Chromosomal distribution of the transposable elements *Osvaldo* and *blanco* in original and colonizer populations of *Drosophila buzzatii*

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Summary

Chromosomal distribution of transposable elements (TEs) *Osvaldo* and *blanco* in *D. buzzatii* was studied in three original natural populations from Argentina (Berna, Puerto Tirol and La Nostalgia) and a colonizer population from the Iberian Peninsula (Carboneras). The Spanish population showed significant differences for *Osvaldo* and *blanco* copy numbers when we compared the X chromosome and the autosomes; but it is mainly the accumulation of copies in chromosome 2, where most sites with high insertion frequency were located, that causes the discrepancy with the negative selection model. We found no significant differences in TE frequency between chromosomal regions with different exchange rates, and no evident accumulation of TE was detected within chromosomal inversions where recombination rate is reduced. The Carboneras population shows euchromatic sites of *Osvaldo* and *blanco* with high occupancy and others with low copy number. On the contrary, Argentinian populations show only a generalized low occupancy per insertion site. Moreover, the mean copy number of both elements is higher in Spain than in Argentina. All these results suggest an important role of the colonization process in the distribution of TEs. The increase in the copy number of the TEs analysed and their elevated frequency in some chromosomal sites in Carboneras is, most probably, a sequel of the founder event and drift that took place at the time of the colonization of the Old World by *D. buzzatii* from the New World some 300 years ago.

1. Introduction

Transposable elements (TEs) are universal components of all organisms investigated so far. In *Drosophila melanogaster* 10% of the total DNA consists of about 50 families of moderately repeated TEs (Finnegan, 1992). Such elements are capable of moving in the host genome, becoming an important source of mutation when they insert into or near transcription units (Finnegan, 1985). However, the role of TEs as a source of genetic variation, and the forces responsible for their maintenance in natural populations, are still controversial issues (Biémont *et al.*, 1997; Charlesworth *et al.*, 1997). Theoretical and experimental studies lead to the conclusion that TEs are maintained as a balance between transposition increasing TE copy number, and opposing forces including excision, regulation of transposition rate, and selection against deleterious insertions and chromosomal rearrangements (Charlesworth & Charlesworth, 1983; Charlesworth & Langley, 1989; Charlesworth *et al.*, 1992*a*, *b*; Vieira & Biémont, 1996*a*; Yoder *et al.*, 1997; Simmen *et al.*, 1999). The relative importance of the factors maintaining the different TE families remains to be ascertained. It seems that each TE has a particular behaviour (Biémont, 1992; Biémont *et al.*, 1994), but it is unclear whether this specificity depends on element peculiarities, history and characteristics of the host populations or both.

D. buzzattii, a member of the *repleta* group originated in South America, has spread within historical times to the Canary Islands, the Mediterranean region, Australia, and several other places throughout the world (David & Tsacas, 1980; Barker *et al.*, 1985; Fontdevila, 1989). Colonization consequences of this species were detected by a reduced allozyme and mtDNA polymorphism (Fontdevila,

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1989; Rossi et al., 1996). Moreover, previous studies (Labrador et al., 1998) have shown that the high occupation of Osvaldo element found in Iberian populations compared with Argentinian ones could be a consequence of a colonization process. As a result of a founder effect, TE copies that arrived at the Iberian Peninsula are at high frequency today, and all other insertions are the result of transposition events.

In this work we test the colonization hypothesis using different Argentinian populations of D. buzzatii and a new LINE type element named blanco (Plata-Rengijo, 1995) as well as Osvaldo element (Labrador & Fontdevila, 1994; Pantazidis et al., 1999). The results suggest that the colonization process is responsible for the distribution frequency of the two TEs studied more than the element peculiarities.

2. Materials and methods

(i) Drosophila strains

Control line 63 42/7 F81 was originated from D. buzzatii Bu42 (28/7) collected in Argentina (Labrador & Fontdevila, 1994; Labrador et al., 1998) and was maintained by brother–sister matings during 63 generations, and thereafter by small mass-cultures to increase viability. In addition, periodical in situ hybridization tests for the insertions of Osvaldo and blanco elements were carried out on this line, the last one just before starting the experiment. At that time, the line was devoid of Osvaldo in euchromatic regions, and the insertion profile of blanco element displayed high stability over generations, exhibiting hybridization labelling at chromosomal sites XG4e, XG3a, 3F3d, 3F4a and 4E1c.

Natural populations of D. buzzatii were collected in November 1995 in NE Argentina (Berna, Puerto Tirol and La Nostalgia) and in July 1996 in SE Spain (Carboneras). These populations were analysed immediately after arrival in the laboratory.

(ii) Mating system

Wild males were crossed individually with virgin females from the control line 63 42/7 F81. To prevent the occurrence of high larval density, mating partners were kept in vials no longer than 4 days. Only female larvae were analysed, allowing the study of the complete male haploid genome (X chromosome included). The TE insertion site pattern of each F1 female larva examined was the sum of the two parental patterns. The blanco insertion profile of each male was obtained by subtracting the stable profile of the control line 63 42/7 F81 from the profile of the F1 larva. In the case of Osvaldo this step was not necessary because the control line was devoid of this element. The method gave a complete haploid genome

per wild male. A minor drawback of this method is that the common sites of the control line and the wild male were excluded from the study. The method was chosen, however, because it is easy to carry out, requires only one generation to analyse individuals, and is more reliable than crosses using balancer chromosomes (García Guerreiro & Biémont, 1995), in which transposition may occur.

