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A test for the role of natural selection in the stabilization of transposable element copy number in a population of *Drosophila melanogaster*

ELIZABETH MONTGOMERY, BRIAN CHARLESWORTH[1] AND CHARLES H. LANGLEY

Laboratory of Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 27709 and [1]*Department of Biology, The University of Chicago, Chicago, IL 60637*

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Summary

The numbers of members of three families of *copia*-like elements were counted on twenty *X*, 2nd and 3rd chromosomes collected from a natural population of *Drosophila melanogaster*. Theoretical predictions were computed for two models of copy number stabilization: (1) element frequencies are regulated by a simple genetic process such as copy number dependent transposition or excision, independent of chromosomal location; (2) elements are eliminated by natural selection against mutational effects of their insertion into the chromosome. Since insertions into the *X* can be expected to suffer more selection than autosomal insertions, due to expression of mutant phenotypes in the hemizygous state, hypothesis 2, called the disproportional model, predicts that the proportion of elements on the *X* will be smaller than the proportion of the genome contributed by the *X*, while hypothesis 1, called the equiproportional model, predicts that this proportionality will be unaffected. Two of the elements, *297* and *roo*, showed no evidence for deficiency of *X*-linked elements, but the data for a third element, *412*, were consistent with the prediction based on the selective model.

These results indicate that simple selection against mutational effects of insertions of transposable elements is not generally adequate to account for their distribution within populations. We argue that a mechanism such as recombination between elements at different chromosomal sites, leading to rearrangements with highly deleterious, dominant effects could play a role in stabilizing copy number. This process would lead to a higher abundance of elements in genomic regions with restricted crossing over. We present some data indicating such an effect, and discuss possible interpretations.

1. Introduction

One of the central questions in the population biology of transposable genetic elements concerns the nature of the processes that control the distribution of copy numbers among individuals in natural populations. Copy number-dependent rates of transposition or excision, and selection against the mutational effects of insertions of transposable elements, can stabilize copy number distributions in large populations (Langley, Brookfield & Kaplan, 1983; Charlesworth & Charlesworth, 1983; Charlesworth, 1985*b*; Charlesworth & Langley, 1986). Although the mode of replicative transposition varies considerably, from germline specific DNA-mediated replicative insertion (Engels, 1983) to retroviral infection and germline integration (Rowe & Kozak, 1980; Jaenisch, 1980; Jenkins & Copeland, 1985), the mechanisms of stabilization of element numbers in populations could be similar. At present, empirical evidence that might enable us to discriminate among the various possibilities is scanty.

Data on transposable element frequencies at individual chromosomal sites in *Drosophila* populations, both from *in situ* hybridization of labelled probes to polytene chromosomes (Montgomery & Langley, 1983) and from restriction maps of defined genomic regions (Langley, Montgomery & Quattlebaum, 1982; Leigh Brown, 1983; Aquadro *et al.* 1986), suggest that element frequencies at individual sites are generally very low (Langley, Brookfield & Kaplan, 1983;

Corresponding author: Elizabeth Montgomery, Laboratory of Genetics, NIEHS, Research Triangle Park, N C 27709, USA.

Kaplan & Brookfield, 1983; Charlesworth & Charlesworth, 1983; Charlesworth, 1985*b*). Insertions of transposable elements can cause mutations with gross phenotypic effects in a wide variety of organisms (Shapiro, 1983), and the vast majority of such mutations reduce the fitness of their carriers (Simmons & Crow, 1977). Thus selection against the presence of elements may play an important role in limiting copy numbers. In fact, restriction map surveys of *Drosophila* populations show that transposable elements are rarely found within transcriptional units, presumably because deleterious effects of such insertions prevent them from rising to the frequencies observed for insertions in non-coding regions (Langley *et al.* 1982; Leigh Brown, 1983; Aquadro *et al.* 1986). In addition, Golding, Aquadro & Langley (1986) showed that constant rates of transposition and excision are inconsistent with the distribution of transposable element insertions among *Adh* region haplotypes in a sample from a natural population of *D. melanogaster*. They concluded that additional forces must be acting, but it is unclear whether these involve selection or copy number-dependent transposition or excision.

In this report, we present another test of the hypothesis that element frequencies are affected by selection against the effects of insertional mutations. Under this hypothesis, one would expect to find relatively few elements on the *X* chromosome compared with the autosomes, since recessive or partially recessive deleterious effects of *X* chromosome insertions would be fully expressed in hemizygous male carriers (Haldane, 1927). We develop a model that predicts the proportions of copy numbers under each of two alternative hypotheses: (1) The turnover of elements occurs at equal rates on the *X* chromosome and the autosomes, as would be the case if copy numbers were stabilized by copy number-dependent or constant transposition or excision (the equiproportional hypothesis); (2) There is differential removal of elements on the *X* chromosome relative to the autosomes, due to selection against mutations caused by insertions (the disproportional hypothesis). In order to test these competing models, we extracted recessive lethal-free *X*, 2nd and 3rd chromosomes from a wild population of *D. melanogaster* and counted the numbers of each of three *copia*-like transposable elements on the *X* chromosome and homozygous autosomes. Analysis of our results indicates that the equiproportional hypothesis 1 is compatible with the observed distribution in two of the families tested (*roo* and *297*), but not with the distribution in a third family (*412*), which fits the expectation under the disproportional hypothesis 2.

