The accumulation of P-elements on the tip of the *X* chromosome in populations of *Drosophila melanogaster*

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Summary

Little information exists about the mechanisms that determine the fate of mobile elements in natural populations. In this study we catalogue the distribution of 638 P-elements across 114 *X* chromosomes in samples drawn from three natural populations of *Drosophila melanogaster*. There is an extremely high occurrence of elements at the tip relative to the rest of the euchromatic chromosome. We demonstrate that the distribution of *de novo* insertions of the P-element on a specific laboratory chromosome is markedly different; no P-elements were recovered at the tip in the 243 insertion events recorded. In contrast, insertion data for the π_2 chromosome suggests an elevated rate associated with the tip site although it does not appear sufficient to explain the large differential accumulation on wild chromosomes. This raises the issue of inter chromosome (or tip) variation in relative rates, as well as the possibility that rates of elimination are lower at the tip.

1. Introduction

In this study we examine via *in situ* hybridization the distribution of P-elements across the *X* chromosome in samples from three natural populations. The results show a high incidence of elements at the tip of the chromosome. To examine further this unusual observation, we collected and cytologically localized a large sample of *de novo* insertions generated through a dysgenic cross. For this chromosome we see no apparent differential accumulation of elements at the tip.

The P-element has been the focus of intense interest in studies of *Drosophila* transposable elements. This element causes a syndrome manifest in specific interstrain crosses and which depends on the cytotype of the parental lines (Bregliano *et al.* 1980; Engels, 1983, 1988; Finnegan & Fawcett, 1986). The manifestation of cytotype depends in a complex fashion on the presence or absence of functional P-element copies as well as defective KP and Q elements (see Black *et al.* 1987; Nitasaka, Mukai and Yamazaki, 1987). The geographical distribution of *P*, *M*, and *Q* cytotypes has been well characterized (see Kidwell, 1983; Anxolabéhère *et al.* 1985), but only the studies by Ronsseray & Anxolabéhère (1987) and Eanes *et al.* (1988) have examined via *in situ* hybridization the genomic distribution of the P element in samples from

natural populations. Therefore, despite an enormous amount of information on the molecular biology of the P element, the genomic distribution of the element in natural populations is not well described.

Recent empirical studies (Montgomery & Langley, 1983; Montgomery, Charlesworth & Langley, 1987; Ronsseray & Anxolabéhère, 1987; Leigh-Brown & Moss, 1987) have described the distribution of several *copia*-like transposons on chromosomes drawn from natural populations. From the density of element insertions along the chromosome they concluded that most element insertions are unique. That is, recognizing the cytological limits of *in situ* resolution, it appears that each recorded insertion site has a very low *occupation frequency* (Charlesworth & Charlesworth, 1983); high frequency polymorphisms or 'fixations' at specific chromosomal sites appear to be very rare. This observation suggests that site-specific rates of element loss (either by excision or selection) are high relative to rates of insertion, implying a rapid turnover of elements of natural populations.

The differential distribution of elements across the genome will reflect the balance between introduction (insertion) and loss via physical excision or selection. However, directly measuring these rates for different sites across the genome is nearly impossible since selection may be weak, though sufficient, and rates of excision and transposition are very small (Charlesworth & Charlesworth, 1983).

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transposons, the P-element constitutes a unique model system, because the rate of transposition is increased in P-M dysgenic crosses; it is therefore impossible to examine insertion and excision processes and their consequences, whereas these events occur at effectively immeasurable rates for other types of elements. We assume that the same transposition mechanisms operate in both dysgenic and non-dysgenic backgrounds, the former being only an amplified version of the latter. Dysgenic transposition was exploited by Eanes *et al.* (1988) to estimate the average impact on fitness of *de novo* insertions, and is used here to generate a *de novo* distribution of insertions that may be contrasted with the distribution of P-elements seen on chromosomes recently screened from natural populations, presumed to be at equilibrium for these processes.