(iii) DNA probes

We used a probe containing a fragment of Osvaldo element (2·1 kb) including the pol gene, inserted at the KpnI site of the plasmid Bluescript KS+ (Labrador & Fontdevila, 1994), and a probe containing a fragment of 3·5 kb corresponding to the new, still undescribed, mobile element blanco (Plata, 1995). This last probe contains an additional genomic fragment that hybridized to the B4E region of the X chromosome (region of the white gene) that was used as positive hybridization control.

(iv) In situ hybridization

Polytene chromosome squashes from salivary glands of third-instar larvae were prepared as described in Labrador et al. (1990), and then hybridized with the above probes labelled with digoxigenin 11-dUTP (Boehringer–Mannheim) using a random primer reaction. Prehybridization processes, hybridization solutions and post-hybridization washes were conducted following the protocol of Schmidt (1992) edited by Boehringer–Mannheim.

3. Results

(i) Distribution of transposable elements

Distribution of Osvaldo and blanco was analysed in all D. buzzattii chromosomes. Some examples of this distribution are presented in Figs. 1 and 2, which depict the distribution of Osvaldo on chromosomes 2 and 5, respectively, and in Figs. 3 and 4, which depict the distribution of blanco on chromosomes X and 2, respectively. It is shown that chromosomal distributions of the elements varied geographically. While the Argentinian populations had a very low insertion site frequency, a high frequency was observed in the Spanish population of Carboneras. In the latter, Osvaldo element was present at site 5A4b of chromosome 5 in 54 gametes of the 85 analysed, and also has a frequency of 26, 31 and 22 copies in sites 2B2a, 2F4a and 2G2h of chromosome 2, respectively. Even the blanco element, whose total number of copies was very low in all populations, showed a high insertion frequency in chromosomes X and 2 in Carboneras, in
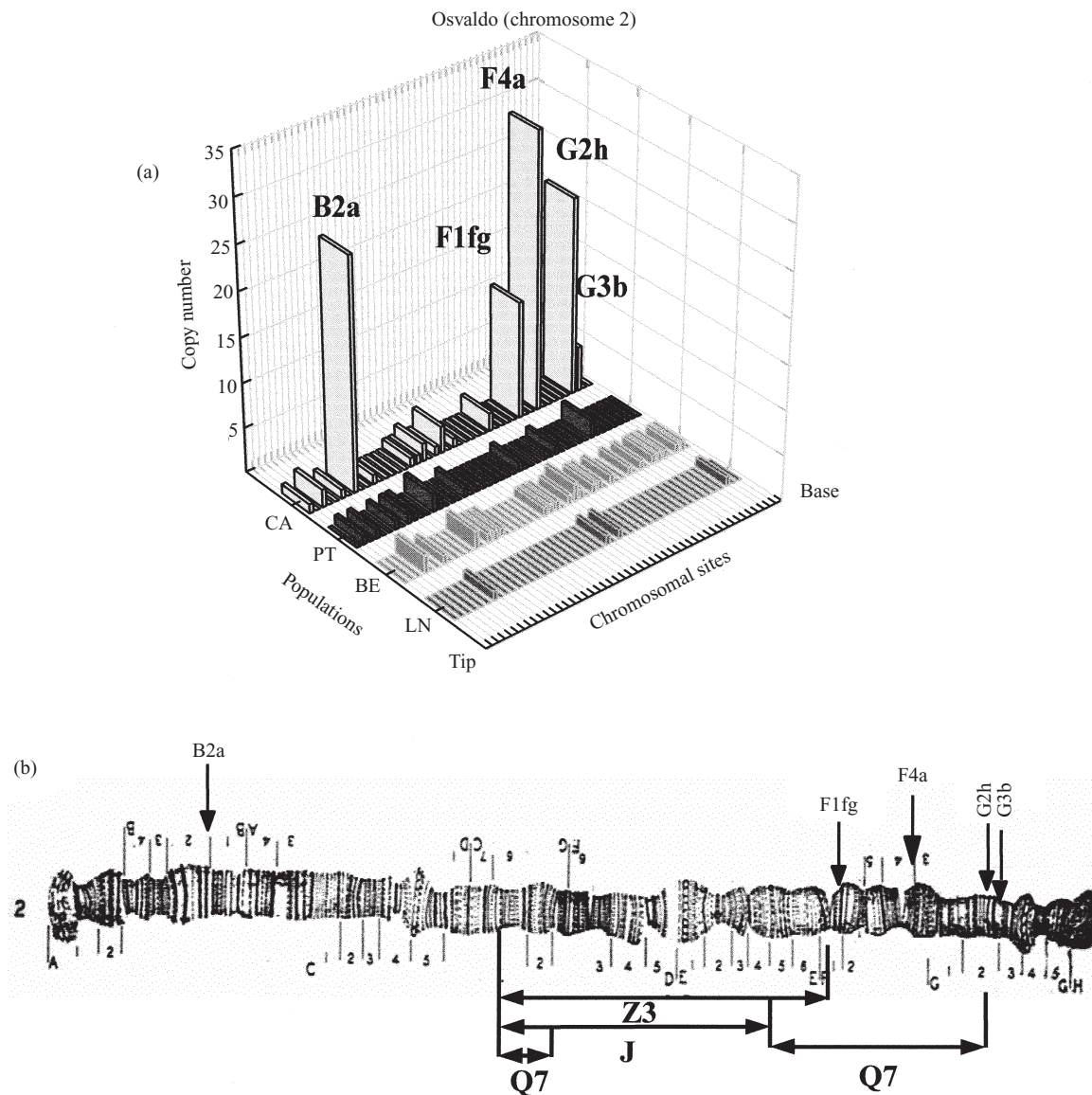
Fig. 1. (*a*) Distribution of *Osvaldo* element in sites of chromosome 2 from the tip to the base of the chromosome. Letters in bold correspond to sites with high insertion frequency. CA, population of Carboneras (Spain); PT, Puerto Tirol (Argentina); BE, Berna (Argentina); LN, La Nostalgia (Argentina). (*b*) Chromosomal location of the high insertion frequency sites on the cytological map. The limits of inversions J, Z3 and Q7 are indicated by horizontal arrows.

which the sites XF1a and 2F4f were occupied 7 and 31 times, respectively. Also site XF1a was present at a quite high frequency in Argentinian populations.