2. Materials and methods

(i) *Strains*

The lines used in this study were derived (avoiding the 'obviously dysgenic' crosses) from isofemale lines, which had been recently established from *D. melanogaster* females from a Raleigh, N.C. Farmer's Market population. The *X* chromosome lines were established and maintained by crossing single males from twenty of the isofemale lines to a stock carrying an attached-*X* chromosome (*C(1)DX, y f/FM7, π²/y⁺Y*) that had the *P* cytotype. Only male larvae were sampled. Homozygous 2nd and 3rd chromosome lines were established by isolating single 2nd or 3rd chromosomes from females belonging to twenty of the isofemale lines. The marked balancer stocks, *Pu²*, *Elp*, *Sp/SM5* for the 2nd chromosome and *TM3*, *p^p*, *Sb/TM6*, *Tb*, *ca*, *e* for the 3rd chromosome were used in the chromosome isolations. Flies were cultured on standard corn meal/ agar medium at 22–25 °C. Since lethal-free autosomes are common ($\geqslant 70\%$), recessive lethals are mappable single mutations and linkage disequilibrium is low across the chromosomes, little correlation between total transposable element number and recessive lethals is expected. Therefore only a small bias was possibly introduced by excluding recessive lethal bearing chromosomes.

(ii) *Chromosome preparations*

The preparation of salivary gland chromosomes for *in situ* hybridization was essentially as described in Pardue & Gall (1975), with modifications noted below. Microscope slides were prepared as described in Brahic & Haase (1978). Third instar larvae were dissected in 45% acetic acid, the salivary glands were removed and incubated for 4–5 min in a mixture of 1 part lactic acid, 2 parts acetic acid and 3 parts water (J. Lim, pers. comm.), after which they were squashed under a coverslip and left to incubate overnight at 4 °C in the residual lacto-acetic acid solution. The coverslip was removed by freezing the slide in liquid nitrogen and the slides were immersed in 95% ethanol for at least 2 h. Prior to hybridization, the slides were allowed to dry and incubated for 30 min in 2 × SSC at 65 °C. The slides were twice dehydrated in 70% ethanol for 5 min each and once in 95% ethanol for 5 min. Chromosomes were denatured by treating the slides with 0·07 N-NaOH for 2 min. After rinsing with three 5 min washes of 2 × SSC, followed by the dehydration series above, the slides were allowed to dry and the chromosomes were hybridized to probe DNAs *in situ*.

(iii) *Nick translation and hybridization*

The cloned transposable element DNAs used in the study were nick translated (Rigby *et al.* 1977) using biotinylated dUTP as the label (both Enzo biotin-16-dUTP and BRL biotin-11-dUTP were used with equal success) (Langer, Waldrop & Ward, 1981). Concentrations and reaction time were adjusted to give optimal incorporation (20–40% substitution). Our conditions were as follows: dATP, dCTP, dGTP,

25 μM; DTT, 10 mM; gelatin, 100 μg/ml; $MgCl_2$, 10 mM; Bio-dUTP, 20 μM; DNA, 20 μg/ml; DNAse, 1·25 μg/ml; Kornberg DNA polymerase, 10 units/100 μl reaction. The reaction was incubated at 12–14 °C for 90 min and then heat-killed at 65 °C for 5 min. The DNA was then ethanol precipitated, dried and resuspended in hybridization buffer containing 50% formamide, 2 × SCC, 10% dextran sulfate, 2·5 mg/ml salmon sperm DNA, with a final probe DNA concentration of 2 μg/ml. The DNA was denatured in hybridization buffer by heating for 15 min at 65 °C and was then cooled briefly on ice. Twenty microlitres of this hybridization solution was applied to each squash, which was then covered with a coverslip and incubated overnight in a moist chamber at 37 °C.

(iv) *Detection of probe DNA and scoring*

After the hybridization period, slides were washed in 2 × SSC twice for 5 min each at 37 °C and twice for 5 min each at room temperature, followed by 5 min in PBS at room temperature. Ten microlitres each of biotinylated horseradish peroxidase H and Avidin-DH (ABC kit from Vector Laboratories) were added to 1·25 ml of a solution of 50 mM Tris, pH 7·6, 4% BSA, and this mixture was allowed to incubate for 5 min. Twenty microlitres of this was applied to each slide and covered with a coverslip. Slides were incubated for 30 min at 37 °C, and then washed for 30 min in PBS with 3 changes. A solution of 3,3′diaminobenzidine tetrachloride (0·5 mg/ml in 50 mM Tris, pH 7·6) containing hydrogen peroxide (2 μl/ml of a 30% hydrogen peroxide solution) was applied to each slide, using enough to cover the squash area (approx. 0·5 ml per slide). After a 30 min incubation at 37 °C, the slides were rinsed in PBS for 5 min and the chromosomes were then stained with Giemsa.

The transposable element DNAs used were *cDm* 4006 (*297*), *cDm* 2042 (*412*), and *cDm* 2173 [containing *roo* (Meyerowitz & Hogness, 1982) or *B104* (Scherer *et al.* 1982)], and additionally for the inversion breakpoint data, *pD* 75·3 (Levis & Rubin, 1982). *cDm* 4006 and *cDm* 2042 each contain complete copies of the elements *297* and *412* respectively, cloned into PBR 322 (Rubin *et al.* 1981). *cDm* 2173 (Strobel, 1982) shows homology to the 3′6·5 kb region of *roo* (C. F. Aquadro & P. S. Davis, pers. comm.). *pD* 75·3 contains a foldback (*FB*) sequence. DNAs were prepared by techniques described in Maniatis, Fritsch & Sambrook (1982).

