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High transposition rates of *Osvaldo*, a new *Drosophila buzzatii* retrotransposon*

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Abstract Transposition of a new *Drosophila* retrotransposon was investigated. Total genomic Southern analysis and polytene in situ hybridizations in *D. buzzatii* strains and other related species using a 6 kb *D. buzzatii* clone (cDb314) showed a dispersed, repetitive DNA pattern, suggesting that this clone contains a transposable element (TE). We have sequenced the cDb314 clone and demonstrated that it contains all the conserved protein sequences and motifs typical of retrovirus-related sequences. Although cDb314 does not include the complete TE, the protein sequence alignment demonstrates that it includes a defective copy of a new long terminal repeat (LTR) retrotransposon, related to the *gypsy* family, which we have named *Osvaldo*. Using a *D. buzzatii* inbred line in which all insertion sites are known, we have measured *Osvaldo* transposition rates in hybrids between this *D. buzzatii* line and its sibling species *D. koepferae*. The results show that *Osvaldo* transposes in bursts at high rate, both in the *D. buzzatii* inbred line and in species hybrids.

Key words Transposable elements · Retrotransposons Transposition rates · *Drosophila buzzatii* Interspecific hybridization

Introduction

The causes of transposable element (TE) mobilization are not well understood. However, since the early work of McClintock (1951) on maize, episodes of increased transposition have been related to a set of genetic instabilities in certain crosses and strains in different organisms. High mutation rates and hybrid sterility are the most important, but not the only, manifestation of this instability. Perhaps the best documented cases of instability, designated as hybrid dysgenesis, are those elicited by intraspecific crosses between certain strains of *Drosophila*. At present, hybrid dysgenesis is known to be caused by high rates of transposition of P, I or *Hobo* elements (Engels 1989; Finnegan 1989; Blackman and Gelbart 1989).

The involvement of transposable elements in speciation processes has been postulated by several authors (Thomson and Woodruff 1978; Rose and Doolittle 1983), as a consequence of their ability to generate genetic instability and sterility under stressful conditions, such as hybridization. However, the direct demonstration of such an involvement has remained elusive. In particular, if this hypothesis is true one should find genetic instabilities in species hybrids. Several examples of genetic instability, evidenced by increased mutation rates, have been reported in species hybrids, ranging from early observations of high rates of lethal and visible mutations in *Drosophila* hybrids (Sturtevant 1939; Miller 1950) to more recent reports of increased production of chromosomal rearrangements in hybrids of *Nicotiana* (Gerstel and Burns 1967) and *Drosophila* (Naveira and Fontdevila 1985) and in subspecies hybrids of grasshoppers (Shaw et al.1983) and *Chironomus* (Hagele 1984). These genetical disorders are similar to those produced during hybrid dysgenesis and they may be produced by TEs. However, no direct estimates of transposition rates have ever been reported in species hybrids; however, evidence of transposition has been reported in hybrids between *D. virilis* and *D. littoralis* for the pDv111 element (Evgen'ev et al. 1982), between two subspecies of *Chironomus thummi* for *Cla* elements (Schmidt 1984) and between *D. simulans* and *D. mauritiana* for the *mariner* element (Haymer and Marsh 1986). On the other hand, Hey (1989) found no increase in the rate of occurrence of visible mutations in *D. affinis* subgroup hybrids and Coyne (1989) did not detect high

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* This paper is dedicated posthumously to Osvaldo A. Reig in recognition of his contributions to evolutionary biology and his early appreciation of the role of transposable elements in evolution

rates of germ-line excision for the *mariner* element in interspecific hybrids of the *D. melanogaster* subgroup, although he found high somatic excision rates in the same hybrids. These considerations suggest that hybridization could be considered as a release mechanism for mobilization.

Although some workers initially postulated that different retrotransposons were mobilized by hybrid dysgenesis (Gerasimova et al. 1985), it has been demonstrated that no other TEs differing from P are mobilized during the syndrome (Eggleston et al. 1988). However, this result does not demonstrate that other types of hybridization are incapable of mobilizing retrotransposons. Although we do not know how retrotransposons regulate their transposition, there are some results showing an increase of transposition in response to the copy number or to a particular genotype in yeast (Wilkie et al. 1992). Also, it is known that there is a complex mechanism of transcriptional control for different *Drosophila* retrotransposons (Parkhurst and Corces 1987; Arkhipova and Ilyin 1992).We also know that one or more retrotransposons are mobilized at high rates after certain outcrosses with particular stocks (Gerasimova et al. 1984, 1985; Leigh-Brown et al. 1989; Nével-Ninio et al. 1989; Georgiev et al. 1990; Pasyukova and Nuzhdin 1993) and in several unstable strains (Kim et al. 1990). Most interestingly the *Ulysses* element, a long terminal repeat (LTR) retrotransposon, is mobilized in *D. virilis* during a syndrome similar to hybrid dysgenesis (Lozovskaya et al. 1990; Scheinker et al. 1990).

These data favour the hypothesis that retrotransposons are under genetic control and that outcrossing may also induce their mobilization. A putative role of interspecific hybridization in mobilizing TEs was suggested by Fontdevila (1987, 1988) based on the observation that hybrids between *D. buzzatii* and *D. koepferae*, two sibling species of the repleta group (Wasserman 1982; Fontdevila et al. 1988), yielded progenies with high frequencies of new chromosomal rearrangements (Naveira and Fontdevila 1985). Here we show that the cDb314 clone from *D. buzzatii* contains a defective copy of a new LTR retrotransposon, which we have named *Osvaldo*. We have measured *Osvaldo* transposition rates in the whole genome during one or a few generations by analysing a large sample of larvae, and demonstrated that *Osvaldo* transposition takes place in bursts at high rates, suggesting that transpositions are related to species hybridization and/or inbreeding.

Materials and methods

Drosophila stocks

The clone cDb314 was obtained from the *D. buzzatii* stock BU-24 from Vipos (Argentina). *D. buzzatii* stocks used for Southern blots and in situ hybridizations came from: Argentina (BU-6, BU-35 and B-SL from San Luis, BU-17 from Quilmes, BU-40 from Ticucho, BU-23 and BU-39 from Arroyo Escobar, BU-42 from Maimara and BU-43 from Tapia); Bolivia (BU-20 from Los Negros); Chile (BU-10 from Melocotón); Spain (BU-13 from Adeje, Canary Islands; BU-11 and BU-30 from Carboneras and BU-1 from Portosín), and Tunisia (BU-26 from Kariouan).

The remaining species stocks were: KO-4 (*D. koepferae* from Vipos, Argentina); KO-2 and KO-SL (*D. koepferae* from San Luis, Argentina); SD-14 (*D. serido* from Cafarnaum, Brazil, BG no.15081–1431.4); BM-1 (*D. borborema* from Cafarnaum, Brazil, Bowling Green stock no.1508–1281); SM-1 (*D. starmeri* from Guaca, Venezuela); UN-5 (*D. uniseta* from La Boca, Venezuela); MA-4 (*D. martensis* from Guaca, Venezuela); VZ-6 (*D. venezolana* from Los Roques, Venezuela); MU-2 (*D. mulleri* from Lake Travis, Tex., USA, BG no.1371.0)

All the lines used for in situ hybridization originated from a single pair mating from one of the original stocks (listed above) and were maintained subsequently by successive single brother-sister matings in each generation. We designate the resulting lines by the original stock name followed by the number of inbreeding generations and the specific line number (in parenthesis). Thus, BU-30(28/4) means the inbred line number 4 after 28 generations of inbreeding since the foundation of the line from the BU-30 original stock.

Nucleic acid preparations

Plasmid DNA for general purposes was extracted by the alkaline lysis procedure as described in Maniatis et al. (1982). Double-stranded supercoiled plasmid DNA of high quality was obtained for sequencing using Magic Minipreps (Promega). Single-stranded M13mp18 or mp19 DNA for sequencing was extracted according to Sambrook et al. (1989). Genomic DNA extractions from *Drosophila* adults were made according to Piñol et al. (1988).

