THE RELATIVE MUTABILITIES OF DNA IN REGIONS OF THE X CHROMOSOME OF *DROSOPHILA MELANOGASTER*¹

GEORGE T. RUDKIN

The Institute for Cancer Research, Philadelphia, Pennsylvania

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MULLER (1927) and MULLER and PAINTER (1932) made the observation that the frequencies with which X-ray induced mutations occurred in regions of the X chromosome of *Drosophila melanogaster* fitted better the relative physical lengths of the regions than the genetic map lengths based on recombination. The frequency of mutation plotted as a measure of chromosome length they called a "mutation map" of the chromosome. The discrepancies between the genetic (recombination) and the mutation maps pointed out by them have been confirmed by others in the four decades since their major paper on the subject appeared (HARRIS 1929; OLIVER 1932; SPENCER and STERN 1948; IVES 1959).

The discovery of the salivary gland giant chromosomes placed cytogenetic mapping on a far more precise physical basis than the ganglion metaphases available to MULLER and PAINTER (1932), DOBZHANSKY (1932) or HEITZ (1933). But extrapolation from polytene to meiotic stages (except for a common linear order) has been tenuous without a detailed knowledge of the growth of the giant chromosomes from the haploid chromosomes of the blastoderm. Some, as CHARLES (1938), concerned with recombination frequencies, measured lengths of chromosomes on BRIDGES' (1938) map and assumed that they were proportional to meiotic chromosome lengths while others, as IVES (1959), used the number of BRIDGES' bands to estimate the number of loci in a given region.

It is the purpose of the present paper to introduce DNA content as a basis on which to express the relative frequencies with which mutations are observed in different chromosome regions. Studies of early development in progress in our laboratory justify the assumption that the distribution of DNA along the X chromosome in salivary gland nuclei represents its distribution along the euchromatic portions of the meiotic chromosomes (RUDKIN 1964 and unpublished). This communication presents a preliminary "DNA map" of the X chromosome euchromatin giving the relative amounts of DNA between eleven cytogenetically mapped loci and compares the DNA map with "mutation maps" published for sex-linked lethals produced by X rays, by *in situ* H³ thymidine or P³² and by a mutator gene. A similar study of relevant data on the distribution of mutants induced by chemical agents is in preparation.

The "apparent relative mutabilities" with respect to X-ray induced lethals of the DNA of seven regions covering all of the euchromatin of the X chromosome

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turn out to be equal within the limits of error of the possible comparisons. The reported H^3 thymidine and P^{32} induced lethals are too few to permit firm conclusions to be drawn, but were distributed in proportion to DNA content insofar as a statistical test could be applied.

MATERIALS AND METHODS

DNA distribution within the X chromosome: Four chromosomes were measured, designated A and B from female late larvae and C and D from male early prepupae, all isogenic with the inbred P_1 Oregon-R stock in our laboratory. The techniques for raising larvae, for cytological preparation for microspectrophotometry and for the photographic method of measuring integrated absorbance (A_T) are described elsewhere (RUDKIN *et al.* 1955; RUDKIN 1960, 1961). The chromosomes measured here in detail are from larvae fed H^3 thymidine early in larval life as described in previous publications and upon which other measurements have been reported (SCHULTZ and RUDKIN 1960b; RUDKIN and SCHULTZ 1961; RUDKIN in press). They were treated with ribonuclease (1.0%, pH 6.5, 2 hr, 37°C) before the measurements were undertaken at 257 nm in the ultraviolet.

In two instances the chromosome did not lie completely free. When it was crossed by another segment of chromosome, half of the integrated or total absorbance (A_T^{257}) of the intersection was assigned to each of the crossing segments. One chromosome (D) was contaminated by cytoplasm in sections 5 and 6 and in subsections 10B and 10C. The remainder of the chromosome (containing 84% of its A_T^{257}) was measured and the missing portions were estimated to be proportional to measured homologous segments in chromosome B. The locus of *spl* could not be accurately demarcated on chromosome C; the *w-ec* segment was measured and divided between the *w-spl* and *spl-ec* regions in proportion to the ratio observed in the other chromosomes.

The regions of the X chromosome: The regions in which the comparisons can be made are necessarily limited by the position and number of the genes that have been localized on the salivary gland chromosome. Those that we used are listed in Table 1 with their genetic symbols, loci on the genetic map (from BRIDGES and BREHME 1944), and cytogenetic loci on BRIDGES' map.

The "subregions" between *γ* and *ec* are of relatively little importance to the present paper because so few of the published data on lethal mutations are classified with respect to them. For the most part, seven regions consisting of *γ-ec* and the other six bounded by the rest of the gene loci in Table 1 have been of use.

TABLE 1

*Marker genes and their symbols, genetic and cytological loci**

Name of gene	Symbol	Genetic locus	Chromosome locus
yellow	<i>γ</i>	0.00	1A5,6
zest	<i>z</i>	1.0	3A3
white	<i>w</i>	1.5	3C2
split	<i>spl</i>	3.0	3C7
echinus	<i>ec</i>	5.5	3F1,2
cut	<i>ct</i>	20.0	7B3,4
vermilion	<i>v</i>	33.0	10A1,2
garnet	<i>g</i>	44.4	12B-12C†
forked	<i>f</i>	56.7	15F1,2
carnation	<i>car</i>	62.5	18D1,2
bobbed	<i>bb</i>	66.0	20A-D

* Collated from BRIDGES and BREHME (1944), *Drosophila* Information Service, personal communication from D. L. LINDSLEY and other sources.

