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Accumulation of P elements in minority inversions in natural populations of *Drosophila melanogaster*

WALTER F. EANES[1]†, CEDRIC WESLEY[1] AND BRIAN CHARLESWORTH[2]
[1] *Department of Ecology and Evolution, State University of New York, Stony Brook, N.Y. 11794*
[2] *Department of Ecology and Evolution, 1101 East 57th Street, University of Chicago, Chicago, IL 60637*

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Summary

The accumulation of a transposable element inside chromosomal inversions is examined theoretically by a mathematical model, and empirically by counts of P elements associated with inversion polymorphisms in natural populations of *Drosophila melanogaster*. The model demonstrates that, if heterozygosity for an inversion effectively reduces element associated production of detrimental chromosome rearrangements, a differential accumulation of elements is expected, with increased copy number inside the minority inversion. Several-fold differential accumulations are possible with certain parameter values. We present data on P element counts for inversion polymorphisms on all five chromosome arms of 157 haploid genomes from two African populations. Our observations show significantly increased numbers of elements within the regions associated with the least common, or minority arrangements, in natural inversion polymorphisms.

1. Introduction

While transposable elements have been implicated in a large number of classical Mendelian mutations in *Drosophila* (Finnegan & Fawcett, 1986), their contribution to the spectrum of mutation in natural populations is unknown. It is believed that transposable element insertions will often produce detrimental effects on fitness (see Charlesworth & Langley, 1989). If this is true, and detrimental transposon insertions are expressed in fully to partially recessive fashion, then lower densities of elements are expected on the X chromosome when compared to the autosomes. However, published studies of *copia*-like and P elements in *Drosophila melanogaster* which have contrasted numbers of elements on these chromosomes failed to observe a consistently lower density on the X chromosome (Montgomery, Charlesworth & Langley, 1987; Eanes *et al.* 1988). A study of nine families shows a significant deficiency of elements on the X, but not as large as that predicted by the above mechanism (Charlesworth & Lapid, 1989). This inconsistency can be explained if many insertion events are either neutral, or have phenotypic effects that are dominant with respect to fitness, so that the effective selection intensity is not increased by male hemizygosity. Dominant fitness is likely if the detrimental consequences of the presence of transposon insertions are due to chromosomal rearrangement generated by ectopic exchange between elements, since the resultant aneuploidy will often have dominant effects on fitness. Therefore, the most significant component of element elimination in natural populations may be due to ectopic exchange between transposons (Langley *et al.* 1988).

Both simple unequal exchanges between non-homologous element copies (Goldberg *et al.* 1983; Davis, Sheen & Judd, 1987), and dramatic chromosomal rearrangements related to transposon-associated mutator systems, are observed in *Drosophila* (Berg, Engels & Kreber, 1980; Engels & Preston, 1981; Lim *et al.* 1983). It is not known if these secondary forms of mutational change are important in element elimination in natural populations, or if it is the disruption of gene expression by simple element insertion that eventually results in element loss. Unfortunately, direct measurement of the dominant lethal mutation rate is not possible, but one prediction of the hypothesis that lowered fitness due to element-associated rearrangement contributes to element copy loss is that genomic features that suppress meiotic recombination, or eliminate the meiotic transmission of such exchanges, should be associated with the accumulation of elements.

† Corresponding author.

Several recent papers have reported the accumulation of transposons in the telomeric and centromeric regions of the genome that are associated with reduced meiotic recombination (Montgomery, Charlesworth & Langley, 1987; Langley *et al.* 1988; Charlesworth & Lapid, 1989). There is, however, little evidence for an accumulation of elements in the telomeric regions of chromosomes (Charlesworth & Lapid, 1989, and in preparation; Aguade, Miyashita & Langley; Beech & Leigh-Brown, 1989; Eanes, Labate & Ajioka, 1989), except for the study of Ajioka & Eanes (1989) showing an excess of P elements at the tip of the X chromosome. This latter result may be due to site-specificity of insertion ability.

Since chromosome inversions reduce recombination, it also has been proposed that transposable elements should accumulate around inversion break points, and this has been reported for balanced lethal inversions in laboratory stocks (Montgomery, Charlesworth & Langley, 1987). Another expectation in natural populations is that element accumulation should be frequency-dependent with respect to inversion type; rarer inversions should carry more elements. This is because the opportunity for recombination is suppressed in heterozygotes; the rarer the inversion in a population, the greater the proportion of the inversion's copies that are present in heterozygous state and the lower the potential recombination rate among copies of that arrangement. If element loss is correlated with recombination, by any mechanism, then minority inversions should carry higher numbers of affected element families.

