Genet. Res., Camb. (2000), **75**, *pp.* 275–284. *With 3 figures.* Printed in the United Kingdom © 2000 Cambridge University Press

Rates of movement of transposable elements on the second chromosome of *Drosophila melanogaster*

XULIO MASIDE, STAVROULA ASSIMACOPOULOS† AND BRIAN CHARLESWORTH*
ICAPB, University of Edinburgh, Ashworth Laboratories, Edinburgh EH9 3JT, UK

(*Received 16 June 1999 and in revised form 21 October 1999*)

Summary

The rates of movement of 11 families of transposable elements of *Drosophila melanogaster* were studied by means of *in situ* hybridization of probes to polytene chromosomes of larvae from a long-term mutation accumulation experiment. Replicate mutation-accumulation lines carrying second chromosomes derived from a single common ancestral chromosome were maintained by backcrosses of single males heterozygous for a balancer chromosome and a wild-type chromosome, and were scored after 116 generations. Twenty-seven transpositions and 1 excision were detected using homozygous viable and fertile second chromosomes, for a total of 235056 potential sources of transposition events and a potential 252880 excision events. The overall transposition rate per element per generation was $1{\cdot}15 \times 10^{-4}$ and the excision rate was $3{\cdot}95 \times 10^{-6}$. The single excision (of a *roo* element) was due to recombination between the element's long terminal repeats. A survey of the five most active elements among nine homozygous lethal lines revealed no significant difference in the estimates of transposition and excision rates from those from viable lines. The excess of transposition over excision events is in agreement with the results of other *in situ* hybridization experiments, and supports the conclusion that replicative increase in transposable element copy number is opposed by selection. These conclusions are compared with those from other studies, and with the conclusions from population surveys of element frequencies.

1. Introduction

Transposable elements (TEs) form a significant fraction of the genome of most species that have been characterized at the molecular level (Berg & Howe, 1989; Capy, 1997). The forces controlling their abundance within their host genomes have been the subject of much empirical and theoretical research (Charlesworth *et al*., 1994*b*; Capy, 1997). Population surveys of TEs in *Drosophila melanogaster* have consistently shown that, with a few exceptions, element insertions are present at very low frequencies at euchromatic sites into which they are capable of inserting (Charlesworth & Langley, 1991; Bièmont, 1992; Bièmont *et al*., 1994). Comparisons of the observed properties of the frequency distributions of insertions, as determined by *in situ* hybridization of TE probes to polytene chromosomes isolated from natural populations, yield the conclusion that TE frequencies per site are held to these low values by deterministic forces that tend to prevent elements from spreading (Charlesworth & Langley, 1991; Bièmont, 1992). While the nature of these forces is still controversial (Hoogland & Bièmont, 1996; Bièmont *et al*., 1994, 1997; Charlesworth *et al*., 1997), the possibilities include self-regulation of transposition rate (such that the rate per element per generation declines with the number of copies of the element family in question) (Charlesworth & Charlesworth, 1983; Langley *et al*., 1983), selection against deleterious mutations caused by new insertions (Charlesworth & Charlesworth, 1983; Kaplan & Brookfield, 1983), direct deleterious effects of transposition on fitness (Brookfield, 1991; Brookfield, 1996), and deleterious fitness effects of chromosome rearrangements induced by ectopic exchange between TEs of the same family (Langley *et al*., 1988).

* Corresponding author. Tel: +44 (0)131 650 5750. Fax: +44(0)131 650 6564. e-mail: Brian.Charlesworth@ed.ac.uk
† Present address: Department of Pharmacology and Physiology, University of Chicago, Chicago, IL 60637, USA.

Estimates of the magnitude of the forces reducing element frequencies, multiplied by $4N_e$ (where N_e is the local effective population size), are usually of the order of 10 or more (Charlesworth & Langley, 1991; Bièmont 1992; Bièmont *et al*., 1994), suggesting that these forces are substantially more effective than genetic drift. If the accepted value for the effective size of a local population of North American flies is used (Mukai & Yamaguchi, 1974), the mean rate of elimination of elements in a population from Maryland is estimated to be approximately $1{\cdot}8 \times 10^{-4}$ per element per generation (Charlesworth *et al*., 1992*a*). If this population is approximately in equilibrium, this rate must be the same as the mean rate of transposition per element per generation (Charlesworth, 1991).

In order to test the validity of the population genetic models on which these conclusions are based, it is important to have independent estimates of the rates of movement of TEs in *D. melanogaster*. Surprisingly few quantitative studies of movement rates for a representative set of element families have been carried out, excluding cases of elements inducing hybrid dysgenesis (whose high rates of transposition are presumably unrepresentative of the natural situation) (Eggleston *et al*., 1988; Harada *et al*., 1990; Nuzhdin & Mackay, 1995; Dominguez & Albornoz, 1996). There is evidence for variability in the rates of movement of some *D. melanogaster* retrotransposons, among both host and element genotypes (Bièmont *et al*., 1987; Pasyukova & Nuzhdin, 1993; Kim *et al*., 1994; Prud'homme *et al*., 1995; Nuzhdin *et al*., 1998). Nevertheless, it seems likely that retrotransposons, which constitute the majority of *D. melanogaster* TE families (Bingham & Zachar, 1989; Finnegan, 1992), usually transpose at a relatively low rate consistent with the above estimate, and that their transposition rates generally greatly exceed their rates of spontaneous excision (Nuzhdin *et al*., 1997). We present here a further study of rates of TE movement, for a set of elements that have previously been extensively used in population surveys (Montgomery & Langley, 1983; Charlesworth & Lapid, 1989; Charlesworth *et al*., 1992*a*, *b*), with results that are consistent with these two generalisations.