† The cytological boundary between the *v-g* and *g-f* region was taken between 12B10 and 12C1,2.

Treatment of published mutation rates: SPENCER and STERN (1948) and KAPLAN *et al.* (1964) published the map location of each mutant they reported. When the locus of a mutant coincided with the standard map position of one of our marker genes (Table 1) it was counted half in each of the regions separated by the marker gene.

IVES (1959) published the number of mutations localized in each 5-unit section of the genetic map. When, as in most cases, our marker gene locus fell within a 5-unit space the number of mutants reported in the space was divided between the regions adjacent to the marker by linear interpolation on the genetic map (but see the Appendix for treatment of coarser grouping in Ives' data and effects of his mutator gene *hi*).

HARRIS (1929) and OLIVER (1932) presented their data as histograms showing mutations per map unit for each 5-map-unit interval and gave also the total number of lethal mutations on which the histograms were based. We reconstructed their results in terms of the number of mutations observed in each 5-unit space and treated them from then on in the same manner as the data of IVES. HARRIS presented separately the intervals from 0.0 to 2.5 and from 2.5 to 5.0 map units.

WALEN (1962) grouped the lethals induced by X rays or P³² into only four regions. No interpolation was attempted.

RESULTS

Intrachromosomal distribution of DNA: The observed distribution of DNA varied somewhat among the four chromosomes measured (Table 2). The widest

TABLE 2
Percent of DNA in regions of the X chromosome between marker genes*

Region	X chromosome				Combined	Percent of genetic map
	A(♀)	B(♀)	C(♂)	D(♂)		
<i>γ-z</i>	11.6	12.1	13.2	12.3	12.1	1.5
<i>z-w</i>	0.8	1.1	0.8	0.9	0.9	0.7
<i>w-spl</i>	1.4	1.8	2.5	1.9	1.8	2.3
<i>spl-ec</i>	3.7	3.8	4.0†	3.2	3.8	3.8
<i>ec-ct</i>	15.5	18.1	18.6†	16.7‡	17.2	22.0
<i>ct-v</i>	18.6	18.8	16.2	14.5	18.0	19.7
<i>v-g</i>	11.3	13.6	13.7	15.5‡	13.0	17.3
<i>g-f</i>	14.9	16.5	13.4	15.3	15.5	18.7
<i>f-car</i>	11.6	10.5	8.3	10.8	10.6	8.8
<i>car-bb</i>	10.6	3.7	9.5	8.9	7.2	5.3
$A_{1^{257}}$ Total	161.7	224.7	69.5	39.6	495.5	66.0
<i>w-2.5§</i>	...	...	...	...	1.20	1.51
<i>2.5§-spl</i>	...	...	...	...	0.59	0.76
<i>γ-2.5§</i>	...	...	...	...	14.23	3.79
<i>2.5§-ec</i>	...	...	...	...	4.34	4.55
<i>γ-ec</i>	...	...	...	...	18.6	7.34

Entries are the individual measurements of four chromosomes; the "Combined" column lists the percentages calculated from the sums of the raw data for individual chromosomes.

* Measured as integrated absorbance ($A_{1^{257}}$) at 257 nm in ribonuclease-treated chromosomes.

† *spl-ct* region measured and divided in proportion to other measurements of the *spl-ec* and *ec-ct* regions.

‡ Sections 5, 6 and 10BC were not measurable in chromosome D. They were estimated to be proportional to the homologous regions in chromosome B.

§ 2.5 units on the genetic map; interpolation between *w* and *spl* proportional to genetic map distances.

|| $A_{1^{257}}$ is the observed integrated absorbance of the whole chromosome at wavelength 257 nm.

deviation ($\pm 48\%$) was in the *car-bb* region for which an exceptionally low value was obtained in chromosome B. The other regions varied less than 15% among the chromosomes with the exception of the very small segments between *z* and *spl* where small absolute deviations are a high percentage of the mean value for a region (16 and 32%). The distribution determined from the combined data (sums of the A_T^{257} in each region), the best estimate at the present time, is the one to be compared with the mutation frequencies.

Presentation of mutation data: The distributions of the mutations from each of the published sources is listed in Table 3 as the percentage of the total published number that we estimated was in each of the indicated regions. (see MATERIALS AND METHODS). The number of mutations in the sample is shown in the "No. mutants" row. Gross chromosomal rearrangements were excluded according to genetic criteria by the original authors from the samples of mutants considered here.

In order to facilitate a direct comparison of the observations by the different authors we have calculated the ratio of percent mutations to percent DNA for each region; we call this number the "apparent relative mutability." The apparent relative mutabilities are plotted in Figures 1 and 2 (y -axis, log scale) against "DNA map" positions of the marker genes (linear scale, x -axis). A reference line has been drawn at an apparent relative mutability of 1.0, expected if recovered lethals were proportional to DNA contents of regions. Statistical treatment of the data is given in an Appendix at the end of the paper.