In this study we present data on the numbers of P elements associated with five inversion polymorphisms in two natural populations of *Drosophila melanogaster*. We also present a general model quantifying the effects of a chromosomal inversion in sheltering element loss, and the subsequent accumulation of elements in the region spanned by the rearrangement.

2. Methods and Materials

(i) *Wild genomes*

Polytene chromosomes were examined from 86 and 71 isofemale lines established from flies collected in August, 1985 in the Luangwa National Park, Zambia and the Okavango Delta, Botswana. These lines carry the P-*cytotype*. *In situ* hybridizations were carried out in the summer of 1986. Wild-type males from each line were crossed with either *C(1)DX* females or females from a line marked with larval visibles $z^a w^{ch}$. Both lines are M-cytotype, lack P elements, and are homozygous for all standard autosomal arrangements. In the former cross, third instar female larvae are heterozygous for wild and strain derived autosomal homologs, but no data are available for the X chromosome as the females carry the *C(1)DX* arrangement. Male larvae from this cross carry the same heterozygous autosomal complement, and a wild-derived X chromosome. For the latter cross only female larvae were screened, and these female progeny are heterozygous for all wild and $z^a w^{ch}$ homologues. P elements appearing on these arms after *in situ* hybridization are present only on the wild homologues. Only a single genome was sampled from each isofemale line. Some lines were inadvertently sampled more than once, and in these instances one observation was sampled at random for inclusion in the data set. Sample sizes for each arm are not equal because not all chromosome arms could be evaluated in each squash.

(ii) *Polytene chromosome preparations and* in situ *hybridizations*

Polytene chromosome preparations were carried out as described in Pardue and Gall (1975). *In situ* hybridizations using biotinylated probe DNA (Langer, Waldrop & Ward, 1981) were carried out using a modified protocol developed by E. A. Montgomery at the N.I.E.H.S., Research Triangle, NC. Biotinylated d-UTP was purchased from BRL laboratories and the streptavidin–peroxidase complex was obtained from ENZO Biochem., Inc. The $p\pi_2 25{\cdot}1$ plasmid was used as a probe (O'Hare & Rubin, 1983). This element was cloned from site 17C, the site of the *hdp* locus (Engels & Preston, 1981). Use of this particular probe precludes identification of elements at this site.

Because P elements are heterogeneous in size, and since the *in situ* method cannot resolve P element derivatives below about 500 bp (Eanes *et al.* 1988), some elements will go undetected. We do not expect this to bias the hypotheses we are examining, since we expect this error to be random with respect to inversion genotype.

(iii) *Statistical tests*

Since the data are not normally distributed, tests were carried out using non-parametric statistics. Wilcoxon two-sample tests on ranked data were carried out on each inversion polymorphism across localities separately. The statistic U_s is then used to compute a t statistic, which is compared to the expected value with infinite degrees of freedom (Sokal & Rohlf, 1981).

3. Results

(i) *Inversion polymorphisms*

The African populations studied show substantial inversion polymorphism. Paracentric inversion polymorphisms with individual frequencies ranging from 4 to 35% were observed on all five chromosome arms in both population samples. Figure 1 illustrates the

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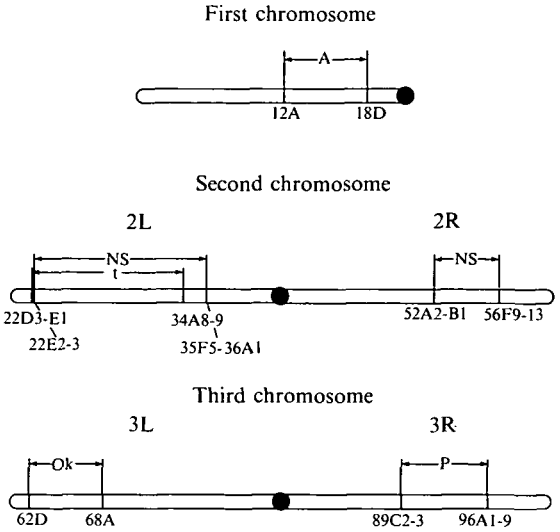