Hybridization procedures

Southern blots were performed as in Maniatis et al. (1982). Two to three micrograms of total genomic DNA from *Drosophila* adults were digested to completion with *Hin*dIII, *Bam*HI, *Sal*I or *Xho*I, electrophoresed in 0.6% agarose gels and transferred to Hybond-C Extra nitrocellulose filters (Amersham). Probes were obtained by random-primed labeling of 300 ng of plasmid DNA with biotin-11-dUTP (Sigma Chemical Co.) or digoxigenin-11-dUTP (Boehringer Mannheim). Details of the hybridization and posthybridization procedures are given in the protocol provided with the BluGENE kit (Bethesda Research labs) and the digoxigenin supplier. Hybridization was performed at 42°C with 50% formamide and the maximum stringency posthybridization washes were carried out at 50°C and 0.16 × SSC (1 × SSC is 0.15 M NaCl, 15 mM sodium citrate). Slides for in situ hybridizations were obtained from mature third instar larvae grown in uncrowded cultures, as described in Labrador et al. (1990). Probes were obtained as described above using digoxigenin-11-dUTP. Prehybridization and chromosome denaturation processes were carried out according to Engels et al. (1986). Hybridization solutions and posthybridization washes were made following the method described by de Frutos et al. (1989).

Cloning of cDb314

A plasmid library was constructed using *D. buzzatii* DNA (BU-24 stock), digested to completion with *Hin*dIII and ligated into the plasmid pUC13. The resulting products were transformed in the DH5 *Escherichia coli* strain. Clone cDb314 was selected based on its repetitive hybridization pattern.

Sequencing of cDb314

Restriction digestions of cDb314 were used to obtain an overlapping series of DNA fragments, subcloned either in pBluescript

KS+ or SK+, or in M13mp18 or mp19 for double- or single-stranded DNA sequencing reactions, respectively. DNA sequencing was performed by the dideoxy chain-termination method (Sanger et al.1977), using the T7 DNA polymerase sequencing kit (Pharmacia) and [^{35}S]dATP (Amersham) or, in two reactions, the ALF automatic sequencing system of Pharmacia. Specific oligonucleotides were designed when no restriction sites were available. DNA and protein sequences were analysed using the PCGENE package of programs and compared with sequences in the EMBL and Genbank databases. The FastA program was used to establish DNA and protein simple alignments between different sequences and the CLUSTAL V (Higgins et al. 1992) program was used to establish the protein multiple alignments.

Experimental design

The experimental design developed to detect new insertions by in situ hybridization on polytene chromosomes is shown in Fig. 1. Inbred lines BU-30(30/4) and KO-SL (5/3) were used as the starting material. At generation 30, 20 offspring larvae from a BU-30(30/4) single pair were analysed by in situ hybridization using the cDb314 probe and all positions occupied by the element were located. These positions are, with high probability, homozygous (fixed) for the element, due to the inbred condition of this line. Inbred line KO-SL (5/3) was characterized by hybridizing in situ three offspring larvae from a single pair. This allowed us to detect three euchromatic positions which, as in the case of BU-30(30/4) line, were present in all analysed larvae. It is possible that some positions were not detected in the *D. koepferae* line, due to the low number of hybridized larvae. This may represent a difficulty in scoring new positions in mass hybrids (see below), but not in single mating hybrids where undetected KO-SL(5/3) positions will show a 1:1 segregation in the offspring, distinct from the unique presence of a new position in only one larva. Additionally, the mass mating design does not discriminate between new insertions produced between the generation of characterization of the line and the generation of the screening. These difficulties with mass mating crosses, and problems associated with the characterization of previous variability have repeatedly occurred in previous experiments by other authors, and we have designed the single mating scheme of crosses in order to avoid them.

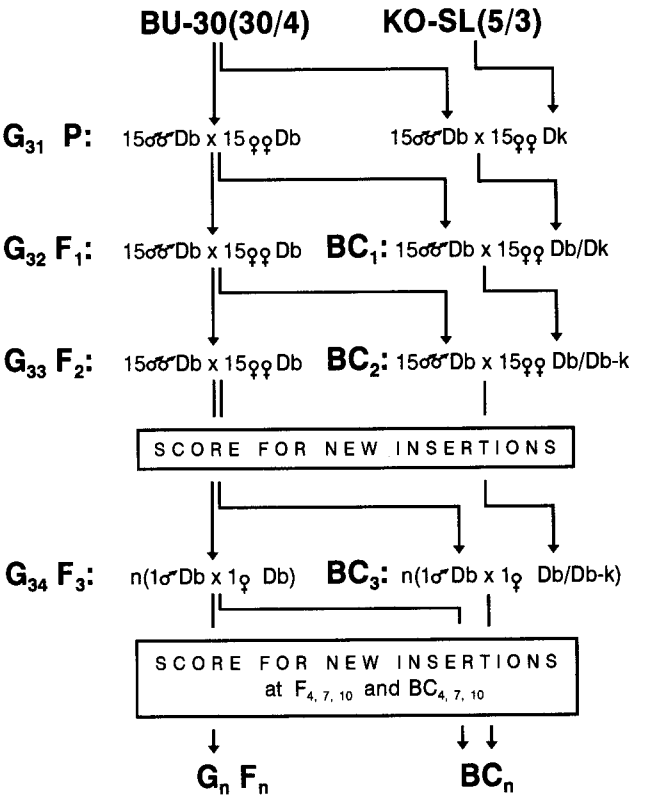


Fig. 1 Experimental design used to detect new insertions. Female F_1 hybrids are designated by Db/Dk (*Drosophila buzzatii*/*D. koepferae*), and segmental hybrids with one or several Dk introgressed segments by Db/Db-Dk. G_n denotes the generation number since the establishment of the *D. buzzatii* line. P, F_n and BC_n are, respectively, the parental cross, subsequent crosses of the *D. buzzatii* line and subsequent backcrosses of hybrid females. The mass cross design was carried out from G_{31} to G_{33}. New insertions were scored in the F_2 and BC_2 larvae. Single-pair mating crosses were started at F_3 and BC_3 with many (*n*) single-pair vials in each generation. New insertions were scored in F_4, F_7, F_{10}, BC_4, BC_7 and BC_{10} offspring

Mass mating design

The first interspecific cross (P) was performed by mass mating 15 BU-30(30/4) offspring males with 15 KO-SL(5/3) offspring females. At the same time an intraspecific mass mating line was started with 15 pairs from the BU-30(30/4) offspring. The interspecific cross yielded a few hybrids, consisting of sterile males and fertile females. Fifteen hybrid females (Db/Dk) were backrossed (BC_1) to 15 *D. buzzatii* males from the intraspecific line. The offspring of the BC_1 contains a mixture of hybrid genotypes with different introgressed recombinant chromosomal segments (Db/Db-k, segmental hybrids), ranging from pure *D. buzzatii* (Db/Db) to whole hybrids (Db/Dk). Fifteen segmental hybrid females were backrossed (BC_2) to 15 *D. buzzatii* males from F_1 offspring. Eighty larvae from each BC_2 and F_2 offspring (a total of 160) were scored for new insertions.

Single pair mating design

At generations F_3 and BC_3 inbred and hybrid lines could be established. The inbred line was maintained subsequently by sib-pair matings. Hybrid lines (segmental hybrid) were maintained by crossing one inbred male with one segmental hybrid female each generation. Actually, both hybrid and non-hybrid females from the hybrid lines were individually mated, but only hybrid female crosses were selected to maintain the hybrid lines. This selection was performed by asynapsis observation in the polytene chromosomes of offspring larvae

(for a more detailed explanation of the selection procedure see Naveira and Fontdevila 1985, 1986). A total of 463 offspring larvae were scored for new insertions, 269 from hybrid lines and 194 from inbred lines (Table 2).

Interspecific chromosomal markers

Chromosomal asynapsis was used as a marker for introgression of *D. koepferae* chromosomal segments into the *D. buzzatii* genome. The consistent asynapsis along hybrid regions allows us to detect any introgressed *D. koepferae* chromosomal segment, regardless of size and position. This procedure is reliable and can reveal even a small number of polytene bands from *D. koepferae* chromosomes introgressed in a *D. buzzatii* genomic background (Naveira and Fontdevila 1986; Labrador et al. 1990) and now is a routine procedure in our laboratory (Naveira and Fontdevila 1985, 1991a, 1991b; Naveira et al. 1986).

Determination of new insertions

New insertions were detected by comparing offspring site distributions with the fixed positions in the characterized BU-30(30/4) line. Positions located in hybrid chromosomal segments were also compared with the characterized positions of KO-SL(5/3). As stated above, in segmental hybrids from mass mating lines some positions in the hybrid chromosomal segment could be old, undetected KO-SL(5/3) positions. In the following paragraph, we give reasons believing that in our data such positions are new; nevertheless, we have omitted these positions from the data used to compute rates of transposition.