If all potential insertion sites have the same probability of hosting one TE, and the insertions of the TEs in two different sites are independent events, the number of copies per chromosomal site should follow a binomial distribution. When the number of sites is high and the probability of insertion per site is low, this distribution becomes Poisson. Table 1 shows the means and variances of chromosomal site number occupied by *Osvaldo* and *blanco* in the natural

populations analysed. In this study we consider only the euchromatic bands, disregarding the pericentromeric ones because they usually accumulate elements. The total numbers of sites (n) were 225, 364, 298, 268, 284 per chromosomes X, 2, 3, 4, 5, respectively as in Labrador *et al.* (1998). Departures from Poisson distribution were tested with the estimator $DC(n-1)$ that, under the null model, follows a χ^2 with $n-1$ degrees of freedom, where DC (variance/mean) represents the dispersion coefficient.

The chromosomal distribution of TEs in chromosomal sites does not fit a Poisson distribution in
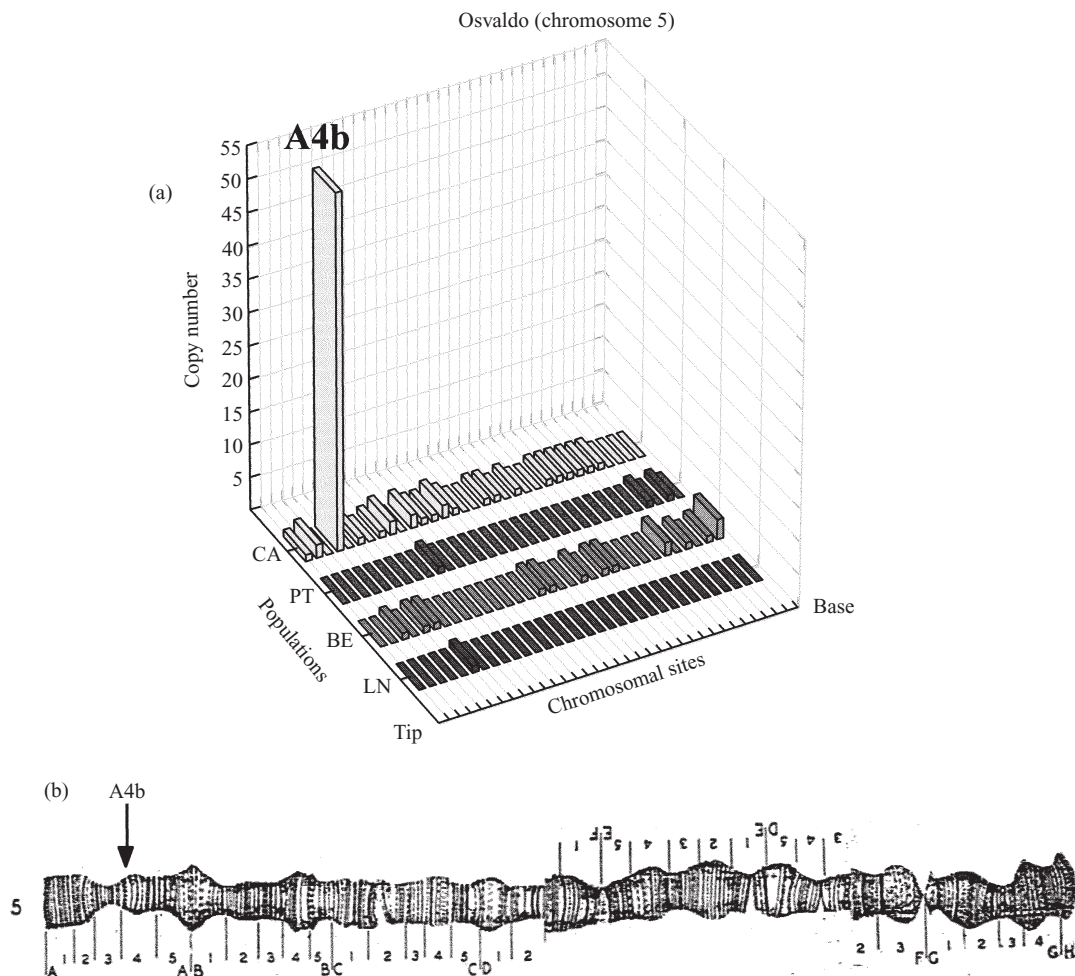
Fig. 2. (*a*) Distribution of *Osvaldo* element in sites of chromosome 5. Letters in bold correspond to sites with high insertion frequency. CA, population of Carboneras (Spain); PT, Puerto Tirol (Argentina); BE, Berna (Argentina); LN, La Nostalgia (Argentina). (*b*) Chromosomal location of the high insertion frequency sites on the cytological map.

Carboneras. In Argentinian populations, *Osvaldo* follows a Poisson distribution in all chromosomes except chromosomes 3 and 5 in Berna, and the X in Puerto Tirol. The distribution of *blanco* fits a Poisson distribution in chromosomes 2, 3, 4 of the Berna population of Argentina. In the case of Puerto Tirol this analysis was not done due to the small sample size. These results show, particularly in Carboneras, an extraordinarily high insertion frequency in some sites. For example 57 % of *Osvaldo* insertions are located in four sites of chromosome 2 and one site of chromosome 5. These results suggest a tendency for accumulation of *Osvaldo* in certain chromosomal regions in Carboneras. This may result, however, from apparent multiple site occupancy in closely neighbouring sites, which are beyond the resolution power of the *in situ* technique. Although we cannot discard this possibility, it is unlikely that chance alone

can account for the clustered distribution of the element insertions in the same region.

