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The distribution of transposable elements within and between chromosomes in a population of *Drosophila melanogaster*. III. Element abundances in heterochromatin

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Summary

The total genomic copy numbers of ten families of transposable elements of *Drosophila melanogaster* in a set of ten isogenic lines derived from a natural population were estimated by slot-blotting. The numbers of euchromatic copies of members of each family were determined for each line by *in situ* hybridization of element probes to polytene chromosomes. Heterochromatic numbers were estimated by subtraction of the euchromatic counts from the total numbers. There was considerable variation between element families and lines in heterochromatic abundances, and the variance between lines for many elements was much greater for the heterochromatin than for the euchromatin. The data are consistent with the view that much of the β-heterochromatin consists of sequences derived from transposable elements. They are also consistent with the hypothesis that similar evolutionary forces control element abundances in both the euchromatin and heterochromatin, although amplification of inert sequences derived from transposable elements may be in part responsible for their accumulation in heterochromatin.

1. Introduction

The nature of the evolutionary forces affecting transposable elements and other types of repeated DNA sequences is a major problem of evolutionary genetics. One approach to solving this problem is to examine the distribution of such sequences in various parts of the genome, which differ with respect to fundamental properties such as the rate of recombination. The results can then be compared with the expected influence of these properties on the evolutionary dynamics of the sequences in question, under different assumptions about the forces influencing these dynamics. Conversely, understanding of the dynamics of repeated sequences may shed light on such long-debated questions as the significance of heterochromatin (Charlesworth *et al.* 1994).

There is a good deal of evidence that *Drosophila* transposable elements (TEs) are disproportionately abundant in the proximal euchromatin. This evidence comes primarily from the use of *in situ* hybridization of labelled TE probes to localize the positions of elements on the polytene maps of chromosomes sampled from natural populations or laboratory stocks (Ananiev *et al.* 1984; Charlesworth & Langley, 1989; Charlesworth *et al.* 1992*b*). Charlesworth *et al.* (1992*b*) estimated that the proximal euchromatin of the major autosomes (defined roughly as the three most proximal polytene chromosome divisions) contains about 60% more elements belonging to nine families, than is expected if elements are distributed randomly within the euchromatin. A similar overabundance relative to the other autosomes is found for the entire fourth chromosome. The proximal region of the X shows an even larger excess abundance (about 160% of expectation). The proximal euchromatin of the major chromosome arms shows strong suppression of meiotic crossing over, and the fourth chromosome completely lacks it (Ashburner, 1989, chap. 11).

Detailed analyses suggest that the patterns of distribution of elements between and among chromosome arms are mostly consistent with the hypothesis that element abundance is in part regulated by a balance between transposition and selection against deleterious chromosome rearrangements created by ectopic exchange between homologous TEs located at different sites in the genome (Montgomery *et al.* 1991; Charlesworth *et al.* 1992*b*). The frequency of such exchanges is likely to be lower in regions, such as the

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proximal euchromatin, in which meiotic exchange is reduced in frequency, so that such regions should experience a lower rate of elimination of elements. This hypothesis may also explain the build-up of TEs on the non-recombining neo-Y chromosome of *D. miranda* (Ganguly *et al.* 1992; Steinemann & Steinemann, 1992), and on low-frequency polymorphic inversions of *D. melanogaster* (Eanes *et al.* 1992; Sniegowski & Charlesworth, 1994). Currently, the only feature of the distribution of TEs that is hard to explain on the ectopic exchange hypothesis is the lack of accumulation of elements at the distal tip of the X chromosome (Charlesworth *et al.* 1992*b*), where meiotic exchange is strongly suppressed (Ashburner, 1989, chap. 11; Sniegowski *et al.* 1994). With this one exception, there is a strong correlation between element abundance and low frequencies of meiotic recombination within the euchromatin of *D. melanogaster*. Processes other than ectopic exchange, such as Muller's ratchet, may also play a role in the accumulation of elements in some of these cases, especially when recombination is completely suppressed, as with neo-Y chromosomes (Charlesworth *et al.* 1994).

The centric heterochromatin of *Drosophila* is essentially inert as far as meiotic recombinational exchange is concerned (Schalet & Lefevre, 1976; Szauter, 1984). Hence, any process that favours the accumulation of elements in regions of the genome with restricted recombination should also cause a build-up in the heterochromatin, provided that element insertions can occur in heterochromatin. In addition, the relative dearth of expressed genes in much of the heterochromatin (Hilliker *et al.* 1980; Pimpinelli *et al.* 1986; Miklos & Cotsell, 1990) may also mean that the spread of elements into heterochromatin is not as strongly opposed by selection against deleterious insertional mutations as in the euchromatin, further contributing to a build-up of elements in heterochromatin (Charlesworth, 1991). Since heterochromatin cannot be resolved into well-defined bands in polytene chromosome preparations (Heitz, 1934; Miklos & Cotsell, 1990), counts of element insertions from *in situ* hybridization preparations cannot be used to ask whether this is indeed the case.

Nevertheless, a variety of methods have provided evidence for an excess of elements in the heterochromatin. Intense signal for TE probes is often detected in the neighbourhood of the chromocentre in preparations of polytene chromosomes (Young, 1979; Finnegan & Fawcett, 1986; Montchamp-Moreau *et al.* 1993), despite the lack of clear banding in this heterochromatic region. Molecular characterization of clones derived from the β-heterochromatin (the section of the proximal heterochromatin that adjoins the euchromatin, and which is visible in polytene chromosome preparations) has revealed a high density of TEs or TE-derived sequences in these regions (Kidd & Glover, 1981; Roiha *et al.* 1981; Miklos *et al.* 1984, 1988; Vaury *et al.* 1989; Devlin, Bingham & Wakimoto, 1990; Nurminsky *et al.* 1994). One family of *D. melanogaster* TEs, *HeT*, is found exclusively in telomeric regions, proximal heterochromatin, and on the Y chromosome (Valgeirsdottir *et al.* 1990; Danilevskaya *et al.* 1993). Another family, *TART*, has recently been shown to be confined to the telomeric regions of *D. melanogaster* chromosomes (Levis *et al.* 1993).

Although these studies mostly concur in suggesting that elements are more abundant in the heterochromatin than would be expected from the fact that it represents only about one-third of the genome of *D. melanogaster* (Miklos & Cotsell, 1990), there have been relatively few quantitative studies of the extent of this over-representation. Estimates of copy numbers from reassociation kinetics for such TEs as *copia*, *412* and *297* in the whole genome for a single wild-type laboratory stock, and comparison of these estimates with the euchromatic counts obtained from *in situ* hybridization, failed to indicate a disproportionate representation of these elements in the non-euchromatic section of the genome (Potter *et al.* 1979; Strobel *et al.* 1979). No quantitative studies have been done using material from natural populations comparable to that used in the *in situ* hybridization surveys mentioned above, apart from a study of the 60-kb SCLR component of the X-chromosomal heterochromatin (Nurminsky *et al.* 1994). There is essentially no information on the extent of variation between genotypes in the abundance of elements in heterochromatin.

