Lim *et al.*, 1983; Georgiev *et al.*, 1990; Pasyukova & Nuzhdin, 1993), and it was demonstrated that several episodes of TE mobilization causing instability in diverse families were related to the RNAi (RNA interference) pathways that control transposition (Desset *et al.*, 1999; Kalmykova *et al.*, 2005; Mével-Ninio *et al.*, 2007). In our experiment, two unstable lines accumulating a great number of insertions have been detected, one line (line 275 belonging to the controls of the O' set) was identified in 2005 and was lost shortly after, but the other one (line 203 of the O' set, raised at high temperature during 75 generations) is still accumulating insertions (see Fig. 3). The instability of these lines indicates that transposition is regulated and that mutations causing instability can arise at a non-negligible rate (two unstable mutants/13 828 generations, giving  $1.4 \times 10^{-4}$  unstable mutations per line and generation). In nature, unstable mutations are presumably eliminated from large populations by selection but, in small populations, it is possible that they can be fixed by drift and hence they constitute an important source of variation. The unstable line 203 was previously shown to present a high rate of structural mutation

that affected different families of TEs (Alonso-González *et al.*, 2006) suggesting that this might affect nets of TEs in the genome. The expression of chimeric sequences from TE nets in  $\beta$ -heterochromatin may co-suppress transcripts of TEs located in euchromatic arms (Bergman *et al.*, 2006). Thus, it may be speculated that the mutations causing instability in lines 203 and 275 might be related with the RNAi pathways controlling transposition.

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