2. Methods

(i) *Stocks and crosses*

The second chromosomes used in this study were derived from the mutation-accumulation (*MA*) lines described by Houle *et al*. (1994). These involved crosses of single males, heterozygous for the balancer *SM1* (Lindsley & Zimm, 1992) and a wild-type second chromosome descended from a single ancestral chromosome, to females heterozygous for *SM1* and *Pm* on a background of X and third chromosomes

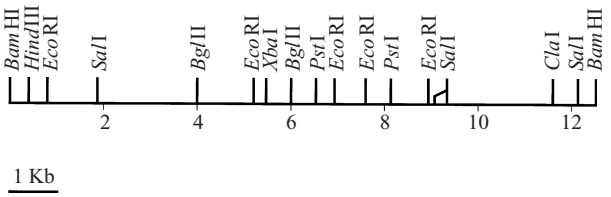

Fig. 1. Restriction map of the genomic DNA from *Drosophila melanogaster* cloned into *cDm 2244*.

derived from the outbred laboratory stock *IV* (Charlesworth & Charlesworth, 1985). The males shared the same background. The fourth chromosome was made homozygous for the recessive marker spa^{pol}, as a precaution against contamination. Crosses were carried out in standard cornmeal vial cultures, with two replicates of each subline set up each generation as an insurance against loss. Three *SM1/Pm* females were used per vial. Until generation 55, the lines were maintained at 25 °C, but thereafter were kept at 19 °C to minimize effort in stock maintenance. In generation 116, viable second chromosomes were made homozygous by intercrossing *SM1/+* females and males from the same subline; lines carrying lethals were detected because of the absence or near-absence of wild-type homozygotes in the progeny, and were retained as balanced cultures, as were homozygous sterile lines. A total of 36 out of 78 lines were lethal. Using a Poisson correction for multiple events, we obtain a lethal mutation rate of 0·0053 per generation, which is consistent with earlier estimates of 0·005 for the lethal mutation rate per second chromosome per generation in males in non-dysgenic crosses (Crow & Simmons, 1983), although Fry *et al*. (1999) report a value of 0·01.

(ii) *Probes and* in situ *hybridization methods*

Probes for the elements *mdg-1*, *opus*, *copia*, *297*, *412*, *jockey*, *roo* and *17·6*, described by Charlesworth *et al*. (1994*a*), were used. In addition, the probe *cDm2244* containing an unknown element or elements was used (Strobel, 1982), and a probe for *pogo* was kindly provided by Dr Ian Boussy. The restriction map of *2244* is shown in Fig. 1. DNA sequence data showed that *cDm2158* (Charlesworth *et al*., 1994*a*) contains a nearly complete copy of *1731*, along with other sections which show high homology to various known repetitive sequences and TEs, such as *Responder* (*Rsp*), *Stalker*, *412* and *mdg-1* (Maside & Charlesworth, unpublished data). In order to avoid mixed results, a 2·6 kb *Pst*I–*Sal*I fragment from *cDm2158*, which is internal to *1731*, was used as a probe for this element. The elements *opus* and *jockey* are retrotransposons which lack long terminal repeats (LTRs), while *pogo* is

https://doi.org/10.1017/S0016672399004474 Published online by Cambridge University Press

a class II transposable element with inverted terminal repeats; the remaining elements are retrotransposons with LTRs (Finnegan, 1992).

Probes were labelled with biotinylated dUTP by random primer labelling, and visualized after hybridization by the diaminobenzidine/peroxidase method. Minor modifications of previously described protocols (Sniegowski & Charlesworth, 1994) were used for these purposes. Probes were hybridized to polytene chromosome squashes of salivary glands from third-instar larvae. After hybridization, slides were air-dried and stained with Giemsa as described by Montgomery *et al.* (1987), or examined directly after hybridization under a ×40 phase contrast objective. Homozygous viable *MA* lines were scored using larvae homozygous for the *MA*-derived wild-type second chromosome; lethal lines were scored by crossing *SM1*/+ females to a stock (*IS-25*) that had been made isogenic for the X chromosome and the major autosomes, as described by Charlesworth *et al.* (1994*a*). This stock was homozygous for the same spa^{pol} fourth chromosome marker as the *MA* lines. The locations of element insertions on the second chromosome of the *IS-25* stock were determined, and in all cases differed from those inferred for the ancestral *MA* chromosome, as expected from the generally low frequencies of element insertions in *D. melanogaster* (Charlesworth & Langley, 1991). The sites contributed by the *MA* chromosomes could therefore be determined from the hybridization patterns in the heterozygotes with *IS-25*.

PCR primers for amplifying a short (401 bp) sequence from the LTR of *roo* were designed, and used to make a probe for this sequence. The primer sequences (5′ to 3′) were as follows: LTR plus strand, CACACATGAACACGAATA, LTR minus strand ATCCCAAATAAGACT.

3. Results

(i) *Events in homozygous viable lines*

The numbers of new sites observed for each element in the homozygous viable *MA* lines, together with the numbers of sites that were shared by all the *MA* second chromosomes and which hence must be ancestral, are shown in Table 1. The number of lines scored for each element varied somewhat, with an average of 20·9 over all 11 elements. Replicate slides were made in many cases, to check that the process of isogenization of the second chromosome was effective. There were no cases of inconsistency between replicates, and the data as a whole were consistent with the interpretation that all 27 new sites of hybridization were the result of new insertions of elements. The positions of all new insertions on the photographic polytene chromosome map (Lefevre, 1976) are shown in Fig. 2. Only one case of an apparent excision of an element (*roo*, from position 29A/B of line *MA*-102) was detected. Replicate slides of this stock gave consistent evidence of lack of detectable hybridization at this site. Hybridization with a probe for the LTR (see Section 2.ii) of this element showed faint but consistent hybridization at this site, whereas hybridization with a probe that contained only internal sequence gave no signal. This implies that the excision of *roo* was due to a recombination event involving the LTRs, which is known to occur in *Drosophila* (Bingham & Zachar, 1989).

In order to determine rates of transposition, the means of the numbers of copies of elements in the euchromatin of the chromosomes other than the second were determined for the lines scored for the second chromosomes (Table 1). There was very little variation between lines, implying that elements were close to fixation within the *SM1*/*Pm* stock used for backcrossing. The copy number estimates were therefore obtained on the assumption of complete homozygosity of elements; residual heterozygosity would mean that these estimates are slightly inflated.

Since the males used in the backcrosses were *SM1*/+ in genotype, and there is evidence that old-established balanced chromosomes may accumulate TEs (Montgomery *et al.*, 1987), it was necessary to determine the numbers of elements in the balancer chromosome. This was done by crossing *SM1*/+ males to a standard isogenic stock (either the *MA-16* line, which was ancestral in state for all elements scored, or *IS-25*), and scoring the numbers of elements on the second chromosomes of larvae heterozygous for *SM1*. Owing to the complex configurations of these chromosomes, this was a laborious procedure, and is subject to error. Since these numbers contribute to a large denominator in the estimates of transposition rates (see below), the errors will have only minor effects on these estimates. One slide per element was used to generate the data. Subtraction of the numbers of elements in the wild-type second chromosomes used in the cross yields the values shown in Table 1 for the balancer.