Fig. 1. The cytological size and distribution of the six polymorphic chromosome inversions observed in the two African samples.

positions and sizes of the regions spanned by the observed inversions on each arm, as well as positions of the break points. The routinely described cosmopolitan inversions, *In(2L)t*, *In(2R)NS*, and *In(3R)P* were observed on three autosomal arms. There is also an inversion polymorphism on the X chromosome with break points 12A–18D, and this is designated *In(1)A*. In *D. melanogaster* X linked inversions are very rare, and polymorphic inversion frequencies have never been reported in any population. This constitutes an exceptional polymorphism with frequencies 0·11 and 0·13 in the Luangwa and Okavango collections respectively. There is also a polymorphism for a unique inversion with break points 62D–68A, designated here as *In(3L)Ok*. The frequency of this inversion is estimated at 33 and 22% at the two localities, and the cosmopolitan *In(3L)P* inversion usually seen on this arm is not present. Ashburner & Lemeunier (1976) reported a single observation of an inversion with similar break points in an isofemale line from Malawi, and we suspect that this is the same inversion. The presence of each polymorphism at both sites, which are separated by over 1000 kilometres, indicates that these inversions are geographically widespread in this region, for which few data on inversion frequencies exist. Several other rare inversions were observed on each arm, and exclusion of these chromosomes results in unequal sample sizes per arm.

(ii) *P element numbers and inversions*

The data for both localities consist of P element counts for the paracentric inversions on five chromosome arms. The inversion frequencies, P, distribution of element counts, mean numbers of P elements within the inverted n_l, and standard regions, n_s, and t-statistics associated with the non-parametric rank tests are given in Table 1. Because of the mixed scheme used to sample genomes, there are fewer data

Table 1. *The numbers of chromosomes associated with different numbers of* P *element copies within regions spanned by the shown polymorphic chromosome inversions*

		P elements									
Arrangement	P	0	1	2	3	4	5	6	n	$n_l:n_s$	t
LUANGWA											
Standard	—	16	17	16	7	—	—	—	1·25	1·14	+0·45
In(1)A	0·11	—	5	1	1	—	—	—	1·43	—	—
Standard	—	11	28	10	11	1	—	—	1·37	1·88	+3·97***
In(2L)t	0·24	—	2	11	3	4	1	—	2·57	—	—
Standard	—	42	17	3	—	—	—	—	0·41	2·32	+3·78***
In(2R)NS	0·23	4	12	3	—	1	—	—	0·95	—	—
Standard	—	12	29	9	2	3	—	—	1·13	0·66	−2·04*
In(3L)Ok	0·33	14	7	5	1	—	—	—	0·74	—	—
Standard	—	19	18	14	14	11	3	1	1·91	1·40	+0·91
In(3R)P	0·04	—	1	—	1	1	—	—	2·67	—	—
OKAVANGO											
Standard	—	12	22	9	9	—	1	—	1·36	1·65	+2.29*
In(1)A	0·13	—	1	5	1	1	—	—	2·25	—	—
Standard	—	12	18	8	6	1	—	—	1·27	1·55	+1·99*
In(2L)t	0·35	1	11	6	5	1	—	—	1·79	—	—
Standard	—	31	23	7	2	1	—	—	0·73	2·73	+3·08***
In(2R)NS	0·10	—	3	1	3	—	—	—	2·00	—	—
Standard	—	24	10	10	7	2	1	—	1·19	1·29	+0·87
In(3L)Ok	0·22	4	3	3	1	2	—	—	1·54	—	—
Standard	—	11	25	9	5	—	—	—	1·18	1·83	+2·90***
In(3R)P	0·20	1	2	4	4	1	—	—	2·17	—	—

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

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for the X chromosome. The data for inversions *In(2L)t* and *In(2L)NS* have been pooled because the inversion break points are very close, but only two observations of the latter inversion were made. In the haploid genomes surveyed, the regions spanned by the polymorphic inversion average 0·41–2·57 P elements depending on inversion size (Fig. 1). The model predicts that the minority arrangement will possess a higher number of elements. Since there are ten pairs of element counts, there are ten non-parametric tests of the null hypothesis of equal count ranks between inverted and standard arrangements, against the alternative hypothesis of greater numbers in the rare inversions, as predicted by the model. The probabilities shown are for one-tailed *t*-tests. In each case the standard arrangement is the most common. In nine of ten cases the minority arrangement is observed to possess a greater number of P elements, and in six tests the difference is statistically significant. The one case of reversal is also statistically significant ($P < 0{\cdot}05$).

