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Transposable Element Numbers in Cosmopolitan Inversions From a Natural Population of *Drosophila melanogaster*

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ABSTRACT

Population studies of the distribution of transposable elements (TEs) on the chromosomes of *Drosophila melanogaster* have suggested that their copy number increase due to transposition is balanced by some form of natural selection. Theory suggests that, as a consequence of deleterious ectopic meiotic exchange between TEs, selection can favor genomes with lower TE copy numbers. This predicts that TEs should be less deleterious, and hence more abundant, in chromosomal regions in which recombination is reduced. To test this, we surveyed the abundance and locations of 10 families of TEs in recombination-suppressing chromosomal inversions from a natural population. The sample of 49 chromosomes included multiple independent isolates of seven different inversions and a corresponding set of standard chromosomes. For all 10 TE families pooled, copy numbers were significantly higher overall within low frequency inversions than within corresponding regions of standard chromosomes. TEs occupied chromosomal sites at significantly higher frequencies within the *In(3R)Mo* and *In(3R)K* inversions than within the corresponding regions of standard 3R chromosomes. These results are consistent with the predictions of the ectopic exchange model.

WHY transposable elements (TEs) persist in natural populations is an issue of considerable speculation and interest. Do TEs persist because their activity provides fitness benefits to host individuals or populations (*e.g.*, NEVERS and SAEDLER 1977; CAMPBELL 1983; MCCLINTOCK 1984; SYVANEN 1984; SHAPIRO 1992; MCDONALD 1993)? Or, do TEs persist simply because of the replicative advantage conferred by transposition; that is, are TEs a form of selfish DNA (DOOLITTLE and SAPIENZA 1980; ORGEL and CRICK 1980)?

Although there is suggestive evidence in some taxa for the involvement of TEs in host developmental and regulatory processes (see, *e.g.*, MCDONALD 1993), the phenomena in question may represent parasitic subversion of host functions rather than contributions to host fitness. There is no evidence that the mutational spectra generated by TEs favor adaptive changes. Mobilization and insertion of *P* elements in *Drosophila melanogaster* can lead to increased variation for quantitative traits (MACKAY 1987; MACKAY *et al.* 1992) and enhanced response to artificial selection (MACKAY 1985; but see TORKAMANZEHI *et al.* 1988), but average fitness clearly declines as a result of *P* element insertional activity (MACKAY 1986; EANES *et al.* 1988). Laboratory studies of evolution in *Saccharomyces cerevisiae* have provided evidence connecting Ty element activity to fitness gains (ADAMS and OELLER 1986; WILKE and ADAMS 1992; WILKE *et al.* 1992), but, again, the average fitness of evolved strains declines with increasing Ty copy number (WILKE *et al.* 1992).

Studies of the genomic distributions and frequencies of TE insertions in natural populations of *D. melanogaster* have provided evidence consistent with the view that TEs are selfish DNA. Frequencies of TE insertions at occupied sites are low in Drosophila populations, suggesting that such insertions are seldom beneficial (CHARLESWORTH and LANGLEY 1989). A combination of empirical and theoretical studies has suggested that TEs are maintained in Drosophila populations at a balance between increase through transposition and decrease as a result of one or more opposing deterministic forces, including natural selection (MONTGOMERY *et al.* 1987; LANGLEY *et al.* 1988; CHARLESWORTH and LANGLEY 1989; CHARLESWORTH and LAPID 1989; CHARLESWORTH *et al.* 1992a,b).

How natural selection opposes the spread of TEs in the Drosophila genome is not completely understood. Selection against TE insertions into coding and regulatory regions is an obvious possibility, since such insertions can have deleterious effects (SHAPIRO 1983). Selection against insertional mutations, however, is inadequate to explain the observed copy numbers of TEs and their pattern of distribution on the Drosophila chromosomes (CHARLESWORTH and LANGLEY 1989; CHARLESWORTH and LAPID 1989; CHARLESWORTH *et al.* 1992b). A model that does not rely on selection against insertions was suggested by LANGLEY *et al.* (1988). They proposed that ectopic meiotic exchange between TEs,

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which can produce deleterious chromosome rearrangements, contains TE copy number increase by reducing the fitness of genomes with higher copy numbers. This model predicts that TEs will be less deleterious, and hence more abundant, in chromosome regions that have reduced levels of meiotic exchange. The distribution of TEs on the Drosophila chromosomes provides support for the model. Many families of TEs are significantly overabundant in the heterochromatin (MIKLOS *et al.* 1988; MIKLOS and COTSELL 1990; B. CHARLESWORTH, P. JARNE and S. ASSIMACOPOULOS, unpublished results) and on the fourth chromosome and in the basal euchromatin of the major chromosomes (MONTGOMERY *et al.* 1987; LANGLEY *et al.* 1988; CHARLESWORTH and LANGLEY 1989; CHARLESWORTH and LAPID 1989; CHARLESWORTH *et al.* 1992b). Meiotic exchange is reduced in all of these regions (LINDSLEY and SANDLER 1977). An exception to this pattern is the observation that TEs are generally not over-represented at the tip of the *X* chromosome, even though meiotic exchange is greatly reduced in this region as well (CHARLESWORTH and LAPID 1989).

A possible problem in comparing TE abundances between chromosome regions with different meiotic exchange rates is that these regions might differ in some other property (*e.g.*, DNA conformation) that could affect TE abundance. This problem can be eliminated by comparing TE abundance between identical chromosome regions with different rates of recombination. The naturally occurring paracentric chromosomal inversions in Drosophila are a source of such material, since inversion heterozygotes exhibit highly reduced meiotic exchange (ROBERTS 1976). If ectopic exchange involving TEs within inversions is also reduced, inversions would be expected to harbor more TEs than corresponding standard (majority) chromosome arrangements (EANES *et al.* 1992). This effect should be strongest for low frequency inversions, since these will almost always pair in heterozygous combination with standard chromosomes. A survey of paracentric inversions from an African population of *D. melanogaster* found that *P* elements were overabundant within low frequency inversions, in agreement with this prediction (EANES *et al.* 1992). Here we report a more comprehensive survey of the abundance of ten TE families in second and third chromosome inversions from a wild North American *D. melanogaster* population.

MATERIALS AND METHODS

Genetic stocks and breeding procedures: All chromosomes were obtained from a set of 600 lines descended from fertilized females captured near Beltsville, Maryland, by JERRY COYNE and sent to our laboratory in 1986. We screened these isofemale lines for segregating inversions by inspecting salivary chromosome squashes from 8–15 larvae of each line using a standard aceto-orcein protocol (ASHBURNER 1989). We extracted inversion chromosomes only from lines observed to be segregating for a single inversion, and standard chromosomes only from lines not segregating for any inversion. Chromosomes were extracted using the standard, multiply inverted laboratory balancer stocks *SM1,Cy/Pm* and *TM6,Ubx/Sb* (LINDSLEY and ZIMM 1992). The balancer stocks carried the P/I cytotype, introduced by repeated backcrossing to the outbred wild-type stock IV (CHARLESWORTH and CHARLESWORTH 1985), in order to avoid hybrid dysgenesis in crosses to wild lines; they were marked, in addition, with the fourth chromosome recessive spa^{pol}. To extract homozygous chromosomes, individual males from wild lines were crossed to virgin balancer females. Single F_1 male progeny heterozygous for the wild (+) chromosome and a balancer were then backcrossed to balancer stock females; in the next generation, balancer stock females were crossed to *+/balancer*; spa^{pol}/spa^{pol} males. Female and male progeny heterozygous for the balancer and the single extracted wild chromosome and homozygous for spa^{pol} were then crossed. Their +/+; spa^{pol}/spa^{pol} progeny were crossed to establish a stock homozygous for the original wild chromosome and marked with spa^{pol} as a precaution against contamination during subsequent laboratory maintenance. Extracted lines that were recessive lethal or infertile were discarded. All other extracted lines were retained and maintained in mass culture. To verify that the extracted lines carried the desired chromosome arrangements, we crossed males from these lines to virgin females from the wild-type IV stock, which is free of inversions, and inspected salivary chromosome squashes from larvae of these matings for inversion loops (ROBERTS 1976).