For this reason, it seemed worthwhile to re-investigate this question. A set of isogenic lines of *D. melanogaster*, constructed from chromosomes isolated from natural populations, was used as a source of independent genotypes representative of natural variation. In this paper, we report the results of estimating total genomic numbers of TEs in each line by slot-blotting (Brown *et al.* 1983; Lyckegaard & Clark, 1991). These estimates were partitioned into heterochromatic and euchromatic numbers by deducting from the total genomic numbers the euchromatic numbers obtained from *in situ* preparations of larvae from the same lines. Our data suggest that many families of *Drosophila* TEs are indeed over-represented in heterochromatin, although there is a high variance in abundance between elements and genotypes. If elements accumulate primarily in the β-heterochromatin adjacent to the euchromatin, as proposed by Miklos & Cotsell (1990), our results imply that a very high density of elements is present in this region of the genome.

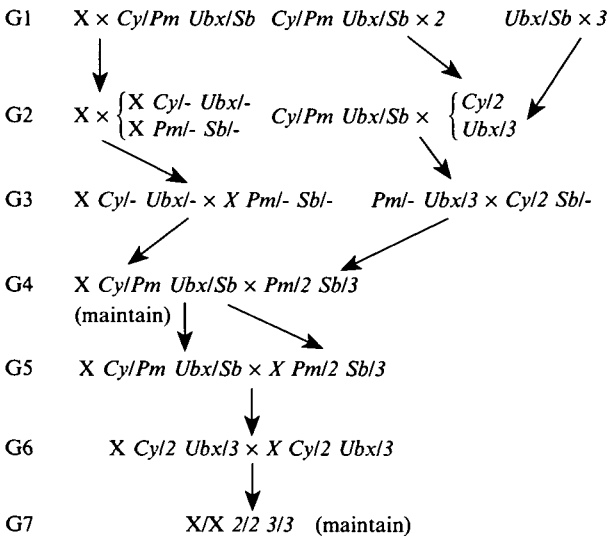

Fig. 1. The breeding programme used to produce the isogenic lines. Wild-derived *X*, second, and third chromosomes are denoted by *X*, *2* and *3*. The gene symbols used are explained in the text. The fourth chromosome in all the stocks was homozygous for the recessive marker spa^{pol}.

2. Materials and methods

(i) *Genetic stocks and breeding procedures*

In order to determine the relation between heterochromatin and TE abundance for chromosomes representative of a natural population, it was necessary to construct a set of stocks that are isogenic for sets of the major chromosomes (the X chromosome and autosomes 2 and 3), isolated originally from a natural population. These stocks provide the material for the determination of element copy numbers described below. Sets of homozygous viable and fertile X, second and third chromosomes were isolated from a population at Beltsville, Maryland, as described by Charlesworth & Lapid (1989) and Charlesworth, Lapid & Canada (1992*a*). The extracted chromosomes were placed singly on a background homozygous for the fourth chromosome recessive marker spa^{pol}, as a precaution against contamination. Many of the resulting stocks were used in the experiments described by Charlesworth & Lapid (1989) and Charlesworth *et al.* (1992*a*, *b*). The Y chromosome of each isogenic stock came from a different initial single wild male from the Beltsville population.

In order to construct the isogenic stocks, spa^{pol} was crossed into balancer stocks containing the second chromosome balancer *SM1* (marked with *Cy*), and the third chromosome balancer *TM6* (marked with *Ubx*). These balancers are described by Lindsley & Zimm (1992). The balancer chromosomes were maintained over the markers *Pm* and *Sb* respectively, on a background of X chromosomes derived from the *IV* random-bred stock described by Charlesworth & Charlesworth (1985). A double balancer stock *IV*; *Cy/Pm*; *Ubx/Sb*; spa^{pol}/spa^{pol} was constructed by intercrossing the two single-balancer stocks. Each balancer stock has the cytotype *P/R* with respect to the *P* and *I* families of transposable elements associated with hybrid dysgenesis, and will therefore not cause mobilization of these elements when crossed to wild-caught males from the USA, which are invariably of this cytotype (Engels, 1989).

The isogenic stocks were constructed using the breeding procedure shown in Fig. 1. All chromosomes of the final constructs, other than the fourth, should be isogenic for chromosomes from wild-caught males, each stock containing independent sets of chromosomes. The 15 stocks originally constructed were subject to a rigorous program of brother–sister mating for over a year, in order to force homozygosity for loci that the balancers failed to handle. Given the possibility that exchange of genetic material between the balancers and the wild-type chromosomes may occur, and the possibility of cross-contamination between lines with similar marker phenotypes, it was essential to verify the genetic status of these lines for the major chromosomes before embarking on the experiments. This was done primarily by examining the salivary gland chromosome locations of transposable elements (see below) for at least two replicate larvae from each stock (see Sections 2(ii) and 3(i)). Prior to this the lines were checked for their karyotype by salivary chromosome squashes of at least eight larvae from each, in order to detect any gross rearrangements introduced by crossing over with the balancer chromosomes. Several lines were found to be segregating for inversions, and were discarded. Eleven stocks survived this screening procedure. Ten of these were used in the experiments described below. One of these (line 10) proved very difficult to use for *in situ* work, and another (line 8) provided only very low yields of DNA for slot-blotting. Accordingly, there are only eight lines for which both *in situ* and slot-blot estimates of copy numbers are available. Line 8 gave poor results for the *in situ* studies of probe *cDm2156*, and was accordingly not used for this probe in the results reported below.

(ii) *Preparation and scoring of* in situ *slides*

Procedures for preparing and scoring slides for *in situ* hybridization of clones of transposable elements to polytene salivary gland chromosomes were as described by Charlesworth & Lapid (1989) and Charlesworth *et al.* (1992*a*). The set of ten TE families studied by Charlesworth & Lapid (1989) were used in the experiments (see (vi) below). The locations of members of each family on the chromosomes of each isogenic line were determined on the Lefevre (1976) photographic map, as described by Charlesworth *et al.* (1992*a*). All sites distal to and including 20A on the X, 40B on *2L*, 41A on *2R*, 80C2 on *3L*, and 81F on