It has been argued that some experiments involving transposition of mobile elements cannot exclude the possibility that the new positions were due to contaminations instead of true new insertions (Engels 1989). We have dealt with this problem in different ways. Firstly, we used chromosomal rearrangements as genetic markers of our lines. BU-30(30/4) is fixed for the 2J and 4ST rearrangements, and no other inversions have been observed during the whole experiment (more than 623 larvae have been checked in this way). Secondly, the experimental results with single pair lines argue against the contamination hypothesis. More explicitly, if there is contamination in one of these lines we expect each contaminated position to segregate, or to be present in different derived lines, depending on whether contamination occurred in the previous generation or several generations before the in situ hybridizations. On the other hand, if contamination happened in the same generation as the in situ hybridization, we expect either two different patterns of in situ hybridization, in the case of an intruder female, or segregation of the positions contaminated, in the case of a strange mating male. None of these offspring distributions were ever observed in the experiment. All new positions reported were found only once in each single pair mating line offspring. Note that the probability of a heterozygous position in one parent being detected in the offspring is 1/2, given that it behaves as a dominant allele in in situ hybridizations. As the minimum number of larvae analysed per single pair mating was 12 (in those matings that showed new insertions), the probability of detecting a pre-existing position in at least two larvae is ≥ 0.997. This means that we can consider all newly observed positions as new, recent insertions.

Transposition rates

Insertion rates were calculated as new insertions per genome, element and generation. However, in contrast to previous work, in which rates were computed by accumulating transpositions across many generations in a single chromosome, we have observed transpositions in the whole genome for one or a few generations. Only euchromatic positions were considered. We have detected seven original euchromatic sites and considered that both male and female contribute equally to transposition. Moreover, transposition rate was divided by 3 in the mass mating lines because we consider that any of the new insertions could be produced in the three previous generations elapsed from the time of BU30(30/4) characterization.

Results

Characterization of cDb314

Southern blot hybridizations of clone cDb314 to digested genomic DNA were performed. Using total DNA from 13 *D. buzzatii* stocks of different geographical origin, 9 stocks of species of the *D. buzzatii* and *D. martensis* clusters and one stock of *D. mulleri*, a more distantly related species (Wasserman 1982), we have demonstrated that cDb314 contains a repetitive sequence present in all stocks and species tested (Fig. 2a and b). Comparisons of hybridization bands on Southern blots of *Hin*dIII digests revealed a striking homogeneity of patterns among stocks (Fig. 2a) and even among species (Fig. 2b). The most prominent bands form a ladder common to all stocks and each species cluster is marked by a strong band that is shared by different members of the cluster (Fig. 2a and b). To investigate the nature of this ladder of conserved bands we performed Southern blots using three additional restriction enzymes for which different numbers of restriction sites exist inside the cDb314 clone. Figure 2c shows a Southern blot comparing the hybridization patterns of several *D. buzzatii* stocks and *Drosophila* species using *Bam*HI, an enzyme for which no restriction sites exist inside the cDb314

clone. Southern blots in Fig. 2d and 2e were carried out with DNA from three *D. buzzatii* stocks digested with *Sal*I (two restriction sites) and *Xho*I (one restriction site), respectively. Results with *Bam*HI (Fig. 2c) and *Sal*I (Fig. 2d) are similar to those obtained with *Hin*dIII, with most of the prominent bands shared by all tested *D. buzzatii* stocks and also with some bands common to several species. However, digestions with *Xho*I gave different hybridization patterns when stocks were compared, although a strong 3.6 kb band, probably an internal restriction fragment, appeared in the three lanes (Fig. 2e).

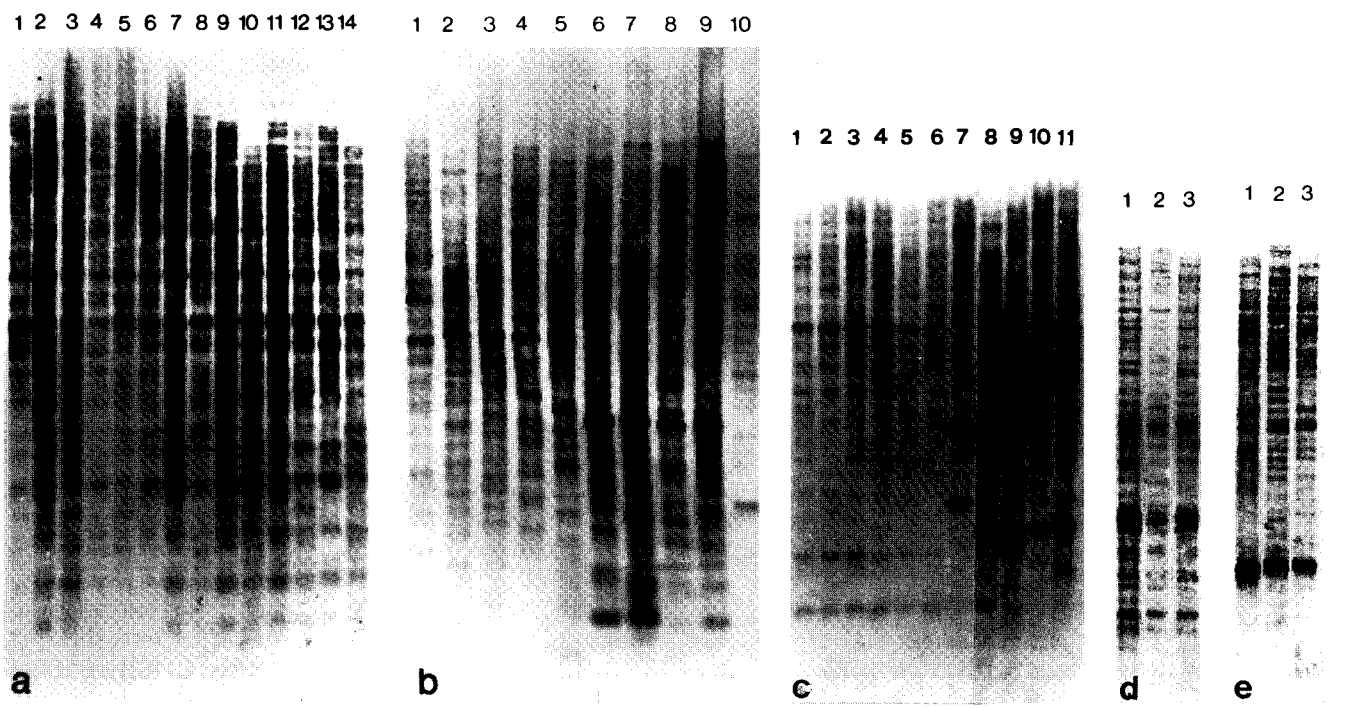
The TE contained in the cDb314 clone shows a dispersed pattern by in situ hybridization experiments. More than 50 different positions have been determined using *D. buzzatii* stocks of different geographical origin (data not shown). Figure 3 shows the differences of in situ hybridization found between three different inbred lines. Note that both euchromatic and β-heterochromatic positions were found, the β-heterochromatic hybridizations being the most abundant. The β-heterochromatic hybridization signal is particularly large in the X chromosome, extending through subdivisions G and H of the Wharton (1942) map. Figure 3a shows the hybridization pattern of the BU-42(28/7) line, in which no euchromatic positions have ever been detected. Figure 3b and c shows the hybridization patterns of the BU-30(28/11) and BU-30(28/4) lines, respectively. Differences in the hybridization patterns among lines in these and also in other derived lines (data not shown) most probably indicate that the original BU-30 stock had some position polymorphisms. The differences were taken as a preliminary suggestion of the mobility of the TE included in the cDb314 clone.

cDb314 sequencing

The restriction map, the sequencing strategy, a schematic representation of the open reading frames (ORFs) and the putative gene products encoded by the cDb314 sequence are shown in Fig. 4. The cDb314 clone consists of 6017 bp, of which 3120 bp are shown in Fig. 5. A display of the stop codons in the sequence (Fig. 4B) shows that there are only two putative coding regions, consisting of 329 and 735 amino acids, respectively. Translation of the ORFs results in protein sequences with a high degree of similarity to vertebrate retrovirus and retrotransposon gene products.