TABLE 3

*Percent of mutations in regions of the Drosophila X chromosome, induced by X rays and by H³ thymidine**

Region	X-ray induced					H ³ thymidine
	SPENCER and STERN	HARRIS	OLIVER	IVES	IVES	KAPLAN <i>et al.</i>
				300r	12.5kr	
<i>γ-z</i>	10.26	17.14	15.45	18.89	13.41	15.00
<i>z-w</i>	1.31					0.00
<i>w-2.5†</i>	1.31	0.00				
<i>2.5†-spl</i>	0.66	0.00				
<i>spl-ec</i>	3.93	0.00				
<i>ec-ct</i>	12.67	11.49	20.17	11.67	16.14	2.50
<i>ct-v</i>	21.18	18.63	12.96	15.74	16.82	22.50
<i>v-g</i>	17.03	9.97	9.10	15.19	11.05	20.00
<i>g-f</i>	19.21	15.98	17.52	19.54	20.34	17.50
<i>f-car</i>	7.42	16.17	12.57	10.01	13.73	5.00
<i>car-bb</i>	5.02	6.69	12.67	8.95	8.52	17.50
No. mutants	229	175	233	324	176	40
<i>γ-ec</i>	17.47	21.08				15.00
<i>γ-2.5†</i>	12.88					15.00
<i>2.5†-spl</i>	4.59					0.00

* References are found in LITERATURE CITED. See MATERIALS AND METHODS for methods of calculation.

† 2.5 map units on the genetic map.

FIGURE 1.—Apparent relative mutability of DNA in regions in the X chromosome with respect to X-ray induced lethal mutations. The relative mutability is the ratio of the percent of sex-linked lethals in a region to the percent of DNA of X euchromatin in the region. The percent of DNA was measured spectrophotometrically in salivary gland chromosomes. The percentages of lethals in the regions are taken from the following sources: ○ SPENCER and STERN (1948), all regions represented, 229 mutants; □ HARRIS (1929), only 0–2.5 and 2.5–5.0 on the *genetic* map represented to the left of echinus, 174 lethals; ▽ OLIVER (1932), γ -*ec* treated as a single region, 233 lethals; △ Ives 300r, 324 lethals, ▲ Ives 12.5kr, 176 lethals (1959), γ -*ec* treated as a single region. References are given in LITERATURE CITED.

X-ray induced mutations: SPENCER and STERN (1948) give their mapped locations of 229 sex-linked mutants of which about 25 to 30 are visibles, the rest lethals. The mutations include those that arose from control cultures and from males given various doses from 25 to 4000r. Their distribution along the chromosome, shown in Table 3, is the only one that can be compared with DNA in all of the marked chromosome segments. Deviations from a relative mutability of 1.0 can be seen in Figure 1 (circles) to be both positive and negative more or less at random along the chromosome. Fluctuations in the *z-ec* region could be related to the relatively small absolute values of A_T^{257} , map distance and number of mutants on which the ratios are based. The relative mutability of the γ -*ec* region, treated as a unit for comparison with the other X-ray data, is 0.94, very close to 1.0.

Of the other regions, *ec-ct*, *f-car* and *car-bb* show a low relative mutability here but higher values are found in the data of others (below). The *g-f* region appears to be the most mutable, and is the only one in which the deviation from 1.0 is statistically significant.

HARRIS' (1929) 174 X-ray induced (about 1000r) sex-linked lethals were readily allotted to the regions to the right of echinus, but to the left he distinguished only the intervals 0–2.5 and 2.5–5.0 map units. We interpolated both his data and the DNA content on the assumption that genetic map distance is proportional to DNA content over the short ranges involved (*w-spl* is 1.5 map units or 2.3% of the genetic map and contains 1.8% of the DNA of euchromatin).

FIGURE 2.—Apparent relative mutability of DNA (see Figure 1) in seven regions of the X chromosome with respect to mutations induced by X-rays and H³ thymidine. Key: ●, combined X-ray mutants of Ives (1959) and SPENCER and STERN (1948) from Figure 1 (729 mutations) with the extreme values at the ends of vertical bars; ×, KAPLAN *et al.* (1964), 40 lethals from sperm labeled with H³ thymidine. The six H³-thymidine mutants to the left of *ec* were all between *y* and *z*; calculation on that basis gives apparent relative mutabilities as follows: *y-z*, 1.2; *z-w*, 0.0; *w-spl*, 0.0; *spl-ec*, 0.0. References are given in LITERATURE CITED.

The resulting distribution, shown in Table 3, is compared with the DNA distribution of Figure 1 (squares). It follows the trends in the data of SPENCER and STERN, deviating from 1.0 more in some places, less in others. The *y-ec* region, treated as a unit, has an apparent relative mutability of 1.14, somewhat higher than 1.0 and than the SPENCER and STERN value for the same region. Significant deviation is found only in the region from *f* to *car*.

OLIVER (1932), working in the same laboratory as HARRIS, reported the locations of 233 lethals which arose from a series of X-ray dosages from about 50 to about 1500r at various intensities and times. In his data we have only seven regions distinguishable; all of our markers to the left of *ec* are within the first five map unit section into which he grouped his data. The resulting distribution of the relative mutabilities (triangles point down in Figure 1) varies around 1.0 to the same extent as the data of SPENCER and STERN and of HARRIS, but not consistently following either; it is sometimes below the others (*ct-v*), sometimes above (*ec-ct*). Significant deviation occurs in the region *car-bb*.

The largest single population of mutants comes from Ives (1959) who located 500 lethals, 324 of which resulted from an X-ray dose of 300r, the rest from a dose of 12,500r. He shows that the distributions in 5-map-unit segments are not statistically different for the two doses. His data (triangles point up in Figure 1)

are in the range already established by the other three sets. It was necessary to treat γ -*ec* as a single region; his localizations were published only to the nearest five map units. The *ec-ct* and *g-f* regions deviate significantly from expected at 300r, none at 12.5kr.