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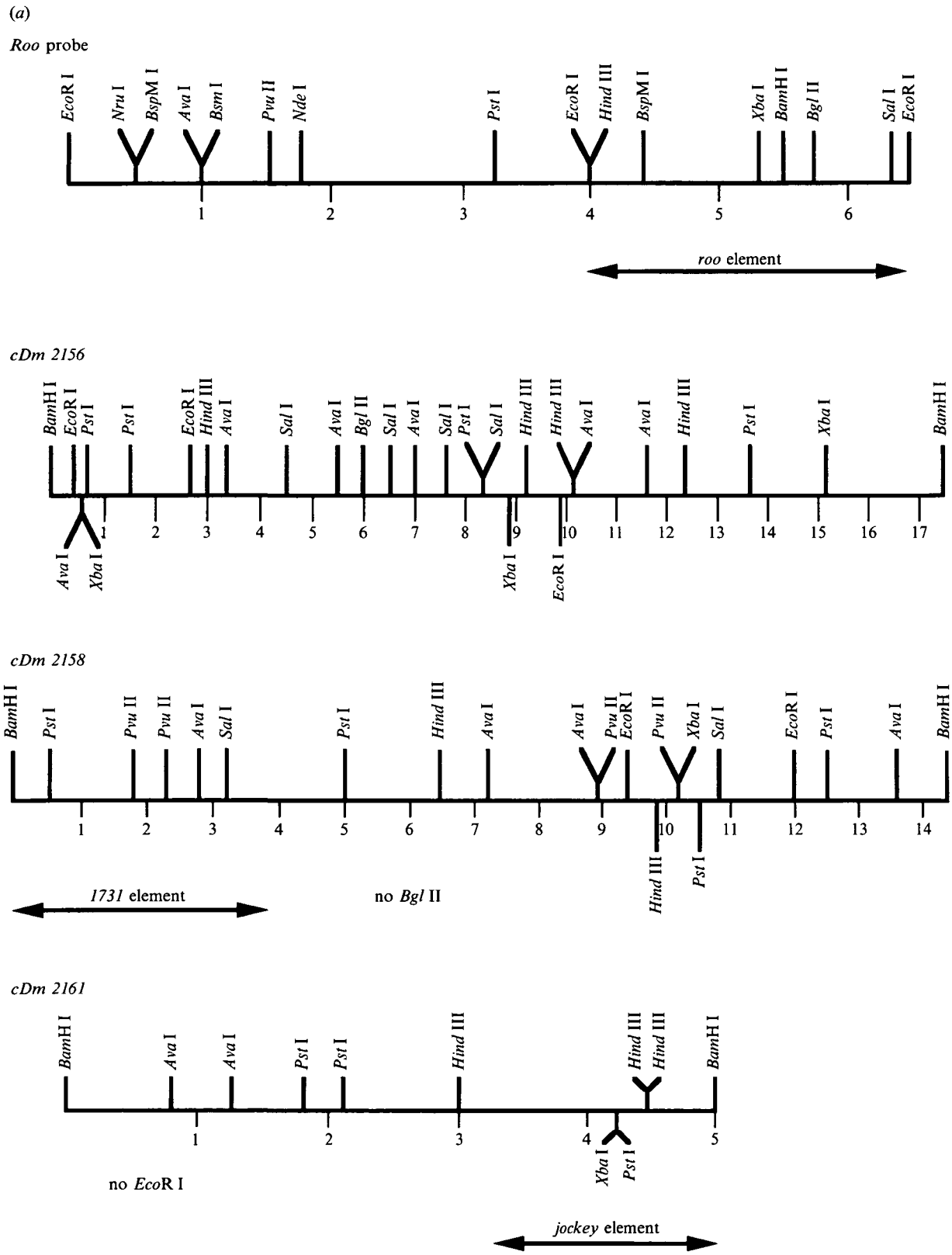

Fig. 2*a–c*. (*a*) Restriction maps of the clones of genomic DNA from *Drosophila melanogaster* used as probes for transposable elements.

3R were scored, and are treated as euchromatic in the analyses described below.

At least two replicate slides were read for each isogenic line. In all but two cases, both involving *copia*, there was consistency between the readings for each line. Examination of further slides for these lines (2 and 6 in the coding used in Section 3) suggested that *copia* had undergone several mobilization events since

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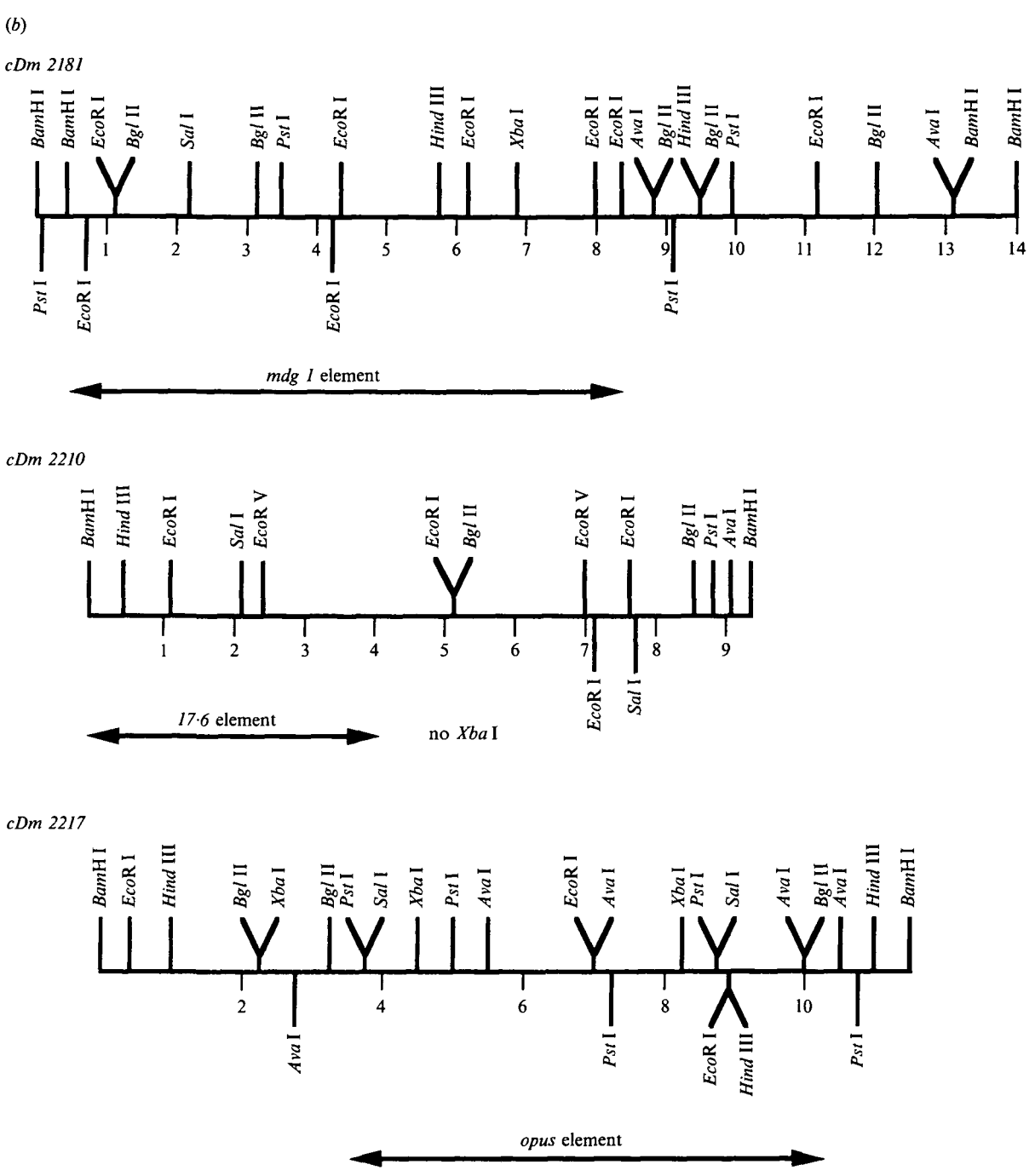

Fig. 2(*b*). For legend see p. 186.

the construction of the lines, and so the lines were not used in the subsequent analyses of *copia*. The copy numbers of elements in the euchromatic portions of the major chromosomes were calculated for each element and each line (with the exceptions noted above). There are six sites at which elements are apparently fixed in the distal sections of sampled chromosomes (*copia* is fixed at 5A/B on the X chromosome, and the element in probe *cDm2210* (*17·6*) is fixed at 30D1/2 on *2L*; fixation sites for the elements in probes *cDm2156*, *cDm2158*, *cDm2181* and *cDm4006* are described by Charlesworth *et al.* (1992*a*)). Charlesworth *et al.* (1992*a*) argued that such cases of fixation are likely to be due to hybridization of flanking sequences in the probes with sites from which the elements were originally cloned, and should be omitted when estimating copy numbers. This is known to be the case for our *copia* probe, *cDm5002* (Dunsmuir *et al.* 1980). For the sake of caution, the fixation sites for all of these elements have been omitted from the euchromatic counts reported below. Inclusion of these sites would in any case make no significant difference to our conclusions.