Probably cDb314 contains only the central region of the element and lacks the 5′ LTR terminal region (Figs. 4 and 5). The first identifiable amino acid sequence (between nucleotides 370 and 573) bears two putative nucleic acid-binding protein (NBP) domains, defined by the CX2CX4HX4C sequence motif. These domains have been included in the nucleocapsid (NC) protein and appear in retrovirus, non-LTR retrotransposon and *copia*-like retrotransposon *gag* genes (McClure 1991). Figure 6A shows the alignment of the

Fig. 2a–e Southern blots of *Hin*dIII- (**a** and **b**), *Bam*HI- (**c**), *Sal*I- (**d**) and *Xho*I- (**e**) digested genomic DNA from different *D. buzzatii* stocks and related species probed with the cDb314 clone. Stocks in **a** are: BU-6 (1), BU-17 (2), BU-40 (3), BU-23 (4), BU-39 (5), BU-43 (6), BU-20 (7), BU-10 (8), BU-13 (9), BU-26 (10), BU-11 (11), BU-30 (12), BU-1 (13) BU-35 (14). Species stocks in **b**, from the *D. buzzatii* cluster are: BU-SL (1), KO-4 (2), KO-2 (3), SD-14 (4), BM-1 (5); and from the *D. martensis* cluster: SM-1 (6), UN-5 (7), MA-4 (8), VZ-6 (9); MU-2 (10) is a *D. mulleri* stock. *D. buzzatii* stocks in **c**: BU-30 (1), BU-10 (2), BU-20 (3), BU-40 (4), BU-39 (5) and BU-6 (6). Species stocks in (c): KO-2 (7), SM-1 (8), UN-5 (9), MA-4 (10) and VZ-6 (11). *D. buzzatii* stocks in **d** and **e**: BU-11 (1), BU-20 (2) and BU-SL (3)



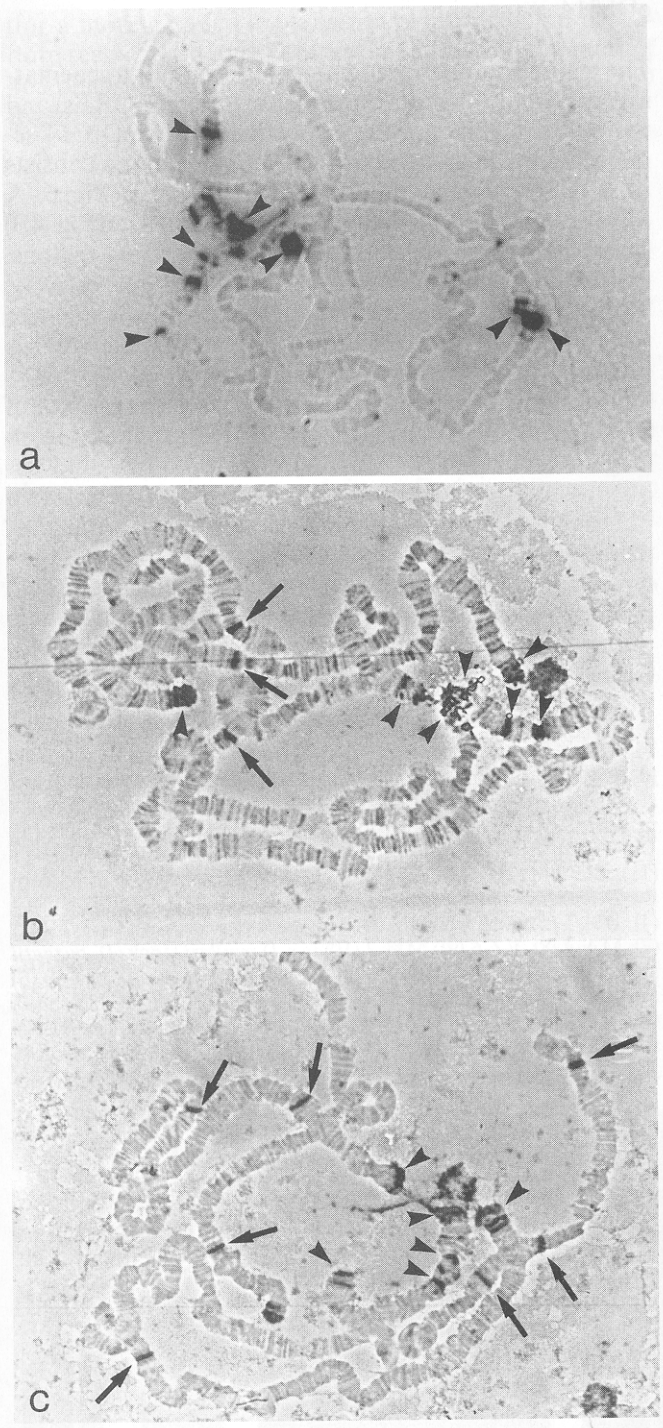


Fig. 3a–c Polytene chromosomes hybridized with the digoxigenin-labeled cDb314 probe. **a** BU-42(28/7) line; **b** BU-30(28/11) line; and **c** BU-30(28/4) line (hybridization sites are indicated in Table 1, in which this line is referred to as BU-30(30/4). *Arrowheads* show strong hybridization signals at pericentromeric areas. *Arrows* show euchromatic hybridizations. Note the lack of euchromatic sites in **a** and the differences in hybridization sites between **b** and **c**, two lines from the same original stock

cDb314 NC with those of the *mag* retrotransposon (Michaille et al. 1990) and the *HIV2* retrovirus (Hirsh et al. 1989). The end of the *gag* polyprotein coding region is not signalled in cDb314 by a stop codon or a frameshift, and the characteristic first *pol* gene product (the putative protease) is in the same frame as the *gag* gene. Retroviral proteases have very low levels of similarity (McClure 1991, 1992). However, as is shown in Fig. 6B for *412*, *TED* and cDb314 sequences, the amino-terminal and carboxyl-terminal ends, which are related to proteolytic processing of *gag* and *pol* polyproteins (Youngren et al. 1988), are conserved domains (McClure 1991, 1992).

All cDb314 *pol* sequences with conserved domains, i.e. reverse transcriptase (RT), ribonuclease H (RNaseH) and integrase (IN) have been described (Figs. 4 ,5 and 6). Although a nonsense mutation in-frame at the end of RT was found (Fig. 5), all coding regions are in the same order and can be perfectly aligned with their counterparts in other retroids (Fig. 6). Alignment of these putative products closely relate cDb314 with the *gypsy* group of LTR-containing retrotransposons from *Drosophila* such as *gypsy*, *17.6*, *297* and *412* (Doolittle et al. 1989; Xiong and Eickbush 1990). Figure 6C, D and E shows the alignment of enzymatic products of ORF2 and their equivalents from *412* (Yuki et al. 1986a) and *TED*, a retrotransposon from the moth *Trichoplusia ni* (Friesen and Nissen 1990). The highest scores and identities were obtained when simple alignments with *TED* and *412* were made; identity levels of 38.5% and 36.8% (RT), 48.5% and 46.1% (RH), and 22.5% and 23.8% (IN) were obtained for *TED* and *412*, respectively. The highest identity levels are always associated with the *TED* element, except when IN protein is compared. Alignment of the putative IN amino acid sequence gives the highest identity with the *412* element, with which cDb314 shares a very similar organization of HH/CC and adjacent domain (Fig. 6E). This domain, a putative zinc finger motif, contributes to specific recognition of LTRs in the cutting and binding reactions and demonstrated a high conservation between related sequences (Khan et al. 1990).

Because LTR retrotransposons of the *gypsy* group have a third coding region (the *env* gene), an in situ hybridization experiment was performed to establish the approximate limits of the element in the clone. The following probes were obtained with the different cDb314 subclones: 2.1 kb *Kpn*I, 0.6 kb *Spe*I-*Eco*RI, 0.6 kb *Hinc*II-*Kpn*I and 1.2 kb *Sal*I-*Hin*dIII (Fig. 4). All probes were hybridized against the BU-30(*n*/4) isofemale line, well characterized for cDb314 hybridization sites (Fig. 3), and found to hybridize at the same sites as the complete cDb314 probe. Consequently, all euchromatic sites detected by this probe belong to a single TE. We have named this element *Osvaldo*.