Direct comparison of all of the X-ray data requires that the same regions be represented in each set; that is, that the segment from γ to *ec* be treated as a single region (all of the other regions are represented in all sets of data). Statistical analysis has been carried out to test the agreement between the sets of data and between the observed distributions of mutations and the distributions expected if relative mutability were proportional to DNA content. (See Appendix for an outline of the statistical treatment.)

The mutation data alone do not pass the chi-square test for homogeneity so not all would be expected to agree with any one hypothesis. However, it does seem important that the two most recent sets (IVES 1959; SPENCER and STERN 1948) are homogeneous by the chi-square test while the older ones (HARRIS 1928; OLIVER 1932) do not agree with each other or with the later data. Inaccuracies in our reconstruction from the graphical presentation of the older data may contribute to the deviations. However, in view of the refinements in genetic and physical techniques that were developed during the two decades that separated the older from the later experiments, it seems reasonable to regard the combined results of SPENCER and STERN and of IVES as the best estimates of the distribution of recovered X-ray induced lethals along the X chromosome. Their combined results are plotted in Figure 2 as solid circles with the extreme values indicated by vertical bars for comparison with the distribution of mutations produced by tritiated thymidine. In the combined data, *ec-ct* shows significantly fewer, *g-f* significantly more mutants than expected.

H³-thymidine induced lethals: Recently KAPLAN and SISKEN (1960) reported the production of lethal mutations by tritium incorporated into the DNA of *Drosophila* sperm via H³ thymidine. Later KAPLAN *et al.* (1964) gave the genetic map location of 40 such lethals. The distribution along the X chromosome (Table 3) is at first glance strikingly different from the distribution of the DNA. However, the difference is of marginal significance because of the small numbers expected in many of the regions. The most extreme deviations are in the *z-ec* regions where less than one mutant would be expected in each of its three parts and none were found. One was found where 6.9 were expected between *ec* and *ct*, 2 were found where 4.2 would be expected between *f* and *car* and 7 were found where 2.9 were expected between *car* and *bb*. The deviations from proportionality with DNA are wide as shown on Figure 2 (X's) but can only be regarded as suggestive of possible differences in the responses of the separate regions to the *in situ* radioactivity. When regions were combined to give numbers large enough to carry out a chi-square test (γ -*ec-v-f-bb*), the observed distribution of lethals did not differ significantly from that expected from the distribution of DNA (see Appendix).

Mutator gene: IVES (1959) reported the distribution of sex-linked lethals and visibles that arose in the presence of his mutator gene *hi*, but only classified

their locations within the four chromosome regions separable in his tester stock, γ *ct*⁶ *ras*² *f*. The deviations from a relative mutability of 1.0 are significant for lethals, not for visibles (see Appendix). It is, unfortunately, not possible to compare the set of *hi* mutations in detail with the others.

*P*³² induced lethals: WALEN (1962) localized 99 lethals which arose in males treated with *P*³² and 76 lethals from males given 1000r of X rays. Since she reported her localization only within the four regions used by IVES (γ *ct* *ras* *f* *bb*) the same restrictions apply to her data as to the mutations found in the mutator stock *hi*. However, while her X-ray data deviated significantly from proportionality with DNA (too few were found between *ct* and *ras*), the *P*³² induced lethals conformed well to an apparent relative mutability of 1.0 (see Table A-3, Appendix). The *ras-f* region had a value consistently but not significantly greater than 1.0.

DISCUSSION

The apparent relative mutabilities of the chromosome regions studied here are functions of two independent processes: the mutation mechanism which governs the frequency of recoverable recessive lethals induced in sperm and the growth of polytene chromosomes during which the relationship between the DNA contents of chromosome regions in sperm and in salivary gland chromosomes is determined. The value of 1.0 which we found to be characteristic for X-ray induced lethals in most of the X chromosome would be established if, in the two chromosome types, proportionate amounts of DNA were found in the different regions and if the probability of inducing a recoverable lethal were proportional to the amount of DNA in a region. If either of those conditions did not hold, then a compensating change would have to be postulated in the other; hence their mutual validity constitutes the simplest hypothesis.

It should be pointed out that the extent of damage to the chromosome and to the DNA in particular is not known for the sets of lethal mutations considered here. Routine tests were carried out by the experimenters to detect and eliminate chromosomal aberrations large enough to seriously affect linkage relationships. The remainder is a class of recovered lethals that can be mapped and could include a wide range of types from the alteration of single base-pairs to the deletion or rearrangement of extensive chains of DNA within a cytogenetic unit (or band). The more extensive the damage, the more likely it becomes that linkage relationships near the mutant locus would be disturbed (without, of course, significantly affecting the frequencies of exchange with more distant loci). In some cases the result could be to shift the apparent genetic locus of a mutation from the region in which it arose to an adjacent one. It seems doubtful that the apparent relative mutability between *g* and *f* could have been appreciably increased by such a mechanism in the absence of consistent compensating low values in the two adjacent regions.

A knowledge of the frequency distribution of the different degrees of damage would aid in interpreting our result. It appears that in *Drosophila* the majority of X-ray induced lethals fall into the class of "point mutations" at the resolving

power of standard mapping procedures, while in maize, for example, the work of STADLER (1951), especially, has shown that nearly all X-ray induced mutations are associated with demonstrable deletions (see MULLER 1954a,b for review). The lethals recovered by the authors whose work is considered here may have included a large percentage of what might be termed "intra-band" deletions as well as small deletions involving a few bands (see, e.g., DEMEREC and KAUFMANN 1937), a consideration of importance to the discussion of possible redundant segments within cytogenetic units below.