(iii) *A model*

The model developed here is based on that proposed by Langley *et al.* (1988) for the study of the expected distribution of elements between the X chromosome and the autosomes of *Drosophila melanogaster* with ectopic exchange between transposable elements. An inverted sequence, I and a standard sequence, S, located on one of the two major autosomes, are assumed to be segregating in a natural population with frequencies P and Q respectively. Hardy–Weinberg frequencies, P^2, $2PQ$, and Q^2 are assumed for the genotypes I/I, I/S, and S/S respectively. Exchange between homologous elements located at different chromosomal sites is assumed to be meiotic, and hence confined to females. The second model of Langley *et al.* (1988), in which exchange occurs only between elements located in nearby chromosomal regions, will be assumed, since this appears to have the greatest empirical support in *Drosophila* (Davis, Sheen & Judd, 1987). Our qualitative conclusions are unlikely to be seriously affected by this assumption. In order to construct a simple model of the transport of elements between different genomic locations, we assume that elements are located only in the euchromatic regions of the X chromosome and autosomes. This is consistent with the finding that P elements are generally absent from heterochromatin (Engels, 1988). The behaviour of the system is then described by the haploid mean copy numbers for the X chromosome and non-inverted autosome (n_X and n_A), together with the haploid mean copy numbers for the region of the chromosome polymorphic for the inversion that is unaffected by the presence of the inversion (n_B), for the region of inversion-carrying chromosomes where exchange is restricted in inversion heterozygotes (n_I), and for the corresponding region of the standard chromosomes (n_S) (see Table 2). Recombination is assumed to be sufficiently free outside the regions affected by inversion heterozygosity that the I and S chromosomes have the same copy number for those regions.

Table 2. *Parameters and variables used in the model of* ectopic exchange

n_X	haploid mean copy number for the X chromosome
n_A	haploid mean copy number for the autosome that is chromosomally monomorphic
n_B	haploid mean copy number for the region of the chromosomally polymorphic autosome that is unaffected by the inversion
n_I	haploid mean copy number for the region of the chromosomally polymorphic autosome that is affected (spanned) by the inversion in inversion carrying chromosomes
n_S	haploid mean copy number for the region of the chromosomally polymorphic autosome that is affected (spanned) by the inversion in standard sequence
k_{AA}	probability of ectopic exchange per element for the chromosomally monomorphic autosome
k_{II}	probability of ectopic exchange per element for the region affected by the inversion, in inversion carrying chromosomes
k^{IS}	probability of ectopic exchange per element for the region affected by the inversion, in inversion heterozygotes
k_{BB}	probability of ectopic exchange per element for the region of the chromosomally polymorphic autosome that is unaffected by the inversion

These considerations lead to the following system of equations that describe the changes in mean copy number per generation (see detailed description in the Appendix):

$$\Delta n_X = -n_X(v + 0{\cdot}667k_{XX}\, n_X) + u(0{\cdot}170n_X + 0{\cdot}207[n_A + n_B + Pn_I + Qn_S]),$$

$$\Delta n_A = -n_A(v + 0{\cdot}5k_{AA}n_A) + u(0{\cdot}311n_X + 0{\cdot}422[n_A + n_B + Pn_I + Qn_S]),$$

$$\Delta n_B = -n_B(v + 0{\cdot}5k_{BB}n_B) + u(1-p)(0{\cdot}311n_X + 0{\cdot}422[n_A + n_B + Pn_I + Qn_S]),$$

$$\Delta n_I = -n_I(v + 0{\cdot}5n_I[Pk_{IS}n_S + Qk_{IS}n_S]) + up(0{\cdot}311n_X + 0{\cdot}422[n_A + n_B + pn_I + 0{\cdot}5Q(n_S + n_I)]),$$

$$\Delta n_S = -n_S(v + 0{\cdot}5n_S[Pk_{IS}n_I + Qk_{II}n_S]) + up(0{\cdot}311n_X + 0{\cdot}422[n_A + n_B + 0{\cdot}5P(n_S + n_I) + Qn_S]).$$