2. Materials and methods

(i) *Wild chromosomes*

To examine the distribution of P-elements on wild X chromosomes, a single X chromosome was genetically extracted using attached-X, $C(1)DX$, $y\ w\ f$ females (Lindsley & Grell, 1968) of P-cytotype. This line was converted to P-cytotype by backcrossing for five generations to the π_2 strain (Engels, 1983). Three collections of isofemale lines were used in the study. These include 40 wild isofemale lines established from a collection taken in Homestead, Florida in April 1983, a sample of 23 lines taken from Port Jefferson Station, N.Y. in August 1985, and a sample of 51 isofemale lines collected in Botswana, Africa in 1985. Each isofemale line was initially established from a single wild caught female. A single male from each line was crossed with several attached-X females to extract its X chromosome. Element positions were determined in larval males the next generation by *in situ* hybridization to polytene chromosomes using a P-element probe.

(ii) *In situ hybridization*

We have used a modified protocol developed by E. A. Montgomery at the N.I.E.H.S., Research Triangle, NC that uses biotinylated DNA probes (Langer-Safer, Levine & Ward, 1982) visualized by a streptavidin-peroxidase complex and staining with diamino-benzene. We obtained biotinylated d-UTP from Bethesda Research Laboratories and the streptavidin-peroxidase complex from ENZO Biochem, Inc. We have used as a probe the p π_2 25·1 plasmid described in O'Hare & Rubin (1983). This clone contains a complete 2·9 kb P-element and 1·8 kb of flanking single copy DNA homologous to the *hdp* locus at band 17C. Use of this particular probe precludes identification of insertions at this site on our chromosomes, yet serves as an important internal hybridization control. Lines which at first failed to show strong hybridization at this site were repeatedly sampled.

(iii) *Generation of* de novo *insertions*

An M-cytotype strain donated by P. M. Bingham and marked with the X-linked visible mutations $z^a w^{ch}$ was used as a source of an element-free X chromosome. A stock homozygous for a single marked X chromosome was created by extracting a single chromosome with a $FM6/N^{264-84}$ balancer stock obtained from the Bowling Green Stock Center. Males (P-cytotype) from the π_2 strain (Engels, 1983) were crossed with homozygous $z^a w^{ch}$ females (M-cytotype) to create dysgenic hybrid males bearing the $z^a w^{ch} X$ chromosome. Hybrid males were individually mated with females from the $FM6/N^{264-84}$ balancer stock (P-cytotype backcrossing for five generations to the π_2 strain) and a single X chromosome was genetically extracted from the progeny of each male. This design avoids multiple recoveries of insertions as premeiotic germline clonal events, thereby ensuring the independence of individual insertions. Thus, individual chromosomes were subjected to a single dysgenic generation, while the *in situ* hybridizations were carried out one to two generations later. Independent readings were made on all slides by both authors, and disparities were re-examined.

3. Results

(i) *The distribution of P-element insertions on wild chromosomes*

All observed element insertions were localized according to Bridges' (1938) polytene map, which divides the X chromosome into approximately 120 numbered and lettered regions according to banding pattern. Further distinction into the numbered bands *within* lettered sections was not made. We pooled all insertions for section 20, and can make no statements concerning insertions at 17C. This results in a potential classification to 115 sampling intervals.

We are probably underestimating the number of P-elements on the X chromosome. Many P-elements are found as partly deleted copies within the genome (Rubin, Kidwell & Bingham, 1982; O'Hare & Rubin, 1983), and it is unlikely that the *in situ* procedure can identify copies below a critical size. We have *in situ* hybridized this probe to several mutations at the *G6pd* locus which are derived from partial deletions of the P-element of known size. These results show that we can detect partial elements as small as 500 bp. Elements smaller that this will be missed. This does not upset our basic observation that many X chromosome tips in natural populations contain P-elements, although it is possible that some *de novo* insertions at the tip have been missed.

Table 1. *The numbers of X chromosomes observed with different P-element counts in the collections from three populations. Below each distribution in brackets is the G-statistic associated with the goodness-of-fit test to Poisson expectation*

Population	Elements/chromosome										Total chromosomes	Mean/chromosome
	1	2	3	4	5	6	7	8	9	10		
Botswana	0	4	3	6	11	12	5	7	3	0	51	5·61
	[G = 7·423; D.F. = 6, $P < 0·1$]											
Homestead, FL	1	2	3	10	5	11	2	4	0	2	40	5·28
	[G = 9·596; D.F. = 6, $P < 0·1$]											
Port Jefferson Station, NY	1	0	1	3	3	5	3	5	1	1	23	6·13
	[G = 0·796; D.F. = 3, $P < 0·5$]											

We observe most insertions to fall within chromomeres (bands), not interband regions. This simply reflects the fact that chromomeres represent the regions of high DNA content. These bands become the defined sampling intervals, and are the cytological limit of *in situ* resolution. We cannot resolve more than one element per band. Because of the potential for multiple insertion sites per band at the molecular level, the possibility arises that some observations identified as single copy insertions could be unresolved multiple insertions within each interval (Kaplan & Brookfield, 1983). We can also not resolve whether elements identified within the same band in two independent chromosomes have precisely the same insertions sites within that interval.

