Letter to the Editor

Transposable Element Distributions in Drosophila

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SURVEYS of transposable elements (TEs) in populations of *Drosophila melanogaster* have shown that the frequencies of elements at individual sites within the genome are nearly always very low. Detailed statistical studies of element distributions indicate that selection must be acting to oppose the transpositional spread of elements (CHARLESWORTH and LANGLEY 1991; BIÉMONT 1992). While this conclusion seems to be widely accepted, the nature of the selective agent is still a matter for debate (CHARLESWORTH *et al.* 1994b). Two main possibilities have been widely discussed in the older literature (i) selection against the deleterious fitness effects of mutations caused by TE insertions into or near genes and (ii) selection against chromosomal rearrangements caused by ectopic recombination between homologous TEs inserted into nonhomologous chromosomal locations (CHARLESWORTH and LANGLEY 1991). It has also recently been suggested that the spread of some rapidly transposing elements may be contained as a result of deleterious effects on fitness that are directly caused by processes associated with transposition events (BROOKFIELD 1991, 1996; NUHZDIN *et al.* 1996).

HOOGLAND and BIÉMONT (1996) have recently reanalyzed some data sets on element distributions derived by *in situ* hybridization of TE probes to chromosomes isolated from populations of *D. melanogaster*. They concluded that their results support mechanism (i) and are inconsistent with (ii). Their main argument is that the ectopic exchange model predicts a negative correlation between element abundance in a given region of the genome and the rate of meiotic recombination in this region (LANGLEY *et al.* 1988). They failed to find a consistent pattern of such negative correlations for the elements included in their survey (which are a subset of those for which data are available).

We question this conclusion, for the following reasons. First, it ignores the shortcomings inherent in mechanism (i) as the sole force for containing the spread of TEs. Elements with deleterious fitness consequences can only persist in the population at sites where the selection coefficient against an insertion is of the same order as the rate of transposition (CHARLESWORTH 1991). The low rates of transposition associated with most families of Drosophila TEs, of the order of 10^{-4} or less per element per generation (NUHZDIN and MACKAY 1995), thus imply that selection coefficients of similar magnitude are associated with TEs found segregating in natural populations. But several lines of evidence suggest that deleterious mutations recovered from natural populations of *D. melanogaster* have an average heterozygous selection coefficient of 1–2% (CROW and SIMMONS 1983; CHARLESWORTH and HUGHES 1998). A large deleterious mutational effect of many insertion events is also consistent with direct laboratory measurements of the effects on viability of *P*-element insertions (MACKAY *et al.* 1992; CABALLERO and KEIGHTLEY 1994; LYMAN *et al.* 1996).

Elements associated with such large selection coefficients will clearly not readily be found in natural populations, in conformity with the observation that TE insertions are rarely found in or near coding sequences in genomes sampled from nature (CHARLESWORTH and LANGLEY 1991). Elements can be maintained in populations only if there is an abundance of sites at which selection against insertions is very weak, such as intergenic regions. But the observed low element frequencies per site in natural populations require a very precise balance between transposition rate and selection coefficient, since elements accumulate at very high abundances at sites where selection coefficients are much smaller than the transposition rate (CHARLESWORTH 1991). This is contrary to the observations on Drosophila. It is not clear how such a balance can be achieved under the insertional model (CHARLESWORTH 1991), but it is a natural consequence of ectopic exchange, because of the probable quadratic dependence of the probability of ectopic exchange on the number of elements per genome for a given family (LANGLEY *et al.* 1988). While selection against the direct deleterious fitness effects of insertions is undoubtedly very effective

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at removing many newly inserted elements from the population, it can only explain the low frequencies of elements at sites where they persist in the population if *ad hoc* assumptions about the distribution of selection coefficients are made.

Second, HOOGLAND and BIÉMONT ignore several lines of evidence that support the ectopic exchange model. In the first place, there is direct evidence for the generation of chromosomal rearrangments by ectopic exchange between Drosophila TEs, at a rate that is consistent with the low frequency of events required to balance transposition (MONTGOMERY *et al.* 1991; LIM and SIMMONS 1994). There is also evidence that TEs accumulate in euchromatic regions sheltered from regular meiotic exchange, such as in low frequency inversions which are predominantly present as heterozygotes and so are sheltered from recombination (MONTGOMERY *et al.* 1987; EANES *et al.* 1992; SNIEGOWSKI and CHARLESWORTH 1994), and on chromosome *4* (CHARLESWORTH *et al.* 1992b). Several patterns in the distribution of elements within the genome are also more readily explained by the ectopic exchange model than by the other models. For example, the density of elements in the mid-section of the *X* chromosome is on average lower than in comparable regions of the autosomes (MONTGOMERY *et al.* 1987; BIÉMONT 1992; CHARLESWORTH *et al.* 1992b). This is predicted by both models (i) and (ii), but is not an obvious consequence of the model invoking direct fitness effects of transposition. No such effect is seen for the proximal section of the chromosomes, which is inconsistent with the insertional mutation model (CHARLESWORTH *et al.* 1992b). HOOGLAND and BIÉMONT also dismiss the finding that elements accumulate at the bases of the major chromosomes, which we interpreted in terms of the ectopic exchange model (LANGLEY *et al.* 1988; CHARLESWORTH and LANGLEY 1991), on the grounds that these regions are heterochromatic, and hence intrinsically different from the rest of the chromosome. While this is a formal possibility, it ignores the evidence that the basal region of the *X* chromosome has a similar gene density to the rest of the *X* chromosome euchromatin and is thus far from being genetically inert (MIKLOS and COTSELL 1990), as HOOGLAND and BIÉMONT imply. The main difference between the β-heterochromatin and euchromatin of Drosophila is the high density of TEs in β-heterochromatin (MIKLOS and COTSELL 1990; CHARLESWORTH *et al.* 1994a), as expected if ectopic exchange is absent in this region.