Chromosomes used for *in situ* hybridization: We examined a total of 49 chromosomes, comprising eight isolates each of standard sequence second chromosomes (2L and 2R), seven isolates each of standard sequence third chromosomes (3L and 3R), three isolates each of chromosomes with the paracentric inversions *In(3L)P*, *In(3R)P*, *In(3R)C*, *In(3R)K*, *In(2L)t* and *In(2R)NS*, and four isolates of chromosomes with the *In(3R)Mo* inversion. The standard chromosomes and chromosomes with the inversions *In(3R)P*, *In(2L)t* and *In(2R)NS* were drawn randomly from larger collections of isolates without prior knowledge of their TE copy numbers and distributions. All isolates of chromosomes with the inversions *In(3L)P*, *In(3R)C*, *In(3R)K* and *In(3R)Mo* were examined.

Inversions: Table 1 lists the inversions used for the study, their cytological breakpoints, and the number of bands within each region visible in the *in situ* preparations. The relative sizes and positions of the inversions, along with the total number of bands on each chromosome arm, are illustrated in Figure 1.

Three of the seven inversions, namely *In(3R)Mo*, *In(3R)C* and *In(3R)K*, have been found at very low frequencies in the southern East Coast region of the United States from which our isofemale lines were collected, and, in general, are quite rare (at frequencies of 1% or lower) in samples from across North America (METTLER *et al.* 1977; their Table 2). Of the

TABLE 1

Chromosomal inversions used in the study

Inversion	Cytological breakpoints	Visible bands	No. of isolates
In(3R)P	89C2-3;96A18-19	96	3
In(3R)Mo	93D;98F2-3	81	4
In(3R)C	92D1-E1;100F2-3	101	3
In(3R)K	86F1-87A1;96F11-97A1	133	3
In(3L)P	63C;72E1-2	110	3
In(2L)t	22D3-E1;34A8-9	93	3
In(2R)NS	52A2-B1;56F9-13	56	3
In(3R) overlap region	93D1-96A9	61	10

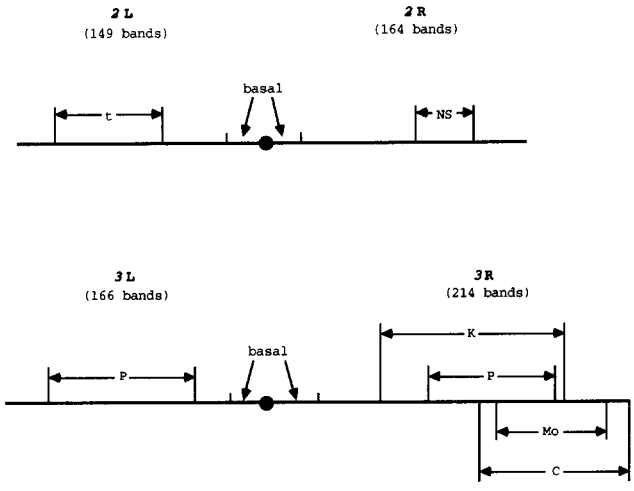

FIGURE 1.—Chromosomes *2* and *3*, showing relative sizes and positions of the inversions and basal euchromatic regions used in the study. The scale is based upon the number of visible bands per region in the polytene chromosomes.

remaining four inversions, *In(2L)t*, *In(2R)NS* and *In(3L)P* are represented by somewhat higher frequencies (often higher than 1%) in the samples of METTLER *et al.* One inversion, *In(3R)P*, is apparently relatively common, being present at frequencies well above 10% in samples from many regions in North America (METTLER *et al.* 1977, their Table 2, column 8). While we did not collect data on inversion frequencies in our original sample of isofemale lines, it was clear during our efforts to isolate inversion chromosomes that *In(3R)P* was relatively common in our source population, whereas the other inversions–particularly *In(3R)K*, *In(3R)Mo* and *In(3R)C*–were much less common.

For *In(3L)P*, *In(3R)C*, *In(3R)Mo* and *In(3R)P* heterozygotes, data summarized in LINDSLEY and ZIMM (1992) indicate reductions in recombination fractions to below 1% of normal levels in the inversion regions, with considerable reductions outside the inversions as well.

Probes used for *in situ* hybridization: The 10 TE probes used in this study were originally provided by CHARLES LANGLEY, WILLIAM EGGLESTON and WALTER EANES. Nine of the probes contain genomic sequences of *D. melanogaster* cloned into *pBR322*. The *roo* probe contains a partial *roo* sequence cloned into a *pBR322*-derived plasmid. Four of the 10 probes contain sequences from the long terminal repeat (LTR) retrotransposons *copia*, *297*, *412* and *roo*, respectively, and have been referred to as such in previous studies (CHARLESWORTH and LAPID 1989; CHARLESWORTH *et al.* 1992a,b). The TE sequences contained in the other six probes were previously unknown, and probe names only have been given in previous studies. Recent restriction mapping in our laboratory (B. CHARLESWORTH, P. JARNE and S. ASSIMACOPOULOS, unpublished results) has enabled us to identify five of these TE sequences with known retrotransposons. Four of these are LTR retrotransposons, as follows (TE names in parentheses): *2158* (*1731*), *2181* (*mdg1*), *2210* (*17.6*) and *2217* (*opus*). The probe referred to in previous studies as *2161* contains sequence from the non-LTR retrotransposon *jockey* (C. F. AQUADRO, personal communication; B. CHARLESWORTH, P. JARNE and S. ASSIMACOPOULOS, unpublished results). In this report, we use the names of the cloned TEs in referring to data obtained using the above nine probes. Descriptions of these known TE families are found in LINDSLEY and ZIMM (1992). The remaining probe, *2156*, remains uncharacterized at present.

***In situ* hybridization methods:** Preparation and *in situ* hybridization of salivary chromosome squashes followed a modification of the protocol described in MONTGOMERY *et al.* (1987). Probe DNAs were labeled with biotinylated dUTP (Bio-11 dUTP: Enzo Diagnostics) using random primer extension (FEINBERG and VOGELSTEIN 1983). Sites of hybridization were detected by staining with diaminobenzidine and peroxidase. Chromosomes were stained in 5% Giemsa (Gurr).

We performed hybridizations on batches of 16 slides at a time. In order to control for possible variation among batches, each batch consisted of inversion and standard chromosomes.

Data collection and analysis: We read the slides using an Olympus microscope with a 100× oil immersion objective lens and 10× oculars. The band locations of all hybridization sites were marked on photocopies of LEFEVRE's (1976) photographic map of the salivary chromosomes (see CHARLESWORTH *et al.* 1992a). We read one slide for each particular TE probe used on a given homozygous inversion line; the presence of the inversion and the spa^{pol} marker in these lines was considered an adequate guard against line contamination. To check for contamination in each standard chromosome line, we read two independent slides for one of the higher copy number TE probes (*roo* or *jockey*); completely identical profiles of insertion sites between the two slides were taken to indicate that the lines remained homozygous for the original standard chromosome. (The same test was employed in those cases in which inversion chromosomes showed unusually high TE abundance, to test whether one homozygous inversion line had been contaminated by another line homozygous for a different isolate of the same inversion. No such contamination was discovered.)

For numerical analysis, all the visible bands for each chromosome arm were assigned numbers in sequence, and the presence or absence of a TE at a given band was recorded as a 1 or a 0 in a computer file (see CHARLESWORTH *et al.* 1992a).