The total mean number of members of a given element family in the euchromatin that constitute potential sources of new transpositions was calculated as the sum of the number for the third plus fourth chromosomes, plus half the sum of the number for the X, the *MA* ancestral second chromosome, and the *SM1* second chromosome (shown as 'Total' in Table 1). The proportion of the euchromatin represented by the second chromosome was calculated from the DNA estimates of Heino *et al.* (1994) as 0·373 (the Y chromosome was ignored in this calculation). The transposition rate per element per generation for a given family was calculated by dividing the number of observed new insertions for this family by the product of 0·373 × 116 × the total genomic copy number × the

https://doi.org/10.1017/S0016672399004474 Published online by Cambridge University Press

Table 1. *Transposition rates in the mutation accumulation lines*

Element family	Element copy no.[a]						No. of lines analysed	No. of new insertions	Transposition rate per element[c]	Upper 95% confidence limit
	X	2-Bal	2-MA	3	4	Total[b]				
mdg-1 (*2181*)	4·0	7	6	9·3	0·0	17·8	22	3	$1{\cdot}77\times10^{-4}$	
opus (*2217*)	2·0	4	7	11·9	0·0	18·4	21	1	$5{\cdot}98\times10^{-5}$	
copia	4·0	9	12	14·8	0·0	27·3	21	10	$4{\cdot}03\times10^{-4}$	
1731 (*2158*)	0.0	0	0	1·0	0.0	1·0	20	0		$3{\cdot}44\times10^{-4}$
297	3·5	16	8	19·1	1·0	33·9	21	4	$1{\cdot}30\times10^{-4}$	
412	1·0	12	12	8·8	0·0	21·3	23	0		$1{\cdot}41\times10^{-4}$
jockey (*2161*)	4·0	8	24	16·2	1·0	35·2	21	1	$3{\cdot}13\times10^{-5}$	
2244	2·8	9	5	12·2	1·0	21·6	20	1	$5{\cdot}35\times10^{-5}$	
pogo	2·8	3	5	6·1	0·0	11·5	20	0		$3{\cdot}01\times10^{-4}$
roo (*B104*)	16·7	19	24	30·2	0·0	60·1	20	7	$1{\cdot}35\times10^{-4}$	
17·6 (*2210*)	1·6	3	1	8·5	1·0	12·3	21	0		$2{\cdot}68\times10^{-4}$
Total	42·4	90	104	138·1	4·0	260·4	20·9	27	$1{\cdot}15\times10^{-4}$	

[a] The copy number values for X, 3 and 4 are means over lines; the value 2-Bal is the estimate for the SM1 balancer; 2-MA is the count for the ancestral *MA* second chromosome.
[b] Mean total element copy number per haploid genome $=\frac{1}{2}X+\frac{1}{2}(2\text{-Bal})+\frac{1}{3}(2\text{-MA})+3+4$.
[c] Transposition rate = number of new insertions/(total element copy number × number of lines studied × 116 × 0·373); 116 is the number of generation of accumulation, and 0·373 is the proportion of *D. melanogaster* euchromatin included in the second chromosome.

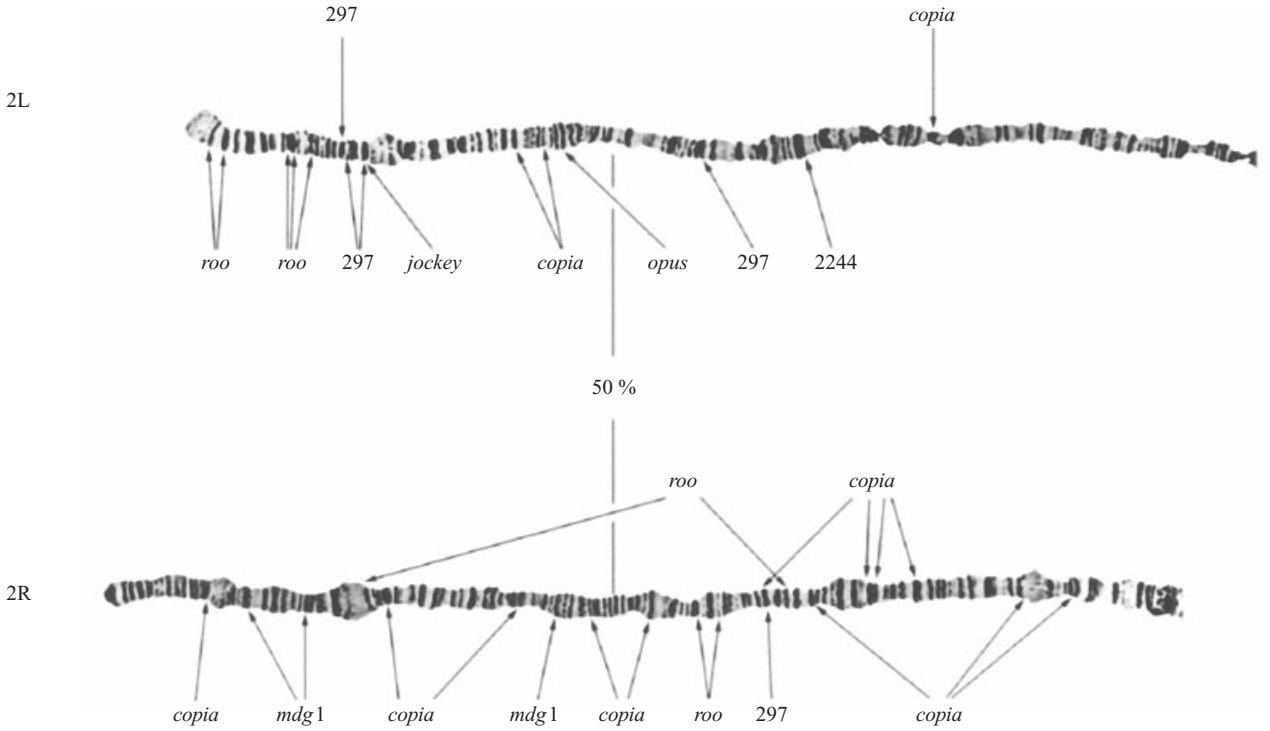

Fig. 2. Location of the new insertion sites found in the viable insertion-accumulation lines (below the chromosomes) and in the lethal lines (above). Adaptation of a figure taken from Lefevre (1976).