The cDb314 3′ region does not show recognizable structural features. Neither have clear ORFs been identified nor does any sequence stretch combine the expected features of an LTR (i.e. polypurine track, short inverted repeats, identifiable promoters or polyadenylation signals) that normally occur in other retrotransposons (Lankenau et al. 1988; Arkhipova and Ilyin 1991). Moreover, the sequence of the *Hin*dIII 3′ terminal fragment (Fig. 4) shows a nucleotide stretch with 63% iden-

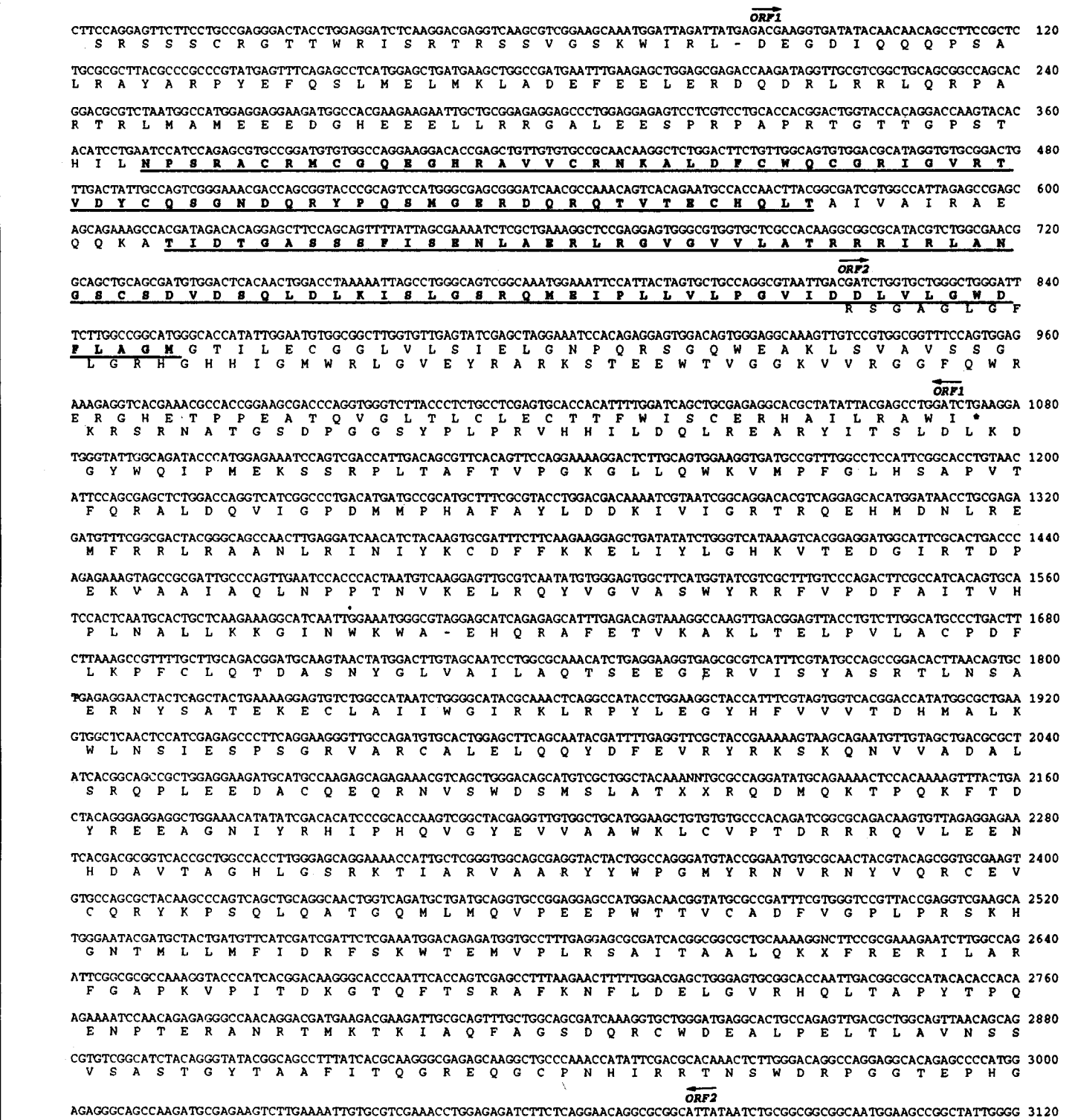


Fig. 5 cDb314 nucleotide sequence and deduced protein sequence. Only the first 3120 nucleotides are shown. Stop codons at the end of the putative ORFs are indicated by *asterisks*. Stop codons in-frame with the putative ORFs are indicated by *hyphens*. *N* and *X* are undetermined nucleotides and amino acid indeterminations respectively. Putative nucleic acid-binding motifs (NBPs) and the protease are *underlined* in the ORF1. Proteins encoded by ORF2 correspond to *POL* gene products and are displayed in Fig. 6

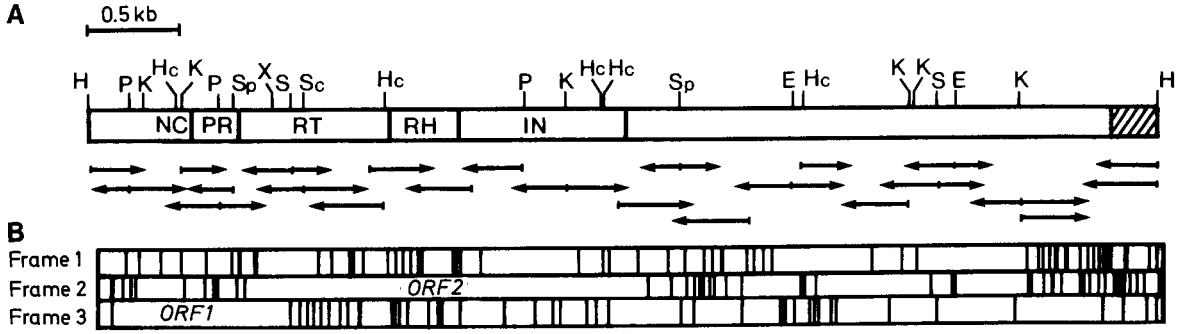


Fig. 4A, B Analysis of cDb314 clone. **A** Restriction map and sequencing strategy. Abbreviations of restriction enzyme sites in the map are: H, *Hin*dIII; P, *Pst*I; K, *Kpn*I; Hc, *Hin*cII; Sp, *Spe*I; S, *Sal*I; Sc, *Sac*I; X, *Xho*I; and E, *Eco*RI. The putative protein products and their gene locations in the clone are indicated in the *open bar*: nucleocapsid (*NC*), protease (*PR*), reverse transcriptase (*RT*), ribonuclease H (*RH*) and integrase (*IN*). The *dashed box* in the open bar shows the presence of a 270 bp DNA sequence similar to *297* and *17.6* TEs from *D. melanogaster*. **B** Display of the stop codons in all possible frames. Note that two putative open reading frames (ORFs) are in the first half of the clone but no clear ORF is found in the 3′ half

Fig. 6A–E Multiple sequence alignment of the putative protein products of **A** *mag* TE from *Bombyx mori*, cDb314 and *HIV2g* and (**B–E**) *412* TE from *Drosophila melanogaster*, cDb314, and *TED* from *Trichoplusia ni*. Amino acid identities between cDb314 and other sequences are indicated in *bold letters*. *Asterisks* show identity with all sequences aligned. *Arabic numerals* in the RT region designate the conserved amino acid domains used to establish phylogenetic relationships between different RT sequences by Xiong and Eickbush (1990). *Roman numerals* in the rest of the *POL* region indicate conserved domains at each one of the retroid-related proteins (McClure 1991, 1992)

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MAG   NC   INFNECSRCGDRRHQAKDCIYKD-YVCSSCHETG-HLRRMCPKNGLKNQAEAAGGSARTGRRGNRGAGGN
314   NC   NPSRACRMCGQEGHRAVVCRNKALDFCWQCGRIGVRTVDYCQSGNDQRYPQSMGERDQR-QTVTECHQLT
HIV2g NC   RKVIRCWNCGKEGHSARQCRAPRRQGCWKCGKTG-HVMAKC-PER-QAGFLGMGPWGKKPRNFPVAQAPP
A

                I                          II                                  III
412   PR   LLDTGADISILKENSDK-FSNIQI---TNKINIQGIGQQKIQSRGQTFIEIQTGKYVIPHDFHLVDKNFPIPCDGIIGIDFIKKY
314   PR   TIDTGASSSFISENLAERLRGVGVVLATRRRIRLANGSCSDVDSQLDLKISLGS---RQMEIPLLVL-PGVIDDLVLGWDFLAGM
TED   PR   LIDTGANQSFISPQAVQKYFSNYSVNYDPFEITNIHGVSRNEHSITLPCFQEFN---ETQDIKLFIYHFHDYFDGLIGLDLLSKW
B