Cytogenetic regions used in comparisons of element abundance: We scored 10 visible bands immediately outside each breakpoint of inversions as if they were inside the inversions, in order to account for suppression of recombination outside the inversion (ROBERTS 1976) and to increase the statistical power of comparisons between inversion and standard regions. The same regions were analyzed in inversion and standard chromosomes, so this adjustment does not bias the results.

The four different inversions on chromosome 3R (*In(3R)P*, *In(3R)K*, *In(3R)Mo* and *In(3R)C*) do not span mutually exclusive regions (see Figure 1). Multiple statistical tests comparing TE numbers in these individual inversions to corresponding regions on the same set of standard 3R chromosomes involve non-independent comparisons for the overlapping areas. To circumvent this problem, we performed an additional analysis of TE numbers in the rare 3R inversions by comparing the region in which these inversions overlap (see Figure 1 and Table 1) to the corresponding region on standard sequence chromosomes.

The same inversion regions as above were used in the calculation of TE occupancy profiles. A previous study in this population showed that element insertions tend to reach higher frequencies in the basal regions of chromosomes than in other regions (CHARLESWORTH *et al.* 1992b). Since this would tend to obscure statistical differences in TE frequencies between inversion and standard chromosomes, we omitted the basal regions of chromosome arms in calculations of occupancy profiles. The numbers of bands in the basal regions are as follows: base of 2L, 22 bands; 2R, 24 bands; 3L, 20 bands; 3R, 34 bands. The sizes and locations of these regions, relative to the inversions, are illustrated in Figure 1. Exact cytological boundaries are given in LANGLEY *et al.* (1988; their Table 3).

TABLE 2

TE copy numbers in the IN *(2L)t*, *In(2R)NS*, *In(3L)P* and *In(3R)P* regions on standard and inversion chromosomes

	2L		*(2L)t*		2R		*(2R)NS*		3L		*(3L)P*		3R		*(3R)P*	
TE	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n
2156	3.13	2.70	3.67	4.33	1.13	0.70	0.33	0.33	2.42	3.95	6.33**	2.33	1.57	1.62	1.66	0.33
1731	0.38	0.27	1.00	1.00	0.25	0.21	0.67	0.33	1.00	1.67	1.00	3.00	0.00	0.00	0.67	0.033
jockey	3.50	2.00	5.33*	0.33	2.25	1.07	3.33	0.33	4.00	2.33	4.33	24.33	2.43	3.29	2.67	0.33
mdg1	1.50	0.86	1.67	2.33	1.13	0.70	1.00	0.00	2.00*	0.67	0.33	0.33	1.14	0.48	1.00	0.00
17.6	0.63	0.55	0.33	0.33	0.00	0.00	0.00	0.00	0.86	1.14	0.00	0.00	0.57	0.62	0.00	0.00
opus	1.88	1.27	1.00	1.00	0.25	0.21	1.67	0.33	1.29	0.57	2.67	6.33	0.57	0.29	1.33	1.33
297	2.25	1.64	3.33	4.33	0.50	0.29	0.00	0.00	2.14*	2.14	0.00	0.00	2.14	1.48	1.67	0.33
412	2.13	2.13	2.67	4.33	1.00	0.29	2.66*	4.33	2.14	1.14	1.67	1.33	1.57	1.62	0.67	0.67
roo	5.75	3.36	6.67	9.33	3.00	1.71	3.33	2.33	5.14	1.81	10.00***(**)	1.00	4.00	4.00	3.33	1.33
copia	2.00	2.29	1.33	1.33	0.88	0.70	0.67	1.33	1.57	1.62	1.67	2.33	1.14	1.14	2.66	2.33
All	23.13	16.13	27.00	97.00	10.38	3.13	13.67*	12.33	22.57	19.29	28.00	112.00	15.28	21.90	15.67	0.33

$\bar{n}$ and V_n denote the mean and variance in copy number. Numbers shown are for eight standard 2L and 2R chromosomes, seven 3L and 3R chromosomes, and three copies of each inversion chromosome. Asterisks indicate significantly higher copy numbers on inversion chromosomes (one-tailed P) or standard chromosomes (two-tailed P). Significance levels: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. Significance levels after Bonferroni correction for multiple tests on chromosome arms are given in parentheses.

Treatment of apparent cases of element fixation at single sites: Previous studies of autosomes from the same natural population revealed five cases in which a particular TE family was present at the same band location in the entire sample (CHARLESWORTH *et al.* 1992a; B. CHARLESWORTH, P. JARNE and S. ASSIMACOPOULOS, in preparation). These apparent fixations were *17.6* at 30D1 on 2L, *mdg1* at 60B1 on 2R, *1731* at 80B on 3L, *2156* at 87C1-2 on 3R, and *297* at 99 E1-2 on 3R. They were observed in the present study as well, on both standard and inversion chromosomes. We have omitted them here in calculating copy numbers and element frequencies, since they may be artifacts due to hybridization of Drosophila flanking sequences cloned along with the TE sequences.

RESULTS

Element copy numbers on inversion and standard chromosomes: Table 2 shows the mean copy numbers and variances for individual TE families and for all families pooled in the inversion regions of *In(2L)t*, *In(2R)NS*, *In(3L)P* and *In(3R)P* chromosomes and the corresponding standard chromosomes. Table 3 shows the same data for the inversion regions of chromosomes with the rare inversions *In(3R)C*, *In(3R)K* and *In(3R)Mo* and the corresponding regions of standard 3R chromosomes, along with pooled data for the region of overlap of these inversions. The data for all TEs pooled (the bottom rows of Tables 2 and 3) are illustrated in Figure 2. TE copy numbers on whole chromosome arms are not tabulated; summary statistics are given in the text, below. Statistical significance was analyzed by t tests, with Bonferroni corrections for multiple tests (RICE 1989) where indicated.

Copy numbers on In(2L)t chromosomes compared to standard 2L chromosomes: The mean copy numbers for all TEs pooled are higher both within the inversion region and on the whole arm of *In(2L)t* chromosomes, compared with 2L chromosomes, but these differences are not statistically significant (Table 2). For the *In(2L)t* region, the mean copy numbers are 27.00 ± 5.69 on inversion chromosomes and 23.13 ± 1.42 on standard chromosomes ($t = 0.98$, 9 d.f., one-tailed $P > 0.05$). For the whole chromosomes, the mean copy numbers are 33.00 ± 6.24 on inversion chromosomes and 28.75 ± 1.94 on standard chromosomes ($t = 0.89$, 9 d.f., one-tailed $P > 0.05$). One TE family, *jockey*, is significantly more abundant in the inversion region of *In(2L)t* chromosomes ($P < 0.05$) before, but not after, Bonferroni correction for multiple tests on 2L chromosomes.

Copy numbers on In(2R)NS chromosomes compared to standard 2R chromosomes: The *In(2R)NS* region of inversion chromosomes harbors significantly more TEs than the corresponding region of standard 2R chromosomes (Table 2). The mean copy numbers for all TEs pooled in the *In(2R)NS* region are 13.67 ± 2.03 for inversion chromosomes and 10.38 ± 0.63 for standard 2R chromosomes ($t = 2.14$, 9 d.f., one-tailed $P < 0.05$). Five of the 10 TE families are more abundant in the *In(2R)NS* region on inversion chromosomes. *412* is significantly more abundant on *In(2R)NS* chromosomes (one-tailed $P < 0.05$) before, but not after, correction for multiple tests on 2R chromosomes. The mean copy numbers for all TEs pooled on whole 2R chromosome arms are 32.00 ± 1.15 on *In(2R)NS* chromosomes and 27.57 ± 1.51 on standard 2R chromosomes. The difference is not statistically significant.