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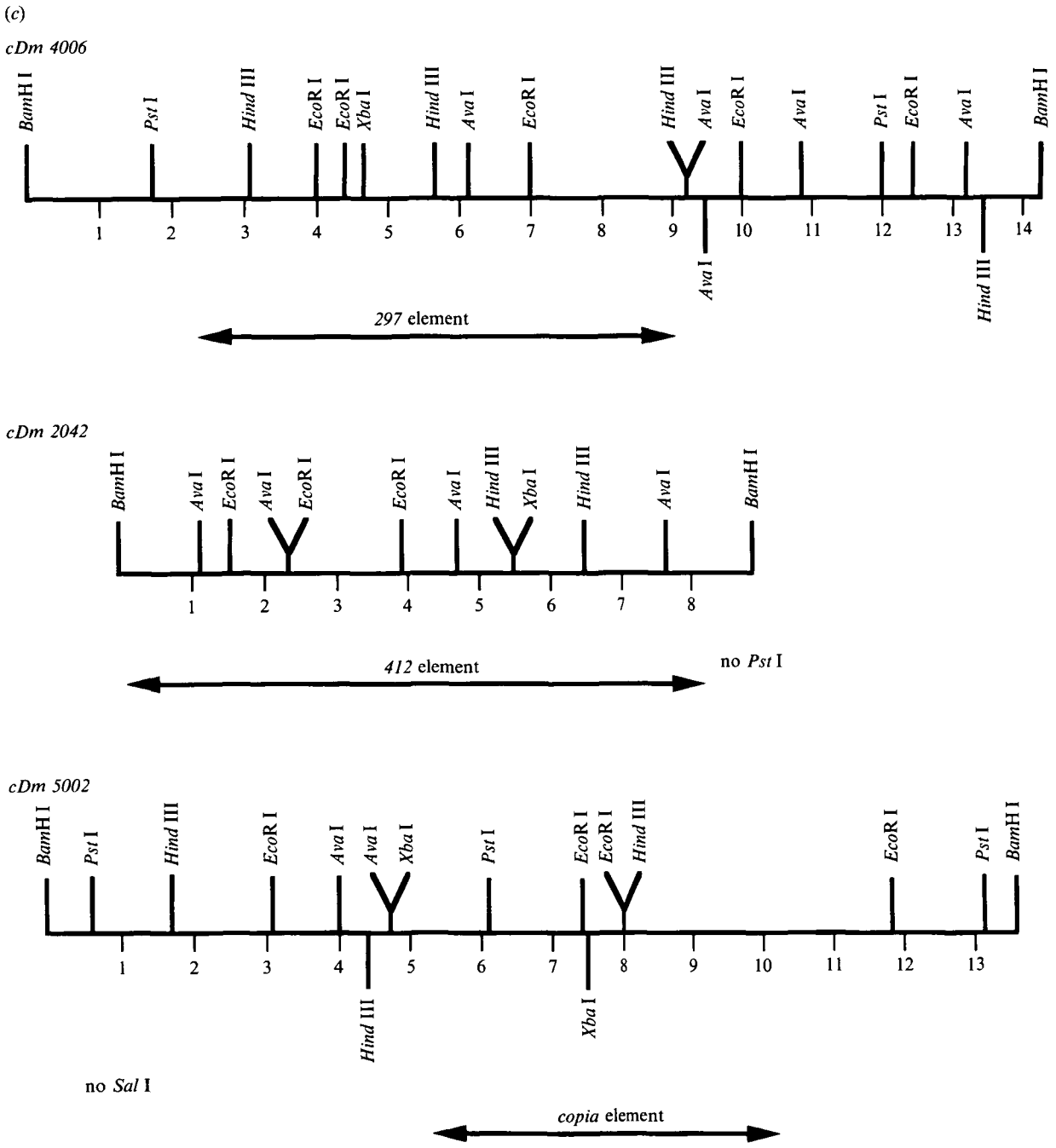

Fig. 2(c). For legend see p. 186.

(iii) *DNA extraction from embryos*

For each of the isogenic lines, several thousand embryos were collected following the method of Wieschaus & Nüsslein-Volhard (1986) and dechorionated in a 5% solution of sodium hypochlorite for one minute (Jowett, 1986). Embryos were used in order to avoid artefactual estimates of copy numbers from polytenised tissue, in which heterochromatin is underreplicated (Ashburner, 1989, chap. 5). Dechorionated embryos were then stored at −80 °C. For DNA extraction, embryo samples were quickly thawed, ground in 1 ml of a solution consisting of 100 mM-Tris-HCl (pH 8), 50 mM-NaCl, 50 mM-EDTA, 1% SDS, 0·15 mM spermine and 0·5 mM spermidine. They were then incubated for 1 h at 50 °C after adding proteinase K to a final concentration of 100 μg/ml. This was followed by an extraction with phenol, phenol/chloroform, and chloroform, and DNA was precipitated with cold ethanol (Sambrook *et al.* 1989). RNA was removed by RNAase digestion (Jowett, 1986).

(iv) *DNA slot-blotting*

A Bio-Dot SF slot-blot apparatus was used to transfer DNA onto Zeta-Probe nylon membranes (Bio-Rad).

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Table 1. *Lengths of inserts used in the experiments and lengths of material derived from TEs*

Probe	Length of insert (kilobases)	Length of TE-derived material
roo (*roo*)	2·3	2·3
cDm2156	17·2	Unknown
cDm2158 (*1731*)	14·3	4·0
cDm2161 (*jockey*)	11·6	1·7
cDm2181 (*mdg1*)	14·4	7·7
cDm2210 (*17·6*)	9·3	4·1
cDm2217 (*opus*)	11·6	6·7
cDm5002 (*copia*)	13·7	5·3
cDm4006 (*297*)	14·2	6·7
cDm2042 (*412*)	8·7	7·8

The names in brackets after the probe designations are the elements identified as being present in the genomic DNA inserts (see Lindsley & Zimm (1992, Chapter by D. J. Finnegan) for details of the elements).

Prior to transfer, the DNA samples were denatured in 0·4 M-NaOH at 100 °C for 10 min and then neutralized by addition of 2 M-NH_4OAc (pH 7). The following types of sample were loaded onto a given membrane: an independently-derived replicate of the genomic DNA of each isogenic line, a dilution series of genomic DNA from one isogenic line, a dilution series of a subset of the TE probes, and a dilution series of a probe consisting of a 4·75-kb *Eco*R 1 fragment of the *Adh* gene of *D. melanogaster*, cloned into *pUC13* (Lyckegaard & Clark, 1991). The other probes used contained a *D. melanogaster* TE cloned into a *pBR322* or derivative plasmid (Montgomery *et al.* 1987; Charlesworth & Lapid, 1989). One sample without DNA was also loaded as a control. The *Adh* probe served as a single-copy control for quantifying the total amount of DNA bound to the membranes. The denatured samples were applied in a randomized block among the 48 slots of the apparatus. Each membrane was replicated using a different randomized block. Samples were loaded and vacuum was applied until the wells were just dry. The wells were rinsed by adding 0·4 ml of 0·4 M-NaOH in each, and vacuum was applied until all wells were quite dry. The membranes were then rinsed in 2 × SSC (Sambrook *et al.* 1989). DNA was cross-linked to the membranes using a UVC 1000 ultraviolet crosslinker (Hoefer) according to the instructions of the manufacturer. Membranes were then air-dried for 10 min prior to hybridization.