Table 2 lists means and variances of copy number per haploid genome for *Osvaldo* and *blanco*. As expected by a Poisson distribution, the average of copy number of *blanco* is approximately equal to the variance in all populations. *Osvaldo* element, however, fits a Poisson distribution only in the Carboneras population. In the Berna and Puerto Tirol populations, a significant deviation from the expected Poisson distribution was detected and this result remained significant after applying the Bonferroni correction. This discrepancy is probably due to an excess of gametes carrying three to five copies in these populations.

Overall, the mean of insertion sites on haploid genomes for both elements was low, ranging from 0·30 to 2·99. *Osvaldo* had a mean copy number per haploid
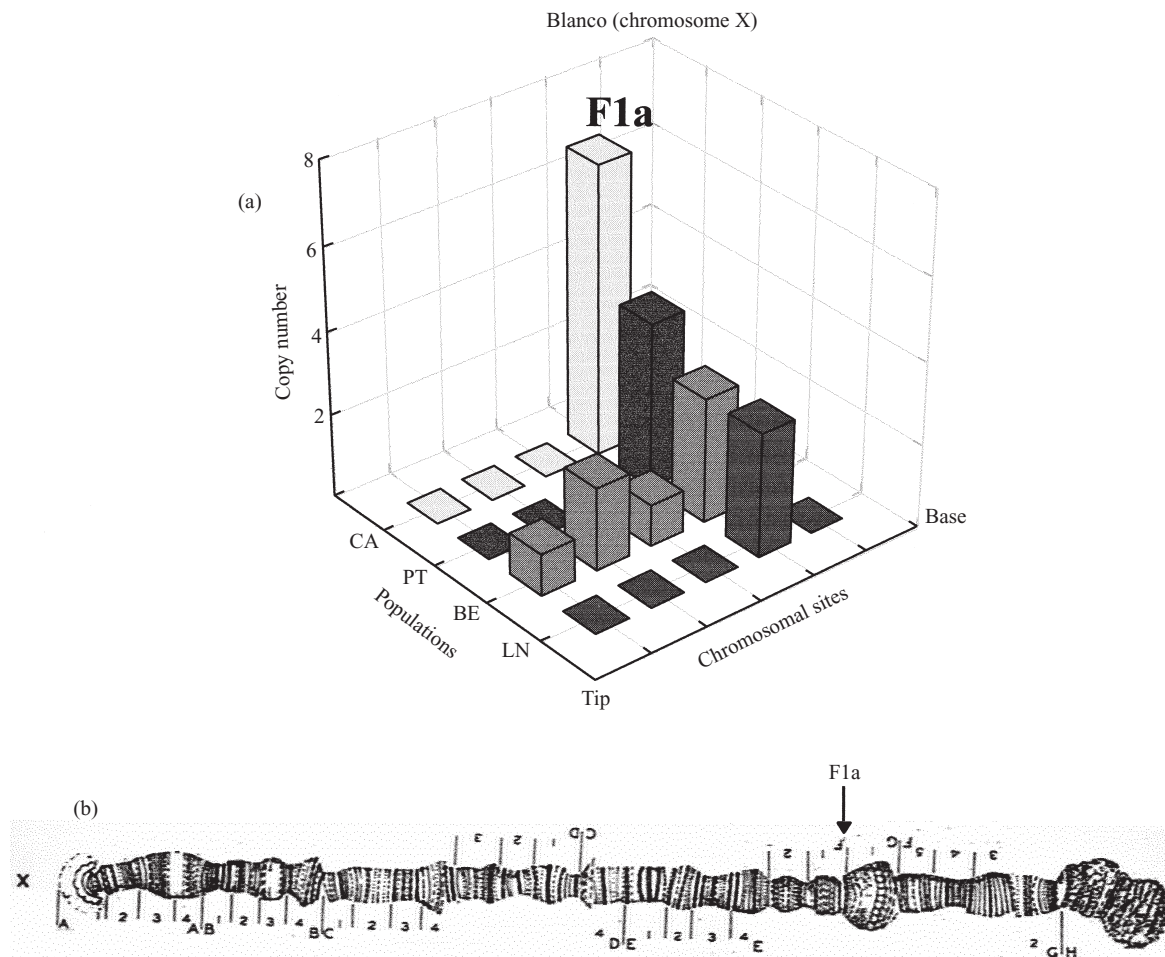
Fig. 3. (*a*) Distribution of *blanco* element in sites of chromosome X. Letters in bold correspond to sites with high insertion frequency. CA, population of Carboneras (Spain); PT, Puerto Tirol (Argentina); BE, Berna (Argentina); LN, La Nostalgia (Argentina). (*b*) Chromosomal location of the high insertion frequency sites on the cytological map.

genome higher than *blanco* in all populations investigated, and the population from Carboneras had the highest mean values for the two elements analysed.