The distribution of element counts across the 112 chromosomes examined is presented for the three localities in Table 1. The average number of element copies observed per chromosome is $6·13 \pm 0·43$ (S.E.), $5·28 \pm 0·32$, and $5·61 \pm 0·26$ for the New York, Florida, and Botswana samples respectively. The variances in P-element copy number were 4·250, 4·096, and 3·44 respectively for the New York, Florida, and Botswana samples.

If there is equal probability of sampling an element at any interval along the chromosome (occupancy frequencies are equal in all intervals), and if all insertion events are independent, then it is expected that the distribution of element counts per chromosome should be Poisson distributed (Charlesworth & Charlesworth, 1983). The distribution of copy number per chromosome does not significantly deviate from a Poisson expectation for the New York, Florida, and Botswana collections (G = 0·796, D.F. = 3, $P > 0·5$ for New York; G = 9·596, D.F. = 6, $P > 0·1$ for Florida; G = 7·423, D.F. = 6, $P < 0·1$). Classes were pooled if the observed number per cell was ⩽ 3.

The distribution of 638 P-element copies by Bridge's subdivisions on 114 wild X chromosomes from the three natural populations is presented in Figure 1 (top). In Table 2, the occupation frequency distribution or number of intervals carrying $i = 0, 1, 2,$

Table 2. *Occupancy profile for all recognized sampling intervals (see text). Data show the number of intervals, n_i, at which i chromosomes carried elements for each locality*

Site	i chromosomes										Total
	0	1	2	3	4	5	6	7	8	>9	
Botswana											51
$n_i =$	40	26[a]	9	18	6	6	2	2	2	0	1 (i = 12)
											1 (i = 14)
											1 (i = 16)
											1 (i = 51)
Homestead, FL											40
$n_i =$	41	31	19	8	4	4	2	3	2	0	1 (i = 29)
Port Jefferson Station, NY											23
$n_i =$	49	32	19	10	2	1	0	0	0	1	1 (i = 19)