Membranes were hybridized using a modification of Lyckegaard & Clark's (1991) protocol. About 0·5 μg of probe DNA was labelled for each element hybridized, which should ensure an excess of labelled probe DNA relative to homologous sites in the genomic DNA. Hybridizations were performed in a hybridization cassette (Bios). The membranes were prehybridized at 65 °C for 20 min in 5 ml of a pre-warmed solution of 1% bovine serum albumin, 1 mM-EDTA, 7% SDS, and 0·5 M sodium phosphate (pH 7·2). The prehybridization solution was removed and replaced with 2 ml of the same solution and the denatured probe. After 18 h, the hybridization step was stopped. The membranes were briefly washed at room temperature in 2 × SSC, 0·1% SDS in the cassette and then washed at 65 °C for 25 min each in 2 × SSC, 0·1% SDS, then in 0·5 × SSC, 0·1% SDS, and finally in 0·1 × SSC, 0·1% SDS. The radioactivity was directly measured on the moist membranes enclosed in a sealed plastic bag using a counter of beta emission (Betascope 603, Betagen). Prior to new hybridization, the previously hybridized probe was stripped off by washing the membranes in 0·4 M-NaOH for 40 min at 65 °C, and then neutralizing with 0·1 × SSC, 0·5% SDS, 0·2 M-tris-HCl, pH 7·5 for 40 min at 65 °C. Correct removal of the labelled probe was checked by an overnight radiographic exposure. Membranes were subsequently hybridized with a different probe as above. Prior to hybridization, all probes were labelled with α^{32}P dATP by nick-translation using a BRL kit.

(v) *Calculation of TE copy numbers*

The copy number for each TE was estimated as follows. We calculated the regression coefficient of the values obtained with the betascope for the dilution series of genomic DNA for a given membrane on the values obtained for the dilution series of a TE-containing probe for the same membrane. This reflects the fraction of the genome represented by the cloned TE for the isogenic line corresponding to the dilution series (Lyckegaard & Clark, 1991). This fraction is converted to a copy number by multiplying by the size of the *D. melanogaster* genome (170000 kb), and dividing by the length of the cloned TE. The copy numbers for the other isogenic lines on the membranes are obtained by calibrating their signal against the signal for the same concentration of DNA in the genomic DNA dilution series. Estimates of error variation in the copy numbers of TEs for each isogenic line can be obtained from the fact that each line is replicated on each membrane, and two membranes are used for each estimation.

(vi) *Probes used in the experiments*

Except for the probe containing *roo* (which has no identifying number), all the probes used contained middle repetitive genomic sequences of *D. melanogaster* cloned into the *Bam H1* site of a standard *pBR322* plasmid in the laboratory of Gerald Rubin (*cDm* clones: Rubin *et al.* 1981; Strobel, 1982; Montgomery *et al.* 1987; Charlesworth & Lapid, 1989). The probe for *roo* contains a partial *roo* sequence cloned into the *Eco*R I site of a deletion derivative of *pBR322*. This probe was supplied to us

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Table 2. *Estimated numbers of copies of elements in the euchromatin, heterochromatin, and whole genome*

	Copy number estimates											
Line	1	2	3	4	5	6	7	8	9	10	Mean	Variance
roo												
Euc.	61	55	75	75	55	64	59	68	61	—	63·7	57·8
											(2·5)	(25·8)
Hc.	191	100	499	316	447	174	378	—	583	—	341	23089**
											(34)	(14130)
Total	252	155	574	391	502	238	437	—	644	120	377	27610**
	(41)	(35)	(35)	(35)	(35)	(35)	(35)	—	(35)	(35)	(33)	(14789)
2156												
Euc.	20	10	10	15	26	11	10	—	20	—	15·2	37·4
											(2·2)	(7·1)
Hc.	21	24	59	30	33	27	36	—	30	—	32·8	50·4
											(2·7)	(76)
Total	41	34	69	45	59	38	46	—	50	32	46·0	56·0
	(7·2)	(7·2)	(7·2)	(7·2)	(7·2)	(7·2)	(7·2)	—	(7·2)	(7·2)	(2·5)	(73)
1731												
Euc.	14	8	9	8	9	9	10	12	15	—	10·4	6·8
											(0·9)	(1·1)
Hc.	27	26	43	36	43	31	29	—	29	—	32·8	−0·3
											(2·0)	(31)
Total	41	34	52	44	52	40	39	—	44	32	42·0	5·99
	(5·5)	(5·5)	(5·5)	(5·5)	(5·5)	(5·5)	(5·5)	—	(5·5)	(5·5)	(1·8)	(28)
jockey												
Euc.	36	37	37	36	32	38	36	34	40	—	36·2	5·2
											(0·8)	(0·9)
Hc.	97	62	187	99	148	61	138	—	95	—	106	555
											(10)	(873)
Total	133	99	224	135	180	99	174	—	135	87	136	737
	(24)	(24)	(24)	(24)	(24)	(24)	(24)	—	(24)	(24)	(9·4)	(831)
mdg1												
Euc.	14	20	23	15	14	16	11	22	23	—	17·6	20·3
											(1·5)	(3·4)
Hc.	15	7	9	17	27	8	20	—	3	—	13·5	54·8**
											(1·8)	(32)
Total	29	27	32	32	41	24	31	—	26	20	28·9	27·2*
	(3·1)	(3·1)	(4·4)	(3·1)	(3·1)	(3·1)	(3·1)	—	(3·1)	(3·1)	(1·4)	(18)
17·6												
Euc.	15	10	8	10	17	9	16	10	10	—	12·5	12·3
											(1·1)	(1·8)
Hc.	21	26	54	34	14	23	31	—	40	—	34·3	104*
											(2·5)	(62)
Total	36	36	62	44	61	32	47	—	50	33	44·7	109***
	(4·8)	(4·8)	(4·8)	(4·8)	(4·8)	(4·8)	(4·8)	—	(4·8)	(4·8)	(2·3)	(59)
opus												
Euc.	12	12	17	13	11	18	23	15	18	—	15·4	15·3
											(1·3)	(2·6)
Hc.	26	21	47	30	38	10	24	—	28	—	29·2	32·1
											(1·8)	(25)
Total	38	33	64	43	49	29	47	—	46	27	40·4	90·8**
	(3·9)	(3·9)	(5·5)	(3·9)	(3·9)	(3·9)	(3·9)	—	(3·9)	(3·9)	(2·1)	(50)
297												
Euc.	22	20	21	20	19	18	28	21	35	—	22·7	29·5
											(1·8)	(4·9)
Hc.	19	9	61	23	57	12	31	—	19	—	29·0	378***
											(3·7)	(194)
Total	41	29	82	43	76	30	59	—	54	32	49·4	343**
	(4·2)	(4·2)	(4·2)	(4·2)	(4·2)	(4·2)	(4·2)	—	(4·2)	(4·2)	(3·4)	(173)
412												
Euc.	26	25	27	24	25	18	18	27	38	—	25·3	34·5
											(2·0)	(5·8)
Hc.	10	−7	70	9	45	13	67	—	18	—	28·2	583
											(8·1)	(410)
Total	36	18	97	33	70	31	85	—	56	10	48·5	696*
	(15)	(15)	(15)	(15)	(15)	—	(15)	—	(15)	(15)	(7·8)	(423)