Duplicate slides were prepared for each line for each DNA probe. Sites of hybridization were counted in as many nuclei as could be located with good stretched chromosomes and clearly visible label. In many cases, there was good agreement among nuclei and between slides. In some cases, a consensus count (the number of sites most often seen) was used for each slide. The second slide was scored independently of the first. Additional slides were prepared if there were significant discrepancies between the original two slides or among different nuclei on the same slide. A single number was arrived at as the final count by (1) using the actual count when there were no discrepancies, (2) taking the higher of two counts (two slides) when the difference was one, or (3) taking the average count when the difference was two. Any larger differences were resolved earlier by re-counting, preparing additional slides, or ascertaining the cytological positions of sites in order to determine if sites actually differed from one nucleus to another. The line numbers used here are independent for *X*, 2nd and 3rd chromosomes, and identical line numbers do not necessarily indicate that the chromosomes were derived from the same isofemale line.

Crosses were made between each second and third chromosome line and a stock containing known standard sequence chromosomes (*cn bw*; *ri e*). Squashes were made from the larvae from these crosses, and the heterozygous second or third chromosomes examined cytologically for the presence of inversions in the wild chromosomes. Of the forty chromosomes examined, only one, 3rd chromosome line no. 9, had a cytologically detectable inversion (polymorphic *In(3R)P*).

3. Theoretical expectations

The models of Charlesworth & Charlesworth (1983) and Charlesworth (1985*b*) for the change in mean copy number of families of transposable elements in infinitely large, random mating populations are adapted here to the problem of predicting the equilibrium distribution of elements between the euchromatic regions of the *X* and autosomes. Let H_X and H_A be the haploid mean copy numbers of a given family of elements in the euchromatic regions of the *X* and autosomes, respectively. We also have to consider the effects of elements located in heterochromatin, since these may contribute by transposition to the pool of elements in euchromatin (Charlesworth, 1985*b*). Since the *Y* chromosome in *D. melanogaster* males is largely heterochromatic, whereas only about 40% of the similarly sized *X* is heterochromatic (Hilliker, Appels & Schalet, 1980), it is necessary to consider females and males separately. Let D_f and D_m be the diploid mean copy numbers of elements in the heterochromatin of females and males respectively.

We assume that elements have a probability of transposition per generation of u, independent of sex or location in euchromatin *vs.* heterochromatin, and a corresponding probability of excision, v. Let P_{Xf}, P_{Af} and P_{Hf} be the probabilities in females of insertion of a newly transposed element into the euchromatin of the *X*, the autosomal euchromatin and the heterochromatin, respectively ($P_{Xf}+P_{Af}+P_{Hf}=1$). The corresponding probabilities for males are P_{Xm}, P_{Am} and P_{Hm}. Finally, let s_X, s_A and s_H be the selection pres-

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sures on mean copy number for these three sectors of the genome; these can be calculated from the formulae of Charlesworth (1985*b*) and standard selection theory (see below). Taking into account the fact that two-thirds of the X chromosomes of the population are carried in females, we obtain the following equations for the changes in H_X and H_A per generation:

$$\Delta H_X = -H_X(s_X+v)+\tfrac{2}{3}uP_{Xf}(H_X+H_A+\tfrac{1}{2}D_f) + \tfrac{1}{3}uP_{Xm}(\tfrac{1}{2}H_X+H_A+\tfrac{1}{2}D_m), \quad (1a)$$

$$\Delta H_A = -H_A(s_A+v)+\tfrac{1}{2}uP_{Af}(H_X+H_A+\tfrac{1}{2}D_f) + \tfrac{1}{2}uP_{Am}(\tfrac{1}{2}H_X+H_A+\tfrac{1}{2}D_m). \quad (1b)$$

Similar equations can be written for D_f and D_m, but these are not used in the subsequent analysis, since *in situ* data on heterochromatic regions cannot be obtained.

At equilibrium we obtain

$$\frac{H_X(s_X+v)}{H_A(s_A+v)} = \frac{4(P_{Xf}+\tfrac{1}{2}P_{Xm})\{1+a_1(H_X/H_A)+a_2(D_f/H_A)+a_3(D_m/H_A)\}}{3(P_{Af}+P_{Am})\{1+b_1(H_X/H_A)+b_2(D_f/H_A)+b_3(D_m/H_A)\}}, \quad (2)$$

where

$a_1 = (P_{Xf}+\tfrac{1}{4}P_{Xm})/(P_{Xf}+\tfrac{1}{2}P_{Xm})$,

$a_2 = P_{Xf}/2(P_{Xf}+\tfrac{1}{2}P_{Xm})$,

$a_3 = P_{Xm}/4(P_{Xf}+\tfrac{1}{2}P_{Xm})$,

$b_1 = (P_{Af}+\tfrac{1}{2}P_{Am})/(P_{Af}+P_{Am})$,

$b_2 = P_{Af}/2(P_{Af}+P_{Am})$,

$b_3 = P_{Am}/2(P_{Af}+P_{Am})$.