number of lines. This has a mean of $9{\cdot}00\times10^{-5}$ and a standard error of $3{\cdot}7\times10^{-5}$ (estimated from the variance in transposition rates among different families). If all elements are pooled, the estimated net rate of transposition is $1{\cdot}15\pm0{\cdot}34\times10^{-4}$, assuming a Poisson distribution for the number of transposition events. (The total size of the experiment, in the sense of Nuzhdin *et al.* (1997), is given by the sum over families of the numbers of elements in the euchromatic genome × the number of generations of accumulation × the number of lines × 0·373, and is equal to 235056.)

The *copia* family is most active, with a transposition rate of $4{\cdot}03\times10^{-4}$. This is nevertheless well below the highest rates of movement that have been reported for this element (Bièmont *et al.*, 1987; Pasyukova & Nuzhdin, 1993; Nuzhdin & Mackay, 1995; Pasyukova

https://doi.org/10.1017/S0016672399004474 Published online by Cambridge University Press

et al., 1998). There was only one excision event, involving *roo*, in contrast to 27 insertions, yielding an excision rate for all elements pooled of $3{\cdot}95 \pm 3{\cdot}95 \times 10^{-6}$ (the total experiment size for this purpose is based on the observed number of elements on the second chromosome, and is equal to 252880). This is in excellent agreement with the pooled estimate of $4{\cdot}05 \pm 1{\cdot}53 \times 10^{-6}$ from other *in situ* experiments, based on a total experiment size of 1729860 (see table 1 of Nuzhdin *et al.*, 1997). Using the Poisson distribution, the 95% probability upper limit for the excision rate in the present experiment is $1{\cdot}88 \times 10^{-5}$, well below the lower 95% confidence limit for the rate of transposition for pooled elements ($6{\cdot}12 \times 10^{-5}$). There is no evidence for any non-random distribution of insertions among the different *MA* lines. Details of the location of the elements will be provided on request.

Various tests of randomness of the distribution of new insertions within chromosome 2 were carried out. Using the Heino *et al.* (1994) estimates of DNA content, the euchromatin (21A–40F) of 2L and of 2R (41A-60F) represent 44·6% and 55·4% of the euchromatin of chromosome 2, respectively. Thirteen insertions into 2L and 15 into 2R were observed, almost exactly the same as expected. An odd feature of the results is that only two insertions into the proximal half (30F–40F) of 2L were observed, compared with 6·5 expected, given that this region represents 50·2% of the chromosome 2 euchromatin ($\chi^2 = 6{\cdot}29$, $P < 0{\cdot}02$). No such difference was observed for 2R, treating 41A–51C as the proximal half, with 7 insertions and 50·2% of the 2R euchromatin. The elements present in the ancestral second chromosome were randomly distributed across these regions, with 30 and 26 insertions in the distal and proximal portions of 2L, and 28 and 24 in the distal and proximal portions of 2R. The result for new insertions in 2L may therefore merely be a statistical fluctuation. The only other indication of non-randomness was the observation that 5 of 7 new insertions of *roo* were found in the 21B to 23E region of 2L, which represents only 7% of the chromosome 2 euchromatin. There is no suggestion of a similar clustering of *roo* elements in the ancestral chromosome. While some evidence for site-specificity of retrotransposon insertions has been reported previously (reviewed by Pasyukova *et al.*, 1998), its causes and implications remain obscure, especially as there is little evidence for clustering of elements in population surveys (Charlesworth *et al.*, 1992*a*).

Another question that can be asked is whether there is any tendency for new insertions to be located close to old ones. This would be expected if elements transpose preferentially to nearby sites, as has been reported for *P* elements (Tower *et al.*, 1993). The locations of new insertions and old insertions on chromosome 2 at the level of salivary chromosome divisions were tabulated; the Kendall rank correlation between the numbers of new and old insertions within each division was calculated, and found to be non-significantly negative ($\tau = -0{\cdot}19$, after correcting for ties; $P = 0{\cdot}08$). This suggests that there is no strong tendency for insertions to be generated at sites close to the elements which generated them. This is confirmed at a finer-scale by inspection of the locations of new and old elements. For example, new *roo* insertions are found in 2L at 21B, 21C, 22F, 23A and 23C, while the ancestral state has *roo* insertions in this region at 21C/D, 22D, 23E and 24E. On 2R, new *roo* insertions occur at 49D/E and 50A, whereas the ancestral chromosome harbours no *roo* elements in these divisions. A similar pattern of lack of close association between new and old elements is seen for the other LTR elements for which movements were detected. On the other hand, the new insertions of *jockey* and *opus* (the only new non-LTR element insertions detected here) are into 25A and 29E/F, respectively, with old insertions being present at 24E and 29D/E. This is suggestive of an association between old and new locations, but the numbers are too small to be truly informative. It is interesting in this context that *jockey* (probe *cDm2161*) is the only element among this set that shows evidence for a non-random distribution within chromosome arms in samples from natural populations (Charlesworth & Lapid, 1989; Charlesworth *et al.*, 1992*a*).