Copy numbers on In(3L)P chromosomes compared to standard 3L chromosomes: The mean copy numbers for all TEs pooled within the *In(3L)P* region are 28.00 ± 6.11 for inversion chromosomes and 22.57 ± 2.54 for the standard chromosomes (Table 2). The difference is not statistically significant ($t = 1.20$, 8 d.f., one-tailed $P > 0.05$). Five of the 10 TE families are more abundant on inversion chromosomes. The difference for *roo* is significant: the mean *roo* copy numbers in the *In(3L)P* region are 10.00 ± 1.00 on inversion chromosomes and 5.14 ± 1.62 on standard chromosomes ($t = 5.79$, 8 d.f., one-tailed $P < 0.01$ with Bonferroni correction for multiple tests on 3L chromosomes). *mdg1* and *297* are sig-

TABLE 3

TE copy numbers in three rare inversions of chromosome 3R on standard and inversion chromosomes

	3R		(3R)C		3R		(3R)K		3R		(3R)Mo		3R		(3R)OL	
TE	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n	$\bar{n}$	V_n
2156	1.86	1.81	2.67	0.33	2.71	3.90	4.33	6.33	1.43	1.95	2.50	5.67	0.57	1.29	1.60	1.38
1731	0.29	0.24	0.00	0.00	0.43	0.29	0.33	0.33	0.28	0.24	0.50	0.33	0.14	0.14	0.20	0.18
jockey	4.57	4.29	3.33	0.33	4.43	4.29	6.33	17.33	2.86	2.14	2.50	1.67	1.86	0.81	1.90	1.66
mdg1	1.86	1.14	1.67	1.33	2.57	0.62	7.00***(***)	0.00	1.43	0.29	4.75***(***)	0.25	1.29	0.24	2.30	2.46
17.6	0.14	0.14	0.00	0.00	1.00	1.00	2.33	6.33	0.13	0.13	0.25	0.25	0.14	0.14	0.30	0.23
opus	0.71	0.57	1.33	2.33	1.88	1.81	2.00	4.00	0.57	0.29	2.00*	2.00	0.43	0.29	1.20*	0.84
297	2.00*	1.00	0.33	0.33	4.29	4.57	4.00	1.00	1.43	0.95	2.75	0.92	1.00	0.67	1.50	1.17
412	2.14	1.81	2.67	4.33	2.14	0.81	5.67***(**)	6.33	2.00	2.00	0.75	0.91	1.14	1.14	1.50	2.10
roo	5.14	6.48	7.00	13.00	6.14	5.14	6.67	6.33	3.57	3.61	7.75*	11.58	2.29	2.24	3.30	4.01
copia	0.71	1.57	1.33	1.33	2.71	4.9	2.67	6.33	0.71	1.57	2.00	2.00	0.57	0.95	0.80	1.73
All	19.43	38.62	20.33	52.33	28.29	58.57	41.33*	30.33	14.43	25.62	25.75***	14.25	9.29	14.29	14.60*	20.93

$\bar{n}$ and V_n denote the mean and variance in copy number. Numbers shown are for seven standard 3R chromosomes, three *In(3R)C* and *In(3R)K* chromosomes and four *In(3R)Mo* chromosomes. The same set of standard chromosomes is employed in comparisons to the three inversion chromosomes. The *(3R)OL* column gives numbers for *In(3R)C*, *In(3R)K* and *In(3R)Mo*, in their region of overlap (10 chromosomes) and the corresponding values for the standard chromosomes. Asterisks indicate significantly higher copy numbers on inversion chromosomes (one-tailed *P*) or standard chromosomes (two-tailed *P*). Significance levels: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. For individual TE families, significance levels after Bonferroni correction for multiple tests on chromosome arms are given in parentheses.

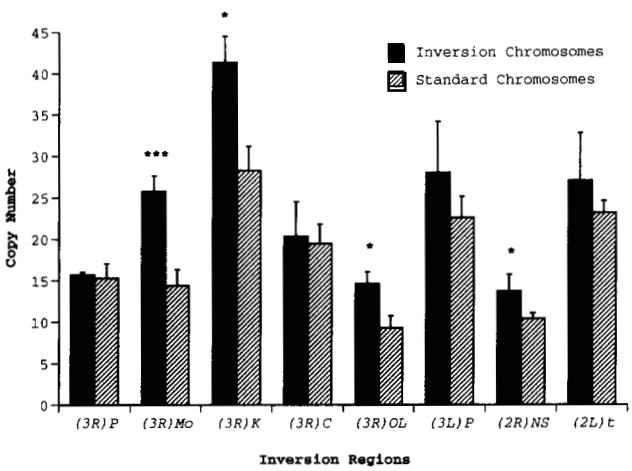

FIGURE 2.—Copy numbers ± standard errors for inversion regions of inversion and standard arrangement chromosomes. *(3R)OL* denotes the overlap region of rare 3R inversions. The same set of seven standard 3R chromosomes was used to calculate copy numbers on standard chromosomes for the four individual inversion regions of 3R. Significance levels: * = 0.05; *** = 0.001.

nificantly over-represented on standard chromosomes (two-tailed $P < 0.05$ in both cases) before, but not after, correction for multiple tests. The mean copy numbers for all TE families pooled on whole 3L chromosomes are 34.66 ± 7.22 for *In(3L)P* chromosomes and 28.71 ± 1.92 for whole standard 3L chromosomes. The difference is not statistically significant.

Copy numbers on In(3R)P chromosomes compared to standard 3R chromosomes: The sample of three *In(3R)P* chromosomes shows little evidence of harboring more TEs than the seven standard 3R chromosomes, either within the *In(3R)P* inversion region or on the whole chromosome arm (Table 2). The mean copy numbers in the *In(3R)P* inversion region for all TEs pooled are 15.28 ± 1.76 on the standard 3R chromosomes and 15.67 ± 0.33 on the *In(3R)P* chromosomes. The mean copy numbers for the whole 3R chromosome arm for all TEs pooled are 40.14 ± 3.76 on 3R chromosomes and 44.00 ± 3.79 on *In(3R)P* chromosomes. Six of the 10 TE families are slightly more abundant within the *In(3R)P* region on inversion chromosomes. *In(3R)P* is apparently present at substantial frequencies across North America (see MATERIALS AND METHODS). For this reason, subsequent analyses in which pooled samples of rare 3R inversion chromosomes are employed omit this inversion.

Copy numbers on the rare inversion chromosomes In(3R)C, In(3R)K and In(3R)Mo compared to standard 3R chromosomes: The three isolates of *In(3R)C* show little evidence of harboring more TEs than the seven standard 3R chromosomes (Table 3). The mean copy numbers for all TEs within the *In(3R)C* region are 20.33 ± 4.18 for inversion chromosomes and 19.43 ± 2.35 for standard chromosomes. For the whole chromosome arm, the mean copy number is 39.67 ± 5.49 for *In(3R)C* chromosomes, as compared with the previously given value of 40.14 ± 3.76 for the standard 3R chromosomes.

A considerable excess of TEs is present on *In(3R)K* and *In(3R)Mo* chromosomes compared to the standard 3R chromosomes. The mean copy numbers of all TEs in the *In(3R)K* region are 41.33 ± 3.18 for inversion chromosomes and 28.29 ± 2.89 for the standard 3R chromosomes. The difference is statistically significant ($t = 2.63$, 8 d.f., one-tailed $P < 0.05$). Seven of the 10 TE families are more abundant on *In(3R)K* chromosomes; *mdg1* and *412* are significantly more abundant after correction for multiple tests (for *mdg1*, one-tailed $P < 0.001$; for *412*, one-tailed $P < 0.01$). For the *In(3R)Mo* chromosomes, the mean copy numbers for all TEs within the inversion region are 25.75 ± 1.89 for *In(3R)Mo* chromosomes and 14.43 ± 1.91 for the standard 3R chromosomes. The difference is statistically significant ($t =$

3.87, 9 d.f., one-tailed $P < 0.001$). Eight of the 10 TE families are more abundant on *In(3R)Mo* chromosomes; *mdg1* is significantly more abundant after correction for multiple tests ($P < 0.001$). Both the *In(3R)K* and *In(3R)Mo* samples also harbor more TEs on the whole chromosome arm than the standard 3R chromosomes. The mean copy numbers on whole chromosomes are 57.00 ± 3.51 for *In(3R)K* chromosomes and 58.75 ± 2.18 for *In(3R)Mo* chromosomes, as compared with 40.14 ± 3.76 for the standard 3R chromosomes.