Numerical values for the constants in equation (2) may be obtained if we assume that elements insert into different genomic regions at a frequency proportional to the fraction of the genome that each region represents. The euchromatin of the X represents $\frac{1}{5}$ of the total euchromatin in females and $\frac{1}{9}$ in males (ignoring the small 4th chromosome). The exact proportions of heterochromatin on each chromosome are not known accurately, but this does not matter for our purposes (see below). We follow the statements of Hilliker *et al.* (1980) and Pimpinelli *et al.* (1986), and assume that the Y is entirely heterochromatic and constitutes 20% of the haploid genome of a male, and that 40 and 22·5% of the X and autosomes respectively are heterochromatic. Using these values, we obtain $P_{Xf} = 0{\cdot}146$, $P_{Af} = 0{\cdot}586$, $P_{Hf} = 0{\cdot}268$, $P_{Xm} = 0{\cdot}075$, $P_{Am} = 0{\cdot}602$, and $P_{Hm} = 0{\cdot}323$. These numbers can be substituted into equation (2), yielding an equation in H_X/H_A whose coefficients depend on the (assumed) values of D_f/H_A and D_m/H_A. A convenient way to express these is to write

$$k_f P_{Hf} = D_f/\{D_f+2(H_X+H_A)\}, \quad (3a)$$

$$k_m P_{Hm} = D_m/\{D_m+H_X+2H_A\}, \quad (3b)$$

where k_f and k_m are the ratios of the equilibrium frequency of elements in the heterochromatin to the proportion of the genome that it contributes, in females and males respectively. (These coefficients also reflect any differences in transposition rates of heterochromatic elements relative to those for euchromatic ones.) Substitution into equation (2) yields the following quadratic in $x = H_X/H_A$:

$$ax^2+bx+c = 0. \quad (4)$$

where

$a = b_1+2b_2c_1+b_3c_2$,

$b = 1-a_1c_3+2c_1(b_2-a_2c_3)+2c_2(b_3-\tfrac{1}{2}a_3c_3)$,

$c = -c_3(1+2a_2c_1+2a_3c_2)$, $c_1 = k_fP_{Hf}/(1-k_fP_{Hf})$,

$c_2 = k_mP_{Hm}/(1-k_mP_{Hm})$,

$c_3 = 4(P_{Xf}+\tfrac{1}{2}P_{Xm})(s_A+v)/3(P_{Af}+P_{Am})(s_X+v)$.

The relevant solution to equation (4) is $x = [-b+\sqrt{(b^2-4ac)}]/2a$.

When the equilibrium proportion of elements in heterochromatin is equal to the proportion of the genome that is heterochromatic ($k_f = k_m = 1$), the predicted frequency of elements on the X, given by $H_X/(H_X+H_A)$, equals 0·173 when there is no selection ($s_X = s_A = 0$). We shall therefore take 0·17 as the expected value for the proportion of elements on the X in the simplest version of hypothesis (1). If elements are being selectively removed because of the deleterious effects of mutations induced by their insertion, and the mean selection coefficient on hemizygous or homozygous mutations is s, with a dominance coefficient h for heterozygotes, we can use the standard results of Haldane (1927) for selection on rare sex-linked alleles, together with the model of transposable elements of Charlesworth (1985*b*), to write $s_X \approx s(\frac{2}{3}h+\frac{1}{3})$, $s_A \approx sh$. A value of h of approximately 0·35 is suggested by the data on viability mutations in *Drosophila* (Simmons & Crow, 1977), so that we have $s_A/s_X \approx 0{\cdot}618$ if viability is representative of net fitness. Assuming similar constants to those used above for hypothesis (1), and neglecting excision rates in equation (4), the expected proportion of euchromatic elements on the X chromosome in the simplest version of hypothesis (2) is 0·11. The effect of non-negligible excision rates is to bring the expectation closer to that of hypothesis (1). An analysis (not shown) of the consequences of variation in the dominance, h, of individual insertions indicates that variance in h may move the equilibrium ratio closer to the expectation under hypothesis (1).

By examining solutions to equation (4) over a range of values of the variables k_f and k_m (which reflect the proportion of elements in the heterochromatin and/or their rates of transposition relative to euchromatic elements), it can be shown that the expected propor-

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Table 1. *The effect of heterochromatic elements on the equilibrium proportion of elements on the* X *chromosome*
(See text for explanation.)

Value of k...	0	0·5	1·0	1·5	2·0	2·5	2·75	3·0
No selection	0·175	0·173	0·171	0·168	0·163	0·151	0·138	0·105
Selection	0·115	0·114	0·112	0·110	0·106	0·098	0·089	0·068

tion of elements on the X chromosome is insensitive to variation in these parameters except when there is a large differential in favor of heterochromatic elements. Table 1 gives the equilibrium proportions of X-linked elements for the case $k_f = k_m = k$, using the above values of P_{Xf}, etc. A similar analysis shows that the expected proportions of X-linked elements are insensitive to sex differences in transposition rates.

4. Results

(i) *Distribution of elements between chromosomes*

Sites of *in situ* hybridization with each of three *copia*-like transposable elements, *412*, *297* and *roo* were counted in twenty hemizygous X chromosomes, twenty homozygous 2nd chromosomes and twenty homozygous 3rd chromosomes. The counts for each chromosome arm are reported in Table 2. Fig. 1 presents the mean number and 95% confidence interval for each element on each of the three chromosomes.

Since the chromosomes (not the arms) were sampled independently, the initial analysis and test of the two hypotheses use the counts on whole chromosomes as the raw data. On the basis of our earlier results (Montgomery & Langley, 1983; Kaplan & Brookfield, 1983), we expect element frequencies at individual sites to be low, so that the number on any one chromosome will be Poisson-distributed (Langley *et al.* 1983; Charlesworth & Charlesworth, 1983) in the absence of non-random associations between frequencies at different sites (linkage disequilibrium). Before presenting the results of the tests of our data against the predictions made by the model, we will first examine the data, in a model-independent test, for consistency and homogeneity.