Under models of copy number regulation which invoke a stable equilibrium under negative selection and transposition, higher transposition rates should lead to higher equilibrium abundances, since stability requires that log fitness falls off faster, the larger the number of elements carried by an individual (Charlesworth & Charlesworth, 1983; Charlesworth *et al.*, 1994*b*). If the fitness function is similar for different families, element families with high transposition rates should therefore have higher mean copy numbers than families with low rates. The numbers of new element copies per family are positively correlated with family abundance ($\tau = 0{\cdot}56$, $P < 0{\cdot}02$, using a one-tailed Kendall rank correlation test), as might be expected on almost any model (Fig. 3). If *copia* is excluded, on the grounds that it is an outlier as far as its transposition rate is concerned, the τ value is increased slightly to 0·60 ($P < 0{\cdot}02$). Transposition rate itself is significantly correlated with copy number if *copia* is included ($\tau = 0{\cdot}40$, $P < 0{\cdot}05$), but the correlation is non-significant if *copia* is excluded ($\tau = 0{\cdot}41$, $P = 0{\cdot}06$). Excluding *copia*, the number of transposition events involving the five most abundant families (13) is not significantly different from the number of events involving the five least abundant families (4), assuming that the expected numbers of transpositions observed are proportional

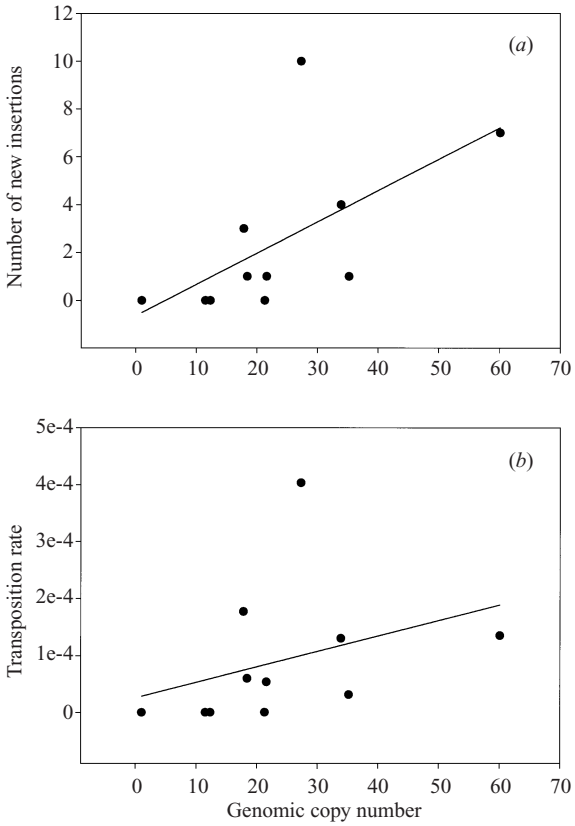

Fig. 3. Relation between (a) the number of new insertion sites and (b) the transposition rates of the 11 element families studied, and their genomic copy number. The lines are the linear regression lines.

to the total mean euchromatic copy numbers for the two classes (172 and 61, respectively). These data do not suggest a strong relation between transposition rate and abundance, and indicate that differences in transposition rates contribute at most only partially to differences in abundance among element families. Other factors, such as differences in susceptibility to ectopic exchange, may be more important.

(ii) *Screening of lethal MA lines*

It is possible that the estimates of rates of movement derived from homozygous viable and fertile *MA* lines may be biased by elimination of lines with TE insertions or imprecise excisions that cause recessive lethal or sterile mutations (Berg & Howe, 1989). For this reason, we conducted a survey of nine lines where the *MA* chromosome was homozygous lethal. The method of scoring such chromosomes is described in Section 2.iii. The five most abundant element families were used for this survey, with the results shown in Table 2. The mean and standard error of the transposition rate for these lines was $1{\cdot}26 \pm 0{\cdot}88 \times 10^{-4}$, compared with $1{\cdot}40 \pm 0{\cdot}71 \times 10^{-4}$ for these elements among the non-lethal lines, representing 22 of 27 of the total transposition events observed. No excision events were detected; the maximum proportion of excisions among movement events compatible with this result at the 5% probability level is 0·11. These results suggest that the above sources of bias are negligible.

4. Discussion

The estimated total number of transpositions per second chromosome per generation is $27/(116 \times 20{\cdot}9) = 0{\cdot}011$ (SE 0·002) for the 11 families of elements which we have followed here. Dividing by 0·373, the proportion of the genome represented by the second chromosome, we get an estimate of $0{\cdot}030 \pm 0.006$ for the haploid euchromatic genome as a whole. Assuming that this set of 11 elements represents about one-quarter of the total (Finnegan, 1992), the total rate of transposition per haploid euchromatic genome would thus be of the order of 0·12. This is likely to be far too high, since low copy elements will not contribute as much to detectable insertion events as high copy number elements (see Fig. 3), and most low copy number families are not included in this study. It is, however, substantially lower than the estimate of 0·20 obtained by Nuzhdin & Mackay (1995), from a study of 17 families of elements in sib-mated isogenic lines from the Harwich strain. The difference largely reflects the fact that their study included two families that transposed at unusually high rates, of the order of 10^{-3} (*copia* and *I*): insertions of these elements

Table 2. *Transposition rates in the lethal lines*

Element	Mean total copy no. per haploid genome[a]	No. of new insertions	Transposition rate per element[a]
copia	27·3	5	$4{\cdot}70 \times 10^{-4}$
297	33·9	1	$7{\cdot}59 \times 10^{-5}$
412	21·3	0	0·00
jockey (*2161*)	35·2	0	0·00
roo (*B104*)	60·1	2	$8{\cdot}55 \times 10^{-5}$
Total	177·7	8	$1{\cdot}16 \times 10^{-4}$

[a] See legend to Table 1.

https://doi.org/10.1017/S0016672399004474 Published online by Cambridge University Press

contributed 67% of the 327 events they observed. In our study, *copia* did not move at such a high rate, although its rate was higher than that of other elements (Table 1).

There is evidence for considerable between-line variability in the rate of movement of *copia* (Bièmont *et al*., 1987; Pasyukova & Nuzhdin, 1993; Charlesworth *et al*., 1994*a*; Nuzhdin *et al*., 1998). It seems likely that host or element genotypes with very high rates tend to be relatively infrequent in nature: only two out of 10 isogenic lines with genotypes representative of independent haploid genomes from the Beltsville, Maryland natural population showed evidence for unusually high rates of *copia* movement (Charlesworth *et al*., 1994*a*). It thus seems probable that the lower rate for *copia* which we have obtained is more representative of the normal situation. *I* might reasonably be excluded from consideration, given its involvement in hybrid dysgenesis, which suggests that its unusually high rate of movement even in non-dysgenic crosses may reflect an lack of coadaptation with the host genome, consistent with the strong possibility that it is either a recent introduction from another species or a recent reactivation of members of an inactive family of elements (Finnegan, 1989). The pooled rate of transposition for the eight families other than *copia* in common between the two experiments was $8{\cdot}94 \pm 3{\cdot}2 \times 10^{-5}$ for the present experiment, and $1{\cdot}79 \pm 0{\cdot}63 \times 10^{-4}$ for that of Nuzhdin & Mackay (1995). These are significantly different ($P < 0{\cdot}02$, on a normal deviate test), in the direction of a higher rate in the Nuzhdin/Mackay experiment (we have omitted their results for *2158* from this calculation, since they also used another probe for *1731*).

