Interspecific Hybridization Increases Transposition Rates of *Osvaldo*

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Several authors have postulated that genetic divergence between populations could result in genomic incompatibilities that would cause an increase in transposition in their hybrids, producing secondary effects such as sterility and therefore starting a speciation process. It has been demonstrated that transposition largely depends on intraspecific hybridization for *P, hobo,* and *I* elements in *Drosophila melanogaster* and for several elements, including long terminal repeat (LTR) and non-LTR retrotransposons, in *D. virilis.* However, in order to demonstrate the putative effect of transposable elements on speciation, high levels of transposition should also be induced in hybrids between species that could have been originated by this process and that are still able to interbreed. To test this hypothesis, we studied the transposition of the LTR retrotransposon *Osvaldo* in *Drosophila buzzatii–Drosophila koepferae* hybrids. We used a simple and robust experimental design, analyzing large samples of single-pair mate offspring, which allowed us to detect new insertions by in situ hybridization to polytene chromosomes. In order to compare transposition rates, we also used a stock recently obtained from the field and a highly inbred *D. buzzatii* strain. Our results show that the transposition rate of *Osvaldo* is 10^{-3} transpositions per element per generation in all nonhybrid samples, very high when compared with those of other transposable elements. In hybrids, the transposition rate was always 10^{-2}, significantly higher than in nonhybrids. We show that inbreeding has no effect on transposition in the strains used, concluding that hybridization significantly increases the *Osvaldo* transposition rate.

Introduction

In general, transposable elements show very low transposition and excision rates. It is commonly accepted that transposition is, on average, 10^{-4} per element per generation or lower (Eggleston, Johnson-schlitz, and Engels 1988; Charlesworth and Langley 1989; Harada, Yukuhiro, and Mukay 1990; Nuzhdin and Mackay 1994, 1995; Suh et al. 1995; Nuzhdin, Pasyukova, and Mackay 1996), although some elements showing transposition rates close to 10^{-3} have been described (Labrador and Fontdevila 1994; Nuzhdin and Mackay 1995; Vieira and Biemont 1997). In a few instances, transposable elements become unstable, and transposition is associated with striking genetic and physiological traits such as high mutation rates, nondisjunction, high recombination rates, chromosomal rearrangements, and male and female sterility. In *Drosophila,* this phenomenon is generally known as hybrid dysgenesis. Hybrid dysgenesis is frequently related to outcrosses involving stocks from different geographical origins. Several transposable elements, both DNA and retrotransposons, are known to be directly involved in independent and unrelated systems. Furthermore, transposition may be limited to a particular element, like *P, I,* or *hobo* (Engels 1989; Finnegan 1989; Blackman and Gelbart 1989; Bucheton 1990), or involve several elements, such as in the hybrid dysgenesis system described for *Drosophila virilis* (Petrov et al. 1995; Evgen'ev et al. 1997; Vieira et al. 1998).

These findings lead to the assumption that at least some transposable elements, regardless of the element class (DNA elements or retrotransposons), will become unstable, increasing transposition rates several orders of magnitude following crosses between strains that are genetically divergent for the factors controlling transposition. After the discovery of hybrid dysgenesis, using an extension of the previous argument, it was proposed that early stages of speciation could be established by hybrid dysgenesis-like mechanisms following the crossing of individuals from genetically divergent populations (Thompson and Woodruff 1978; Rose and Doolittle 1983; Fontdevila 1992). These claims were sustained by the initial observation of high mutation rates in *Drosophila* hybrids as well as in other interspecific hybrids (Sturtevant 1939; Miller 1950; Gerstel and Burns 1967). This idea has been discouraged, mostly due to the inability to reproduce some of the initial results and because of the indirect evidence of no transposition in interspecific hybrids (Hey 1989; Coyne 1989). Nonetheless, the direct analysis of transposition in interspecific hybrids has been systematically approached so far in only a few instances, and the results were, in general, inconclusive (Evgen'ev et al. 1982; Labrador and Fontdevila 1994; Marin and Fontdevila 1995; Garcia-Guerreiro 1996).

One of the strongest evidences of a hybrid dysgenesis syndrome in interspecific hybrids in *Drosophila* was observed in hybrids between *D. buzzatii* and *D. koepferae.* It was found that these hybrids could yield progenies with a high frequency of new chromosomal rearrangements, similar to those produced by hybrid dysgenesis (Naveira and Fontdevila 1985; Marin and Fontdevila 1998). Furthermore, we have previously shown that the retrotransposon *Osvaldo* is actively transposing in *D. buzzatii* (Labrador and Fontdevila 1994). Our data also suggested that transposition was more frequent in hybrids than in nonhybrids. However, the strains used in our experiments were highly inbred, making our results inconclusive, because, as has been suggested, inbreeding could be a stressful condition, capable of triggering genetic instabilities promoting transposition. In

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Key words: LTR retrotransposons, *Drosophila,* transposition rate, interspecific hybridization, speciation.