Copy numbers in the overlap region of rare 3R inversion chromosomes: The mean copy numbers within the overlap region for all TEs pooled are 14.60 ± 1.45 for the rare inversion chromosomes and 9.29 ± 1.43 for the standard chromosomes (Table 3). The difference is statistically significant ($t = 2.52$, 15 d.f., one-tailed $P < 0.05$). All 10 element families are more abundant in the overlap region on the inversion chromosomes (on a one-tailed sign test, $P = 0.001$), although none of the individual differences is significant after correction for multiple tests.

For the whole 3R chromosome arms, the mean copy numbers for all TEs are 52.80 ± 3.33 for rare inversion chromosomes and 40.14 ± 3.76 for standard chromosomes. This represents a significant excess of elements on rare inversion chromosome arms ($t = 2.49$, 15 d.f., one-tailed $P < 0.05$). Nine of the 10 TE families are more abundant on whole inversion chromosomes ($P = 0.0107$ on a one-tailed sign test), although none of the individual differences is significant when the sequential correction for multiple tests is applied.

It is of interest to ask whether the excess in copy number on rare inversion chromosomes relative to standard chromosomes is confined only to the inversion regions or extends to uninverted regions also. A test for this is provided by examining mean copy numbers on a portion of chromosome 3R that is well away from any of the regions analyzed for the three rare inversions. The cytological boundaries of this region are 81F1 (the base of 3R) and 85F3, which is 10 visible bands (approximately one chromosome division) outside the proximal breakpoint of *In(3R)K*. This region contains 51 visible bands, compared with the 61 bands contained in the overlap region. (We omit the distal portion of 3R from this analysis, since the distal breakpoints of *In(3R)Mo* and *In(3R)C* are near the tip of the chromosome.) The mean copy numbers for all TEs pooled in this region are 11.80 ± 1.14 on inversion chromosomes and 11.00 ± 1.07 on standard chromosomes. The lack of a significant difference for this region suggests that the copy number difference between inversion and control chromosomes is confined largely to regions within and adjacent to inversions.

Overall comparison of copy numbers between inversions and corresponding standard regions: Figure 2 shows the mean copy numbers and standard errors for all of the inversion regions on inversion and standard chromosomes. TEs are more abundant within inversions in all cases. The hypothesis that TEs are more abundant in the aggregate within low frequency inversions may be tested by combining the probabilities of the independent tests for the individual inversions, according to the method of FISHER (1958). As above, we omit *In(3R)P* from this comparison, since this inversion is present at much higher frequency in the population than the others. Combining exact one-tailed probabilities from the *t* tests comparing pooled TE copy numbers in *In(3L)P*, *In(2R)NS*, *In(2L)t* and the *In(3R)* overlap region to corresponding regions on standard chromosomes gives $\chi^2 = 23.27$, 8 d.f., $P < 0.01$, indicating a significant excess of TEs overall within the low frequency inversions.

Copy number variances in relation to means: If element frequencies are low at occupied sites and there is no linkage disequilibrium between members of the same element family, TE copy numbers are expected to follow the Poisson distribution, with equality of mean and variance (CHARLESWORTH and CHARLESWORTH 1983). For the whole arms of standard 2L, 2R, 3L and 3R chromosomes, the ratios of mean copy number to variance in copy number are 0.78, 1.02, 0.85 and 1.07; the average over all four sets is 0.93, in reasonable accord with Poisson expectation. Some variance-to-mean ratios for individual TE families differ markedly from unity, but this is difficult to interpret, given the large number of individual copy numbers and variances calculated and the small number of standard chromosomes in the sample. A previous study in this population, employing a larger set of chromosomes (14 second chromosomes and 13 third chromosomes), found good agreement with Poisson expectation for the TE families used here (CHARLESWORTH *et al.* 1992a).

The average variance-to-mean ratios for TE copy numbers on the inversion chromosomes are somewhat more variable. For the whole chromosomes carrying the inversions, these values over all 10 TE families are: *In(3R)P* chromosomes, 0.88; *In(3R)Mo* chromosomes, 1.13; *In(3R)K* chromosomes, 1.51; *In(3R)C* chromosomes, 1.58; *In(3L)P* chromosomes, 1.72; *In(2R)NS* chromosomes, 0.86; *In(2L)t* chromosomes, 1.00; the average over all seven inversion chromosomes is 1.24. For just the inversion regions the average variance-to-mean ratios are: *In(3R)P*, 0.43; *In(3R)Mo*, 0.97; *In(3R)K*, 1.46; *In(3R)C*, 1.03; *In(3L)P*, 1.83; *In(2R)NS*, 0.76; *In(2L)t*, 1.10; the average over all seven inversions is 1.08. The strong departure of some of these values from unity suggests that there may be some linkage disequilibrium between element insertions on the inversion chromosomes, or variance in element frequency between sites. The small samples of each inversion, however, render direct statistical tests infeasible.

TE occupancy profiles: Tables 4–8 give occupancy profiles for all TE families on the standard and inversion chromosomes. These profiles list the number of times

TABLE 4

Occupancy profiles for 10 families of elements on eight standard 2L and 2R chromosomes

	2L								2R							
Element	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
2156	16	5	1	0	0	0	0	0	15	3	0	0	0	0	0	0
1731	2	1	0	0	0	0	0	0	5	1	0	0	0	0	0	0
jockey	15	6	3	0	0	0	0	0	19	7	1	0	0	0	0	0
mdg1	12	2	0	0	0	0	0	0	18	0	0	0	0	0	0	0
17.6	7	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
opus	13	3	0	0	0	0	0	0	10	1	0	0	0	0	0	0
297	18	2	0	0	0	0	0	0	17	3	0	0	0	0	0	0
412	15	3	1	0	0	0	0	0	18	1	0	0	0	0	0	0
roo	30	7	3	0	0	0	0	0	39	6	3	0	0	0	0	0
copia	16	2	0	0	0	0	0	0	18	3	0	0	0	0	0	0

TABLE 5

Occupancy profiles for 10 families of elements on seven standard 3L and 3R chromosomes

	3L							3R						
Element	1	2	3	4	5	6	7	1	2	3	4	5	6	7
2156	23	1	0	0	0	0	0	26	1	0	0	0	0	0
1731	7	0	0	0	0	0	0	5	0	0	0	0	0	0
jockey	24	4	0	0	0	0	0	32	7	1	1	0	0	0
mdg1	18	1	0	0	0	0	0	15	3	1	0	0	0	0
17.6	7	0	0	0	0	0	0	7	0	0	0	0	0	0
opus	11	0	1	0	0	0	0	16	0	0	0	0	0	0
297	12	3	0	0	0	0	0	30	2	1	0	0	0	0
412	17	2	0	0	0	0	0	18	3	0	0	0	0	0
roo	38	3	0	0	0	0	0	47	10	1	0	0	0	0
copia	12	2	0	0	0	0	0	17	0	1	0	0	0	0

TABLE 6

Occupancy profiles for 10 families of elements on *In(2L)t* and *In(2R)NS* chromosomes

	In(2L)t						*In(2R)NS*					
	Inside inversion			Outside inversion			Inside inversion			Outside inversion		
Element	1	2	3	1	2	3	1	2	3	1	2	3
2156	12	0	0	1	0	0	2	0	0	6	0	0
1731	3	0	0	0	0	0	3	0	0	4	1	0
jockey	14	1	0	2	0	0	10	0	0	12	0	0
mdg1	3	1	0	1	0	0	3	0	0	0	0	0
17.6	1	0	0	0	0	0	0	0	0	2	0	0
opus	3	0	0	2	0	0	5	0	0	4	0	0
297	10	0	0	1	0	0	0	0	0	3	0	0
412	6	1	0	3	0	0	8	0	0	5	0	0
roo	16	2	0	4	1	0	8	1	0	13	0	0
copia	4	0	0	1	0	0	2	0	0	4	0	0

that sites are occupied 1, 2, 3, . . . , n times by a member of a given TE family in a sample of n chromosomes. The standard chromosome occupancy profiles are similar to those previously published and based on larger samples of chromosomes from the same population (CHARLESWORTH *et al.* 1992a).