The growth of the salivary gland chromosomes is open to experimental study by independent means, currently employed in our laboratory. It has been found that the well known "deficiency" in centric heterochromatin characteristic of polytene nuclei must be established very early in development (RUDKIN and SCHULTZ 1961) and that it probably arises by failure of centric heterochromatic segments to replicate in definitive salivary gland cells (RUDKIN 1964 and unpublished). The DNA of euchromatin which is under consideration here does appear to be replicated in a classic geometric series with a common ratio of 2 (RUDKIN 1964 and unpublished, supported also by data of WELCH 1957 and SWIFT 1962). Although most of the available measurements have been made on whole nuclei, proportionate synthesis of all DNA in euchromatin is the simplest hypothesis by the same argument employed above, i.e., if DNA in some regions were to be replicated to more than the average amount, then a compensating deficiency in synthesis would have to be postulated for some other regions. The matter is not settled for it has been reported that deviations may occur in very short regions (SCHULTZ and RUDKIN 1960a); they are, from the evidence at hand, minor compared to whole nuclei or to the regions considered here. Thus, additional support is given to the hypothesis that recessive lethals are detected in proportion to the amount of chromosomal DNA investigated. For the one region in which proportionality was consistently not observed, either a high incidence of lethals or a restriction in the replication of salivary gland DNA (or both) may be postulated. It should be pointed out, however, that other factors independent of either mutation rate or polytene growth may be overriding for that region, for example, factors affecting the recovery and mapping of mutants.

Satisfying as an apparent mutation frequency proportional to DNA might seem to be, the many factors which enter between an initial mutational event and a recovered lethal prevent a simple interpretation. If we assume that all induced chromosomal changes which are potential recessive lethals have an equal chance of surviving to the generation in which the tests for lethals are made, their distribution on the X chromosome could be a function of the organization of DNA in the chromosome. The DNA, as revealed in the polytene state, is packaged into bands. Since the genetic content of a band is uncertain, we found it convenient to designate them cytogenetic units in connection with earlier measurements (RUDKIN *et al.* 1956). The implication is the classical one that the separate units embody separate functions, the functions attributable to the genes that have been localized within them (as in Table 1). (See also BEERMANN 1962; EDSTRÖM 1964.) The different bands or units contain widely different

amounts of DNA. The range for single bands is an order of magnitude, but the inclusion of double bands and capsules, so designated by BRIDGES (1935), extends the total range over several orders of magnitude (RUDKIN *et al.* 1956; RUDKIN 1961). On the premise that DNA replication is proportionate in euchromatin during polytene chromosome growth we have calculated the amount of DNA present per cytogenetic unit in haploid chromosome strands, i.e., in the chromosomes of sperm where the lethals were induced. The data essential to determine the growth factor involved (degree of polyteny in euchromatin) were obtained with the collaboration of DR. SCHULTZ, and that factor, combined with a recent estimate of the DNA content of a diploid nucleus (RUDKIN 1964), necessitates a revision of the previously published figures (RUDKIN *et al.* 1956; SCHULTZ and RUDKIN 1960b; RUDKIN 1961). The smallest measurable band corresponds to 5×10^{-6} picogram DNA or 5×10^3 nucleotide pairs; the average amount for the 1012 bands in the X chromosome is 3×10^{-5} picogram DNA or 3×10^4 nucleotide pairs per haploid chromosome strand. The average is close to 6×10^{-5} pg found for *Chironomus* by EDSTRÖM and BEERMANN by a quite different approach (EDSTRÖM 1964). These values are large compared to estimates for the cistrons of microorganisms but are quite comparable to larger functional units such as the histidine "operon" of P22 phage with 1.3×10^4 nucleotide pairs (see AMES and HARTMAN 1963).

If a recessive lethal were to involve a change in any one of a random sample of such cytogenetic units, then one of two relationships might be expected. First, if the probability of receiving an effective hit were the same for all units, then the distribution of lethals would be proportional to the number of bands whatever their DNA contents. It is easily shown that the combined data of IVES and of SPENCER and STERN do not fit the distribution of lethals expected on that basis ($P < 0.01$) whether double bands are counted as one or two bands each. However, the main discrepancy is found in the low (300r) dose in IVES' data ($P < .001$) while his high dose (12.5kr) group and SPENCER and STERN's group (0-4000r) deviate much less (at the 5% level or not significantly). This brings us to the second postulated relationship, namely, that the probability of a "lethal hit" is proportional to the DNA content of the cytogenetic unit. In that case, the distribution of lethals would follow the DNA contents, as we observe. At high doses of X rays, multiple hits would be more frequent in the larger units and the effect of the size of a unit on the probability of a "lethal hit" would diminish without altering the overall proportionality between DNA content and recovered lethals. Thus, the overall agreement of the observations with theory are consistent with the packaging of DNA in chromosomes.