          1                    2                              3                                  4
412   RT   LLVPKKSSPNSDKKKWRLVIDYRQINKKLLADKFPLPRIDDILDQLGRAKYFSCLDLMSGFHQIELDEGSRDITSFST-SNGSYRFTRLPFGLKIAPNSF
314   RT   WTVGGKVV--RGGFQWRKRS--RNATGSDPGGSYPLPRVHHILDQLREARYITSLDLKDGYWQIPMEKSSRPLTAFTVPGKGLLQWKVMPFGLHSAPVTF
TED   RT   WVVPKKIDA-SGKQKWRLVVDFRKLNEKTIDDKYPIPNISDVLDKLGKCQYFTTLDLASGFYQVEMDPQDISKTAFNV-EHGHFEFLRMPMGLKNSPSTF

          4                    5                              6                    7
412   RT   QRMMTIAFSGIEPSQAFLYMDDLIVIGCSEKHMLKNLTEVFGKCREYNLKLHPEKCSFFMHEVTFLGHKCTDKGILPDDKKYDVIQNYPVPHDADSARRF
314   RT   QRALDQVIGPDMMPHAFAYLDDKIVIGRTRQEHMDNLREMFRRLRAANLRINIYKCDPFKKELIYLGHKVTEDGIRTDPEKVAAIAQLNPPTNVKELRQY
TED   RT   QRVMDNVLRGLQNNICLVYLDDIIVYSTSLQEHLENLERVFQRLRESNFKIQMDKSEFLKLETAYLGHIISRDGIKPNPDKISAIQKYLIPKTPKEIKQF

412   RT   VAFCNYYRRFIKNFADYSRHITRLCKKNVPFEWTDECQKAFIHLKSQLINPTLLQYPD
314   RT   VGVASWYRRFVPDFAITVHPLNALLKKGINWKWAXEHQRAFETVKAKLTELPVLACPD
TED   RT   LGLLGYYRKFIPDFARLTKPLTQCLKKGSKVTLSPEYVNAFEHCKTLLTNDPILQYPD
C

                I                                  II                                  III
412   RH   FSKEFCITTDASKQACGAVLTQNHNGHQLPVAYASRAFTKGESNKSTTEQELAAIHWAIIHFRPYIYGKHFTVKTDHRPLTYLFSMVNPSSKLTRIRLEL
314   RH   FLKPFCLQTDASNYGLVAILAQTSEEGERVISYASRTLNSAERNYSATEKECLAIIWGIRKLRPYLEGYHFVVVTDHMALKWLNSIESPSGRVARCALEL
TED   RH   FTREFNLTTDASNFAIGAVLSQGPIGSDKPVCYASRTLNESELNYSTIEKELLAIVWATKYFRPYLFGRKFKILTDHKPLQWMMNLKDPNSRMTRWRLRL

              IV          V
412   RH   EEYNFTVEYLKGKDNHVADALSRITIKELK
314   RH   QQYDFEVRYRKSKQNVVADALSRQPLEEDA
TED   RH   SEYDFSVVYKKGKSNTNADALSRVEIHTTE
D

                          HHCC REGION                                                  II
412   IN   AILSTLHDDPIQGGHTGITKTLAKVKRHYYWKNMSKYIKEYVRKCQKCQKAKTTKHTKTPMT-ITETPEHAFDRVVVDTIGPLPKSENGNEYAVTLICDL
314   IN   QVLEENHDAVT-AGHLGSRKTIARVAARYYWPGMYRNVRNYVQRCEVCQRYKPSQLQATGQM-LMQVPEEPWTTVCADFVGPLPRSKHGNTMLLMFIDRF
TED   IN   DIIRHYHDGKT--NHRGINECYLALSKRYYWPRMKDQITKFINECTICGQAKYDRNPIRPQFNIVPPATKPLETVHHDLFTV------QNEKYITFIDVF