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Table 2. (*cont.*)

Line	Copy number estimates 1	2	3	4	5	6	7	8	9	10	Mean	Variance
copia												
Euc.	11	—	18	12	14	—	15	18	14	—	14·6 (1·0)	7·3 (1·6)
Hc.	29	—	72	37	59	—	30	—	33	—	44·0 (4·4)	240* (179)
Total	40 (7·4)	32 (6·4)	90 (6·4)	49 (6·4)	73 (6·4)	38 (6·4)	45 (6·4)	—	47 (6·4)	26 (6·4)	49·2 (3·9)	353*** (192)

Euc. indicates the copy number of a line for the euchromatin, derived from *in situ* hybridization.
Hc. indicates the mean value of the estimated copy number of a line for the heterochromatin, derived by subtracting the euchromatin value from the estimate of the total number of elements.
The bracketed terms are the standard errors of the estimates. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

by Walter Eanes. Restriction maps of each of the TE probes were constructed, in order to determine the sizes of the inserts containing DNA from the elements. Standard enzymes and procedures were used. The restriction maps are shown in Fig. 2.

In addition, the restriction maps were used to determine the nature of the inserts into the probes for all except *cDm2156*. The identifies of *copia*, *297* and *412* had been established earlier (Rubin *et al.* 1981). The restriction maps of the other probes were compared with the published maps of *Drosophila* TEs, and with maps deduced from available element sequences in Genbank. The identities of the elements and the estimated lengths of element-derived sequences are shown in Table 1 and Fig. 2. Since the exact positions of the termini of the elements cannot be established from the restriction maps, the lengths shown are slight overestimates of the element insertion sizes, in order to provide conservative estimates for the abundance of elements in the heterochromatin (see below). For *cDm2156*, where we have been unable to establish the identity of the element, we assumed an insert size of 8 kb, which is towards the upper limit for *Drosophila* TEs (Lindsley & Zimm, 1992). Our copy numbers for this element should therefore be underestimates.

In the case of *cDm2161*, partial sequencing of naturally occurring 3′ inserts into the *Adh* locus, which show homology with *2161* on Southern blots, reveals sequence homology with *jockey* (C. F. Aquadro, pers. comm.), confirming the restriction map results. The numbers of inserts homologous to *2161* determined by *in situ* hybridization (a mean of 36 per haploid genome) are consistent with *jockey* being the only element contained in the probe. The sites of *in situ* hybridization of *cDm2181* have been found to correspond with those of *mdg1* (S. V. Nuzhdin, pers. comm.), in accordance with the restriction map identification. This gives confidence in the identifications of the remaining elements (*1731* in *cDm2158*, *17·6* in *cDm2217* and *opus* in *cDm2217*). All of the elements are retroviral-like TEs with long terminal repeats, with the exception of *jockey*, which is a retroviral-like TE with no LTR's (Lindsley & Zimm, 1992).

3. Results

(i) *Means and variances of copy numbers*

Table 2 shows the copy numbers of each line for euchromatic sites derived by *in situ* hybridization. In addition, the estimates of mean copy numbers per line over membranes and replicates within membranes from slot-blotting are shown, together with their standard errors derived from the analyses of variance described below. These provide the estimated total numbers for each line. Estimates of the number of elements in the heterochromatin are obtained by subtraction of the euchromatin counts. As can be seen from the table, in most cases little confidence can be placed on the slot-blot values of the copy number of an element family for a given line. This is due to the considerable error of estimation of copy number, revealed by the variances between replicates and between membranes.

Table 2 also shows the mean copy number and its standard error for each element for the euchromatin, heterochromatin, and whole genome. In addition, the variances between lines are shown. For heterochromatin and the whole genome, the variances were obtained from the between-line variance components derived from hierarchical analyses of variance, which included between-line effects, effects of membranes within lines, and replicates between membranes. The significance levels for between-line effects are indicated. (Although some of the ANOVA's are slightly unbalanced, due to missing data, the effects on expected mean squares are small, so that the significance tests are nearly unbiased.) The standard errors

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Table 3. *Abundances of elements in euchromatin* vs *heterochromatin*

	Mean deviations of euchromatic numbers from expected			Ratios of heterochromatin TE density to euchromatin TE density		
Element	1	2	3	1	2	3
roo	−202**	−293***	−313***	10	45	95
	(40)	(54)	(57)	(1·8)	(8·1)	(17·1)
2156	−16·7***	−27·1***	−30·1***	5·1	23	48
	(3·1)	(3·8)	(4·0)	(1·1)	(5·0)	(11)
1731	−18·6***	−28·6***	−30·7***	7·0	31	65
	(1·8)	(2·2)	(2·4)	(0·8)	(3·7)	(7·9)
jockey	−62·2***	−96·0***	−103***	6·2	28	59
	(10)	(14)	(15)	(0·9)	(4·1)	(8·7)
mdg1	−3·29	−10·2**	−11·8**	1·9	8·3	17
	(2·3)	(2·7)	(2·8)	(0·5)	(2·2)	(4·7)
17·6	−19·0***	−29·6***	−32·0***	6·4	29	60
	(2·8)	(3·6)	(3·8)	(1·2)	(5·3)	(11)
opus	−13·7**	−23·7***	−25·9***	4·0	18	37
	(2·7)	(3·5)	(3·7)	(0·7)	(2·9)	(6·2)
297	−11·7*	−23·6**	−26·1**	2·7	12	25
	(4·8)	(6·3)	(6·7)	(0·7)	(3·3)	(6·9)
412	−10·6	−22·9*	−25·6*	2·5	12	24
	(7·0)	(9·2)	(9·7)	(1·0)	(4·4)	(9·2)
copia	−24·4**	−37·6**	−40·5**	6·2	27	58
	(4·7)	(6·5)	(6.9)	(0·7)	(3·3)	(7·0)

of the between-line variance components derived from the ANOVA's are also shown; they are so high that little reliance can be placed on the estimates of individual variance components. In several cases, there was evidence for statistically significant variances in heterochromatic copy numbers between lines (*roo*, *mdg1* and *297*). In most cases, the estimated variances for heterochromatic copy numbers and total copy numbers are much higher than the means, suggesting that there are deviations from a Poisson distribution of copy number between lines for the heterochromatic elements.

Membrane effects significant at the $P < 0{\cdot}01$ level were detected for *roo* and *jockey*, one membrane gave meaningless results for *412*, and so no information on variation between membranes is available for this element. The significance of the ratios of variance to mean for the euchromatic counts for each element were compared by the Poisson index of dispersion test (Hoel, 1962, pp. 255–259); the ratio for *jockey* was significantly smaller than expected on the null hypothesis of a Poisson distribution of copy number between lines ($P < 0{\cdot}01$). *2156* shows an ostensibly significant deviation for euchromatin copy number from Poisson expectation ($\chi^2 = 17{\cdot}2, P < 0{\cdot}05$), but the large number of tests performed means that this is probably a result of sampling error. In the other cases, there was no evidence of departure from Poisson. The result for *jockey* is surprising, in view of the fact that previous studies of individual chromosome arms yielded no evidence for departure from Poisson (Charlesworth & Lapid, 1989; Charlesworth *et al.* 1992*a*). We have no explanation for this.