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Mol. Biol. Evol. 16(7):931–937. 1999

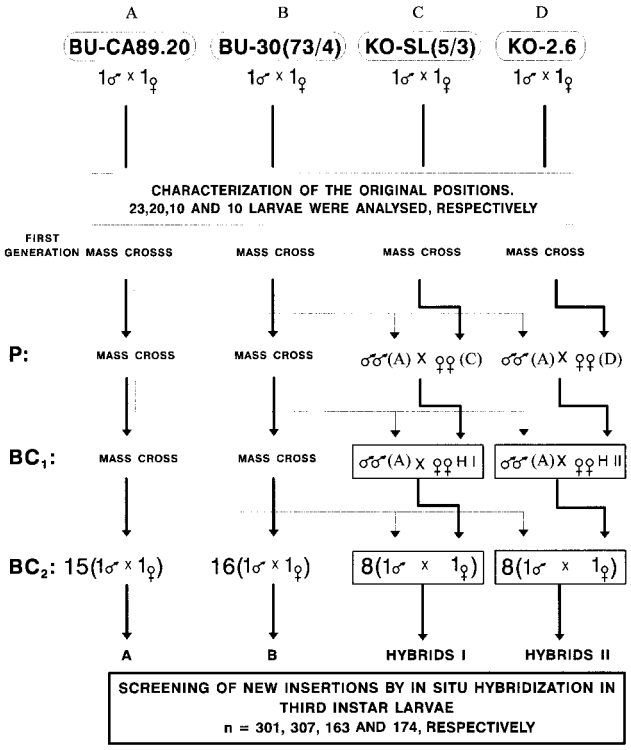

FIG. 1.—Flow chart of the crosses performed to detect *Osvaldo* transposition (*A*) with outbred lines, (*B*) with inbred lines, and (*C* and *D*) with interspecific hybrids.

the present work, we used an outbred stock directly obtained from a natural population to avoid experimental interference due to the putative effect of inbreeding on transposition. A comparison of transposition rates between samples of hybrids and nonhybrids (10^{-2} and 10^{-3} transpositions per element per generation, respectively) shows that transposition is always significantly higher in hybrids, irrespective of the inbred nature of the stock used.

Materials and Methods

Drosophila Stocks

Four *Drosophila* stocks were used in this experiment (fig. 1). BU-CA89.20 is a stock founded by a single inseminated female from the population of Carboneras, Spain, and shortly thereafter maintained by mass crossing. BU-30 (73/4) is an inbred stock that was also used in experiments by Labrador and Fontdevila (1994). This stock is different from the one used by Labrador and Fontdevila (1994) because of the accumulation of 43 additional generations of inbreeding, adding up to 73 generations of continuous sib pair crossing. Stocks KO-SL(5/3) and KO-2.6 are two *D. koepferae* laboratory stocks originally from San Luis, Argentina.

In Situ Hybridization

In situ hybridization to polytene chromosomes was carried out using standard procedures, as in Labrador and Fontdevila (1994). We used Digoxigenin (Boheringher Mannhein) as a nonradioactive label. A *Kpn*I fragment of 2.1 kb which comprises the coding region of the Pol gene, including reverse transcriptase, of *Osvaldo* (Labrador and Fontdevila 1994) was used as a probe.

Characterization of Preexisting *Osvaldo* Sites

In order to determine all the positions of the *Osvaldo* element in the original stocks, we started the experiment using a single pair per stock as parents (fig. 1). In situ hybridization of at least 10 F_1 larvae was used to characterize the original positions of *Osvaldo* for each line (fig. 1). A study of natural populations from the Iberian Peninsula showed that the average number of *Osvaldo* copies per *D. buzzatii* haploid genome is three, with a maximum of eight (Labrador, Seleme, and Fontdevila 1998). As the expected copy number per larva is very low, we calculated the probability of detecting any occupied site in the parents considering that the characterized positions detected by in situ hybridization behave as dominant markers and segregate in the offspring as Mendelian factors. The probability of detecting a position in the less favorable case, when only one parent is heterozygous and the other parent does not contain the element at this position, is very high when 10 or more larvae are analyzed. This position will be found in 50% of the offspring, assuming Mendelian segregation. The probability of detecting this position in at least one of the offspring larvae out of a sample of n is $P = 1 - 0.5^n$. It follows that for a sample of $n = 10$, $P = 0.999$, and for a sample of $n = 20$, $P \approx 1$. Therefore, we can be confident that we have detected all the positions present at the beginning of the experiment.

Figure 1 shows the schematic crosses carried out in this study. After the characterization of the original positions, the remaining offspring of each line was used to establish the parental generation (P). This step was necessary in order to obtain enough individuals to carry out the interspecific cross. In BC1, hybrid I was obtained from an interspecific mass cross between A and C lines, and hybrid II was obtained similarly, by mass crossing lines A and D (fig. 1). In BC_1, female hybrids were mass backcrossed to *D. buzzatii* males of line A. Due to the low viability and fertility of the F_1 hybrid females, we were forced to carry out the backcrossing procedure in order to obtain sufficient hybrid mothers to set up the single-pair crosses. BC_1 virgin offspring females were backcrossed individually in pairs with single males from the A line (BC_2). Simultaneously, single-pair crosses using virgin females from lines A and B were also performed (nonhybrid- outbred and nonhybrid-inbred samples). A sample (n) of BC_2 offspring larvae per single-pair cross was analyzed, using the same *Osvaldo* probe, to detect the new insertions produced.