[a] Indicates that there are 26 intervals where only a single element was observed in the total collection of 51 chromosomes.
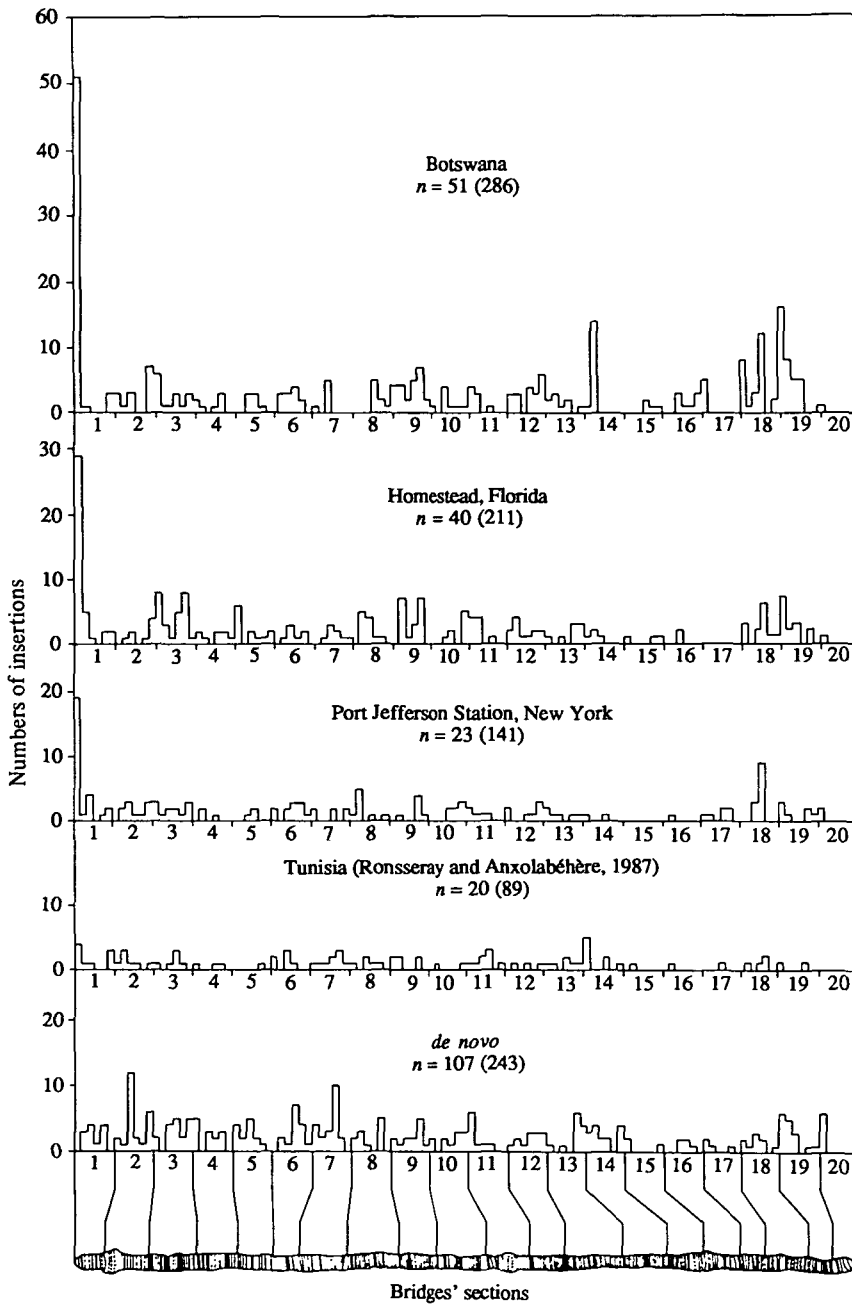
Fig. 1. The distribution of 638 P-element insertions on 114 wild chromosomes (top) collected from Botswana, Africa, Homestead, Florida and Port Jefferson Station, N.Y. The Tunisia data ($n = 20$ chromosomes) from Ronsseray & Anxolabéhère (1987) are also plotted. The number of insertions is given in parentheses after the sample size. In addition the distribution (bottom) of 243 independent *de novo* insertions recovered from replicates of a single stem chromosome passed through a P-M dysgenic cross are shown. Element positions are shown relative to Bridges' polytene map which is divided here into his numbered and lettered subdivisions. Subdivision distinctions could not be made in section 20.

3 ... *n* elements in the samples of *n* chromosomes from each collection is summarized. Each collection of chromosomes contains one specific interval where the occupancy frequency is very high. In each case this site is at the distal end of the *X* chromosome. The published data for Tunisia (Ronsseray & Anxolabéhère, 1987) also summarized in Figure 1, shows only 4 of 20 chromosomes possessing P-elements at the tip of the *X* chromosome.

(ii) *The distribution of* de novo *P-element insertions on the X chromosome*

A total of 107 independent *X* chromosomes was recovered from the described dysgenic cross and screened by *in situ* hybridization for P-element insertions. We recorded 243 *de novo* insertions or an average rate of 2·27 element insertions per *X* chromosome. This rate of insertion is approximately twice

that reported for other studies (Engels, 1983). We partly attribute this to residual dysgenesis in the one to two subsequent generations following the primary dysgenic generation and prior to our *in situ* hybridization. The inheritance of cytotype is clearly complex (Engels, 1983) and we suspect the *FM6/N²⁶⁴⁻⁸⁴* stock is variable for cytotype. The number of insertions per chromosome does not fit a Poisson distribution (G = 64·1, D.F. = 9, $P \ll 0.005$). We assume the overdispersed distribution reflects heterogeneity in the amount of dysgenesis created in independent germ cell lines. All recovered chromosomes were sheltered over the *FM6* balancer chromosome to reduce selection bias against subvital insertions. Figure 1 (bottom) summarizes the distribution of the *de novo* insertions on these chromosomes.