The equilibria generated by these equations were determined by computer calculations of population trajectories. It was verified that when $k_{IS} = k_{II}$ the equilibrium abundance of elements in the region

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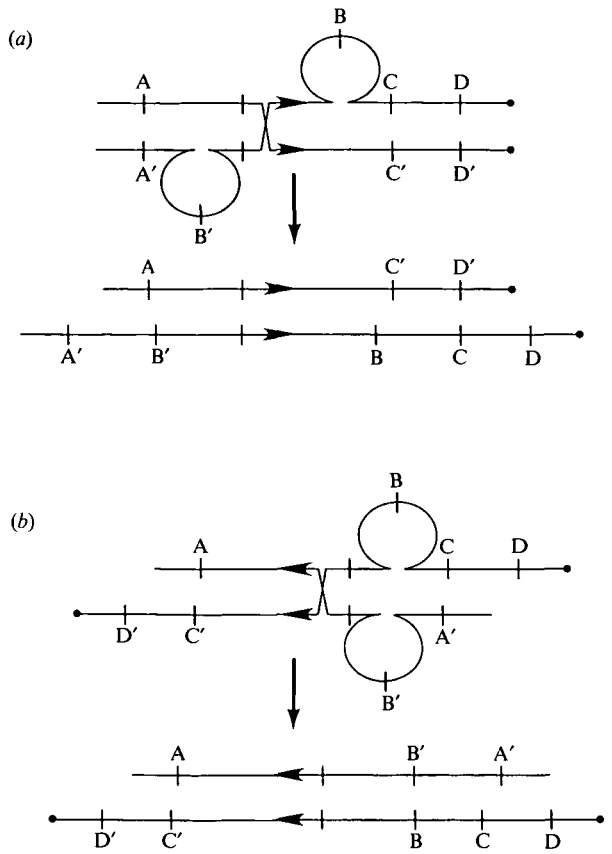

Fig. 2. The consequences of ectopic exchange between elements located on homologous chromosomes when the elements are oriented in (*a*) direct and (*b*) reverse fashions. The position of an element is denoted by a vertical line followed by an arrow. In an inversion homozygote exchange between elements in direct orientation results in a duplicated and deficient strand (*a*) and in a deficient, acentric and a duplicated, dicentric strand in (*b*). Conversely, in inversion heterozygotes exchange between elements in reverse orientation results in a deficient, acentric strand and a duplicated, dicentric strand as in (*a*), and in direct orientation results in duplicated and a deficient strand as in (*b*).

affected by the inversion was equal to the fraction of the chromosome affected by the inversion (p), so that the inversion had no effect on the rate of unequal exchange. In other cases, the region affected by inversion heterozygosity equilibrates such that the proportion of elements found in this region was higher than p, for both I and S chromosomes. The effect is larger for the chromosome that is rarer (arbitrarily assumed to be I). When the inversion frequency P is low, there can be a strong differential accumulation of elements in the affected region. The differential is highest when the rate of exchange in inversion heterozygotes is zero for the region in question ($k_{IS} = 0$), and the excision rate is zero ($v = 0$), as might be expected intuitively when this mechanism is the only one responsible for element loss.

The question arises as to how much suppression of ectopic exchange is likely to occur in inversion heterozygotes. Unfortunately, it is difficult to give a clear cut answer to this question at present. Since mitotic exchange is much less frequent than meiotic exchange in *Drosophila* (Ashburner, 1989, Chapter 29), it seems reasonable to assume that meiotic events are the main source of ectopic exchanges. [Recent evidence from yeast indicates that ectopic and regular exchanges have similar causal pathways (Steele, Morris & Jinks-Robertson, 1991).] Ectopic exchanges may generate a diverse array of chromosomal rearrangements, depending on the orientation of the repeated elements involved, and on whether the exchanges involve sister-chromatid, intrachromosomal or interchromosomal events [see Fig. 2 of Petes & Hill (1988)]. Meiotic intrachromosomal or sister-chromotid ectopic exchanges will not be affected by inversion heterozygosity. It is not known how frequent such exchanges are in relation to ectopic exchange between homologues in *Drosophila*, although evidence in yeast suggests strongly that inter-homologue exchanges are the most frequent class of event (Jackson & Fink, 1985; Petes & Hill, 1988), and regular meiotic sister strand exchange is infrequent in *Drosophila* (Ashburner, 1989, Chapter 22). Regular meiotic exchange is reduced in inversion heterozygotes in female *Drosophila*, both because of inhibition of pairing between inverted and standard sequences, and because of the elimination of single exchange products into the polar body due to linear orientation of meiotic products in *Drosophila* (Roberts, 1976). The former effect can be large for the relatively small inversions characteristic of natural populations (Roberts, 1976), and would probably cause a large reduction in the frequency of meiotic interchromosomal ectopic exchange in inversion heterozygotes, extending beyond the limits of the inversion itself.