In order to test whether the distributions of elements within families and within chromosomes are random (as assumed from previous studies; see above), the following hypothesis was tested, $H_0: E(n_{ijk}) = n_{ij}/20$, where n_{ijk} is the observed number of ith element in the jth chromosome in the kth sample ($n_{ij} = \Sigma_k n_{ijk}$). The χ^2 for this test was 202·3 with 175 D.F. Accepting that the numbers of each element within chromosomes are Poisson distributed, the following test of interaction between element families and chromosomes can be performed. The hypothesis tested was $H_0: n_{ij} = n_i \ldots n_j \ldots /n$. The χ^2 with 4 D.F. was 13·4 ($P < 0{\cdot}01$). This indicates that the three families are not distributed among the X, 2nd and 3rd chromosomes in the same proportions. In order to test whether the primary difference involved the X chromosome, the following hypothesis was tested, $H_0: n_{Xj} = n_X \ldots n_j \ldots /n$. The χ^2 (with 2 D.F.) for this test was 8·45 ($P < 0{\cdot}02$). The effect is thus due to the X *vs.* autosome contrast. The hypothesis $H_0: n_{X412} = n_{412} \ldots n_X \ldots /n$, was tested and gave a χ^2 equal to 8·26 (1 D.F., $P < 0{\cdot}01$). This shows that the major source of heterogeneity is associated with the proportion of *412* elements on the X chromosome.

The model-dependent tests for the expected proportion of elements on the X chromosome (see theoretical expectations) predicts that if selection acting on insertions of transposable elements is the main force in stabilizing frequencies, then 11% of the elements in a given family will be found on the X (the disproportional model). If other forces are primarily responsible (the equiproportional model) 17% of elements in a given family should be found on the X. Fig. 1 presents the results of these tests, where n designates the expectation under the equiproportional model, and s designates the expectation under the disproportional model.

We can also test these two hypotheses for the expected proportion of elements on the X chromosome for each element separately. These tests are presented in Table 3. The proportions on the X chromosome for two of the elements, *roo* and *297*, are consistent with the equiproportional model (hypothesis 1). The proportion of *412* on the X is, however, in agreement with the disproportional model (hypothesis 2), and is significantly different from that expected in hypothesis 1.

(ii) *Other findings*

Analysis of the data for each chromosome arm revealed several other interesting deviations from the simple expectation of a Poisson distribution across samples. (1) There is a significantly less variation than expected among X chromosomes in the number of *roo* elements ($\chi^2 = 6{\cdot}52$, D.F. = 19, $P > 0{\cdot}995$); (2) There is significantly greater variation than expected among the left arms of the second chromosomes in the observed numbers of *roo* elements ($\chi^2 = 37{\cdot}58$, D.F. = 19, $P < 0{\cdot}01$). The correlations between the two arms of the same chromosome in the numbers of the various elements (e.g. *297*/2L *vs.* *297*/2R and *297*/2L *vs.* *roo*/2R) were also examined. In one of the eighteen cases, *roo*/2L *vs.* *roo*/2R, there was a significant association ($r = 0{\cdot}51$; $P < 0{\cdot}02$). (This may not be meaningful in view of the large number of tests performed.) The possible causes and implications of these deviations are discussed below.

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Table 2. *The number, mean and variance of transposable elements*, 297, 412 *and* roo, *on the chromosome arms* (X, IIL, IIR, IIIL *and* IIIR; *sample size = 20*)
(See text for explanation.)

	X			*IIL*			*IIR*			*IIIL*			*IIIR*		
Line	*297*	*412*	*roo*	*297*	*412*	*roo*	*297*	*412*	*roo*	*297*	*412*	*roo*	*297*	*412*	*roo*
1	4	1	10	3	7	13	4	7	13	7	5	12	6	4	10
2	6	6	10	4	4	22	6	4	12	6	4	12	7	3	10
3	3	1	13	1	3	10	3	4	10	5	3	6	6	7	8
4	3	2	11	3	7	20	4	3	12	7	4	15	3	2	20
5	6	7	14	9	0	11	4	6	5	4	8	9	9	6	11
6	8	1	12	3	8	11	6	6	13	4	4	13	3	4	18
7	4	2	9	4	7	12	1	6	9	4	7	9	5	7	16
8	2	4	10	5	6	8	2	1	13	6	2	17	6	7	16
9	2	4	12	8	2	13	7	6	11	6	5	12	8	10	17
10	8	2	12	6	6	15	0	1	18	2	4	9	3	3	21
11	5	0	11	3	3	16	6	4	11	6	4	11	10	6	14
12	7	4	14	4	5	20	5	4	11	5	7	12	5	6	13
13	7	2	11	4	6	12	2	2	13	4	4	10	5	5	12
14	3	3	12	3	4	10	1	0	9	2	4	8	2	6	6
15	4	4	15	3	3	12	7	4	13	2	3	12	7	5	16
16	7	3	13	2	4	21	7	3	12	6	1	12	6	7	13
17	3	4	10	7	3	10	6	4	14	7	4	8	6	7	15
18	4	0	10	2	4	5	1	2	8	3	3	13	4	3	11
19	2	2	7	4	6	18	3	9	15	3	5	14	6	2	16
20	1	1	13	1	3	5	4	3	11	4	1	9	7	6	13
Means	4·45	2·65	11·45	3·95	4·55	13·20	3·95	3·95	11·65	4·65	4·10	11·15	5·70	5·30	13·80
Variances	4·68	3·50	3·73	4·58	4·15	24·80	5·10	5·00	7·50	2·87	3·25	7·08	4·22	4·22	14·91