No *de novo* insertions were recovered at the tip of the *X* chromosome. If it is assumed that for a sample of chromosomes the number of insertions for any site or interval is Poisson distributed, then the upper 95 percent confidence limit for the real proportion of *de novo* insertions associated with the tip is less than three per cent of the total *de novo* insertions on the entire *X* chromosome.

4. Discussion

The studies on *copia*-like transposons (Montgomery & Langley, 1983; Ronsseray & Anxolabéhère, 1987; Leigh-Brown & Moss, 1987), report element insertions to occupy all intervals at low frequency. The distribution of P-elements on the *X* chromosome conforms to those previous observations (low occupancy frequency per site) with one unique exception. We observe a high frequency of P-elements at the tip of the *X* chromosome in samples from three different natural populations. This was not observed by Ronsseray & Anxolabéhère (1987) for their Tunisian chromosomes. In contrast, our study of *de novo* insertion into a single chromosome showed no insertions into the tip, and no suggestion of non-random distribution across the remainder of the chromosome. The *de novo* data would suggest that elevated insertion does not explain the high frequency of P-elements at the tip of wild *X* chromosomes in our samples.

It is possible that the failure to recover *de novo* insertions at the tip of the $z^a w^{ch}$ stem chromosome reflects interchromosomal variation for insertion at this site, and the particular chromosome we selected possesses no tip insertion sites. There is cytological evidence for structural heterogeneity of *X* chromosome tips (see Roberts, 1979) in *Drosophila melanogaster*. Several other observations are pertinent to this question. None of the 18 P-element insertions recovered on the hybrid dysgenic Canton-S *X* chromosome was at the tip (Bingham, Kidwell & Rubin, 1982). However, Benz (personal communication) has kindly provided *de novo* insertion data for 55 sublines

of the π_2 chromosome which were maintained for up to 18 generations in a continuously dysgenic background. Out of 562 total insertions, 34 were identified at the tip. Using the raw data on total *de novo* insertions for the two chromosomes ($z^a w^{ch}$ and π_2) it can be shown that there is a statistically significant difference in tip insertion frequencies (0 and 243 for $z^a w^{ch}$, 34 and 528 for π_2; Fisher's exact test, $P < 0.0001$). Adjusting for the occurrence of multiple insertions into the tip in each line, Benz estimates the rate of insertion into that site to be about 0·052 per generation, or about 7 % of all *de novo* insertions on the *X* chromosome. From the comparisons of insertion frequencies into the tip for these three independent *X* chromosomes, it clearly appears there is variation in the rate of insertion into the tip. But it is not clear whether this variation is sufficient to generate the high occupancy frequencies in the wild chromosomes. The low occupation of this site in the Tunisian chromosomes also suggests that there could be *X* chromosome variation in the potential of the tip to accumulate P-elements, and this extends to geographical variation. It is desirable to repeat the *de novo* study with a large collection of independent *M* chromosomes.

Why should the tip or 'telomeric' sites *per se* possess such a high frequency of P-elements? The answer must involve elevated insertion or reduced loss. The principal features associated with the tip are so-called beta-heterochromatin and greatly reduced genetic crossing over. Heterochromatic regions could serve as element 'sinks' because they carry other middle repetitive sequences (Miklos *et al.* 1988) that may provide a high density of the consensus target sequence (O'Hare & Rubin, 1983), and so make insertion more probable, or because insertion in heterochromatin has relatively benign effects on fitness. The lack of crossing over could lead to the accumulation of elements by preventing the production of defective gametes via asymmetric synapsis (see Davis, Shen & Judd, 1987; Goldberg *et al.* 1983 Langley *et al.* 1988), or because physical excision involving recombination dependent mechanisms may be reduced in heterochromatic regions. At least three mechanisms may account for our observations.

We have screened the autosomal arms on these same genomes, and although we have not classified the insertions to the fine detail reported here for the *X* chromosome, we can confidently state that none of the four autosomal tips possesses elements at a similar frequency. Therefore, this excess is apparently not associated with telomeres *per se* but, rather, seems specific to the tip of the *X* chromosome. Furthermore, if suppression of crossing over were the primary mechanisms, we would expect to see an accumulation of elements throughout Bridges' first section, since recombination is substantially suppressed across this entire region relative to other sections. It appears that the answer to this problem may require analysis of the tip insertion sites at a molecular level.

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