The consequences of exchange in inversion heterozygotes are harder to gauge. Figure 2 shows the effects of inter-homologue ectopic exchange in structural homozygotes. Ectopic exchange between elements in direct orientation (*a*) leads to chromosomes carrying duplications and deficiencies, which would have severely deleterious fitness effects and lead to element loss. Exchange between elements in reverse orientation (*b*) leads to the formation of acentric and dicentric chromosomes, which would be passed into the polar body in the same way as regular exchanges in inversion heterozygotes and hence not contribute to any loss in fitness. The same formation of acentric and dicentric chromosomes occurs with ectopic exchange between elements in direct orientation in inversion heterozygotes, and shelters elements from loss. However, ectopic exchange in inversion heterozygotes between elements in reverse orientation leads to the production of duplication/deficient chromosomes, with a consequent reduction in fitness. Since the orientation of elements on different chromosomes is presumedly random, any effect of inversion heterozygosity on reducing ectopic exchange through this path depends

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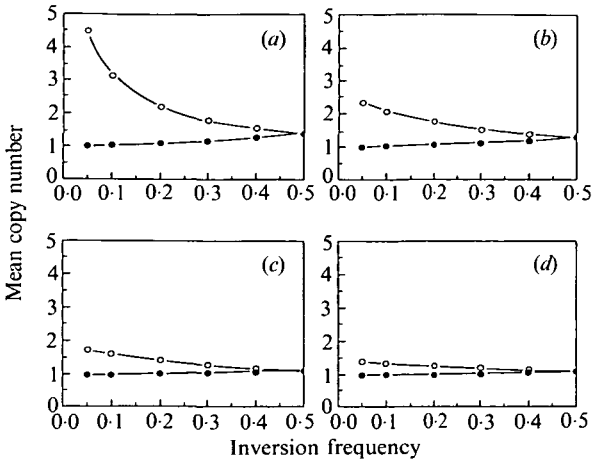

Fig. 3. The upper curves in each plot show the equilibrium mean numbers of elements on the inversion (I) chromosomes that are located within the region affected by inversion heterozygosity, as a function of the equilibrium frequency of I. The lower curves show the corresponding mean numbers for the standard (S) chromosomes. The transposition rate (u) is 0·001 for Fig. 3*a*, *c*, and 0·0015 for 3*b*, *d*. The excision rate (v) is 0 for Fig. 3*a*, *c*, and 0·0005 for 3*b*, *d*. The coefficient for the effect of unequal exchange for the autosome lacking the inversion (k_{AA}) is 0·0002 in each case, corresponding to the haploid mean copy number of 10 per chromosome in the absence of the inversion. The mean copy number for the X chromosome is somewhat less than one half this, and the exchange coefficient k_{XX} is 0·0004. The proportion of the other autosome affected by the inversion (p) is 0·1, so that the coefficient for the effect of unequal exchange for this region in chromosomal homozygotes (k_{II}) is 0·002. The coefficient of unequal exchange for this region in chromosomal heterozygotes (k_{IS}) is assumed to be zero in Fig. 3*a*, *b*, and $0{\cdot}5k_{II}$ in Fig. 1*c*, *d*.

on whether ectopic exchanges between elements in reverse orientation are less frequent than between elements in direct orientation. It seems more difficult topologically to achieve ectopic pairing between elements in reverse orientation, since the reversed loop similar to that required for pairing in an inversion heterozygote must be formed in this case, but no direct evidence is available.