The main difference, other than genetic background, between the two experiments are (a) that our experiment can only detect transpositions in males, whereas their experiment detected transpositions in both sexes, and (b) that our experiment was partly conducted at a lower temperature than theirs. Transposition rates for rapidly moving *copia* elements in males are higher than in females (Pasyukova *et al*., 1997), whereas rapidly transposing *Doc* elements are thought to move predominantly in females (Pasyukova *et al*., 1998). No solid data are available for other retrotransposons. Despite these differences among experiments in estimates of rates of movement, the various estimates concur in suggesting that transposition rates are typically of the order of 10^{-5} to 10^{-4}, in broad agreement with estimates from fits of population genetic models to population surveys of element locations (Charlesworth *et al*., 1992*a*).

The lethal mutation rate per second chromosome was about 0·005 (Section 2.i), so we would expect that at most 0·005/0·011 (45%) of insertions could lead to a lethal mutation. The true frequency is probably much lower, especially given the lack of evidence for a significant enrichment of element insertions in the lethal lines. This is consistent with the estimate that approximately 10% of *P* element insertions cause recessive lethals (Engels, 1989), and the lack of evidence for enhanced frequencies of recessive lethal mutations in lines with high rates of *copia* transpositions (Pasyukova *et al*., 1998). The mutation rate for visible mutations in *D. melanogaster* is about 10^{-5} per locus (Drake *et al*., 1998); with 15000 genes, the total mutation rate per haploid genome would thus be about 0·15 for mutations which severely disrupt gene function. Our upper bound to the haploid rate of transposition per genome of 0·12 would imply that a maximum of 80% of these are due to TE insertions. This is in reasonable agreement with the estimate that about 50% of visible mutations are due to TE insertions (Finnegan, 1992).

Our data have added three more families (*opus*, *jockey*, and the unknown element present in the *2244* probe) to the list of elements that have previously been observed to transpose in laboratory lines of *D. melanogaster* (see table 2 of Nuzhdin *et al*., 1997). Given the generally low rates of transposition discussed above, it is likely that much apparent variation in rates of transposition between experiments is due to the fact that there is a high chance that a given family will fail to generate a new insertion in any one experiment, rather than to real variability in rates of movement. Without very detailed investigations of transposition rates in different lines, which has been done in only a few cases (e.g. Pasyukova *et al*., 1998), caution should be exercised in interpreting apparent differences in transposition rates. For example, comparison of the results of Nuzdhin & Mackay (1995) with the present data show that none of the five families that did not transpose in their experiment, but which were mobile in ours, can be regarded as having significantly lower rates of movement by Fisher's exact test, after correcting for multiple comparisons.

In common with other studies using *in situ* hybridization to polytene chromosomes to detect movements, we have found an excess of transposition over excision events (Eggleston *et al*., 1988; Harada *et al*., 1990; Nuzhdin & Mackay, 1995) (see table 1 of Nuzhdin *et al*., 1997). Such an excess is consistent with the great stability of mutations induced by insertions of retrotransposons (Bingham & Zachar, 1989). This supports the selfish DNA model for the maintenance of transposable elements in host genomes, consistent with the evidence from population surveys (Charlesworth & Langley, 1991), since it implies that the replication of elements within the genome would lead to an increase in mean copy number in the population as a whole, unless there are forces that oppose the spread of elements. This result also would seem to exclude the possibility that self-regulation of the rate of transposition can be solely

responsible for the maintenance of stable copy number of retrotransposons (Charlesworth & Charlesworth, 1983; Langley *et al*., 1983); since the copy numbers found in the stocks used for these experiments are similar to those reported for genotypes isolated from natural populations (Charlesworth *et al*., 1994*a*), near-equality of transposition and excision rates would be expected under the self-regulation model (Charlesworth & Langley, 1991). Other data that seem to be inconsistent with this model are reviewed by Pasyukova *et al*. (1998).

Given the rates of movement estimated in these experiments, the mean number of TEs in the *D. melanogaster* haploid genome would be expected to increase initially from its present value by at least 0·03 per generation, if unopposed by selection. It would take approximately 6301 generations to double the present haploid copy number (269) for the set of elements studied here, i.e. about 315 years assuming 20 generations per year in nature. This makes it understandable how large differences in TE copy number can evolve over relatively short periods of geological time, as has been documented in many different cases (Capy, 1997), if the effectiveness of selection against TEs can be altered by genetic or ecological factors. There is no need to appeal to episodes of unusual transposon activity to explain such differences.

The only study which has generated results that disagree with these conclusions is that of Dominguez & Albornoz (1996), based on the use of Southern hybridization to detect element movements throughout the whole genome. They found rates of movement that are much lower on average than those reported here and by the other workers cited above, using *in situ* hybridization. But they found a strong tendency for gains of bands to be accompanied by losses, even for the retrotransposons *copia* and *297*. One possible interpretation is that they are detecting rearrangements involving pairs of elements, rather than true transpositions or excisions (Dominguez & Albornoz, 1996). This would imply even lower rates of transposition than were ostensibly found, and is still hard to reconcile with the results from the *in situ* experiments if the rearrangements involve euchromatin.

A possible resolution of this difficulty is that the Southern hybridizations mainly detected heterochromatic insertions under the loading conditions that were employed by Dominguez & Albornoz (1996), and that transposition rate estimates which are biased towards heterochromatic copies are very low, due to the disproportionate abundance of defective TEs in the heterochromatin (Vaury *et al*., 1989). Dominguez & Albornoz (1996) attempted to determine heterochromatic copy numbers by comparing blots using digests of DNA from salivary glands of third-instar larvae with DNA from adults. But it is not clear how sensitive this method is, since it assumes that heterochromatin is underreplicated in salivary glands but not in adult tissues, whereas the beta-heterochromatin that contains the bulk of heterochromatic TEs is known to be extensively replicated during polytenization (Dimitri, 1997). The fact that Dominguez & Albornoz found rather small numbers of heterochromatic relative to euchromatic copies, in contrast to what is commonly reported in the literature (Charlesworth *et al*., 1994*a*; Dimitri, 1997), is consistent with this interpretation. The simultaneous gains and losses of elements they observed may therefore reflect rearrangements involving heterochromatic elements. Further experiments comparing Southern and *in situ* results for the same lines are needed to resolve this issue.