The evolution of new units by the duplication of smaller ones and subsequent mutation at random in the duplicate parts was proposed by BRIDGES (1935) to explain a number of features of salivary gland chromosome morphology (see MULLER 1951 for historical review). Some redundancy would be expected as chromosomes evolve in that manner, redundancy that should render functional a locus in which only one of two or more duplicate nucleotide sequences had been altered to a "lethal allele." What our result implies is that if such masking

of potential lethal mutations does exist it occurs to the same extent in all of the regions observed with the reservation that intra-band deficiencies referred to above would obscure the effects of replicate DNA. We obtain a measure of the possible intra-band redundancy by calculating the average percent of chromosomal DNA per band for each region and then compare it with the average relative number of mutations per band (combined data of SPENCER and STERN and of Ives). It turns out that the highest rate per band is in the *γ-ec* region (percent mutations/percent bands=1.37) where the bands have the highest DNA content and the next highest rate per band in the *car-bb* region (1.12) where the bands contain the second largest amount of DNA of any of the regions considered (Table A-4, Appendix). There is no suggestion here that the DNA content of a cytogenetic unit is related to genetic redundancy but the analysis is consistent with the view that the larger bands represent functional units, "operons" perhaps, that encompass longer chains of DNA than the smaller bands, perhaps more cistronic units. EDSTRÖM (1964) was led by the evidence he considered to the view that not all of the DNA of a band was functional in protein synthesis, a view not excluded for all bands by the present argument.

Redundancy, if present, might be at the level of whole bands, not within bands, and could be represented in part by the structures that BRIDGES called double bands, the presence of which reenforced the theory of evolution by duplication and mutation. If mutations were randomly distributed with respect to band type, regions with the highest proportions of doublets would be expected to have the most redundancy which would in turn reduce the frequency of recovered lethal mutations. In point of fact, the region with the highest rate of recovery, *g-f*, has a very high ratio of double to single bands (counted on BRIDGES' map): 1.76 compared to a mean of 1.21 for the whole chromosome and to a range from 0.63 (*γ-ec*) to 2.41 (*car-bb*) in the regions studied (see Table A-4). However, the recovered lethals might all occur in single bands which outnumber them in most sets of data. These considerations do not constitute arguments against duplication as a factor in chromosome evolution but would, insofar as they countermand redundancy, imply that in structures which might be presumed to encompass originally duplicate sequences of DNA, the sequences have now diverged sufficiently to be governing different functions in the species as it exists today.

It should not be surprising that the present analysis does not reveal any especially "hot spots" comparable to those highly mutable sites reported by BENZER (1961) in T2 bacteriophage although it could be that a similar phenomenon contributes to the variability in proportion of mutations found between different pairs of loci. Whereas BENZER was dealing with a few hundred nucleotide pairs we have on the average one seventh of the X euchromatin which contains 3×10^{-2} picograms (3×10^7 nucleotide pairs) of DNA or about 5×10^6 nucleotide pairs per region (calculated for the haploid strand as described above). If hot spots for recessive lethals are present, as SPENCER and STERN (1948) suspect, then they must be distributed at random in the DNA of most of the euchromatin.

Short regions, particularly single bands, would have to be studied to detect

the kind of regional differences sought by KAPLAN *et al.* (1964) in their experiments with H^3 thymidine. Our observation that mutations were induced in proportion to the DNA contents of rather long regions still leaves room for wide variations in relative mutabilities, possibly related to local variations in the thymidine content of chromosomal DNA as pointed out by KAPLAN *et al.* Such variations are suggested by the points plotted in Figure 2 but more data would be required to determine their validity. The four regions in which we could make statistically valid comparisons involved of the order of 10^7 nucleotide pairs each. It seems unlikely that differences from the average chromosomal thymidine content would be detected in such large samples, as indeed they were not.

While the foregoing considerations suggest that the apparent relative mutabilities are consistent with certain features of the organization of the genetic material in *Drosophila*, their most important function is to indicate directions in which future research might go. The genetic data at hand are restricted as to the type of mutations investigated, the X-ray dosages employed and the detail with which the nature and genetics of each mutant was investigated for, of course, the original data were collected for other purposes. More restrictive are the long stretches of chromosome between marker genes which, although they reduce the sample of DNA under investigation by an order of magnitude compared to the whole chromosome, still present the analyst with groups of 100 to 200 cytogenetic loci and of the order of over ten times that many cistronic units within which the events he studies cannot be correlated with their chromosomal DNA. The possibility for increasing the resolving power by two to three orders of magnitude is in the material, and the effort involved in reaching that goal would probably not be proportionately great.

SUMMARY

The relative DNA contents of ten regions of the X chromosome in four salivary gland nuclei of fully grown larvae and prepupae were determined by cytophotometric measurements of ultraviolet absorbance ($\lambda = 257$ nm) of chromosomes treated with ribonuclease. The regions were bounded by the cytogenetic loci of the 11 marker genes, γ , z , w , spl , ec , ct , v , g , f , car and bb .

The "apparent relative mutability" of a chromosome region is defined as the ratio of the percentage of all mutations that were found to occur in the region to the percentage of DNA in the region. Apparent relative mutabilities were determined for seven to ten regions on the basis of eight published sets of sex-linked lethal and visible mutations: five sets produced by X-ray treatment, one set by a "mutator gene," one set by incorporated H^3 -thymidine and one set by incorporated P^{32} . The apparent relative X-ray sensitivities of the regions were not demonstrably different from one another. Lethal mutations induced by Ives' mutator gene *hi* were not randomly distributed among four chromosome regions; visibles were. Differences between the susceptibilities of the regions to the different mutagenic treatments are suggested but not established by the data.

The theory of chromosome evolution by duplication would predict redundancy

of genetic function in duplicate regions after the duplication event and until the two regions had diverged by mutation to have different functions. No evidence for different degrees of redundancy was found, for the distribution of the observed mutations was not related to the number of bands, the ratio of single to double bands, or to the average DNA contents of the bands in the regions analyzed.