Determination of New Insertions

New insertions were detected by in situ hybridization to polytene chromosomes of BC_2 offspring larvae. Since the cross was performed using single pairs as parents, we applied the same criteria as in the initial characterization process in order to have a very high probability of detecting all segregating positions. Therefore, the minimum sample size analyzed per cross was 10

Table 1
Euchromatic Sites of the *Osvaldo* Element at the Start of the Experiment in the Four Different Lines

		Chromosomes[a]				
Line	N[b]	X	2	3	4	5
BU-CA89.20 . . .	23	A2a; C3cd; E3d	B2a; D5a	A3d; F1f; G4a	—	A4ab
BU-30 (73/4) . .	20	—	A4a; F4a; F4f	—	F3b	A4ab; C5b; F1f
KO-SL (5/3) . . .	10	—	E3e/4a	F1cf	F2fg	—
KO-2.6.	10	—	—	B4e; F1; D5	—	F3cf

[a] As described by Wharton (1945) and Ruiz, Fontdevila, and Wasserman (1982).
[b] Number of analyzed larvae.

larvae. Only those positions different from the ones described at the beginning of the experiment were considered new insertions. We observed that all the positions different from those initially characterized always appeared only once among the offspring of a single pair. The probability of finding a position with this frequency when only one parent is heterozygous is very low with the analysis of a large offspring sample. Considering that the frequency of this site in the F_1 follows a binomial distribution with $P = 0.5$, the probability of finding that position in only 1 larva out of a sample of 10 is $P = 0.0098$, and that of finding it in only 1 larva out of 20 is $P = 1.9 \times 10^{-5}$. We used sample sizes of a minimum of 10 and an average of 20 larvae per single-pair cross. (The difference was due to the fact that not all slides were readable, and some of them were discarded during the reading process.) Therefore, we conclude that unique positions in the offspring indicate unambiguously new insertions, ruling out the possibility that these positions were previously present in the parents.

Transposition Rates

Transposition rate, defined as the number of transpositions per element per generation, is computed by dividing the number of new insertions per generation by the transposition opportunities. Transposition opportunities are the number of times that each euchromatic position has passed through a chromosomal generation. They were calculated for each single-pair cross, taking into account all euchromatic *Osvaldo* positions from both parents. If a position is fixed, the number of opportunities for transposition is simply the number of fixed sites times the number of analyzed larvae times the number of genomes contributing to the offspring (a diploid genome from the father and another from the mother). When a position is polymorphic, calculations have to be performed in order to estimate the number of times it is present in the parents (homozygous, heterozygous, etc.) from the segregation numbers in the offspring larvae from each single-pair cross.

Results

Characterization of the Initial Populations

A detailed characterization of the *Osvaldo* sites in each *Drosophila* line was obtained. Table 1 shows all the initial euchromatic positions. With the exception of the inbred line BU-30 (73/4), in which all sites were fixed, all the stocks showed position polymorphisms for most of the sites. Only position B2a in the second chromosome of the BU-CA89.20 line showed hybridization in all the larvae of that line. We concluded that this position was fixed in the chromosomes of the single pair used as parents. On the other hand, line BU-30 (73/4) showed exactly the same positions previously described (Labrador and Fontdevila 1994). Therefore, *Osvaldo* sites were more polymorphic in the BU-CA89.20 stock than in the BU-30 (73/4) stock according to differences in inbreeding. After this result, we expected that most positions (excepting those coming from BU-30 (73/4)) would segregate in the course of the experiment.

Frequency Distributions and Transposition Rates

Four different crosses were performed in order to obtain information about the transposition rate of *Osvaldo* in distinct genomic backgrounds. Two nonhybrid backgrounds, one highly inbred (BU-30 (73/4)) and another outbred (BU-CA89.20), and two hybrid backgrounds (BU-CA89.20/KO 2.6 and BU-CA89.20/KO-SL(5/3) were analyzed (fig. 1). A total of 945 larvae have been successfully hybridized and analyzed. From each of them, all the *Osvaldo* sites detected were mapped and located on *D. buzzatii* polytene chromosome maps (Wharton 1942; Ruiz, Fontdevila, and Wasserman 1982). The analysis of these positions allowed us to distinguish between new and preexisting transpositions using the criteria described in *Materials and Methods*

Table 2 shows the distribution of the new insertions per larva in each of the samples. The χ^2 in table 2 measures the deviation of the coefficient of dispersion (DC) from the expected value if the number of new insertions per larva follows a Poisson distribution (DC = 1). All the samples deviate significantly, showing that the new insertions often occurred in clusters of several insertions per larva, such as those described in our previous experiment (Labrador and Fontdevila 1994).We concluded that *Osvaldo* transposition occurs by bursts, as most of the larvae with new positions contained more than one transposition (see, e.g., fig. 2). As shown in Labrador and Fontdevila (1994), the non-Poisson distribution of the number of insertions per larva precludes standard chi-square comparisons between different samples. Consequently, we tried to fit our results to those theoretical distributions normally used when the observations are clustered (e.g., the negative binomial distribution). The best fit was obtained with the ''Poisson with zeroes''