Occupancy profiles on inversion chromosomes: Tables 6–8 give occupancy profiles for the inversion chromosomes. For visual comparison, profiles for regions inside and outside inversions are given separately in each table. In general, the inversion chromosome occupancy profiles are similar to profiles for standard chromosomes; the majority of occupied sites occur once, reflecting the prevailing rarity of TEs. There are some instances of apparent fixation at individual sites on inversion chromosomes, however, that were not observed on standard chromosomes. *mdg1* was present at the same site (94A1) within the *In(3R)Mo* inversion on all four *In(3R)Mo* chromosomes, and was observed at this site only once in the sample of seven standard 3R chromosomes. *jockey* occupied the same single site (87D1) on all three *In(3R)C* chromosomes, and was not observed at this site

TABLE 7

Occupancy profiles for 10 families of elements on *In(3L)P* and *In(3R)P* chromosomes

	In(3L)P						*In(3R)P*					
	Inside inversion			Outside inversion			Inside inversion			Outside inversion		
Element	1	2	3	1	2	3	1	2	3	1	2	3
2156	13	3	0	6	0	0	3	1	0	13	0	0
1731	3	0	0	0	0	0	3	0	0	4	0	0
jockey	13	0	0	1	0	0	8	0	0	9	4	0
mdg1	1	0	0	0	0	0	4	0	0	3	1	0
17.6	0	0	0	0	0	0	0	0	0	2	0	0
opus	8	0	0	1	0	0	4	0	0	3	0	0
297	0	0	0	0	1	0	5	0	0	7	0	0
412	3	1	0	1	0	0	2	0	0	7	0	0
roo	21	3	0	6	0	1	10	0	0	14	2	0
copia	5	0	0	1	0	1	8	0	0	10	0	0

TABLE 8

Occupancy profiles for 10 families of elements on *In(3R)C*, *In(3R)K* and *In(3R)Mo* chromosomes

	In(3R)C						*In(3R)K*						*In(3R)Mo*							
	Inside inversion			Outside inversion			Inside inversion			Outside inversion			Inside inversion				Outside inversion			
Element	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	4	1	2	3	4
2156	8	0	0	7	1	0	7	3	0	4	0	0	6	2	0	0	11	1	0	0
1731	0	0	0	1	0	0	1	0	0	2	0	0	2	0	0	0	2	1	0	0
jockey	6	2	0	7	0	1	13	3	0	2	3	0	4	0	2	0	16	4	0	0
mdg1	1	2	0	5	0	0	11	5	0	3	0	0	9	3	0	1	12	2	0	0
17.6	0	0	0	3	0	0	5	1	0	0	0	0	1	0	0	0	4	0	0	0
opus	4	0	0	3	0	0	6	0	0	2	0	0	6	1	0	0	6	1	0	0
297	1	0	0	9	1	0	6	3	0	3	1	0	6	1	1	0	11	1	0	0
412	8	0	0	2	0	0	15	1	0	2	2	1	1	1	0	0	4	0	0	0
roo	13	4	0	13	3	0	16	2	0	11	0	0	14	7	1	0	13	7	0	0
copia	2	1	0	1	0	0	6	1	0	0	0	0	6	1	0	0	16	2	0	0

on any of the seven standard 3R chromosomes. This site is located well away from the *In(3R)C* inversion (compare the breakpoints given in Table 1). *412* was present at 98A1 on all three *In(3R)K* chromosomes, but was not observed at this location on any of the seven standard 3R chromosomes. This site is just over one chromosome division outside the distal breakpoint of *In(3R)K*, and may be within the region that experiences greatly reduced recombination in *In(3R)K* heterozygotes. Since the samples of inversion chromosomes are small, these isolated instances of high element frequency should be regarded with caution; they may, however, reflect unusual evolutionary dynamics within inversions, or persistence of TE insertions that were present on the original inversion chromosome at the time the inversion arose (see DISCUSSION).

Comparisons of TE frequencies on inversion and standard chromosomes: Previous studies (CHARLESWORTH and LAPID 1989; CHARLESWORTH *et al.* 1992a) have provided evidence that TE frequencies at occupied sites are elevated in the proximal regions of chromosomes, where recombination is reduced. It is of interest to examine whether element frequencies are also higher within inversions than within corresponding regions of standard chromosomes. Two measures of frequency can be calculated from the information in the TE occupancy profiles. The first is the proportion of the chromosome bands in a region that are occupied at least once by a TE (simple occupancy). The second is the proportion of occupied bands in a region that are multiply occupied (conditional multiple occupancy); this measures the tendency for TEs to be present at higher frequencies at sites that are occupied.

Comparisons of occupancy between inversion chromosomes and the full sets of standard chromosomes are not possible, since the differences in sample size are expected to affect the observed number of occupied bands differently, regardless of any true differences in TE frequencies. To equalize the sample sizes, we drew (without replacement) appropriate-sized random subsets of standard chromosomes from the full sets and used these to calculate measures of occupancy for comparison to inversion chromosomes.

No significant differences in simple or multiple occupancy were observed between *In(2L)t*, *In(2R)NS* and *In(3L)P* chromosomes and the corresponding subsets of standard chromosomes, comparing both inversion and non-inversion regions. Significantly higher multiple occupancy was, however, observed within the *In(3R)K* and *In(3R)Mo* inversions. The limited sample of stand-

ard 3R chromosomes did not allow independent comparisons to all of the 3R inversions; we omitted *In(3R)P* and *In(3R)C* from the comparisons, since TE copy numbers on these chromosomes are similar to those obtained for the standard chromosomes. To analyze TE frequencies on the *In(3R)K* and *In(3R)Mo* chromosomes compared to standard chromosomes, we randomly partitioned the set of seven standard 3R chromosomes into subsets of three and four chromosomes each. For the *In(3R)K* chromosomes compared with three standard chromosomes, the average simple occupancies in the inversion region are 0.086 ± 0.011 for inversion chromosomes and 0.071 ± 0.012 for standard chromosomes. The average conditional multiple occupancies in the *In(3R)K* region are 0.17 ± 0.046 for inversion chromosomes and 0.066 ± 0.031 for standard chromosomes, suggesting higher frequencies at occupied sites in the *In(3R)K* inversion than in the corresponding region on the standard chromosomes (a *t* test of the pairwise difference in conditional multiple occupancies across all TE families gives $t = 2.37$, 9 d.f., one-tailed $P < 0.05$). Outside the *In(3R)K* region, average simple occupancies are 0.077 ± 0.021 on inversion chromosomes and 0.075 ± 0.018 on standard chromosomes. The average conditional multiple occupancies outside the *In(3R)K* region are 0.15 ± 0.080 for inversion chromosomes and 0.075 ± 0.018 for standard chromosomes (the pairwise difference in occupancies across all elements is not statistically significant: $t = 1.24$, 9 d.f., one-tailed $P = 0.124$).