(ii) *Comparison of element copy number among chromosomes*

Montgomery *et al.* (1987) have proposed that selection against insertions should lead to fewer insertions on the X chromosome than on the autosomes, because of the stronger deleterious effect of insertions in hemizygous males. In *D. melanogaster*, the predicted proportion of insertions on the X chromosome under a selection model is 0·13 (Charlesworth & Langley, 1989). Because in *D. buzzatii* we have no data about the selection magnitude, only a proportion of insertions in chromosome X versus autosomes can be compared. The number of chromosomal sites was used as an approximation of the DNA amount to

calculate the expected proportion of TEs on each chromosome arm, as in Labrador *et al.* (1998). For the X chromosome this number is 225/1439 (total number of bands) = 0·16, and so on for the other chromosomes. Table 3 shows the proportions of *Osvaldo* and *blanco* in the different chromosomes. The proportion of *Osvaldo* tended to be higher in chromosome 2 than in the rest of the autosomes in all populations analysed, and the difference deviates significantly in Carboneras. *Blanco* element showed a high proportion of copies on chromosome 2 in Carboneras but not in Puerto Tirol and Berna. Observed and expected proportions were compared using the *G* statistic (Sokal & Rohlf, 1995). This analysis shows no significant difference in the number of copies of *Osvaldo* and *blanco* between chromosome X and autosomes in the Argentinian populations, even when the two populations were pooled ($G = 0·91$). In the Spanish population the numbers of
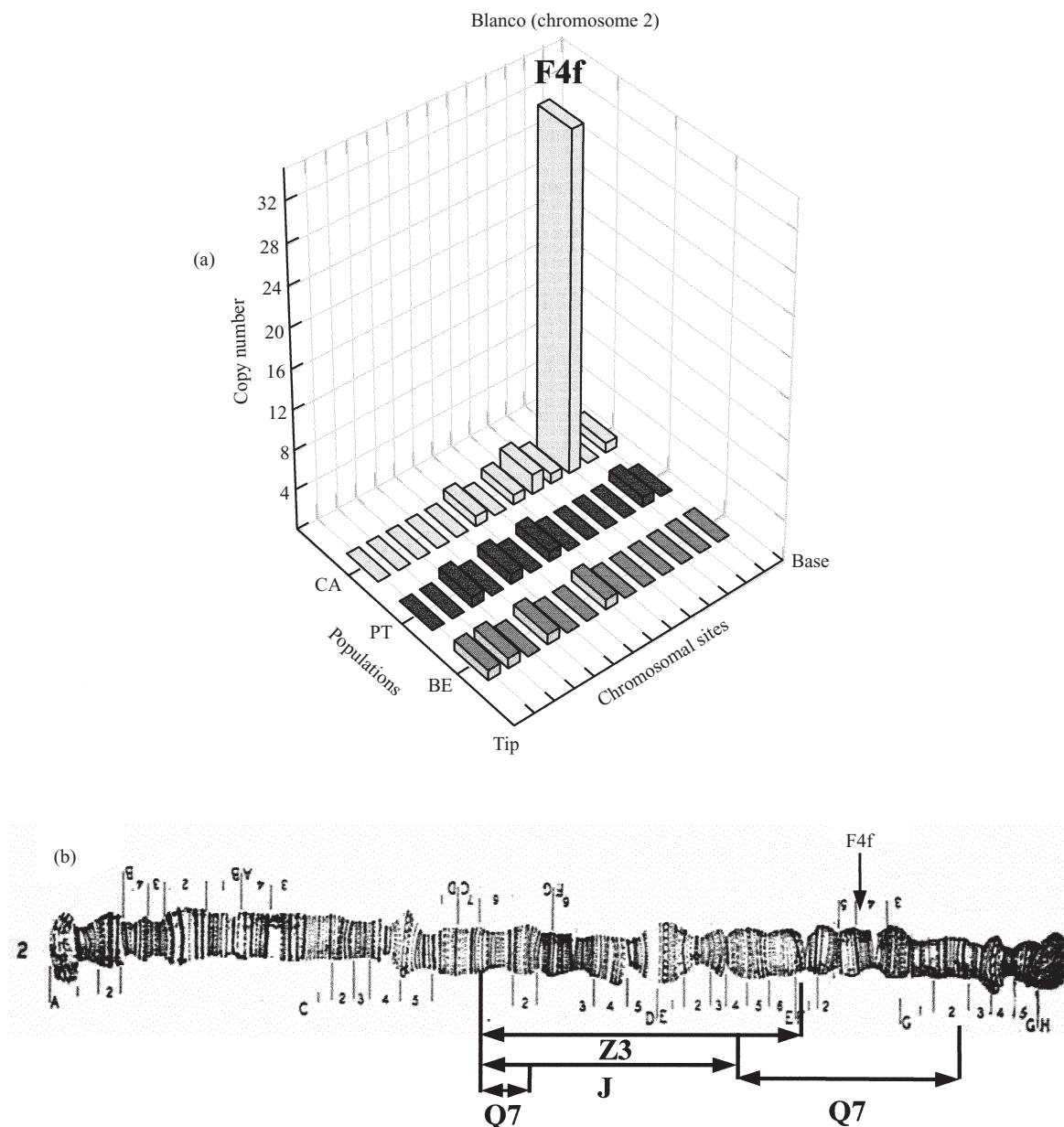
Fig. 4. (a) Distribution of *blanco* element in sites of chromosome 2 from the tip to the base of the chromosome. The site with high insertion frequency is represented in bold. (b) Chromosomal location of high insertion frequency sites on the cytological map. The limits of inversions J, Z and Q7 are indicated by horizontal arrows.

insertions on chromosome X and autosomes were significantly different. This result is due to an accumulation of *Osvaldo* sites with high insertion frequency on chromosomes 2 and 5 rather than a reduced number of insertions in chromosome X. In fact, when these high insertion frequency sites were disregarded the *G* test became not significant.