Table 2
Frequency Distributions of Number of New Insertions per Larva

X	BU-CA89.20	BU-30 (73/4)	Hybrid I	Hybrid II
0	286	299	148	135
1	5	3	10	14
2	6	4	1	11
3	3	1	2	9
4	0	0	0	2
5	0	0	1	2
6	0	0	1	1
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	1	0	0	0
DC	4.23	1.96	3.05	2.47
χ^2	1,268.23	559.92	426.99	493.54

NOTE.—X = number of new insertions. $\chi^2 = ([N - 1]S^2)/\bar{X}$, where S^2 and $\bar{X}$ are the variance and the mean, respectively, and N is the sample size. $P \leq 0.001$ in all samples. Hybrids I and II are as in Figure 1.

distribution (Johnson and Kotz 1969), since none of the samples differ significantly from the expected distribution, using the estimated parameters $\hat{\lambda}$ and $\hat{\omega}$ (table 3). The ''Poisson with zeroes'' distribution is characterized by two parameters, λ and ω, with ω being the proportion of zeroes added to a Poisson distribution of parameter λ. The maximum- likelihood estimator of λ ($\hat{\lambda}$) is obtained by solving numerically the equation $X_{-0} = \hat{\lambda}[1 - e^{-\hat{\lambda}}]^{-1}$, where X_{-0} is the average number of insertions per larva, excluding larvae with zero new insertions, and the maximum-likelihood estimator of ω is given by $\hat{\omega} = (1 - e^{-\hat{\lambda}})^{-1}(P_0 - e^{-\hat{\lambda}})$, where P_0 is the proportion of zeroes in the sample.

An overall test of homogeneity among all distributions showed that the different samples are nonhomogeneous ($P < 0.0001$). Paired tests between individual nonhybrid stocks and each of the hybrids also showed a highly significant heterogeneity in all cases (table 3). Finally, paired tests between nonhybrids showed that they are homogeneous ($P = 0.36$), while the comparison between hybrids showed that the two distributions are only different with $P = 0.015$. Considering the parameters that define the distribution, these differences are accounted for by X_{-0} and P_0. X_{-0} is higher in both hybrid samples than in the BU-30 (73/4) line. The outbred line BU-CA89.20 showed the highest value (table 3). This high value is mainly due to a larva with 10 new insertions (table 2). If we consider this value as an outlayer, the value of X_{-0} in the outbred sample is only 1.857. P_0 is always lower for the hybrid samples.

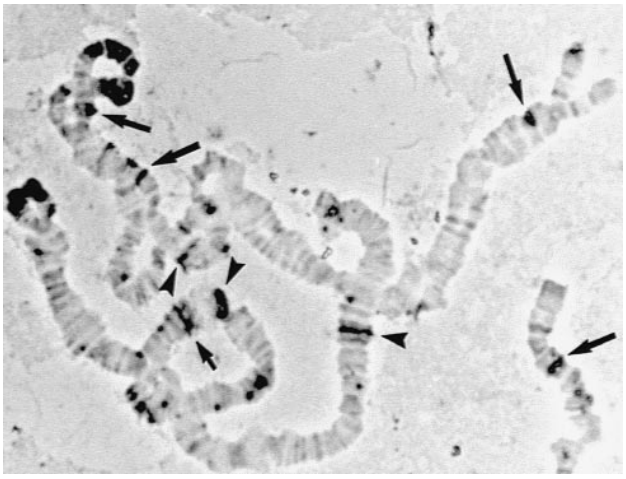

FIG. 2.—In situ hybridization to polytene chromosomes of a hybrid larva, showing four new insertions of *Osvaldo.* Arrowheads show preexisting positions in the stock BU-CA89.20. Arrows show new insertions. Three new insertions are shown in a third chromosome (top) that is completely hybrid, as is shown by the asynapsis all along the chromosome. A second chromosome (bottom) shows a new insertion inside an inversion loop. The tip of another asynapsed third chromosome (bottom right) shows the same insertion as the third chromosome on the top.

Table 3
Parameters of the ''Poisson with Added Zeroes'' Distribution for the Number of Insertions per Larva

X	BU-CA89.20 (A)	BU-30 (73/4) (B)	Hybrid I (C)	Hybrid II (D)
$\bar{X}_{-0}$	2.400	1.750	1.933	2.231
P_0	0.950	0.974	0.908	0.776
$\hat{\lambda}$	2.109	1.247	1.503	1.896
$\hat{\omega}$	0.943	0.964	0.882	0.736
P	0.50	0.25	0.06	0.78

NOTE.—X is number of insertions per larva (see text for parameter explanations). Homogeneity test probabilities: A vs. D, $P < 0.0001$; A vs. C, $P = 0.0098$; B vs. D, $P < 0.0001$; B vs. C, $P = 0.0007$.