As described in Section 3.i, we have evidence that the excision event involving *roo* occurred by recombination among its LTRs. No other excisions were detected. Such 'LTR excisions' have earlier been detected and characterized at the molecular level as causes of revertants of retrotransposon-induced mutations, and appear to be the major cause of loss of such elements apart from ectopic exchange (Bingham & Zachar, 1989). The fact that the excision observed here must have taken place in a male indicates that host meiotic recombination is not required for LTR excision. An X-ray-induced LTR excision of a *copia* insertion in the *white* locus has been described by Carbonara & Gehring (1985).

This work was supported by a BBSRC grant and grant DEB-9527717 from the US National Science Foundation to B.C., and a Marie Curie Fellowship to X.M. B.C. is supported by the Royal Society. We thank Patricia Pignatelli for assistance with fly crosses and *in situ* work, Bryant McAllister for help with designing *roo* LTR primers, and Helen Borthwick for media preparation and washing up. We also thank C. Bièmont, P. D. Keightley, T. F. C. Mackay and two reviewers for their comments on the manuscript.

References

Berg, D. E. & Howe, M. M. (eds.) (1989). *Mobile DNA*. Washington, DC: American Society for Microbiology.

Bièmont, C. (1992). Population genetics of transposable DNA elements: a *Drosophila* point of view. *Genetica* **86**, 67–84.

Bièmont, C., Aouar, A. & Arnault, C. (1987). Genome reshuffling of the *copia* element in an inbred line of *Drosophila melanogaster*. *Nature* **329**, 742–743.

Bièmont, C., Lemeunier, F., Garcia Guerreiro, M., Brookfield, J. F. Y., Gautier, C., Aulard, S. & Pasyukova, E. G. (1994). Population dynamics of the *copia*, *mdg*1, *mdg3*, *gypsy* and *P* transposable elements in a natural population of *Drosophila melanogaster*. *Genetical Research* **63**, 197–212.

Bièmont, C., Tsitrone, A., Vieira, C. & Hoogland, C. (1997). Transposable element distribution in Drosophila. *Genetics* **147**, 1997–1999.

Bingham, P. M. & Zachar, Z. (1989). Retrotransposons and the FB transposon from *Drosophila melanogaster*. In

Mobile DNA (ed. D. E. Berg & M. M. Howe), pp. 485–502. Washington, DC: American Society for Microbiology.

Brookfield, J. F. Y. (1991). Models of transposition repression in P-M hybrid dysgenesis by P cytotype and by zygotically encoded repressor proteins. *Genetics* **128**, 471–486.

Brookfield, J. F. Y. (1996). Models of the spread of non-autonomous selfish transposable elements when transposition and fitness are coupled. *Genetical Research* **67**, 199–210.

Capy, P. (ed.) (1997). *Evolution and Impact of Transposable Elements*. Dordrecht, Netherlands: Kluwer.

Carbonara, B. D. & Gehring, W. J. (1985). Excision of *copia* element in a revertant of the white-apricot mutation of *Drosophila melanogaster* leaves behind one long terminal repeat. *Molecular and General Genetics* **199**, 1–6.

Charlesworth, B. (1991). Transposable elements in natural populations with a mixture of selected and neutral insertion sites. *Genetical Research* **57**, 127–134.

Charlesworth, B. & Charlesworth, D. (1983). Population dynamics of transposable elements. *Genetical Research* **42**, 1-27.

Charlesworth, B. & Charlesworth, D. (1985). Genetic variation in recombination in *Drosophila*. I. Responses to selection and preliminary genetic analysis. *Heredity* **54**, 71–84.

Charlesworth, B. & Lapid, A. (1989). A study of ten transposable elements on X chromosomes from a population of *Drosophila melanogaster*. *Genetical Research* **54**, 113–125.

Charlesworth, B. & Langley, C. H. (1991). Population genetics of transposable elements in *Drosophila*. In *Evolution at the Molecular Level* (ed. R. K. Selander, A. G. Clark & T. S. Whittam), pp. 150–176. Sunderland, MA: Sinauer.

Charlesworth, B., Lapid, A. & Canada, D. (1992*a*). The distribution of transposable elements within and between chromosomes in a population of *Drosophila melanogaster*. I. Element frequencies and distribution. *Genetical Research* **60**, 103–114.

Charlesworth, B., Lapid, A. & Canada, D. (1992*b*). The distribution of transposable elements within and between chromosomes in a population of *Drosophila melanogaster*. II. Inferences on the nature of selection against elements. *Genetical Research* **60**, 115–130.

Charlesworth, B., Jarne, P. & Assimacopoulos, S. (1994*a*). The distribution of transposable elements within and between chromosomes in a population of *Drosophila melanogaster*. III. Element abundances in heterochromatin. *Genetical Research* **64**, 183–197.

Charlesworth, B., Sniegowski, P. & Stephan, W. (1994*b*). The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* **371**, 215–220.

Charlesworth, B., Langley, C. H. & Sniegowski, P. D. (1997). Transposable element distributions in *Drosophila*. *Genetics* **147**, 1993–1995.

Crow, J. F. & Simmons, M. J. (1983). The mutation load in *Drosophila*. In *The Genetics and Biology of Drosophila*, vol. 3c (ed. M. Ashburner, H. L. Carson & J. N. Thompson), pp. 1–35. London: Academic Press.

Dimitri, P. (1997). Constitutive heterochromatin and transposable elements in *Drosophila melanogaster*. *Genetica* **100**, 85–93.

Dominguez, A. & Albornoz, J. (1996). Rates of movement of transposable elements in *Drosophila melanogaster*. *Molecular and General Genetics* **251**, 130–138.

Drake, J. W., Charlesworth, B., Charlesworth, D. & Crow, J. F. (1998). Rates of spontaneous mutation. *Genetics* **148**, 1667–1686.