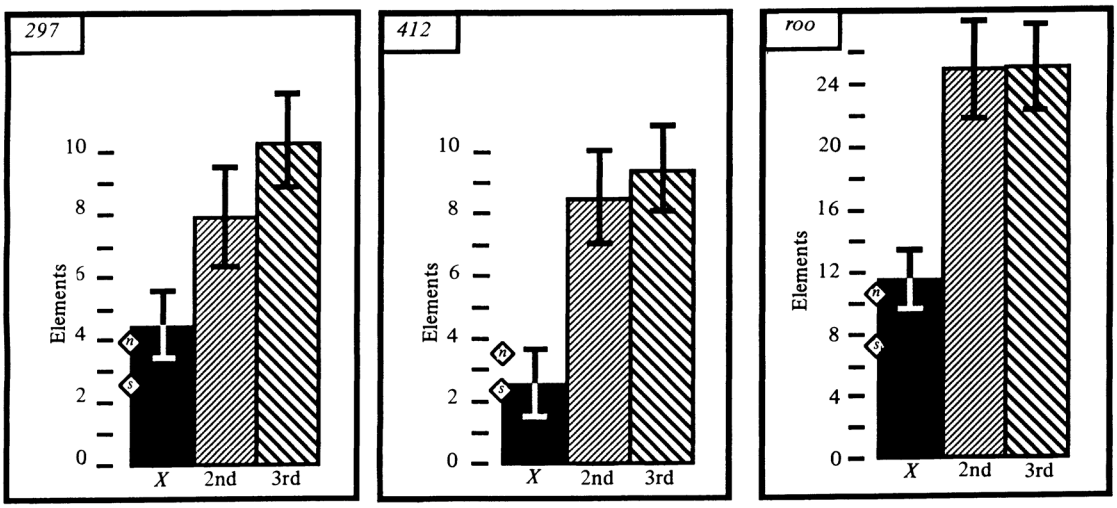

Fig. 1. The mean and 95% confidence interval of the number of transposable elements (*297*, *412* and *roo*) found on the *X*, 2nd and 3rd chromosomes (sample size = 20). *n* indicates the expected proportion if elements on the *X* chromosome are removed at the same rate as those on the autosomes, i.e. hypothesis 1. *s* indicates the expected number on the *X* chromosome under the model that incorporates natural selection against the phenotype caused by the insertion of the transposable element, i.e. hypothesis 2. (See text for explanation.)

5. Discussion

(i) *Distribution of elements on the* X vs. *the autosomes*

The results indicate that the three transposable element families differ in their distribution between the *X* chromosome and the autosomes. *412* occurs on the *X* chromosome in the proportions predicted by the disproportional model of selection against partially recessive deleterious effects of the insertions, and *297* and *roo* occur in proportions consistent with the equiproportional model of no selection. The difference between *412* and the other two families of elements is highly significant in a model-free test (see above). The reason for this difference is not clear. The few possibly relevant characteristics of *412* are (1) its relative conservation in evolution (Brookfield, Montgomery & Langley, 1984); (2) its relatively low copy number; (3) the apparent absence of a distinct target sequence for *412* insertions (Yuki *et al.* 1986; Inouye *et al.* 1986). With further work, it may become clear why *412* is different.

Table 3. *The proportions of* 297, 412 *and* roo *elements on the* X *chromosome and tests against the two proposed models*

(See text for explanation.)

Element	Proportion on *X*	χ^2 [0·17]	χ^2 [0·11]
297	0·20	2·2	31·2***
412	0·13	4·9*	1·0
roo	0·19	2·5	66·8***

* $P < 0{\cdot}05$; *** $P < 0{\cdot}005$.

The conclusion that the insertion of *412* elements may be affected by selection, while insertions of the other two element families apparently are not, depends on several assumptions about the equivalence of the *X* chromosome and the autosomes as targets for insertional mutagenesis. The first assumption is that the genetic and phenotypic effects of insertions are similar for the *X* chromosome and autosomes. Although recessive lethal-bearing *X* chromosomes are rare compared to autosomes ($\approx 30\%$), only a negligible bias could have been introduced by the exclusion of recessive lethal bearing chromosomes from the sample. A particular recessive lethal might be due to (at the most) one transposable element insertion out of hundreds from the various families on each chromosome.

The second assumption is that the proportion of insertions into the euchromatin of the *X* chromosome in a haploid set is one-fifth of all euchromatic insertions. This proportion is derived from extensive genetic and cytogenetic studies of *D. melanogaster*, which have not indicated any great differences between the *X* chromosome and the large autosomal arms, apart from dosage compensation effects. Furthermore, estimates of spontaneous and induced mutation rates are consistent with the approximate equivalence of the *X* and each of the four large autosomal arms (Wallace, 1968; Simmons & Crow, 1977). The overall proportions reported above for *297* and *roo* fit the expected ratio of 1:2:2 for the *X* chromosome and the two large autosomes.

The chromosomes are clearly not equivalent with respect to the cytogenetic measure of band counts in the polytene chromosomes. Lefevre (1976) estimated 804 and 1136 polytene chromosome bands for 2L and 2R respectively. We observed a total of 434 and 391 elements on 2L and 2R, respectively (see Table 2).

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The lack of precision of the many measures of chromosome 'size' and the strictly empirical and approximate correlation between chromosome bands and genetic or physical measures (Judd, Shen & Kaufman, 1972; Wright *et al.* 1981; Lefevre, 1981; Spierer *et al.* 1983), make it difficult to judge the effect of this disparity in chromosome 2 on the interpretation of our results.