Some examples from the model are illustrated in Figure 3. Two extreme cases of the possible effects of inversion heterozygosity are shown. In each case, equilibrium mean copy numbers in the inverted region of the inversion chromosome are compared with the numbers for the corresponding region of the standard chromosome. In Figs 3a, b, ectopic exchange is completely suppressed in inversion heterozygotes. It can be seen that a very large excess of elements in the inversion is maintained in this case, especially with low frequencies of the inversion. In Figs 3*c*, *d*, the more conservative assumption of 50% reduction in ectopic exchange in inversion heterozygotes is assumed. Even in this case there can be a substantial excess abundance of elements in the inversion chromosome, especially with low rates of excision. The strong effect of inversion frequency on the differential between inverted and standard arrangements is clearly evident. It is also apparent that the abundance of elements in the region of the standard chromosome corresponding to the inversion is somewhat larger than the value expected from the size of this region (approximately 1 in this case). This effect is greatest for high frequency inversions, and reflects the effect of inversion heterozygosity on exchange in the standard chromosomes.

4. Discussion

The contribution that transposable elements make to mutation in natural populations, and the way in which their phenotypic effects of fitness determine element number and genomic distribution are unknown. Observations so far indicate that transposable elements create two classes of mutation. Many transposable element insertions probably cause polygenic mutations, reducing fitness by very small increments (Montgomery, Charlesworth & Langley, 1987; Eanes *et al.* 1988). Other insertions may generate mutations of large effect, such as lethal mutations, but most of these will be eliminated as a result of their heterozygous effects. Some mutations may even be neutral as insertion events. However, any insertion, if it becomes the site of a non-homologous exchange event, has the potential to become associated with a dominant lethal mutation, regardless of its initial fitness impact. While direct measurement of the rate at which elements contribute to dominant detrimental mutation through ectopic exchange is prevented by the technical impossibility of recovering dominant lethals, one prediction of this model is that the density of elements should increase in association with genomic features known to suppress meiotic recombination, such as telomeric and heterochromatic regions, and inversions (Langley *et al.* 1988). We report here significant differences in element density between inverted and standard arrangements, with the minority arrangement usually bearing the higher density of P elements. We propose it is the general suppression of recombination in inversion heterozygotes that causes this pattern, as discussed in Section 3(iii).

At the population level, the sheltering of P elements from elimination by suppression of exchanges is expected to be frequency-dependent, since the opportunity to lose elements via this mechanism is limited to karyotypic homozygotes. As an inversion becomes the minority arrangement, an increasing proportion of its copies (equal to the frequency of the other arrangement) are carried in heterozygous condition and the associated element copies are sheltered. Rare inversions should possess a higher transposable element copy number. This intuitive argument is quantified in our model of the genomic distribution of elements associated with inversion polymorphisms. It is important to recognize that the complete sup-

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pression of ectopic exchange is not necessary for significant differential accumulations (see Figs 3*c*, *d*).

The intent of the model is to confirm the frequency-dependency of the accumulation and examine the bounds of the potential accumulation under various parameter values. It should also be recognized that our model is an equilibrium model and if these populations have reached equilibrium is unclear. The model predicts measurable differences in element density, certainly of the order observed here. It is not expected that a rigorous quantitative fit to the model can be carried out, since many parameters are involved and their values unknown. As predicted, the linear regression between transformed observed n_I/n_S values and inversion frequency P is negative, but the slope is not statistically significant from zero. However, the more general *a priori* prediction that the minority arrangement will always possess the higher number of elements is very clear in the data. Nine of ten cases (six statistically significant) are in agreement with this prediction. This is statistically significant by sign test ($P = 0{\cdot}02$; see Sokal & Rohlf, 1981). The sole exception is associated with the inversion polymorphism for *In(3R)Ok* in Luangwa, and it is statistically significant ($t = 2{\cdot}28$, $P < 0{\cdot}05$). That inversion frequency is relatively high, $P = 0{\cdot}33 \pm 0{\cdot}052$ (S.E.). Inversion frequencies in *Drosophila melanogaster* are known to fluctuate significantly over time (Inoue & Watanabe, 1979), and so we do not know the extent to which the observed inversion frequencies for any polymorphism are representative of the long term frequencies. This means that our results must be interpreted with caution.