                                    III                                                  V
412   IN   TKYLVAIPIANKSAKT--VAKAIFESFILKYGPMKTFITDMGTEYKNSIITDLCKYLKIKNITSTAHHHQTVGVVERSHRTLNEYIRSYISTDKTD
314   IN   SKWTEMVPLRSAITAA--LQKXFRERILARFGAPKVPITDKGTQFTSRAFKNFLDELGVRHQLTAPYTPQE-NPTERANRTMKTKIAQFAGSDQRC
TED   IN   TKYGQAYHLRDG-TAISILQALLRFCTHH--GLPITIVTDNGTEFSNQLFSEFVRIHKIIHHKTLPHSPSDNGNIERFHSTILEHIRILKLQHKDE
E
```

tity to the *D. melanogaster* retrotransposons *297* and *17.6* (data not shown), suggesting that the *Osvaldo* element is interrupted, in the cDb314 sequence, at its 3′ end. Translation of this sequence showed fragmented stretches of similarity with *297* and *17.6* RTs, including the highly conserved YXDD motif characteristic of all RTs (Yuki et al. 1986b; Xiong and Eikbush 1990; McClure 1991, 1992); however, this homology stops at around nucleotide 270. The high number of stop codons indicates that this fragment is an inactive *D. buzzatii* element. This is the first time that a sequence similar to the *297* element has been reported outside the *D. melanogaster* subgroup (Martin et al. 1983; Brookfield et al. 1984).

Osvaldo transposition rate

Table 1 shows all hybridizing positions detected (original and new). Excluding two new positions in the F4 subdivision of chromosome 4 (larvae 9 and 12), where polytene bands are diffuse and difficult to tell apart, all new positions appeared only once, in a single larva. This is the result to be expected if transpositions are truly new insertions, since contaminations or segregation of pre-existing positions will yield the same sites in several larvae. Chromosomal asynapsis, as the marker for introgression, allows us to assign unambiguously those new positions located in hybrid chromosomal segments (see Fig. 7a).We found four of these sites (specified in Table 1) in the mass mated offspring. These positions are the only ones that might be occupied by segregating elements undetected in the KO-SL(5/3) line. In order to be conservative, we have disregarded these positions when computing transposition rates (Table 2). However, these positions always appeared associated with other new insertions outside the hybrid segment in the same larvae (Table 1) as would be expected if the phenomenon of transposition occurs in bursts. This intra-larval association of new positions occurred also in the inbred lines and in the hybrid lines of single-pair matings, where the hypothesis of undetected segregating positions from *D. koepferae* can be ruled out. These considerations suggest that these four sites found in hybrid segments are very probably the product of a recent burst of transposition.

Table 2 shows the frequency distributions of new insertions per larva. Frequently, more than one insertion was found per larva (Fig. 7). The analysis of goodness-of-fit shows that insertion frequencies in mass-mated hybrids deviate significantly from those expected of a Poisson distribution ($G=10.373$; $P=6.4\times10^{-4}$). Goodness-of-fit tests could not be carried out with the other samples because grouping of data leads to zero degrees of freedom. However, coefficients of dispersion (CD) for all samples (Table 2) are consistently much greater than 1, as expected for clumped samples that deviate from a Poisson distribution. This means that new insertions are not independently distributed, so comparisons of the number of new insertions are not statistically possible.

To test for differences between samples we used the data for number of larvae with new insertions (see

Table 1 Original positions and new insertions of *Osvaldo* element

	Chromosomes				
	X	2	3	4	5
Original positions					
BU-30(30/4)	G, H	A4a; F4a;H	A4a; H	F3b; H	A4b; C5b; F1f; H
KO-SL(5/3)	G, H	H	B4e; F1; D5; H	H	H
Larva[a]	New insertions				
1		B4b*		F1c	
2	B4a			G3e	
3			F1h	A5f	
4			E2f		
5				C3b	
6	C2b; D4g*; F2j*	G1a; E3e; E5a	A2d*; G2a	C3d	A3h
7				D4b	
8	B1a; B4d; C1b	C7b; F1c		E1e;E2d;E3f	E5g
9				F4	
10	E3-4				D5-4; F3
11	C4a; D1d; E2e		F4g		B4c
12				F4	
13		B4c			
14					F1g
15	B3a	F2b	B4e		G2d
16				E3	

[a] Larvae showing at least one insertion: 1 to 6, from mass mated hybrid lines; 7 to 9, from mass mated inbred lines; 10 to 14, from single-pair mated hybrid lines; and 15 to 16, from single-pair mated inbred lines

* Insertions in hybrid segments, not considered in the computation of insertion rates (Tables 2 and 3)

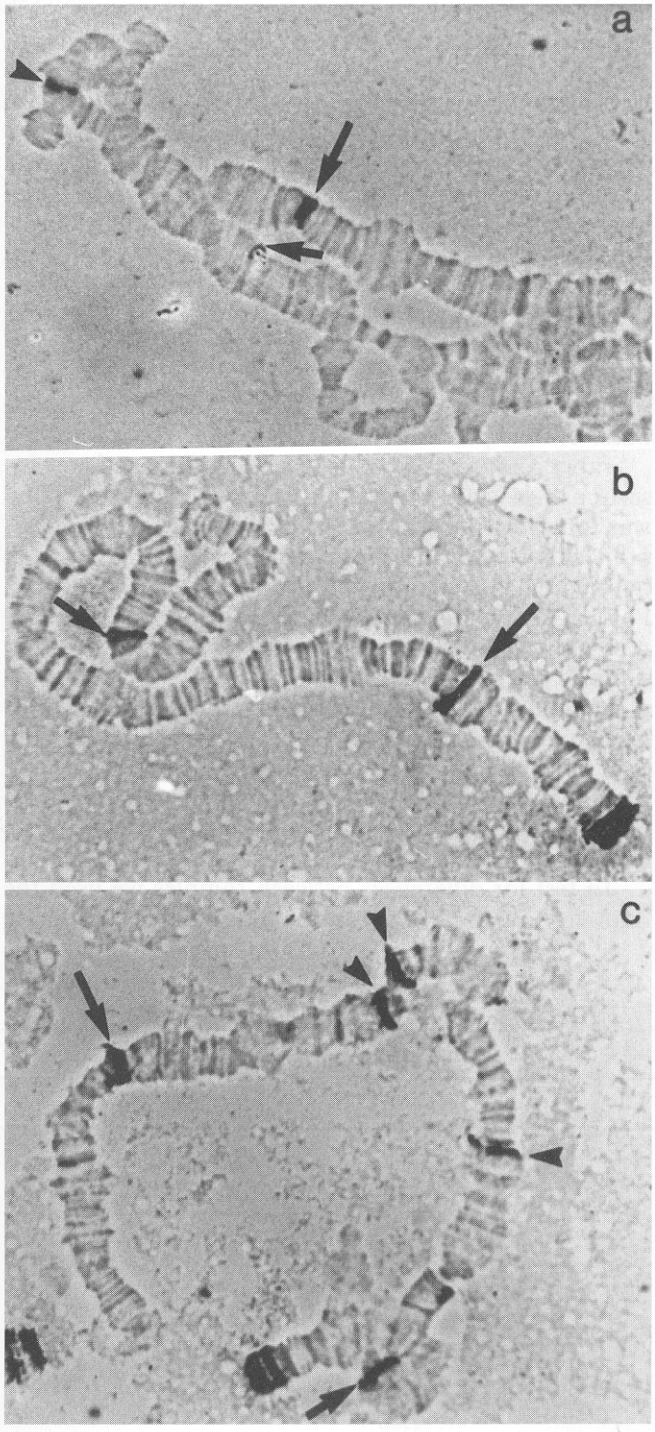

Fig. 7a–c In situ hybridization showing original (*arrows*) and new (*arrowheads*) insertion sites. **a** Second and fifth polytene chromosomes from a backcross hybrid larva (Db/Db-Dk). A new insertion is shown at the tip of the second chromosome. This insertion must have occurred in a *D. koepferae* chromosome in a hybrid female because it maps inside the $2l^9$ inversion (larva 1, Table 1). **b** Second chromosome showing the original sites at the start of the experiment [BU-30(30/4) in Table 1]. **c** Second chromosome showing three new insertions (larva 6, Table 1)

Table 2 Frequency distribution of *Osvaldo* new insertions[a] per larva. (*n* Number of new insertions per larvae, *MH* mass mated hybrids, *MI* mass mated inbred lines, *SPH* single pair hybrids, *SPI* single pair inbred lines)

n	MH	MI	SPH	SPI
0	74	77	264	192
1	3	2	3	1
2	2	0	0	0
3	0	0	1	0
4	0	0	0	1
5	0	0	1	0
7	1	0	0	0
9	0	1	0	0
CD[b]	4.2	7.5	3.3	3.4

[a] Excluding insertions in hybrid segments
[b] CD (Coefficient of dispersion) $= S^2/\bar{Y}$, where S^2 is the variance of the number of insertions per larva (Y) and $\bar{Y}$ is the Y mean

Table 3 *Osvaldo* insertion rates. (N_l Number of analysed larvae, N total number of new insertions, I insertion rates). I calculated as $N/(2N_l \times 7 \times n)$, where n is the number of generations (2 stands for both genomes and 7 for the number of euchromatic original positions; n = 3 for mass matings and n = 1 for single pair matings)

	N_l	N	I
Mass matings			
Hybrids	80	14	4.2×10^{-3}
Inbred lines	80	11	3.3×10^{-3}
Single-pair matings			
Hybrids	269	11	2.9×10^{-3}
Inbred line	194	5	1.8×10^{-3}

Table 2) and performed several 2×2 *G*-tests (Sokal and Rohlf 1981). The results showed that there are no significant differences between the type of cross in inbred lines ($G = 2.08$; $P = 0.1492$ with 1 d.f.), but differences are significant when hybrids are compared ($G = 5.386$; $P = 0.0210$ with 1 d.f.), favouring insertions in mass crosses. The rest of the paired comparisons gave no significant differences. Table 3 shows that more than 10^{-3} insertions per element per generation occurred both in hybrids and inbred lines, although the number of new insertions was always higher in the hybrids. Transposition rates in mass matings have to be considered as underestimates. As described in the Experimental design section, the transposition rates were divided by a factor of 3, considering that three generations have passed between the characterization of original positions and the screening of new positions.

Discussion

Osvaldo is an active TE present in all *D. buzzatii*-related species

cDb314 gives positive signals in Southern blot hybridizations against all *D. buzzatii* stocks and related

species. This result indicates that *Osvaldo* TE is an ancestral component of the genome in the *D. buzzatii* complex. This species distribution of *Osvaldo* TE contrasts with those of other TEs, which are often incongruent with the phylogenetic relationships of their hosts (Dowsett and Young 1982; Stacey et al. 1986). When Southern patterns are compared, a high degree of band conservation is observed, not only among *D. buzzatii* stocks but also among species. The possibility exists that Southern blots reveal *Osvaldo* copies that have remained fixed since the species diverged. This interpretation has been suggested (Vaury et al. 1989) for different repetitive sequences associated with *D. melanogaster* β-heterochromatin. Other interpretations are possible, such as heterochromatic tandem associations of the *Osvaldo* sequence or differential amplification in polytene chromosomes, as suggested by Di Franco et al. (1989) to explain prominent bands in *copia* Southern blot hybridizations.

There are several lines of evidence, however, suggesting that the most intense bands most probably result from restriction enzyme digestion inside the active copies of the element, i. e. those that have well conserved sequences. These sites are frequent in a large proportion of copies in each stock, leading to internal fragments revealed as intense conserved bands in Southern blots. The remaining conserved bands are the result of losses of restriction sites in inactive copies, probably heterochromatic ones. If this were true, we must postulate a large number of sequence classes in *Osvaldo*. In fact, out of a total of 830 bp sequenced from two additional clones only 55% of restriction sites are shared when compared with the cDb314 clone (data not shown). Thus, the number of common bands in Southern blots should be correlated with the number of internal restriction sites. This is in accordance with the results in Fig. 2, if we postulate that the number of restriction sites is high for *Hin*dIII and *Bam*HI, intermediate for *Sal*I and low for *Xho*I. On the other hand, in situ hybridization has shown a high signal density over pericentromeric areas and a low number of dispersed euchromatic sites, highly variable among different strains. This distribution is a feature of some TEs, particularly of those transposing by a reverse transcription mechanism (Vaury et al. 1989; Shevelyov et al. 1989; Charlesworth and Langley 1989), suggesting that this TE is an active element.

DNA sequencing shows that *Osvaldo* is an LTR-containing retrotransposon