The low frequency of *X*-linked *412* elements can be explained by a high concentration of *412* in the heterochromatin and/or a high rate of transposition of heterochromatic *412* elements relative to euchromatic ones (i.e. large values of k, see previous section). The lowest value of k that is consistent at the 95% level with the observations for *412* is approximately 2. The situation of $k \geqslant 2$ corresponds either to a situation when more than 54% of all elements are carried in the heterochromatin (when heterochromatic elements have the same rate of transposition as euchromatic elements) or to a rate of transposition of heterochromatic elements that is at least 2·75 times that of euchromatic ones, or to a combination of these circumstances. The number of *412* elements in heterochromatin can be estimated to be approximately eight by subtracting the euchromatic copy number of 32 (estimated from the *in situ* hybridization data of Strobel, Dunsmuir & Rubin (1979)) from a total genomic copy number of 40 (estimated by Potter *et al.* (1979)). Thus it seems unlikely that the scarcity of *412* elements on *X* chromosomes is due to excess numbers of elements in the heterochromatin. The alternative cause of a high value of k could be an elevated rate of transposition of *412* elements inserted in the heterochromatin (perhaps 2·75 times the rate of euchromatic copies). Since there is no independent evidence for this increased transposition rate nor any *a prior* reason to suspect it, we can reasonably conclude that natural selection against the insertional mutations themselves accounts for the removal of *412* elements from the natural population. Natural selection of this type is not, however, an adequate explanation for the removal of *roo* and *297* elements.

If the deleterious fitness effects of insertions at the genic level do not selectively remove most of the insertions of *297* and *roo*, what mechanisms could be involved? One possibility is that only a proportion of sites available for insertion by members of these families are such that insertions cause deleterious mutations; insertions at other sites are neutral. The first class of sites might correspond to coding sequences and their adjacent regulatory sequences, and the second to non-functional DNA (if it exists). A model of this has been sketched by Charlesworth (1985*b*), who showed that selection on insertions into the first class of sites could stabilize copy numbers of both classes, due to the fact that transpositions from the second class enter the first class in proportion to their abundance in the genome. In this model, one would expect a much lower equilibrium frequency of elements at the first class of sites, compared with the second. The majority of observable elements would therefore be free of the direct effect of selection, and would not show any disproportionality between the *X* and autosomes. Another possibility is the following: Recombination between insertions at different sites would lead to chromosome rearrangements that are generally dominant lethal, or so deleterious that their probability of transmission and/or survival in subsequent generations is substantially reduced when compared with that of a simple insertional mutation. There is good evidence from bacteria, yeast, and mammals for such events (Mikus & Petes, 1982; Roeder, 1983; Maeda & Smithies, in press). In *Drosophila*, there is also some evidence suggesting their occurrence (Goldberg *et al.* 1983; Davis, Shen & Judd, in press). This unequal recombination* process would provide an effective mechanism of selective stabilization of copy number within populations, since the rate of induction of rearrangements will increase with the square of the copy number. Such a relationship between copy number and fitness is required for a stable equilibrium distribution of copy number under selection (Charlesworth & Charlesworth, 1983; Charlesworth, 1985*b*). Selection against dominant lethal and highly deleterious mutant effects will, of course, be equally effective on sex-linked and autosomal elements. Finally, if such meiotic, unequal recombination is correlated with homologous recombination along the various chromosomal regions, then the rate would be expected to be lower near the centromere and the tip of the chromosome in *Drosophila* and other eukaryotes (Charlesworth, Langley & Stephan, 1986). Further, in *Drosophila* meiotic recombination occurs only in females, in which both the *X* chromosome and the autosomes are diploid, so that the effects of unequal recombination on the *X* and autosomes would be expected to be similar.

(ii) *Other findings and their significance*

Our analysis of the data revealed that the variance in the numbers of *roo* elements on the *X* chromosomes was significantly smaller than expected. The most reasonable explanation is that there are sites that occur in high frequency in the sampled *X* chromosomes (Charlesworth & Charlesworth, 1983, eq. [4]; Langley *et al.* 1983). A preliminary examination of six of the *X* chromosomes indicated that 6 out of 43 sites hybridizing with *roo* are indeed labelled in three or more of the chromosomes (data not shown). Interestingly, five of these six are either very near the tip or the base of the chromosome (see below). The num-

* We refer to recombination between homologous sequences at non-homologous locations as 'unequal recombination'. Since this term is commonly associated with recombination between tandemly repeated sequences, there may be some confusion. No appropriate alternative phrase is available, however.

Table 4. *Numbers of transposable elements in the regions of inversion breakpoints in laboratory stocks of* D. melanogaster

(See text for explanation.)

Stock	Element	Total	Observed	Test A Expected	Test A χ^2	Test B Expected	Test B χ^2
			X chromosome inversions				
ClB/dor	*412*	10	7	2·0	15·63***	3·33	6·06*
	297	10	3	2·0	0·63	3·33	0·05
	FB	13	8	2·6	14·02***	4·33	4·66*
	roo	24	14	4·8	22·04***	8·00	6·75**
In(1)dl-49/amx	*412*	13	8	2·6	14·02***	4·33	4·66*
	297	11	7	2·2	13·09***	3·67	4·53*
	FB	9	5	1·8	7·11**	3·00	2·00
			2nd chromosome inversions				
In(2L)(2R)Cy/L	*297*	30	10	3·0	18·15***	5·45	4·64*
	FB	22	8	2·2	16·99***	4·00	4·89*
	roo	54	18	5·4	32·67***	9·82	8·32***

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}005$.

bers of *roo* on 2L also deviate significantly from a Poisson distribution in that the variance is too large. A reasonable explanation is that there are non-random associations between element frequencies at different sites (i.e. linkage disequilibrium; Charlesworth & Charlesworth, 1984, eq. [4]; Langley *et al.* 1983). The apparent correlation in copy number between 2L and 2R for this element is consistent with this interpretation, but we have not yet investigated this possibility. These deviations from the predictions of a Poisson distribution do not compromise the hypothesis tests concerning the relative proportion of elements on the *X* chromosome and whole autosomes.