For the *In(3R)Mo* chromosomes compared with four standard chromosomes, the average simple occupancies in the inversion region are 0.090 ± 0.023 for inversion chromosomes and 0.098 ± 0.025 for standard chromosomes. The average conditional multiple occupancies in the *In(3R)Mo* region are 0.24 ± 0.054 for inversion chromosomes and 0.00625 ± 0.00625 for standard chromosomes; the *t* test of pairwise differences in occupancy across all element families gives $t = 4.44$, 9 d.f., one-tailed $P < 0.001$, indicating higher frequencies at occupied sites within the *In(3R)Mo* region. Outside the *In(3R)Mo* region, the average simple occupancies are 0.12 ± 0.021 for inversion chromosomes and 0.075 ± 0.018 for standard chromosomes. The paired *t* test of the difference in occupancies across all element families gives $t = 2.67$, 9 d.f., one-tailed $P < 0.05$, suggesting a greater proportion of occupied sites in this region on *In(3R)Mo* chromosomes. The average conditional multiple occupancies outside the *In(3R)Mo* region are 0.12 ± 0.040 for inversion chromosomes and 0.053 ± 0.023; the paired *t* test of the difference in occupancies across all element families gives $t = 2.17$, 9 d.f., one-tailed $P < 0.05$, suggesting higher element frequencies at occupied sites in this region on *In(3R)Mo* chromosomes.

To summarize, element frequencies at occupied sites are significantly higher for the *In(3R)K* and *In(3R)Mo* chromosomes, especially within the inversion regions. Since these chromosomes also showed a striking excess in copy number over the standard *3R* chromosomes (Figure 2), it would seem that some of this excess can be accounted for by higher element frequencies, rather than by increases in the proportion of sites at which elements are segregating.

DISCUSSION

Overall, TE copy numbers are significantly higher within low frequency inversions than within the corresponding standard chromosome regions. Three of the low frequency inversions, *In(2R)NS*, *In(3R)Mo* and *In(3R)K*, had significantly higher TE copy numbers when compared individually with standard regions (Figure 2). An inconsistency in the results is that the apparently rare inversion *In(3R)C* does not show significant increases in TE numbers over the standard chromosomes. It is possible that this inversion was not as rare in 1986, the year our isofemale lines were collected, as indicated in the study of METTLER *et al.* (1977); inversion frequencies in *D. melanogaster* are known to fluctuate significantly over time (INOUE and WATANABE 1979). Because of the small number of *In(3R)C* chromosomes surveyed, it is also possible that their lack of excess TEs is a sampling effect.

Low frequency inversions are expected to have lower rates of recombination than corresponding standard regions; in other respects, they are expected to be essentially the same. The results agree, therefore, with previous findings associating TE abundance with reduced recombination (MONTGOMERY *et al.* 1987; LANGLEY *et al.* 1988; CHARLESWORTH and LANGLEY 1989; CHARLESWORTH and LAPID 1989; CHARLESWORTH *et al.* 1992b; EANES *et al.* 1992). This association is predicted if TEs within inversions are less frequently involved in deleterious ectopic exchanges. We discuss possible alternative explanations for the results before considering the plausibility of ectopic exchange.

Biased gene conversion and LTR excision: There are two types of recombinational event that can excise TE copies at individual sites, and which, therefore, might create differences in TE abundance between regions of high and low recombination. These are: biased gene conversion at heterozygous TE insertion sites (HOLLIDAY 1982); and intrachromosomal recombination between the 5′ and 3′ LTRs of a single TE (LTR excision: CARBONARA and GEHRING 1985). These processes, however, cannot account for the low TE frequencies observed at most chromosomal sites on standard chromosomes. The net rate of excision of TEs by these processes rises only linearly (at most) with TE copy number, which is insufficient to maintain a stable equilibrium in copy number in the face of transpositional increase (CHARLESWORTH and CHARLESWORTH 1983; BROOKFIELD 1986).

Generation of inversions by recombination between TEs: Chromosomal rearrangements resulting from in-

trachromosomal recombination between TEs are well documented in laboratory strains of *D. melanogaster* (BINGHAM and ZACHAR 1989; ENGELS 1989; MONTGOMERY *et al.* 1991; SHEEN *et al.* 1993). LYTTLE and HAYMER (1992) have presented evidence that the *hobo* element is closely associated with the breakpoints of some inversions present in natural *D. melanogaster* populations. This suggests that these inversions may have originated through recombination between *hobo* copies at different locations along the same chromosome, and raises the possibility that the association between TE abundance and inversions in the present results could be a consequence of the same phenomenon. There was, however, no statistically significant evidence that elements are more abundant around the breakpoints of the inversions used in the present study, although mean numbers in these regions were often higher on inversion chromosomes (data not shown). The two instances of apparent fixation associated with inversions, *mdg1* inside *In(3R)Mo* and *412* outside *In(3R)K*, are located well away from the actual inversion breakpoints.

Hitchhiking: For *In(3R)K* and *In(3R)Mo*, higher copy numbers of TEs were accompanied by significant increases in element frequencies at occupied sites. This suggests that hitchhiking (MAYNARD SMITH and HAIGH 1974; KAPLAN *et al.* 1989; STEPHAN *et al.* 1992) of TE insertions with beneficial mutations occurring within inversions might account for the observed increases in TE copy number. A previous study has found some evidence for hitchhiking in element frequencies at the bases of autosomes (CHARLESWORTH *et al.* 1992a). Under hitchhiking, the spread of a favorable mutation occurring within a given copy of an inversion will increase (possibly to fixation) the frequencies of TE insertions in that copy, while decreasing (possibly to zero) frequencies of insertions in copies of the inversion not containing the favorable mutation. Compared to standard regions, a sample of inversions is predicted to have higher frequencies of TEs at occupied sites, but fewer occupied sites overall. Though *In(3R)K* and *In(3R)Mo* show significant increases in element frequencies compared with corresponding standard regions, in both these cases the overall proportion of occupied sites is not significantly different.

Further evidence against hitchhiking is provided by a consideration of the numbers of TEs apparently fixed at insertion sites associated with inversions. One site was apparently fixed for a single TE family in each of the *In(3R)Mo*, *In(3R)K* and *In(3R)C* chromosome samples, but not in the standard chromosomes. It is perhaps surprising that more such apparent fixations were not observed, assuming that the inversions are descended from a single, unique ancestor. This is because each such inversion must arise with a unique profile of TE insertions captured in the region of reduced recombination between its breakpoints. In the absence of excision or recombination events, this relict pattern of insertions should persist unaltered on all descendant copies of the inversion. Given the apparently ancient association between the retrotransposons studied here and the genome of *D. melanogaster* (BROOKFIELD *et al.* 1984), it seems reasonable to suppose that currently observed copy numbers of these TEs reflect a long term equilibrium. If this is so, then the expected number of TE insertions associated with the immediate descendants of any unique ancestral inversion must have been approximately equal to the presently observed mean copy number in the corresponding region of the standard chromosomes. Yet the apparent fixations of TEs associated with inversions in the present sample are obviously far fewer in number than the TE copy numbers observed for the inversion regions on the standard chromosomes. This suggests that the magnitude of the forces tending to remove elements from inversions is sufficient to erase the pattern of insertions left by common descent.

Studies of the occurrence of double crossovers or gene conversions in inversion heterokaryotypes suggest that genetic markers are switched or converted between mutually inverted regions at rates of 10^{-4} or lower (PAYNE 1924; SPURWAY and PHILIP 1952; LEVINE 1956; CHOVNICK 1973; MALPICA *et al.* 1987), while the available evidence (EGGLESTON *et al.* 1988; HARADA *et al.* 1990; NUZHDIN and MACKAY 1994) suggests that rates of excision of retrotransposons from occupied sites are 10^{-5} per element per generation or lower. These data would suggest that, in order for patterns of TE occupancy on inversions to show little sign of common descent, the inversions must have persisted for several thousand generations (on the order of the reciprocal of the rate of removal at individual sites: ISHII and CHARLESWORTH 1977) since the last event generating a unique common ancestor. Studies of allozyme associations with inversions have provided data consistent with the notion that such associations decay slowly as a result of recombinational processes (MUKAI *et al.* 1971; ISHII and CHARLESWORTH 1977; MUKAI and VOELKER 1977; LANGLEY *et al.* 1974, 1977; VOELKER *et al.* 1978; YAMAGUCHI *et al.* 1980; MALPICA *et al.* 1987; AGUADÉ 1988; AQUADRO *et al.* 1991).