Eggleston, W. B., Johnson-Schlitz, D. & Engels, W. R. (1988). P-M hybrid dysgenesis does not mobilize other transposable elements in *D. melanogaster*. *Nature* **331**, 368–370.

Engels, W. R. (1989). P elements in *Drosophila*. In *Mobile DNA* (ed. M. M. Howe & D. E. Berg), pp. 437–484. Washington, DC: American Society for Microbiology.

Finnegan, D. J. (1989). The I factor and I-R hybrid dysgenesis in *Drosophila melanogaster*. In *Mobile DNA* (ed. M. M. Howe & D. E. Berg), pp. 503–521. Washington, DC: American Society for Microbiology.

Finnegan, D. J. (1992). Transposable elements. In *The Genome of* Drosophila melanogaster (ed. D. L. Lindsley & G. G. Zimm), pp. 1196–1008. San Diego, CA: Academic Press.

Fry, J. D., Keightley, P. D., Heinsohn, S. L. & Nuzhdin, S. V. (1999). New estimates of the rates and effects of mildly deleterious mutation in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA* **96**, 574–579.

Harada, K., Yukuhiro, K. & Mukai, T. (1990). Transposition rates of movable genetic elements in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA* **87**, 3248–3252.

Heino, T. I., Saura, A. O. & Sorsa, V. (1994). Maps of the salivary gland chromosomes of *Drosophila melanogaster*. *Drosophila Information Service* **73**, 621–738.

Hoogland, C. & Biémont, C. (1996). Chromosomal distribution of transposable elements in *Drosophila melanogaster*: test of the ectopic recombination model for the maintenance of insertion site number. *Genetics* **144**, 197–204.

Houle, D., Hughes, K. A., Hoffmaster, D. K., Ihara, J. T., Assimacopoulos, S. & Charlesworth, B. (1994). The effect of spontaneous mutation on quantitative traits. I. Variances and covariances of life history traits. *Genetics* **138**, 773–785.

Kaplan, N. L. & Brookfield, J. F. Y. (1983). The effect on homozygosity of selective differences between sites of transposable elements. *Theoretical Population Biology* **23**, 273-280.

Kim, A. I., Lyubomirskaya, N. V., Belyaeva, E. S., Shotack, N. G. & Ilyin, Y. V. (1994). The introduction of a tranpositionally active copy of retrotransposon *gypsy* into the Stable Strain of *Drosophila melanogaster* causes genetic instability. *Molecular and General Genetics* **242**, 472–477.

Langley, C. H., Brookfield, J. F. Y. & Kaplan, N. L. (1983). Transposable elements in Mendelian populations. I. A theory. *Genetics* **104**, 457–471.

Langley, C. H., Montgomery, E. A., Hudson, R. R., Kaplan, N. L. & Charlesworth, B. (1988). On the role of unequal exchange in the containment of transposable element copy number. *Genetical Research* **52**, 223–235.

Lefevre, G. (1976). A photographic representation of the polytene chromosomes of *Drosophila melanogaster* salivary glands. In *The Genetics and Biology of Drosophila*, vol. 1a. (ed. M. Ashburner & E. Novitski), pp. 31–36. London: Academic Press.

Lindsley, D. L. & Zimm, G. G. (1992). *The Genome of* Drosophila melanogaster. San Diego, CA: Academic Press.

Montgomery, E. A. & Langley, C. H. (1983). Transposable elements in Mendelian populations. II. Distribution of

three copia-like elements in a natural population. *Genetics* **104**, 473–483.

Montgomery, E. A., Charlesworth, B. & Langley, C. H. (1987). A test for the role of natural selection in the stabilization of transposable element copy number in a population of *Drosophila melanogaster*. *Genetical Research* **49**, 31–41.

Mukai, T. & Yamaguchi, O. (1974). The genetic structure of natural populations of *Drosophila melanogaster*. XI. Genetic variability in a local population. *Genetics* **76**, 339-366.

Nuzhdin, S. V. & Mackay, T. F. C. (1995). The genomic rate of transposable element movement in *Drosophila melanogaster*. *Molecular Biology and Evolution* **12**, 180–181.

Nuzhdin, S. V., Pasyukova, E. G. & Mackay, T. F. C. (1997). Accumulation of transposable elements in laboratory lines of *Drosophila melanogaster*. *Genetica* **100**, 167–175.

Nuzhdin, S. V., Pasyukova, E. G., Morozova, E. A. & Flavell, A. J. (1998). Quantitative genetic analysis of *copia* retrotransposon activity in inbred *Drosophila melanogaster* lines. *Genetics* **150**, 755–766.

Pasyukova, E. G. & Nuzhdin, S. V. (1993). *Doc* and *copia* instability in an isogenic *Drosophila melanogaster* stock. *Molecular and General Genetics* **240**, 302–306.

Pasyukova, E. G., Nuzhdin, S. V., Li, S. V. & Flavell, A. J. (1997). Germ line transposition of the *copia* retrotransposon is restricted to males by tissue-specific control of *copia* RNA level. *Molecular and General Genetics* **255**, 115-124.

Pasyukova, E. G., Nuzhdin, S. V. & Filatov, D. A. (1998). The relationship between the rate of transposition and transposable element copy number for *copia* and *Doc* transposable elements of *Drosophila melanogaster*. *Genetical Research* **72**, 1–11.

Prud'homme, N., Gans, M., Masson, M., Terzian, C. & Bucheton, A. (1995). *Flamenco*, a gene controlling the *gypsy* retrovirus of *Drosophila melanogaster*. *Genetics* **139**, 697–711.

Sniegowski, P. D. & Charlesworth, B. (1994). Transposable element numbers in cosmopolitan inversions from a natural population of *Drosophila melanogaster*. *Genetics* **137**, 815–827.

Strobel, E. (1982). Mobile dispersed repeated DNA elements in the *Drosophila* genome. *Federation Proceedings* **41**, 2656–2658.

Tower, J., Karpen, G. H., Craig, N. & Spradling, A. C. (1993). Preferential transposition of *Drosophila* P elements to nearby chromosomal sites. *Genetics* **133**, 347–359.

Vaury, C., Bucheton, A. & Pelisson, A. (1989). The β-heterochromatin sequences flanking the I elements are themselves defective transposable elements. *Chromosoma* **98**, 215–224.