(iii) *Proportion of elements in different sections of chromosomes*

Langley *et al.* (1988) proposed a model of negative selection against chromosomal rearrangements produced by ectopic crossing-over between homologous

elements located at different chromosomal sites. According to this model, if the actual distribution of TEs is influenced by recombination, an accumulation of elements at the tip and base of chromosomes could be expected, because in these regions the rate of recombination is very low (Lindsley & Sandler, 1977). The procedure described by Langley *et al.* (1988) in *D. melanogaster* has been adapted to *D. buzzatii*, by dividing the polytene chromosomes of this species (Wharton, 1942; Ruiz *et al.*, 1982) into three regions: tip, middle and base (near the centromere). Each region includes the number of bands described in *D. melanogaster* (7 % correspond to the distal region and 12 % to the proximal one) as a minimum.

Table 1. *Test of Poisson distribution of* Osvaldo *and* blanco *TEs in chromosomal bands*

Populations[a]	Mean	Variance	DC[b]
Osvaldo			
BE (87)			
X	0·027	0·026	0·96
2	0·069	0·080	1·16
3	0·047	0·085	1·80**
4	0·041	0·047	1·14
5	0·053	0·078	1·47**
PT (42)			
X	0·027	0·053	1·96**
2	0·036	0·045	1·25
3	0·033	0·032	0·97
4	0·015	0·015	1·00
5	0·014	0·014	1·00
CA (85)			
X	0·049	0·090	1·86**
2	0·310	6·340	20·45**
3	0·100	0·580	5·83**
4	0·086	0·160	1·87**
5	0·270	0·370	1·37**
blanco			
BE (83)			
X	0·035	0·097	2·77**
2	0·011	0·010	0·91
3	0·020	0·026	1·30
4	0·015	0·015	1·00
5	0·042	0·061	1·45**
CA (62)			
X	0·031	0·220	7·09**
2	0·100	2·650	26·51**
3	0·017	0·043	2·55**
4	0·007	0·007	1·00
5	0·018	0·024	1·350**

[a] Capital letters denote the geographic origin of the populations: BE, Berna; PT, Puerto Tirol; CA, Carboneras.
[b] Dispersion coefficient (variance/mean).
*$P < 0.05$; **$P < 0.01$. The numbers of haploid genomes analysed are indicated in parentheses. Bonferroni's correction was applied (0·05/number of test).

Table 2. *Test of Poisson distribution of* Osvaldo *and* blanco *transposable elements on the entire haploid genome of individuals from three natural populations*

	n[a]	Mean	Variance	DC[b]
Osvaldo				
BE	87	0·83	1·16	1·40**
PT	49	0·88	1·30	1·48**
CA	85	2·99	2·98	1·00
blanco				
BE	83	0·40	0·44	1·10
PT	36	0·30	0·38	1·27
CA	62	0·89	0·68	0·76

[a] Number of haploid genomes analysed.
[b] Dispersion coefficient (variance/mean).
*$P < 0.05$; **$P < 0.01$. Bonferroni's correction was applied. See Table 1 for population identification.

Table 4 summarizes the number of sites occupied by *Osvaldo* in the populations of Berna and Carboneras (*blanco* was not considered due to the low insertion copy number of this element). The number of sites in each region was calculated, omitting the regions near the centromere (XG1h-base, 2G5f-base, 3G4d-base, 4G5a-base, 5G4d-base) which were shown to accumulate elements. There are no significant differences in the number of sites occupied by *Osvaldo* among the three chromosomal regions considered, except for the chromosome 5 in Carboneras, which showed a deficiency of occupied sites in the chromosome base. When the total copy number of the *Osvaldo* element (data not shown) was used, instead of the number of occupied sites, the results remained the same for the Berna population, in which the number of occupied sites was close to the copy number, but they changed dramatically for Carboneras, which showed a significant accumulation of *Osvaldo* ($\chi^2 = 29.4**$) in site 2G2h located at the base of chromosome 2. The test was also significant for chromosome 5, but in this case the discrepancy is due to a deficiency of elements in the base compared with other chromosomal regions.

(iv) *Transposable elements and chromosomal inversions*

Different studies suggest that TEs tend to be more abundant in chromosomal regions where crossing-over is reduced as inversions and inversion-breakpoints (Montgomery *et al.*, 1987; Langley *et al.*, 1988; Charlesworth & Lapid, 1989; Charlesworth *et al.*, 1992*a*, *b*; Eanes *et al.*, 1992; Lyttle & Haymer, 1992; Sniegowsky & Charlesworth, 1994). Element accumulation should depend on the inversion frequency, such that rare inversions should carry more TEs. This is because these inversions are more frequently found in the heterozygous state, which suppresses recombination inside the inversions.

D. buzzatii is polymorphic for the second and fourth chromosomes with seven and one inversions, respectively (Ruiz *et al.*, 1984). The frequency of these inversions is highly variable and depends on the inversion type and the origin of the population (Fontdevila, 1982, 1989, 1991; Hasson *et al.*, 1995). In the Argentinian populations only inversion 2J was found, with a frequency varying between 0·06 in PT and 0·19 in Berna. In the Spanish population of Carboneras, however, three different inversions were found (2J, 2JZ3 and 2JQ7) at a frequency of 0·43, 0·20 and 0·05 respectively, in chromosome 2, and one inversion (4S) in chromosome 4 at frequency 0·20. The gametic disequilibrium between *Osvaldo* or *blanco* and the inversions of chromosomes 2 and 4 were estimated by product–moment correlation coefficient for high-frequency positions in Carboneras (Table 5).