The transposition rate measured from each sample is shown in table 4. The differences between hybrids and nonhybrids are even more clear when we compare the values of transposition rates, which in hybrids are always on the order of 10^{-2}, while in nonhybrids they are 10^{-3}. From the comparisons above, and from the differences in transposition rates between hybrids and nonhybrids, we conclude that there is a positive effect of interspecific hybridization on the transposition rate.

Effect of Introgression

In order to test whether the amount of introgression of the *D. koepferae* genome has any effect on the transposition rate of *Osvaldo*, we carried out a nonparametric regression analysis between the fraction of the genome introgressed and the transposition rate (table 5). The hybrid genome fraction has been estimated from direct observations of the in situ- -hybridized polytene chromosomes, using chromosomal asynapsis in order to map the hybrid regions (see Naveira, Pla, and Fontdevila

Table 4
Transposition Rates of *Osvaldo* in Outbred and Inbred Lines and in *Drosophila buzzatii–Drosophila koepferae* Interspecific Hybrids

Samples	N	LNI	NI	TO	TR
Outbred BU-CA89.20	301	15	36	4,224	8.5×10^{-3}
Inbred BU-30 (73/4)	307	8	14	9,824	1.4×10^{-3}
Hybrid I KO-2.6	163	15	29	1,836	1.5×10^{-2}
Hybrid II KO-SL (5/3)	174	39	87	2,230	3.9×10^{-2}

NOTE.—N = number of analyzed larvae; LNI = larvae with new insertions; NI = number of new insertions; TO = number of transposition opportunities; TR = transposition rate.

Table 5
Fraction of the *Drosophila buzzatii* Genome Introgressed with the Genome of *Drosophila koepferae*

Pair Crosses	TR	Introgressed Genome Fraction					
		Chr X	Chr 2	Chr 3	Chr 4	Chr 5	G
HI.1	0.067	0.536	0.000	1.000	1.000	0.000	0.481
HI.2	0.012	0.000	0.000	0.000	0.000	1.000	0.204
HI.3	0.000	1.000	1.000	1.000	0.143	0.182	0.685
HI.4	0.000	0.000	0.000	0.000	0.714	0.000	0.123
HI.5	0.000	1.000	1.000	1.000	1.000	0.000	0.796
HI.6	0.000	1.000	0.000	1.000	0.607	0.000	0.494
HI.7	0.000	0.286	0.000	1.000	0.179	1.000	0.500
HI.8[a]	0.012	0.000	0.000	0.000	0.000	0.000	0.000
HII.1	0.052	1.000	1.000	1.000	0.000	1.000	0.827
HII.2	0.006	0.464	0.000	0.628	0.821	0.212	0.401
HII.3	0.098	0.464	0.000	1.000	0.179	0.000	0.327
HII.4	0.000	0.286	0.000	0.000	0.143	0.000	0.074
HII.5	0.000	1.000	1.000	1.000	1.000	0.000	0.796
HII.6	0.000	1.000	1.000	0.857	0.750	1.000	0.926
HII.7	0.029	1.000	1.000	1.000	0.393	1.000	0.895
HII.8	0.040	1.000	0.053	1.000	0.857	0.000	0.549

Note.—Fraction is given by chromosome (Chr) and by genome (G) in the different hybrid lines (HI and HII, see fig. 1). TR = transposition rates. $P > 0.05$ for all correlations.

[a] Only the dot chromosome was hybrid in this line.

1986; Labrador, Naveira, and Fontdevila 1990). Table 5 shows the fraction of introgressed genome for each cross, per single chromosome and per the complete set. The Kendall's tau correlation coefficient was calculated for each chromosome and for the total amount of hybrid genome versus the transposition rate. The same correlation has been calculated in each hybrid sample independently and in the whole sample. Our results show that the correlation between transposition and the amount of introgression is always very low with different signs and never significant. Although we must be cautious with this result (mainly because of the low number of lines analyzed and the lack of statistical power of the test), it shows that the quantity of the genome that is introgressed does not have a strong effect on the transposition rate. Therefore, a qualitative rather than a quantitative factor is controlling transposition. Consequently, only small portions of the hybrid genome would be responsible for the observed effect. An alternative explanation for this result would be that a maternal effect is determining the observed transposition rate. This would explain the high transposition rate of line H1.8 (table 5) which is only hybrid for chromosome 6 (the dot chromosome). The mothers of analyzed females were hybrid for the entire genome.

Discussion

We addressed two important questions about transposable elements: (1) Can interspecific hybridization influence transposition? And (2) what is the transposition rate of a retrotransposon in a *Drosophila* line taken directly from the field? In order to answer these questions, we used the retrotransposon *Osvaldo* and the species *D. buzzatii* and *D. koepferae* as a model. Our system has the advantage of having a low copy number per genome, facilitating the determination of preexisting positions and the subsequent characterization of newly produced insertions in the entire genome.