Third, there are flaws in the methods of data analysis used by HOOGLAND and BIÉMONT. One measure of TE abundance in a given chromosomal region was to count as only one event a site that was found to be occupied by an element in at least one of the chromosomes in a sample, regardless of the number of times that site was occupied in the sample, yielding an estimate of "insertion site number." This measure is highly dependent on sample size, and does not estimate the population mean number of elements for the region, which is the variable which is controlled by the evolutionary forces affecting TE abundance (CHARLESWORTH and LANGLEY 1991). It is therefore an unsatisfactory measure of element abundance. The reasons given by HOOGLAND and BIÉMONT for using this method (to avoid effects of drift, population subdivision, and site-specificity of insertions) are specious. Estimates of the product of $4N_e$ and the mean selection coefficient against TEs indicate that drift can have little effect on element abundances in natural populations of *D. melanogaster* (CHARLESWORTH and LANGLEY 1991). Measures of allozyme and nucleotide site polymorphisms have repeatedly shown a lack of population subdivision in this species (SINGH and RHOMBERG 1987; BERRY and KREITMAN 1993). There is little evidence for site specificity of insertions at the level of polytene chromosome bands (CHARLESWORTH *et al.* 1992a). Even if these effects were important sources of bias in estimates of element abundances, it is not clear why they should not affect insertion site number as much as any other measure.

It is thus not very surprising that the insertion site number fails to show any relation with recombination rate, even if such a relation were really present. It is interesting to note that the other measure that they use, the frequency of elements per occupied site, shows evidence for negative correlations with recombination rate (their Figure 2 and Table 3), although only 3L shows an overall significant effect for aggregated elements. Charlesworth *et al.* (1992b) also found that this statistic was a more sensitive indicator of the tendency of elements to accumulate at the bases of the chromosomes than the insertion site number. This probably reflects the fact that it gives weight to the element frequencies at occupied sites, unlike the other measure.

In addition, HOOGLAND and BIÉMONT do not examine the power of their Spearman rank correlation tests to detect underlying true correlations, especially given the heterogeneity between elements in their tendency to accumulate in regions of low recombination (CHARLESWORTH *et al.* 1992b; SNIEGOWSKI and CHARLESWORTH 1994). In view of the fact that a correlation as high as 0.5 in their data is not significant after correction for multiple comparisons, it is clear that the power of this test is low and that failure to detect a significant negative correlation in a given instance does not carry much weight. We regard the fact that a highly significant negative correlation between occupancy frequency and recombination rate was detected for the aggregate of elements on 3L, and that several other correlations were moderately large and negative (Table 3 of HOOGLAND and BIÉMONT), as quite supportive of the possible existence of a generally negative association between recombination and element abundance.

Nevertheless, we believe that caution should be exercised in conducting this kind of analysis as a test of the

ectopic exchange model. The primary reason for this is that the details of the relation between regular meiotic exchange and ectopic exchange in Drosophila are largely unknown; while it seems reasonable to assume that gross differences in rates of regular meiotic exchange (such as exist between the proximal euchromatin and the rest of the euchromatin, or between inversion heterozygotes and homozygotes) will be accompanied by corresponding difference in rates of ectopic exchange, there is no guarantee that this is true for finer-scaled differences. Indeed, it is still unclear whether the absence of evidence for accumulation of elements at the tip of the *X* chromosome (LANGLEY *et al.* 1988; CHARLESWORTH and LAPID 1989) reflects a relatively normal frequency of ectopic exchange there, despite the strong suppression of crossing over, or the operation of forces other than meiotic ectopic exchange. Recent evidence from yeast shows that the relation between rates of regular and ectopic exchange at meiosis may be quite complicated, with a high frequency of ectopic exchanges involving telomeric insertions (GOLDMAN and LICHTEN 1996). Further work on the relation between regular and ectopic exchange in Drosophila is needed before the question of the role of ectopic exchange in regulating the abundance of TEs can be regarded as settled.

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