An inherent problem in phylogenetic studies with retroelements is their high rate of DNA evolution. However, homologies are easy to find in amino acid sequences that are conserved across distantly related elements (Doolittle et al. 1989). Analysis of cDb314 DNA sequence showed around 50% identity with other LTR-containing retrotransposons (data not shown). However, this homology was found only when conserved regions were compared. The lack of homology in non conserved regions and the low DNA identity levels in conserved regions indicates that *Osvaldo* can be considered a new element.

Amino acid sequences analogous to retroviral *gag* and *pol* genes are encoded by the two large ORFs of cDb314. Alignment of all conserved protein regions relates the *Osvaldo* element to the *gypsy* retrotransposon group, which includes, among others, the *Drosophila gypsy*, *412*, *297* and *17.6* TEs (Doolittle et al. 1989; Xiong and Eickbush 1990). On the other hand, alignment of the cDb314 nucleocapsid (NC) sequence (Fig. 6A) gives a 48.3% identity along 28 amino acids with human immunodeficiency virus (*HIV2*) and simian immunodeficiency virus (*SIVs*; Hirsh et al. 1989). This high degree of identity is probably due to chance, given the low residue number and the high degree of similarity between all NCs (Covey 1986). Nonetheless, NCs are rare components of the *gag* gene in the group of LTR retrotransposons (McClure 1991) and have been described only in the *mag* element of *Bombyx mori* (Michaille et al. 1990) and in the *micropia* element, where some degenerate HC motifs have been described (Lankenau et al. 1988). Although NC seems implicated in RNA packaging in virus like particles (Covey 1986), retrotransposons such as *TY1* have all the necessary precursors for functional virus-like particle formation (Adams et al. 1987), and lack the coding capability for NC proteins (Clare and Farabaugh 1985). This means that NC is not necessary for transposition. The lack of NCs in the majority of the *gypsy* group retrotransposons can be explained by a trend to lose NC in the ancestral members of this group. Nevertheless, differences among elements of the same group in the sequence content of the *gag* gene, such as the presence or absence of an NC sequence, may be the result of recombinational events or independent gene assortments between different retroid sequences after long evolutionary periods, as suggested by McClure (1991) to account for the phylogenetic tree topologies found among non-LTR retrotransposons.

No ORF with recognizable protein products or DNA motifs has been detected between nucleotides 3080 and 5700. Since all subclones of this region hybridize in the same positions as the 2.1 kb *Kpn*I subclone, which contains all the putative coding information of the *Osvaldo* element, we conclude that this region is a degenerate sequence corresponding to the putative *Osvaldo env*-related gene. This sequence occupies the characteristic *env* position of retroviruses and most LTR-containing retrotransposons. However, amino acid sequence conservation of *env* polyproteins is too low to be detected when different elements are compared (Doolittle et al. 1989; McClure 1991, 1992). The unusual presence in the cDb314 clone of a protease sequence in ORF1, a stop codon at the end of RT and a putative *env* gene, probably truncated by non-functional stop codons, are evidence that this sequence is a non-functional copy of the *Osvaldo* element. Since clones

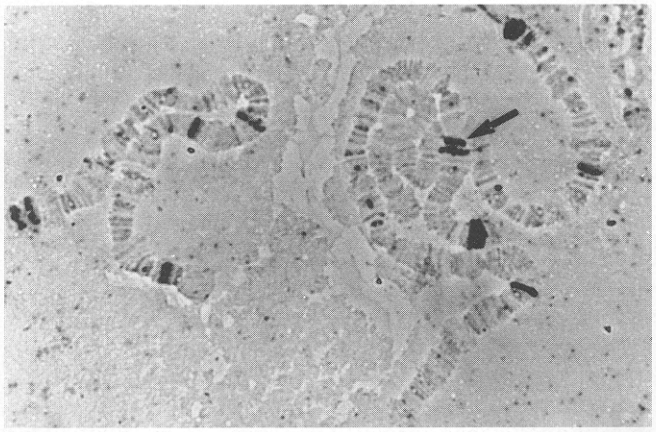

Fig. 8 Hybridization sites in BU-30(55/4) probed with the 2.1 kb *Kpn*I subclone. *Arrow* shows a new position in chromosome 2. All other sites coincide with those previously described at generation 30

from pericentromeric areas frequently carry a heterogeneous mixture of repetitive DNA types and their elements are truncated and non-functional (Vaury et al. 1989), we believe that cDb314 has been cloned from this chromosomal region.

In situ hybridization shows *Osvaldo* transpositions

In situ hybridization analyses demonstrates that all cDb314 subclones tested correspond to the same element, making this clone a suitable probe for TE mobilization experiments. Since the cDb314 clone contains 3′ sequences presumably not belonging to the *Osvaldo* element, it could not be used for these in situ hybridization experiments. To check this possibility, a 2.1 kb *Kpn*I probe containing only coding regions of the *Osvaldo* retrotransposon (Fig. 4) was hybridized in situ to polytene chromosomes of the BU-30(55/4) line [line BU-30(30/4) after 25 generations of further inbreeding]. Figure 8 shows that this internal fragment reveals not only all original positions, but also a new position in chromosome 2, due to a recent transposition. This confirms that cDb314 is a good marker of *Osvaldo* sites. Another possible source of error in using cDb314 as probe may be attributed to the short 297-related sequences included in it. We think that this possibility can be disregarded due to the low probability of in situ hybridization detecting a comparatively small sequence (270 bp) in a 6017 bp probe.

Osvaldo transposes in bursts at high frequencies

Our direct observations have shown that the *Osvaldo* element transposes in bursts. Eight larvae out of 16 showed more than one *Osvaldo* transposition at the same time, and some of them showed 5 to 9 new sites (Tables 1 and 2). This observation indicates that transposition occurs in bursts and is supported by the calculation of CDs which are consistently greater than 1, as expected from clumped distributions. These results substantiate the idea that transposition, at least under certain stressful conditions such as inbreeding or hybridization, is not the result of a constant movement in most individuals, but on the contrary is the outcome of massive changes produced in a few individuals. The similar mode of transposition for different retroid elements (*mdg1*, *mdg3*, *mdg4*, *stalker*, *jockey*) in lines and crosses derived from the family of ct^{MR2} strains (Gerasimova et al. 1985; Georgiev et al. 1989, 1990) and for *copia* elements in inbred lines (Biémont et al. 1987) suggests that transposition in bursts is a common feature of retrotransposons.

Insertion events were always more frequent in mass matings than in single-pair matings, though these differences were only statistically significant across hybrids. One explanation may be the higher average proportion of *D. koepferae* genome introgressed in our mass-crossed hybrid females, compared to single-paired ones. In most cases, the latter were hybrid only for one segment whereas the former carried a mixture of segments, which would be more effective in increasing the transposition rate. This putative introgression effect on transposition may explain the consistently increased rates in hybrids. Nonetheless, differences between hybrids and inbred lines are not statistically significant. This indicates that either differences are too small to be detected with our sample size or they do not exist at all. We have estimated the sample size required to detect true differences such as those reported here and found it to be larger than 1000 larvae in either type of cross (test of Casagrande et al. with $\alpha = 0.05$, $\beta = 0.05$ in Sokal and Rohlf 1981).

Nevertheless, the direct observations of transposition reported here yield insertion rates higher than those normally reported for most TEs (for review see Charlesworth and Langley 1989). Also, using a direct approach, Eggleston et al. (1988) reported transposition rates for 12 different TE families. For dysgenic crosses, excluding the P element which can be considered as a special case, *FB* and *297* elements transpose at rates of 10^{-3}, *copia* and *B104* at 10^{-4} insertions per element per generation, but no transpositions were seen for the rest of the TEs. For non-dysgenic crosses the only element that showed a new insertion was *B104* with a transposition rate of 7.7×10^{-4}. Similarly, Harada et al. (1990) estimated that I element transposition frequency amounts to 7.5×10^{-3} per element per generation and a similar figure holds for *hobo* (both elements probably under dysgenic conditions). No transposition or low insertion rates (10^{-5} to 10^{-4} per element per generation) were detected for *copia*, *412* and *17.6* elements. These data support our contention that the transposition rates reported here are higher than those described for non-dysgenic crosses.

The causes of these high rates may be related to inbreeding and interspecific hybridization. Previous evidence of the inbreeding effect on transposition comes mainly from the massive changes in locations of copia elements observed in inbred lines of *D.melanogaster* by

Biemont et al. (1987) and *mdg-1*, *mdg-3* and *copia* in low-fitness inbred lines of *D. melanogaster* (Pasyukova et al. 1986). On the other hand, data on increased transposition rates in interspecific hybrids are rather controversial and only indirect data have been reported thus far (Evgen'ev et al. 1982; Haymer and Marsh 1986; Coyne 1989; Hey 1989). The results reported here are the first direct estimates of high insertion rates in interspecific hybrids and, thus, are compatible with the hypothesis that interspecific hybridization is a release mechanism of transposition. Nevertheless, the fact that the tested inbred line also shows a high insertion rate suggests that inbreeding (or this particular unstable inbred line) may also be the release mechanism of transposition. Only further work will enable us to decide between these alternatives.

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