The first conclusion on *Osvaldo* transposition comes from the observation that new insertions are not distributed independently. If transpositions were produced at random, the distribution of new insertions per larva will follow a Poisson distribution. Our data show, however, that most transpositions took place in only a few larvae. The DCs of the distribution number of insertions per larva range from 4.23 to 1.96. Dispersion coefficients larger than 1 indicate a contagious distribution in which the presence of a measured event increases the probability of finding a second one. *Osvaldo* transpositions occur in clusters of new insertions inside a larva and clusters of larvae with new insertions in the offspring of single-pair mates. This result corroborates our previous work, in which *Osvaldo* also showed transpositions in bursts (Labrador and Fontdevila 1994). Evidence that elements transpose in bursts is only circumstantial in cases in which a high number of copies are accumulating in a few of the lines studied. This kind of accumulation has been interpreted as true bursts of transpositions and has been previously described for several elements (Biemont, Aouar, and Arnault 1987; Nuzhdin and Mackay 1994).

The mechanisms controlling transposition in these systems are largely unknown. It is known, however, that *gypsy* transposition is controlled by a recessive mutation in the *flamenco* gene and that new insertions result from an infectious process manifested as a maternal effect (Kim et al. 1994; Pelisson et al. 1994; Song et al. 1994, 1997; Prud'homme et al. 1995; Labrador and Corces 1997). In the accompanying paper, we describe the structure of an active copy of *Osvaldo* that exhibits all the structural features of a true retrovirus (Pantazidis, Labrador, and Fontdevila 1999). Although we cannot demonstrate that transposition of *Osvaldo* is also an infectious process, this mechanism may account for the observed distribution of new insertions. Only some mothers from the preceding generation will produce enough particles to generate an infectious process that would be manifested only as transpositions in the germ line of the next generation.

Our experiments comparing transposition rates between outbred stocks and stocks of different degrees of inbreeding suggest that the transposition process of *Osvaldo* is somehow regulated but independent of the inbreeding. In our previous work, the BU-30 (30/4) line showed a transposition rate of about 10^{-3} transpositions per element per generation (Labrador and Fontdevila 1994), suggesting that inbreeding increased transposition because such rates have been described only for genetically unstable strains or hybrid dysgenic crosses (Harada, Yukuhiro, and Mukay 1990; Eggleston, Johnson-schlitz, and Engels 1988; Charlesworth and Langley 1989). Estimations of transposition rates in laboratory strains and in natural populations range between 10^{-4} and 10^{-5} per copy per generation (Eggleston, Johnson-schlitz, and Engels 1988; Charlesworth and Langley 1989; Suh et al. 1995; see also Labrador, Seleme, and Fontdevila 1998). Furthermore, in previous experiments

that showed high transposition rates, the high transposition rates were always related to some kind of inbreeding effect (Biemont, Aouar, and Arnault 1987; Nuzhdin and Mackay 1994). Our present data, using the same strain but with 43 more generations of additional inbreeding (BU-30 (73/4)), indicate that an increase in inbreeding is not necessarily accompanied by an increase in transposition rate. Moreover, a line recently obtained from the wild showed no differences from the inbred strain. This result suggests that inbreeding does not have a specific effect on transposition. The high transposition rate that we found could be explained by particular features of the lines used in this experiment or by the fact that *Osvaldo* transposes at rates higher than those described for other elements in *D. melanogaster*. Our results analyzing natural populations originated by a recent colonization process also suggest that the transposition rate of *Osvaldo* in natural populations must be high (Labrador, Seleme, and Fontdevila 1998). Finally, we observed that the variance in transposition rates is always lower in the inbred line than in the outbred or the hybrid lines. This result suggests that the differences between parents could be due to genetic factors segregating in the lines or due to the variability in the number of active copies of the retrotransposon segregating in the genome. These active copies would be present in all individuals in the inbred strain, decreasing the variance in transposition, and presumably are segregating in the outbred stocks, increasing the variance between lines.

The data that we show here indicate that there is not a correlation between transposition and the fraction of *D. koepferae* genome introgressed in *D. buzzatii* (table 5). This lack of correlation also supports the putative involvement of a maternal effect, as mentioned above. The transpositions that we are observing could have been originally induced in the mothers of the females that we directly tested, which were hybrids for the whole genome. This interpretation could explain the high transposition observed in one of the lines, which was hybrid only for the dot chromosome (table 5).

These results on transposition in hybrids are in complete agreement with those previously obtained in a similar experiment (Labrador and Fontdevila 1994). In that experiment, the use of a single inbred stock and a lower sample size precluded us from strongly concluding that interspecific hybridization was enhancing *Osvaldo* transposition. Now, with two independent experiments using different *D. buzzatii* and *D. Koepferae* strains, we have been able to show that *Osvaldo* systematically transposes more in interspecific hybrids. As stated above, the hybrid dysgenesis syndromes described and the associated sterility in F_1 hybrids early suggested a mechanism of speciation triggered by transposition. In that sense, our results showing an increase of transposition rate in hybrids between *D. buzzatii* and *D. koepferae*, two sibling species, represent a first step in testing the involvement of transposable elements in speciaton processes.