Table 3. *Comparison test of the proportion of* Osvaldo *and* blanco *TEs among chromosomes and between chromosome X and autosomes*

Chromosome	No. of bands	Osvaldo			blanco		
		BE	PT	CA	BE	PT	CA
X	225	0·08	0·16	0·05	0·23	0·36	0·12
2	364	0·35	0·35	0·44	0·11	0·36	0·66
3	298	0·19	0·27	0·12	0·17	0·09	0·10
4	268	0·17	0·11	0·08	0·12	0·09	0·03
5	284	0·21	0·11	0·31	0·35	0·09	0·08
G_1^a		5·50	5·14	101·85**	8·86	5·07	43·67**
G_2^b		3·42	0·03	32·72**	1·55	2·02	0·18

[a] Comparison of the proportion of TEs among chromosomes (G_1).
[b] Comparison of the proportion of TEs between chromosome X and autosomes (G_2).
*$P < 0.05$; **$P < 0.01$.
See Table 1 for population identification.

Table 4. *Proportion of* Osvaldo *insertion sites found in three sections of chromosomes of the Carboneras* (CA) *and Berna* (BE) *populations*

Chromosome	BE				CA			
	S.O.	Tip	Middle	Base	S.O.	Tip	Middle	Base
2	22	0·04 (0·08)	0·72 (0·79)	0·23 (0·11)	17	0·12 (0·08)	0·76 (0·79)	0·12 (0·11)
3	11	0·09 (0·09)	0·82 (0·83)	0·09 (0·07)	17	0·23 (0·09)	0·65 (0·83)	0·12 (0·07)
4	10	0·10 (0·07)	0·80 (0·82)	0·10 (0·10)	17	0·06 (0·07)	0·81 (0·82)	0·13 (0·10)
5	12	0·08 (0·07)	0·66 (0·80)	0·25 (0·10)	17	0·09 (0·07)	0·86 (0·80)	0·05** (0·10)
Total[a]	61	0·06 (0·09)	0·77 (0·81)	0·17 (0·10)	76	0·12 (0·09)	0·78 (0·81)	0·1 (0·10)

S.O., number of sites occupied.
[a] Total number of sites occupied (X chromosome included). The expected values are in parentheses.
**$P < 0.01$.

Table 5. *Correlation coefficients between chromosomal arrangements and high insertion frequency in chromosomal sites of the Carboneras population*

TEs	HF sites	Arrangements				
		Chromosome 2				Chromosome 4
		2St	2J	2JQ7	2JZ3	4S
Osvaldo	2B2a	−0·08	0·09	−0·14	0·11	–
	2D1h	−0·19	−0·17	1***	−0·09	–
	2F1d	0·27	−0·09	−0·08	−0·20	–
	2F4a	−0·05	0·27**	−0·05	−0·29**	–
	2G3b	0·03	0·018	−0·05	0·03	–
	2G2h	0·01	0·13	−0·11	−0·19	–
	4C2g	–	–	–	–	0·28**
blanco	2F4f	0·14	0·3**	−0·26	−0·15**	–

HF, sites with high insertion frequency.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Interestingly, site 2D1h, which was inside the JQ7 arrangement, was completely associated with this arrangement ($r = 1$) in populations where this arrangement was present. A partial positive linkage disequilibrium was also observed between 2J arrangement and site 2F4a. In the case of 2JZ3, this arrangement was negatively associated with 2F4a. In chromosome 4, an association was observed between the arrangement 4S and the chromosomal site 4C2g. Similar results were obtained for *blanco*, where the site F4f was positively associated with 2J and negatively associated with 2JZ3 chromosome arrangement. In spite of the above-mentioned correlations, none of these high-frequency sites, except 2D1h, are included in an inversion.

4. Discussion

(i) *Transposable element frequency at individual sites of chromosomes*

Distribution of the *Osvaldo* and *blanco* TEs in chromosomal sites appears quite random in the Argentinian populations. In contrast, the distribution of these two elements departs from Poisson in the Spanish population of Carboneras, where some sites exhibit a high occupation frequency. These results are in accordance with those of Labrador *et al.* (1998) for *Osvaldo* in populations of the Iberian Peninsula and for NW Argentinian populations 2000 km distant from the Argentinian populations analysed here. Moreover, the insertion sites with high insertion frequency in the Spanish populations were the same in both experiments. Elevated frequencies of occupation of some chromosomal sites have been reported previously. For example, the accumulation of *P* elements in site 1A detected in populations of *D. melanogaster* (Ajioka & Eanes, 1989; Biémont, 1994) and in inbred lines (Biémont *et al.*, 1990) has been associated with transposition regulation (Ronsseray *et al.*, 1991). Furthermore it is known that some chromosomal sites, such as the 42B region of *D. melanogaster*, are characterized by an accumulation of TEs (Biémont & Gautier, 1989). Finally, other sites are characteristic of species, as occurs with site 42C, where the TE 412 is present in all 71 natural populations of *D. simulans* (Vieira & Biémont, 1996*b*).

In our study only the *Osvaldo* site at 3F2b with high insertion frequency could be considered a hotspot. This site had a high insertion frequency in Carboneras and was also present three times in the Berna population (data not shown). This hypothesis is reinforced by the observation that this same site was already present in populations from Argentina and the Iberian Peninsula previously analysed by Labrador *et al.* (1998). With respect to *blanco*, site XF1a of chromosome X is a good candidate to be a hotspot of

transposition because it was present in all populations analysed, even if its frequency was higher in the Carboneras population than in Argentinian populations. Hotspots can result from target site specificity, characteristics of DNA conformation or functional constraints. Local DNA structure can affect insertion specificity because the chromatin structure imposed by nucleosomes or by other proteins can influence the efficiency of element insertion into a particular target DNA (Craig, 1997). In this way, recent investigations have shown that the *gypsy* element must recognize features of the *ovo* locus because a target specificity was observed in some regions of this locus (Kimberley *et al.*, 1998). In a natural population of *D. melanogaster*, *gypsy* showed high occupation frequency in sites 61C and 98B of chromosome 3 (Biémont *et al.*, 1994).