(ii) *Extent of accumulation of elements in the heterochromatin*

The data were also tested for the extent of accumulation of elements in the heterochromatin compared with the euchromatin. Heterochromatin in *Drosophila* can be divided into two main categories, α-heterochromatin and β-heterochromatin (Heitz, 1934; Miklos & Cotsell, 1990). The α-heterochromatin forms the centromere-proximal regions which are cytologically distinct in mitotic preparations, but are not visible in the polytene chromosomes (this includes the Y chromosome). It consists largely of long tandem arrays of satellite-DNA sequences, and appears to be relatively free of TE-derived sequences (Lohe *et al.* 1993). About 23% of the genome of *D. melanogaster* is made up of satellite sequences and rRNA genes (Lohe & Brutlag, 1986; Lohe *et al.* 1993). The more distal β-heterochromatin corresponds to the diffusely staining material of the most proximal division of each of the polytene chromosomes, and is not visible as a distinct entity in mitotic preparations. It contains transcribed genes, and appears to be enriched for TE-derived sequences (Miklos & Cotsell, 1990). Approximately 33% of the genome is conventionally estimated to consist of heterochromatin, if males and females are averaged (Montgomery *et al.* 1987). There is uncertainty about the size of the β-heterochromatin relative to the euchromatin, but the above figures suggest that it must represent at most about 10% of the total genome. From the lengths of the proximal divisions of the polytene chromosomes compared with the total length of these chromosomes (Lefevre,

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1976), it may constitute only 5% of the total DNA that is not contained in the α-heterochromatin. As a rough guide to calculation, we can use 5% and 10% as lower and upper bounds to the fraction of the non-α-heterochromatic genome which is represented by β-heterochromatin.

Two different questions can therefore be asked about the relative abundances of elements in different components of the genome: (a) is there any evidence for a differential accumulation of elements in the heterochromatin as a whole, as opposed to the euchromatin? (b) Is there a differential accumulation of elements in the β-heterochromatin, assuming that the α-heterochromatin is nearly element-free? The first question can be asked by comparing element abundances in the euchromatin with the expectation that it contains 67% of the elements; the second, by using expectations of 90% or 95%.

The results of such analyses are shown in Table 3. The left-hand columns labelled 1, 2 and 3 refer to expectations of concentrations of elements in the euchromatin under the respective null hypotheses that it contains 67%, 90%, and 95% of the total numbers of elements. The tests of significance of the null hypotheses for each element are performed by computing the deviation of the *in situ* count for each line from the product of the expected proportions and the mean total number of copies of the element per line from the slot blot data. The means and standard errors of these deviations over lines are shown in the Table, together with the significance levels from the corresponding *t*-tests against the null hypothesis of a zero mean. The right-hand columns labelled 1, 2 and 3 give the means and standard errors over lines for the ratios of the densities of the element in the euchromatin to the density in the respective parts of the heterochromatin, obtained by dividing the observed numbers in the heterochromatin by the proportions 0·33, 0·10 or 0·05, respectively, and taking the ratio of this to the *in situ* count divided by 0·67, 0·90 or 0·95.

Except for *412*, there is firm evidence that there are more elements in the heterochromatin than expected on the hypotheses that 5% or 10% of elements are located there. Except for *mdg1*, *297*, and *412*, there is also firm evidence for deviations from the hypothesis that 33% of elements are found in heterochromatin. On this model, estimates of the ratio of the density of elements in the heterochromatin to the density in the euchromatin range from 10 (*roo*) to 1·9 (*mdg1*). On the extreme alternative model, that 5% of the genome is available for TE insertions, the ratio ranges from 95 to 17.

4. Discussion

The results described above show that most of the element families we have studied show an excess mean abundance in the heterochromatin over what would be expected if they were partitioned randomly between euchromatin and heterochromatin. The quantitative estimate of their over-representation depends critically on whether the division of *Drosophila* heterochromatin into α-heterochromatin (assumed to be free of TEs) and β-heterochromatin (which may be enriched for TEs) is justified (Miklos & Cotsell, 1990). The results displayed in Table 3 show only a modest enrichment of TEs in the heterochromatin as a whole (an average of a 5·2-fold increase in density relative to the euchromatin). There is a very high enrichment in the β-heterochromatin, if the assumption that TEs are largely absent from the α-heterochromatin is correct (an average of a 23-fold increase in density relative to the euchromatin, assuming that 10% of the non-α-heterochromatic genome is β-heterochromatin).

The evidence seems strong that a large part of the centric heterochromatin is made up of blocks of tandemly-repeated sequences of satellite DNA, corresponding to the cytologically-defined α-heterochromatin. But there is also evidence that the arrays of repeats are sometimes interrupted by other sequences (Miklos & Cotsell, 1990; Lohe *et al.* 1993). In particular, TE insertions (including *copia* and *297*) are occasionally found in clones of satellite DNA (Carlson & Brutlag, 1978; Lohe & Brutlag, 1987; Lohe *et al.* 1993). A recent example of this is the *Bari-1* element, recently described by Caizzi *et al.* (1993), and which is mostly found as a fixed tandem array in the α-heterochromatin of 2R. The assumption that TE insertions never occur in α-heterochromatin is clearly too strong. On the other hand, the assumption that the heterochromatin available for insertions by TEs constitutes 10% of the genome available for TE insertions (used in obtaining the expectations for the columns labelled 2 in Table 3) may well be too conservative, given the small size of the β-heterochromatic regions in polytene chromosome preparations (Lefevre, 1976).

Charlesworth & Langley (1989) estimated that the mean density of TEs per kilobase in the euchromatin is approximately 0·005. If we adopt the 10% value as a working estimate of the proportion of the genome represented by the β-heterochromatin, the estimated 23-fold enrichment of this region for TEs implies that their mean density in the β-heterochromatin is 0·11 elements per kb. If the average size of a *D. melanogaster* element is roughly 5 kb (Lindsley & Zimm, 1992), then this result implies that about 50% of the total DNA in the β-heterochromatin is composed of TE-derived sequences. This agrees quite well with the estimate of 33–50% obtained by Miklos *et al.* (1988) from analyses of cloned material from the base of the X chromosome.

The view that β-heterochromatin contains a very high proportion of TE-derived sequences (Miklos *et al.* 1988; Miklos & Cotsell, 1990; Vaury *et al.* 1989; Nurminsky *et al.* 1994) is thus consistent with our results. The slot-blotting technique will, however, underestimate the numbers of copies of elements in

the heterochromatin, if a substantial proportion of these have suffered deletions, or have diverged greatly in DNA sequence from the probe copies because of immobility (see below). Our estimates of the extent of accumulation of elements in the heterochromatin may therefore be somewhat conservative.

The results shown in Table 2 indicate that the estimated between-line components of variance for copy number in the heterochromatin or total genome are often much greater than the means. There thus appears to be a greater-than-Poisson variance in copy number between lines for the heterochromatin, which is not seen in the euchromatic insertions. Of course, the estimates of the variances in heterochromatic copy numbers have large sampling errors. But there is a probability of only 0·01 of obtaining the observed result that all but one of the variances for total copy number for the well-characterized elements are greater than the corresponding means, on the null hypothesis of equality of means and variances. This raises the question of why such a difference between euchromatin and heterochromatin in the nature of the distribution of copy numbers between genomes should be observed. Most factors that might be imagined to operate in heterochromatin, such as high element frequencies at individual sites or variance in elements frequencies between sites, reduce the variance below Poisson expectation (see equation (1) of Charlesworth & Langley, 1989). Positive linkage disequilibrium in copy number between elements belonging to the same family could generate excess variance, but it is difficult to see how this could be generated by the normal population processes affecting TE abundances, particularly as there is little evidence for such consistently positive linkage disequilibrium in the proximal euchromatin (Charlesworth *et al.* 1992*a*).