Acknowledgments

We thank Tatiana Gerasimova for a critical reading of the manuscript and Ignacio Marín for his helpful comments on a previous version of this manuscript. This work has been supported by Spanish Dirección General de Investigación Científica y Técnica grants PB90/0711, PB93/0843, and PB96-1136 to A.F. and by a Catalonian Comissió Interdepartamental de Recerca i Tecnologia (CIRIT) grant to F.U. A predoctoral fellowship from CIRIT to M.L. is also acknowledged.

LITERATURE CITED

BIEMONT, C., A. AOUAR, and C. ARNAULT. 1987. Genome reshuffling of the *copia* element in an inbred line of *Drosophila melanogaster*. Nature **329**:742–744.

BLACKMAN, R. K., and W. M. GELBART. 1989. The transposable element *hobo* of *Drosophila melanogaster*. Pp. 523–529 *in* D. E. BERG and M. M. HOWE, eds. Mobile DNA. American Society for Microbiology, Washington, D.C.

BUCHETON, A. 1990. I transposable elements and I-R hybrid dysgenesis in *Drosophila*. Trends Genet. **6**:16–21.

CHARLESWORTH, B., and C. H. LANGLEY. 1989. The population dynamics of *Drosophila* transposable elements. Annu. Rev. Genet. **23**:251–287.

CORCES, V. G., and P. K. GEYER. 1991. Interactions of retrotransposons with the host genome: the case of the *gypsy* element of *Drosophila*. Trends Genet. **7**:86–90.

COYNE, J. A. 1989. Mutation rates in hybrids between sibling species of *Drosophila*. Heredity **63**:155–162.

EGGLESTON, W. B., D. JOHNSON-SCHLITZ, and W. R. ENGELS. 1988. P-M hybrid dysgenesis does not mobilize other transposable element families in *Drosophila melanogaster*. Nature **331**:368–370.

ENGELS, W. R. 1989. *P* elements in *Drosophila melanogaster*. Pp. 437–484 *in* D. E. BERG and M. M. HOWE, eds. Mobile DNA. American Society for Microbiology, Washington, D.C.

EVGEN'EV, M. B., G. N. YENIKOLOPOV, N. I. PEUNOVA, and Y. V. ILYIN. 1982. Transposition of mobile genetic elements in interspecific hybrids of *Drosophila*. Chromosoma **85**: 375–386.

EVGEN'EV, M. B., H. ZELENTOSOVA, N. SHOSTAK, M. KOZITSINA, V. BRASKYI, D.-H. LANKENAU, and V. CORCES. 1997. Mobilization of multiple transposable elements during hybrid dysgenesis in *Drosophila virilis*. Proc. Natl. Acad. Sci. USA **94**:196–201.

FINNEGAN, D. J. 1989. The I factor and I-R hybrid dysgenesis in *Drosophila melanogaster*. Pp. 503–517 *in* D. E. BERG and M. M. HOWE, eds. Mobile DNA. American Society for Microbiology, Washington, D.C.

FONTDEVILA, A. 1992. Genetic instability and rapid speciation: are they coupled? Genetica **86**:247–258.

GARCIA-GUERREIRO, M. P. 1996. Behavior of the transposable elements copia and mdg1 in hybrids between the sibling species *Drosophila melanogaster* and *D. simulans*. Heredity **77**:40–46.

GERSTEL, D. V., and J. A. BURNS. 1967. Phenotypic and chromosomal abnormalities associated with the introduction of heterochromatin from *Nicotiana otophora* into *N. tabacum*. Genetics **56**:483–502.

HARADA, K., K. YUKUHIRO, and T. MUKAY. 1990. Transposition rates of movable genetic elements in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **87**:3248–3252.

HEY, J. 1989 Speciation via hybrid dysgenesis: negative evidence from the *Drosophila affinis* subgroup. Genetica **78**: 97–104.

JOHNSON, N. L., and S. KOTZ. 1969. Discrete Distributions. John Wiley and Sons, New York.

KIM, A., C. TERZIAN, P. SANTAMARIA, A. PELISSON, N. PRUD'HOMME, and A. BUCHETON. 1994. Retroviruses in invertebrates: the *gypsy* retrotransposon is apparently an infectious retrovirus of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **91**:1285–1289.

LABRADOR, M., and V. G. CORCES. 1997. Transposable element-host interactions: regulation of insertion and excision. Annu. Rev. Genet. **31**:381–404.

LABRADOR, M., and A. FONTDEVILA. 1994. High transposition rates of *Osvaldo*, a new *Drosophila buzzatii* retrotransposon. Mol. Gen. Genet. **245**:661–674.

LABRADOR, M., H. NAVEIRA, and A. FONTDEVILA. 1990. Genetic mapping of the *Adh* locus in the repleta group of *Drosophila* by *in situ* hybridization. J. Hered. **81**:83–86.