APPENDIX: STATISTICAL CONSIDERATIONS

Direct statistical tests of the agreement of the mutation data with the hypothesis that the frequency of recovered mutations is proportional to DNA content cannot be relied upon for several reasons. First, the methodological errors in the relative DNA measurements are difficult to assess: compromises have been made to obtain a reasonable estimate of all regions in as many as four salivary gland chromosomes (see MATERIALS AND METHODS); the chromosomes on which the measurements were carried out were not optimum spectrophotometric material but were measured because of the possibility of correlation with autoradiographic results; and the observed interchromosomal variations are not the same for all regions.

Second, the precision with which the sex-linked mutants were mapped depends upon many factors including the stocks used for treatment and for mapping and on the sample sizes from which the crossover data were obtained (see, e.g., STERN 1957). For example, the high standards set in the most recent and most extensive X-ray experiment (IVES 1959) do not justify classifying the mutant loci into groups less than five map units long, according to the author. That alone could introduce an uncertainty of the order of 20% per region when only seven regions are demarcated, as in the bulk of the data (Figure 2). Differences between genetic strains, the conditions of irradiation and the doses given constitute additional variables of unknown effect on which, in most cases, we have superimposed a process of interpolation which itself assumes random occurrence of mutants on the *genetic* map within 5-map-unit segments.

In spite of the magnitude of the uncertainties in the raw data, some statistical treatment is desirable to examine the homogeneity of the sets of mutation data and their agreement with the hypothesis that relative mutability is proportional to DNA content. The seven regions shown in Figure 2 were used. Individual apparent relative mutabilities are listed in Table A-1 and marked according to the probabilities that as great or greater a deviation from 1.0 would occur by chance alone. The probabilities were obtained from χ^2 values comparing observed numbers of mutants with the numbers expected if mutation frequency were proportional to DNA content.

TABLE A-1

*Apparent relative mutabilities of DNA in regions of the X chromosome of
Drosophila melanogaster*

Region	HARRIS	OLIVER**	HARRIS and OLIVER* combined	SPENCER and STERN*	IVES* 300r	IVES 12.5 kr	SPENCER and STERN and Ives** combined	Combined X ray**
<i>y-ec</i>	1.13	0.83	0.96	0.94	1.02	0.72	0.92	1.05
<i>ec-ct</i>	0.67	1.17	0.97†	0.74	0.68*	0.94	0.76**	0.82*
<i>ct-v</i>	1.04	0.72	0.86	1.18	0.87	0.93	0.98	0.91
<i>v-g</i>	0.77	0.70	0.73	1.31	1.17	0.85	1.13	0.96
<i>g-f</i>	1.03	1.13	1.09	1.24*	1.26*	1.32	1.27**	1.18**
<i>f-car</i>	1.52*	1.18	1.33*	0.70	0.94	1.29	0.95	1.00
<i>car-bb</i>	0.93	1.71**	1.37*†	0.70	1.25	1.19	1.06	1.22
N	175	233	408†	229	324	176	729	1137†

* and **: 5% and 1% probability levels.

† Not homogeneous, 5% probability level.

The five sets of mutation data are (not surprisingly) not homogeneous when tested against each other in a general contingency table ($0.01 > P > 0.001$); not all of them can be expected to agree with any single hypothetical distribution. Indeed, only two sets and not the combined data did fit the frequencies expected on the basis of the DNA in the regions (Table A-1). The interaction χ^2 is also significant (dagger for combined X ray, Table A-1), indicating that the deviations from expected are different for the different authors. However, those sets of data collected within the last 20 years (SPENCER and STERN 1948; IVES 1959) are internally consistent (Table A-1) even though two of them disagree with the hypothetical distribution. The two sets collected in MULLER's laboratory at the University of Texas nearly 40 years ago (HARRIS 1928; OLIVER 1932) are not in statistical agreement with each other, with the DNA distribution or with the later data (Table A-1). The significance of deviations from expected mutabilities of 1.0 at the 5% level are dubious because the error in the determination of DNA contents has not been taken into consideration (see above); the *inhomogeneity* in the older sets of data is not subject to that uncertainty.

It is clear from these tests that close control of the conditions under which mutation data are collected is necessary to make valid comparisons.

The mutants induced by Ives' mutator gene *hi* were distributed differently from his X-ray induced lethals (IVES 1959). Since he reported their locations only within the four regions marked in his tester stock (*γ ct⁶ ras² f*) the data are not directly comparable with the results of other authors and the "resolution" in terms of chromosome length is poor. We did, however, compare them with the DNA distribution (Table A-2) by equating the locus of *ras* (32.8 on the genetic map) with that of *v* (33.0 on the genetic map). The lethals did deviate significantly from the expected distribution. The small sample of visible mutations, on the other hand, agreed very well. The nonsignificant χ^2 for interaction in a combined table agrees with Ives' observation that the two types of *hi*-induced mutants were not differently distributed among the four regions, but the importance of comparisons with so small a sample of visibles is questionable, aside from the well known personal bias affecting their detection.