The reduced variance in the numbers of *roo* elements on the *X* chromosome in this study is apparently due to the accumulation of *roo* insertions in the centromeric and tip regions. Similarly, Ajioka & Eanes (personal communication) have observed a greatly increased concentration of *P*-elements near the tip of the *X* chromosome in natural populations of *D. melanogaster*. These observations are consistent with the above model of generation of dominant lethal rearrangements by unequal exchanges (assuming that unequal exchange is correlated with normal meiotic crossing-over). We have also determined the abundance of several element families in laboratory stocks carrying the inversions *ClB* and *In(1)dl-49* (*X* chromosome) and *In(2L)(2R)Cy* (2nd chromosome), using the *in situ* technique described above. These inversions are all homozygous lethal and have therefore been maintained for many generations as permanent heterozygotes (Lindsley & Grell, 1968). Since there is a low but significant rate of exchange of alleles between inverted and standard sequences in inversion heterozygotes, due to double crossing-over and gene conversion (Ishii & Charlesworth, 1977), we have compared element abundances in the chromosome as a whole with copy numbers for regions located close to the breakpoints within the inversion, where exchange is virtually absent (Chovnick, 1973).

Table 4 shows the copy numbers for the *X* and second chromosomes in these stocks, together with the numbers of elements located within ± 1 salivary chromosome division (for chromosome 2) or ± 2 divisions (for the *X*) of the breakpoints. (Numbers on the *X* were too small for statistical analysis using only ± 1 divisions; numbers are larger for chromosome 2 because of the presence of two breakpoints on each arm in *In(2L)(2R)Cy*.) Since there are 20 divisions of each chromosome arm, one would expect 20% of *X* chromosome elements to be located within two divisions of a breakpoint, and 10% of 2nd chromosome elements to be located within one division of a breakpoint. The expectations and χ^2 tests in Table 4 under 'Test A' are calculated on this basis. For both the *X* and the 2nd chromosome, the observed number of elements within the regions around the breakpoints is greater than expected in all ten cases. In all but one of these comparisons the observed excess is statistically significant at the 0·05 level or less (see Table 4).

There is thus a significant tendency for elements to be present in excess of random expectation near the breakpoints of inversions in both the *X* and 2nd chromosome balancer stocks. This result could be artefactual, however, since the original inversion must have occurred in a single chromosome and hence have carried a unique complement of elements. The chromosome over which it was balanced would have an array of elements located in different sites from those occupied in the chromosome in which the inversion arose. Thus twice as many sites of hybridization might be expected in regions protected from exchange as in

regions where exchange is free (single chromatid hybridization sites can rarely be distinguished from homozygous sites in paired homologues). Since exchange is not completely free in inversion heterozygotes, even for points far from the breakpoints, a comparison of the numbers near the breakpoints with twice the random expectation provides a conservative test of the null hypothesis of no accumulation of insertions in regions of reduced exchange. The expected numbers of elements near the breakpoints on this basis are shown in Table 4 under 'Test B', together with the corresponding χ^2 values. Again there is an excess near the breakpoints of one or other of the *X* chromosome inversions for *412*, *297*, and *FB*, and clear evidence for *roo*. Similarly there is an excess for *297* and *FB* and *roo* near the breakpoints of *In(2L)(2R)Cy*. These results are consistent with a model of unequal exchange leading to the containment of copy number.

An alternative interpretation of these observations is that the process of random accumulation by genetic drift of deleterious mutations in genomic regions with restricted recombination, known as 'Muller's ratcher' (Felsenstein, 1974; Haigh, 1978), causes the preferential accumulation of transposable elements in such regions of reduced recombination. This has previously been proposed as an explanation of several natural situations in which middle repetitive DNA sequences have accumulated in genomic regions where crossing-over is restricted (Charlesworth, 1985*a*, *b*; Charlesworth *et al.* 1986). Qualitatively, the pattern of association of element abundance with rates of meiotic crossing-over predicted by Muller's ratchet will be similar to that predicted by the model of unequal recombination described above. The major difference between the two is that the unequal recombination model predicts an equilibrium copy number distribution and no differential with respect to numbers on the *X* chromosome, whereas the ratchet model predicts a continuous build-up of elements in regions of reduced crossing-over (probably with a higher rate on the autosomes, due to stronger selection against *X*-linked insertions). The abundance of repetitive DNA in systems such as the chromosome 1 heteromorphism of *Triturus cristatus* (Sims *et al.* 1984) and the neo-*Y* chromosome of *D. miranda* (Steinemann, 1982) are more readily interpreted in terms of the ratchet. Further modelling and experimental work are clearly needed if critical tests are to be applied.

Taken as a whole, the data on the distribution of elements between the *X* chromosome and autosomes indicate that selection against the mutational effects of individual insertions of transposable elements does not provide a general explanation for the stabilization of copy numbers in natural populations, although this hypothesis is consistent with our results for *412*. Other mechanisms, such as copy number-dependent transposition or excision rates or the induction of chromosome rearrangements by unequal crossing-over between elements, must therefore be considered.

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