Muller's ratchet: The stochastic population process called Muller's ratchet (MULLER 1964) has been identified as a possible cause of TE copy number buildup in regions of low recombination (CHARLESWORTH 1985). The specific requirements for the ratchet to work include a very low recombination rate, small effective population size, and a much higher rate of forward mutation than of "back mutation" (FELSENSTEIN and YOKOYAMA 1976; HAIGH 1978; PAMILO *et al.* 1987; CHARLESWORTH *et al.* 1993). Rare inversions are expected to undergo very little recombination, and may also have very small effective population sizes. This raises the possibility that Muller's ratchet can explain the overrepresentation of TEs within rare inversions. However, if the arguments against hitchhiking above are correct,

the rate of removal of TEs from inversions (effectively, the rate of back mutation) is almost certainly too high to allow the ratchet to progress at an appreciable rate (see CHARLESWORTH *et al.* 1993).

Drift of TE insertions to high frequencies: Selection coefficients on the order of 10^{-4}–10^{-5} per element insertion are probably sufficient to contain TE copy numbers and frequencies at the levels observed on standard chromosomes (CHARLESWORTH and LANGLEY 1989). Since effective population sizes for rare inversions could be small, it is conceivable that TE frequencies and copy numbers within inversions are higher as a consequence of genetic drift rather than the effect of sheltering from a specific selective force such as ectopic exchange. Theoretical studies suggest that TE insertions can rise to generally higher frequencies under drift in very small populations (CHARLESWORTH and CHARLESWORTH 1983); drift generates a large dispersion in element frequencies across different sites, which reduces the variance in copy number below Poisson expectation. An extension of this model to the general case of rare inversions indicates that TE frequencies at occupied sites can rise as a result of drift, although the variance in copy number is predicted to be substantially less than the mean in this case as well (B. CHARLESWORTH, unpublished results). TE frequencies are significantly higher at occupied sites within the rare inversions *In(3R)Mo* and *In(3R)K* (but not within the other inversions). Contrary to the expectation under drift, however, the copy number variances are not lower than the means: the variance-to-mean ratios for all TEs pooled are 0.97 for *In(3R)Mo* and 1.46 for *In(3R)K*). This possibility can, therefore, reasonably be excluded.

Ectopic exchange: It is possible that sheltering of TEs from selective elimination incident to ectopic exchange (LANGLEY *et al.* 1988; EANES *et al.* 1992) has contributed to the observed increase in their copy numbers within inversions. In contrast to forces such as LTR excision or gene conversion, the selective force generated by ectopic exchange increases in a faster-than-linear manner with TE copy number (LANGLEY *et al.* 1988), as required for a stable equilibrium copy number when transposition rate exceeds exision rate (CHARLESWORTH and CHARLESWORTH 1983; BROOKFIELD 1986).

There is direct evidence for the occurrence of ectopic change between TEs in Drosophila. GOLDBERG *et al.* (1983) examined the molecular structure of reciprocal duplications and deficiencies produced between heterozygous copies of the *white* locus alleles w^a and w^{a4} and concluded that they were the result of unequal exchange between two copies of a retrotransposon located at least 60 kb apart on the parental chromosomes. The observed rate of occurrence of these exchanges was comparable to that for normal, homologous exchange between DNA segments of the same size as the TEs. Similarly, DAVIS *et al.* (1987) demonstrated that four classes of regularly occurring duplications and deficiencies near the *white* locus occur as the reciprocal products of unequal crossovers among three copies of the retrotransposon *roo*. Most recently, MONTGOMERY *et al.* (1991) identified 25 retrotransposon-mediated rearrangements in the *white* locus region, which they interpret as resulting from ectopic exchange involving copies of the *roo* element. In the latter two studies it was possible to detect excision of *roo* from the *white* locus in some crosses, yet no excision was observed. Retrotransposons apparently possess no endogenous capacity for excision (EICKBUSH 1994), and, as noted previously, observed rates of excision of retrotransposons (by whatever mechanism) are extremely low. Taken together, these data suggest that excision of TEs may be less frequent than TE-mediated ectopic exchange events.

The model of EANES *et al.* (1992), which provides the theoretical basis for expecting that TEs will accumulate within inversions, assumes that ectopic exchange between TEs on homologous chromosomes is reduced within and around inversions, as is observed for normal homologous exchange. Such an assumption seems reasonable, inasmuch as the two types of exchange are likely to have a common mechanistic basis. In yeast, mutants deficient in normal (allelic) exchange also show reduced levels of ectopic exchange (STEELE *et al.* 1991). The mechanistic basis for observed decreases in meiotic exchange in Drosophila inversion heterozygotes is still not well understood, but evidence points to reduction in both the detection and the occurrence of recombination. Single crossovers within inversion heterozygotes yield acentric and dicentric chromosomes, which are segregated to polar bodies, causing biased recovery of non-recombinants (STURTEVANT and BEADLE 1936). Though double crossovers do not yield acentric and dicentric chromosomes, their products are also recovered from inversion heterozygotes much less frequently than expected, indicating that the rate of crossing over is reduced (ROBERTS 1976). A recent study of 37 pericentric inversions in *D. melanogaster* (COYNE *et al.* 1993) revealed many cases in which decreased fertility in heterozygous females was not observed, indicating that the rate of single crossovers was greatly reduced within the inversions. It is possible that failure of synapsis (*i.e.*, heterosynaptic pairing) suppresses recombination in Drosophila inversion heterozygotes. Heterosynaptic pairing has been observed for paracentric and pericentric inversions in several species (MCCLINTOCK 1933; MARTIN 1967; NUR 1968; ASHLEY *et al.* 1981; GREENBAUM and REED 1984; HALE 1986; GABRIEL-ROBEZ *et al.* 1988; BOJKO 1990), but such observations are currently impossible in Drosophila.

Although meiotic recombination is reduced at the tip of the *X* chromosome in *D. melanogaster* (LINDSLEY and SANDLER 1977), TEs are not significantly overrepresented in this region, a finding inconsistent with the prediction based on ectopic exchange (LANGLEY *et al.* 1988; CHARLESWORTH and LAPID 1989). A proposed

explanation for this inconsistency was that meiotic exchange might be disproportionately increased at the tip of the *X* chromosome in natural populations with abundant autosomal inversion heterozygosity (LANGLEY *et al.* 1988; MONTGOMERY *et al.* 1991). A study using inversion and standard chromosomes extracted from our isofemale lines, however, indicates that this is not the case (SNIEGOWSKI *et al.* 1994). We can only speculate that perhaps TEs at the tips of chromosomes are free to interact ectopically with other TEs in the freely recombining middle regions, even though normal meiotic exchange is suppressed at the tip; there are presently no relevant data.

Whatever the contribution of ectopic exchange to the containment of Drosophila TEs, it seems unlikely to be the only selective force involved. The fourth chromosome and the centric heterochromatin, regions in which meiotic exchange is apparently completely absent, harbor a significant overabundance of TEs but do not show the very high levels of accumulation that would be expected if copy number increase were constrained only by the selective effects of ectopic exchange (CHARLESWORTH *et al.*, 1992b; B. CHARLESWORTH, P. JARNE and S. ASSIMACOPOULOS, unpublished results).

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