It seems to us that the most probable explanation for the excess variances for heterochromatic copy numbers is that some of the sampled genomes contain one or more tandemly duplicated arrays of TE families, formed by amplification since initial insertions of elements. This would effectively generate positive correlations between copy numbers at different sites in the genome for a given family, and so will inflate the variance in copy number. Consider, for example, the case of a family on a particular chromosome, in which a fraction p of gametes in the population carry a tandem array of size n, and the remaining fraction carry no elements. The variance to mean ratio for this chromosome is easily seen to be $n(1-p)$, which will be much greater than one if n is large. Apparent cases of such amplification have been described in the literature (Kidd & Glover, 1981; Roiha *et al.* 1981; Di Nocera & Dawid, 1983; Vaury *et al.* 1989; Caizzi *et al.* 1993; Nurminsky *et al.* 1994).

Another indication that amplification may be important is provided by the fact that heterochromatic copy numbers for different element families seem to be correlated, when between-line comparisons are made. Although individual correlations between elements are only rarely statistically significant, the mean Spearman rank correlation between the values of the mean heterochromatic copy number estimates for pairs of elements across lines is 0·466, with standard error 0·047. In contrast, euchromatic copy numbers have a mean rank correlation of $0{\cdot}041 \pm 0{\cdot}056$. The correlations cannot be accounted for by errors of experimental technique such as effects of membranes on copy number estimates, since elements and lines were replicated across membranes (see Section 2(iv)). The only obvious interpretation is that the abundances of different families of elements are influenced in parallel by processes such as amplification or deletion of blocks of heterochromatin, or by variation in the amount of heterochromatin available for element insertion. The data of Nurminsky *et al.* (1994) on variation in the numbers of copies of the SCLR region of the X heterochromatin, which contains a block of TE-derived material, are consistent with the first of these possibilities.

The fact that TEs inserted into the heterochromatin are often defective in structure (Vaury *et al.* 1989) suggests that they tend to lose the ability to transpose. If this is the case, such inert elements would behave as passively replicating 'junk' DNA, subject to the same evolutionary forces as satellite DNA. The absence of crossing over in the heterochromatin is favourable to the persistence over evolutionary time of highly repeated sequences generated by amplification (Charlesworth *et al.* 1994). While there is evidence for transcription of β-heterochromatin (Lakhotia & Jacob, 1974), the fact that variegated position effect is often induced by chromosome breaks that bring a euchromatic locus into proximity to β-heterochromatin (Spofford, 1976) suggests that conditions in this region are often unfavourable for normal gene expression, so that elements may often lose their ability to transpose when inserted into β-heterochromatic. Under these circumstances, there is no selection against loss-of-function mutations, which may therefore accumulate by genetic drift and mutation pressure (Charlesworth & Langley, 1989).

In the introduction, we mentioned possible reasons why TEs should accumulate in heterochromatin. The fact that gene density in the β-heterochromatin (as opposed to α-heterochromatin) does not seem to be much lower than in the euchromatin (Miklos & Cotsell, 1990; Yamamoto *et al.* 1990) militates against the possibility that lack of selection against deleterious effects of insertional mutations is a major factor in causing this accumulation (c.f. Charlesworth, 1991), since the probability that an insertion will be in the neighbourhood of an expressed gene is similar to that for the euchromatin. The lack of genetic recombination in this region is a more plausible factor; both Muller's ratchet and the absence of elimination of elements through ectopic exchange could contribute to a build-up of elements in regions of greatly restricted

recombination (Charlesworth & Langley, 1989; Charlesworth *et al.* 1994).

We can use the argument of Charlesworth & Lapid (1989) to show that the relatively small proportion of the genome represented by the β-heterochromatin of a *Drosophila* chromosome implies that Muller's ratchet is unlikely to operate, based on the fact that this process cannot operate below a certain level of input of mutational events per generation in relation to population size (Haigh, 1978; Stephan *et al.* 1993), and so is ineffective for an isolated small genomic region with restricted recombination. From the data of Charlesworth *et al.* (1992*b*) on the abundances of elements in the autosomal euchromatin, the infinite population equilibrium number of elements per chromosome for the β-heterochromatin, if there were no build-up of elements in the heterochromatin, would at most be of the order of 2·5 for *roo* on chromosome 3, and less for other elements and chromosomes. The expected frequency of 3rd chromosomes with no *roo* elements in the β-heterochromatin is $\exp(-2{\cdot}5) = 0{\cdot}08$. Even in a population of 1000 individuals, the expected number of such chromosomes is 160, which is far greater than the threshold needed for the ratchet to operate at a noticeable rate (Haigh, 1978; Stephan *et al.* 1993). This suggests that Muller's ratchet is unlikely to be a significant factor in explaining the accumulation of elements in heterochromatin. If one accepts the arguments of Charlesworth *et al.* (1992*b*) against other possibilities, such as reduced rates of excision by recombination between long terminal repeats (which in any case does not apply to elements which lack repeats, such as *jockey*), ectopic exchange would seem to be the only viable candidate that remains.

Alternatively, element dynamics may differ qualitatively between the heterochromatin and euchromatin. Some authors have suggested that repetitive sequences in the heterochromatin are functionally significant (e.g. Pimpinelli *et al.* 1986), and the *HeT* and *TART* families have been implicated in telomere functions in *D. melanogaster* (Valgeirsdottir *et al.* 1990; Levis *et al.* 1993). The evidence from population surveys of *Drosophila* is very strong that euchromatic insertions do not in general confer a benefit on their host; it is, for example, very difficult to account for the very low frequencies of elements at nearly all individual chromosomal sites except on the hypothesis that elements are intra-genomic parasites (Charlesworth & Langley, 1989; Charlesworth *et al.* 1992*b*). Since we have little information of this kind for heterochromatic insertions, the question of the significance of heterochromatic TEs cannot be decided on the basis. An indirect method of answering the question is to compare the mean abundances of families of TEs in the heterochromatin with their abundances in the euchromatin. If similar forces affect TEs in both sections of the genome, there should be a positive correlation of mean euchromatic and heterochromatic abundances across different families. In fact, the product moment correlation between euchromatic and heterochromatic copy numbers is 0·94 ($P < 0{\cdot}01$). This is clearly not a conclusive test, but it accords with other evidence that heterochromatic repeated sequences are largely functionless (Miklos, 1985; Miklos & Cotsell, 1990).

Finally, the question of the reason for the apparent relative absence of TEs from α-heterochromatin, in contrast to their over-representation in β-heterochromatin, remains unanswered. Since the evolutionary dynamics of elements is favourable to their accumulation in both regions of the genome, provided that they can insert into α-heterochromatin, the simplest answer is that the simple repeated sequences which make up most of the α-heterochromatin do not provide suitable targets for insertion. There is little evidence on this point for most elements, since new insertions are hard to recognise in the absence of genes whose function can be disrupted.

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