On the other hand, the high insertion frequency of *Osvaldo* and *blanco* observed in some sites may reflect a founder event that took place during the colonization of the Iberian Peninsula by *D. buzzatii* flies from the New World. TE distribution could, thus, be a characteristic of the populations (Labrador *et al.*, 1998). It is known that about 300 years ago *D. buzzatii* colonized the Old World (Fontdevila *et al.*, 1981), and 70 years ago, Australia (Barker, 1982). Population diversification after colonization is highly dependent on the dynamics of founder events in *Drosophila* species (Fontdevila, 1991). A founder event in Old World populations of *D. buzzatii* has been previously postulated from the observed change in inversion frequencies, loss of gene arrangements (Fontdevila, 1991), reduced allozyme polymorphism (Fontdevila, 1989) and distribution of *Osvaldo* element (Labrador *et al.*, 1998). Also, the fact that colonizing populations of the Iberian Peninsula are monomorphic for mtDNA compared with South American populations (Rossi *et al.*, 1996) is an indication of a recent founder event. A strong argument in favour of this hypothesis is the fact that the two elements, *Osvaldo* and *blanco*, investigated in the present study, behave similarly.

(ii) *The role of unequal exchange in accumulation of transposable elements*

According to the unequal exchange hypothesis, TE insertion number should be negatively correlated with rate of recombination. So far, data reported about accumulation of TEs in regions near the centromeres of chromosomes are contradictory. Some studies have detected an accumulation of TEs in the base of the X chromosomes (Charlesworth & Lapid, 1989) but not of the autosomes (Charlesworth *et al.*, 1992*b*). Finally no negative association between TE copy number and recombination rate has been detected in a natural population of *D. melanogaster* (Hoogland & Biémont,

1996). Ultimately the origin of the discrepancies can be traced back in part to whether the centromeric and pericentromeric insertion sites are considered in the analysis. In the present work pericentromeric regions were omitted and we applied the test to regions with different recombination rate. In this way we eliminated the conflicting regions where events of recombination can be mixed with other unknown events due to the heterochromatin structure. Moreover, considering occupied insertion sites instead of copy number of TEs prevents any potential error motivated by attribution of hotspots of transposition to recombination events. It is known that TEs are prone to accumulate in heterochromatin (Miklos et al., 1988; Vaury et al., 1989; Charleworth et al., 1994, Pimpinelli et al., 1995), which has a reduced rate of crossing-over. It is not possible, however, to establish a direct relationship between the accumulation of TEs in heterochromatin and the recombination rate, because accumulation of TEs in heterochromatin of Drosophila is probably a consequence of the peculiar genetic properties of the heterochromatic material itself (Pimpinelli et al., 1995).

Taking into account the suppression of recombination in heterokaryotypes, an alternative way to test the model of ectopic recombination is the comparison of the number of copies in chromosomes bearing rare inversions with standard ones. If the recombination model is verified, we should find more TEs in inverted regions because the recombination events are eliminated. In our case, it is necessary to note, however, that it is not possible to compare element copy numbers in chromosomes that carry inversions 2J and 2JZ3 with the standard one because these inversions were very frequent in the Carboneras population (0·42 and 0·19 repectively), were sometimes homozygous, and recombination was not suppressed. However the 2D1h site, which was occupied by Osvaldo in Carboneras, showed complete association with the 2JQ7 inversion. The sites 2F4a (occupied by Osvaldo) and 2F4f (occupied by blanco) showed significant positive correlation with the 2J inversion and a negative correlation with 2JZ3. These two sites located in the region 2F4 and occupied by two different TEs are curious because they presented the same correlation characteristics with the inversions 2J and 2JZ3. Moreover, this region is known for its high insertion frequency of Osvaldo and blanco in Carboneras and by a recent new Osvaldo transposition in a laboratory line (Labrador & Fontdevila, 1994; Pantazidis et al., 1999).

Hence, globally our results show an absence of significant differences in the number of sites occupied by the Osvaldo element in regions of different recombination rate. They are, thus, in accordance with the absence of a global negative relationship of TE site occupancy with recombination rates along

chromosomes of D. melanogaster as in Hoogland & Biémont (1996).

Distribution of Osvaldo and blanco elements in the Spanish population is a consequence of a founder effect and drift that follow a colonization process. We think that a small effective number of colonizers is implied in this process and consequently sites coming from an ancestral population arrived in Spain at high frequencies and show high frequencies today. The sites at low frequency are the result either of transpositions occurring at the same rate as in the original population, of TE mobilization following environmental stress, or the response to a rapid increase in the population size in the new environment. Vieira & Biémont (1997) explain that differences in 412 copy number observed in D. malanogaster and D. simulans are difficult to explain by different transposition rates in these two species. In the same way, Wisotzkey et al. (1997) found that the distribution of two TEs in Hawaiian Drosophila suggests that colonization of new islands by species of older islands resulted in a increase in TE copy number in a process similar to that proposed for Osvaldo and blanco. It is difficult to imagine that only transposition events are responsible, in Carboneras, for the increase in copy number of two TEs belonging to different classes. Moreover, the hypothesis of transposition increase is difficult to reconcile with the polymorphism of low-frequency sites observed in both original and colonizer populations – unless hotspots of transposition present in the Iberian Peninsula population led to an increase in TE frequency in some chromosomal sites. Taking all the evidence into consideration led us to favour the idea that the increasing of mean copy number of Osvaldo and blanco in the Spanish population compared with Argentinian ones is due mainly to founder effects. However, only the results of sequencing the same insertion site in both original and colonizing populations, now in progress in our laboratory, will allow us to distinguish between founder effects and hotspots of transposition.

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