LABRADOR, M., M. C. SELEME, and A FONTDEVILA. 1998. The evolutionary history of *Drosophila buzzatii* XXXIV. The distribution of the retrotransposon *Osvaldo* in original and colonizing populations. Mol. Biol. Evol. **15**:1532–1547.

MARIN, I., and A. FONTDEVILA. 1995. Characterization of *Gandalf*, a new inverted repeat transposable element of *Drosophila koepferae*. Mol. Gen. Genet. **248**:423–433.

———. 1998. Stable *Drosophila buzzatii-Drosophila koepferae* hybrids. J. Hered. **89**:336–339.

MILLER, D. D. 1950. Observations on two cases of interspecific hybridization with *Drosophila athabasca.* Am. Nat. **84**:81–93.

NAVEIRA, H., and A. FONTDEVILA. 1985. The evolutionary history of *Drosophila buzzatii.* IX. High frequencies of new chromosome rearrangements induced by introgressive hybridization. Chromosoma **91**:87–94.

NAVEIRA, H., J. PLA, and A. FONTDEVILA. 1986. The evolutionary history of *Drosophila buzzatii* XI. A new method for cytogenetic localization based on asynapsis of polytene chromosomes in interspecific hybrids of *Drosophila.* Genetica **71**:199–212.

NUZHDIN, S. V., and T. F. C. MACKAY. 1994. Direct determination of retrotransposon transposition rates in *Drosophila melanogaster*. Genet. Res. **63**:139–144.

———. 1995. The genomic rate of transposable element movement in *Drosophila melanogaster*. Mol. Biol. Evol. **12**:180–181.

NUZHDIN S. V., E. G. PASYUKOVA, and T. F. MACKAY. 1996. Positive association between *copia* transposition rate and copy number in *Drosophila melanogaster*. Proc. R. Soc. Lond. B Biol. Sci. **263**:823–831.

PANTAZIDIS, A., M. LABRADOR, and A. FONTDEVILA. 1999. The retrotransposon *Osvaldo* from *Drosophila buzzatii* displays all structural features of a functional retrovirus. Mol. Biol. Evol. **16**:909–921.

PELISSON, A., S. SONG, N. PRUD'HOMME, P. A. SMITH, A. BUCHETON, and V. CORCES. 1994. *Gypsy* transposition correlates with the production of a retroviral envelope-like protein under the tissue-specific control of the *Drosophila flamenco* gene. EMBO J. **13**:4401–4411.

PETROV D. A., J. L. SCHUTZMAN, D. L. HARTL, and E. R. LOZOVSKAYA. 1995. Diverse transposable elements are mobilized in hybrid dysgenesis in *Drosophila virilis.* Proc. Natl. Acad. Sci. USA **92**:8050–8054.

PRUD'HOMME, N., M. GANS, M. MASSON, C. TERZIAN, and A. BUCHETON. 1995. *flamenco,* a gene controlling the *gypsy* retrovirus of *Drosophila melanogaster*. Genetics **139**:697–711.

ROSE, M. R., and W. F. DOOLITTLE. 1983. Molecular biological mechanisms of speciation. Science **220**:157–162.

RUIZ, A., A. FONTDEVILA, and M. WASSERMAN. 1982. The evolutionary history of *Drosophila buzzatii* III. Cytogenetic relationships between two sibling species of the buzzatii cluster. Genetics **101**:503–518.

SONG, S. U., T. GERASIMOVA, M. KURKULOS, L. D. BOEKE, and V. CORCES. 1994. An Env-like protein encoded by a *Drosophila* retroelement: evidence that *gypsy* is an infectious retrovirus. Genes Dev. **8**:2046–2057.

SONG, S. U., M. KURKULOS, J. D. BOEKE, and V. G. CORCES. 1997. Infection of the germ line by retroviral particles produced in the follicle cells: a possible mechanism for the mobilization of the *gypsy* retroelement of *Drosophila.* Development **124**:2789–2798.

STURTEVANT, A. H. 1939. High mutation frequency induced by hybridization. Proc. Natl. Acad. Sci. USA **25**:308–310.

SUH, D. S., E. H. CHOI, T. YAMAZAKI, and K. HARADA. 1995. Studies on the transposition rates of mobile genetic elements in a natural population of *Drosophila melanogaster.* Mol. Biol. Evol. **12**:748–758

THOMPSON, J. N. JR., and R. C. WOODRUFF. 1978. Mutator genes—pacemakers of evolution. Nature **274**:317–321.

VIEIRA, C., and C. BIEMONT. 1997. Transposition rate of the 412 retrotransposable element is independent of copy number in natural populations of *Drosophila simulans.* Mol. Biol. Evol. **2**:185–188.

VIEIRA, J., C. P. VIEIRA, D. L. HARTL, and E. R. LOZOVSKAYA. 1998. Factors contributing to the hybrid dysgenesis syndrome in *Drosophila virilis.* Genet. Res. **71**:109–117.

WHARTON, L. T. 1942. Analysis of the repleta group of *Drosophila.* Univ. Tex. Publ. **4228**:23–53.

PIERRE CAPY, reviewing editor

Accepted March 22, 1999