An alternative to the ectopic model is, at first sight, suggested by the model of P element excision recently proposed by Engels *et al.* (1990). This envisages the occasional creation of double stranded gaps in DNA, as the result of the excision of a P element from its site of insertion. It is postulated that, in most cases, recombinational repair from the sister chromatid replaces the lost element with the sister copy, resulting in a transposition event if the excised element reinserts, but no element loss from the original site. In some cases, however, the template for repair is the homologous chromosome, which is unlikely to carry an element at the same site, thus resulting in complete loss of the element from its original site. Engels *et al.* (1990) showed that excision of P elements is suppressed by the absence of a homologue, as in the case of the X chromosome in males, or when a rearrangement restricts pairing. Therefore, the failure of pairing in inversion heterozygotes might seem at first sight to reduce the rate of excision and hence lead to higher abundance of P elements in low frequency inversions. However, failure to repair a double-stranded break will lead to dominant lethality of the chromosome in question when it is transmitted to the progeny. Loss of the unrepaired chromosome is equivalent to excision of the P element as far as the dynamics of element abundance is concerned (the element disappears from the population in both cases), and so this process in itself will not contribute to a build-up of elements in minority inversions. But insofar as the induction of double-strand breaks contributes to the production of rearrangements by recombinational processes outlined above, this mechanism could indirectly cause an accumulation of P elements in inversions.

It is not clear if inversion heterozygosity will suppress element loss associated with rearrangements arising at stages other than meiosis. Loss due to premeiotic rearrangement in both sexes may or may not be suppressed by inversion heterozygosity, and it may not shelter elements from rearrangements that arise because of induced recombination in males. However, to the extent that fitness loss due to rearrangements is reduced at any stage by inversion heterozygosity, it would result in the differential accumulation of elements inside inversions because of the frequency-dependent nature of the process. With a more detailed understanding of the mechanistic processes that generate P element associated rearrangement at all stages, it may be possible to propose alternative hypotheses, other than suppression of crossing over, to explain the observed differential accumulation of P elements in minority arrangements.

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Appendix

The probability per generation that a given element produces a new copy that inserts elsewhere is denoted by u and the probability that it is excised or eliminated by selection is v. The mean numbers of new copies produced each generation in females and males are thus $2u(n_X + n_A + n_B + Pn_I + Qn_S)$ and $2u(0{\cdot}5n_X + n_A + n_B + Pn_I + Qn_S)$ respectively. We assume that the X chromosome has the same size as an autosomal arm, so that the probability of a newly transposed element inserting into an X chromosome in a female is 2/10, and the corresponding probability for a male is 1/9. Similarly, the probability that an element inserts into the inversion-free autosome is 4/10 for females and 4/9 for males. If the region affected by the inversion occupies a fraction p of that chromosome, then the probability of transposition into that region is $4p/10$ for females, and $4p/9$ for males; the corresponding probability for the other part of the chromosome are $4(1-p)/10$ for females and $4(1-p)/9$ for males. For the X chromosome, the mean increase in copy number due to transposition is thus given by multiplying the mean total increase for females by 2/10, and the value for males by multiplying their mean total increase by 1/9. Since there are two X chromosomes in each female and one in each male, yielding a total of three per mating pair, the net increase in haploid mean copy number for the X chromosome is obtained by dividing the sum of these two quantities by three. The corresponding values for the inversion-free chromosome and the region of the polymorphic autosome that is unaffected by the inversion are obtained by dividing the equivalent sums by four, taking into account the relevant probabilities of insertion into these regions. The values for the regions of I and S chromosomes that are affected by inversion heterozygosity are calculated similarly, taking account of the fact that these chromosomes each have probabilities P and Q of being present in association with I and S homologs respectively.

The decreases in mean copy numbers due to excision (and selection) events are given by multiplying the corresponding mean copy numbers by v. The decreases due to the ectopic exchange model used here are as follows. The model implies that the probability of an exchange in a female between two elements that leads to their elimination, in a region i of the genome that contains n_i elements is $k_{ii}n_i^2$, where k_{ii} is inversely proportional to the size of region i (Langley *et al.* 1988, equation 13). Since an autosome is twice the size of the X chromosome, the k value for the X chromosome, k_{XX}, is twice the value for the inversion-

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free autosome, k_{AA}. Similarly the k value for the region affected by the inversion in homozygotes for the I or S chromosomes is $k_{II} = k_{AA}/p$, and the value for this region in heterozygotes for the I and S chromosomes is k_{IS}, where in general $k_{IS} < k_{II}$. The value for the region of this chromosome that is unaffected by the inversion is $k_{BB} = k_{AA}/(1-p)$. Furthermore, the absence of recombination in males must be taken into account by multiplying k_{XX} by two-thirds and the k values for the autosomal regions by one-half, in the net terms for decrease in copy number.

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