Interestingly, when only Ives' four chromosome regions are compared, the mutator lethals deviate from proportionality with DNA more than do the X-ray induced ones; in fact, the 12.5kr sample is in good agreement (Table A-2). It would appear that the effect of the mutator gene in inducing lethal mutations were not distributed randomly over chromosomal DNA. Inspection of the data shows an excess of lethals in the two center regions, too few lethals in the two end regions, paralleling the distribution of crossovers (CHARLES 1938). By contrast, the 300r X-ray dose yielded its greatest excess of mutations in the *ras-f* region with compensating low numbers of mutations in the two distal regions. In spite of the fact that the distributions produced at the two X-ray doses were not significantly different from one another (IVES 1949 and Table A-2) only one of them is in statistically significant disagreement with the DNA as we measured it.

The KAPLAN *et al.* (1964) sample of H³ thymidine mutants is too small for the application of

TABLE A-2

Apparent relative mutabilities of DNA in regions of the X chromosome for Ives' mutator gene hi and for two doses of X rays

Region	Mutator gene <i>hi</i>			X-ray induced lethals		
	Visibles	Lethals	Combined	300r	12.5 kr	Combined
<i>γ-ct</i>	0.93	0.59***	0.64***	0.87	0.84	0.86
<i>ct-ras</i>	0.79	1.23***	1.43**	0.84	0.88	0.86
<i>ras-f</i>	1.42	1.36**	1.38***	1.27*	1.08	1.20*
<i>f-bb</i>	0.67	0.70*	0.69*	0.99	1.31	1.10
N	42	250***	292***	324*	176	500**

*, **, ***: 5, 1 and 0.1% probability levels.

the χ^2 test when the data are distributed among all possible regions. Lumping the observations into the four regions bounded by the loci of γ , ec , v , f , and bb gives expected (on the basis of DNA) numbers of mutants between 7.1 and 14.1 per region. The fit is a good one ($\chi^2_3 = 3.1$, $0.5 > P > 0.3$). It is certainly premature to conclude uniformity in the distribution of thymidine within the DNA of the rather long regions involved. The possibility that there are very short regions with a high (or low) thymidine content remains but cannot be adequately tested with the data at hand.

Phosphorus would be expected to be incorporated along the DNA molecule more nearly at random than is the case for a specific base such as thymidine. The P^{32} -induced sex-linked lethals found by WALEN do, indeed, seem to be distributed in proportion to the DNA content of the four regions for which she gives data (see Table A-3). Her X-ray controls (1000r) deviate significantly from an apparent relative mutability of 1.0, but the pattern of deviations looks different from that in IVES' data (reported for the same chromosome regions, Table A-2). The difference in the distributions of lethals in the two sets of data is not significant (homogeneity test) but WALEN's sample is rather small for the demonstration of differences. Insofar as the data go, they are consistent with the assumption that lethals induced by P^{32} and by X-rays bear the same relation to DNA content.

Comparisons with the distribution of BRIDGES' bands are illustrated by the summary given in Table A-4. Statistical tests were carried out on the numbers of mutants observed and those expected if recovered mutants were in proportion to numbers of bands.

TABLE A-3

Apparent relative mutabilities of DNA in regions of the X chromosome for Waleen's P^{32} and X-ray induced lethals

Region	P^{32}	1000r	Combined
γ -ct	0.90	0.92	0.91
ct-ras	0.95	0.44*	0.73
ras-f	1.17	1.15	1.16
f-end	0.97	1.48	1.19
N	99	76*	175

* 5% probability level for deviations from 1.0.

TABLE A-4

Comparison of the distribution of x-ray induced sex-linked lethals with the banding characteristics of the regions in which the lethals occurred

Region	Percent of bands†	Ratio double bands: single bands	Ratio percent DNA: percent bands†	SPENCER and STERN	Percent mutation/percent bands‡		
					IVES		Combined
					300r	12.5 kr	
γ -ec	12.5	0.63	1.49***	1.39*	1.51**	1.07	1.37***
ec-ct	16.8	1.51	1.02	0.62	0.69*	0.96	0.78*
ct-v	18.7	1.14	0.96	1.08	0.84	0.90	0.95
v-g	14.6	1.11	0.89	1.17	1.04	0.76	1.01
g-f	18.2	1.88	0.85*	1.06	1.08	1.12	1.08
f-car	12.4	1.79	0.86	0.60*	0.81	1.10	0.81
car-bb	6.8	2.41	1.06	0.74	1.45	1.25	1.12
Whole X	1030§	1.24	***	*	**		***

† A "double band" counted as two bands.

*, **, ***: 5, 1.0 and 0.1% levels of significance.

§ Number of bands, double band counted as two bands.

LITERATURE CITED

- AMES, B. N., and P. E. HARTMAN, 1963 The histidine operon. Cold Spring Harbor Symp. Quant. Biol. **28**: 349-356.
- BEERMANN, W., 1962 Riesenchromosomen. Protoplasmatologia **6**: 1-161.
- BENZER, S., 1961 On the topography of the genetic fine structure. Proc. Natl. Acad. Sci. U.S. **47**: 403-415.
- BRIDGES, C. B., 1935 Salivary chromosome maps with a key to the banding of the chromosomes of *Drosophila melanogaster*. J. Heredity **26**: 60-64. — 1938 A revised map of the salivary gland X-chromosome of *Drosophila melanogaster*. J. Heredity **29**: 11-13.
- BRIDGES, C. B., and K. S. BREHME, 1944 *The Mutants of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **552**:
- CHARLES, D. R., 1938 The spatial distribution of cross-overs in X-chromosome tetrads of *Drosophila melanogaster*. J. Genet. **36**: 103-126.
- DEMEREK, M., and B. P. KAUFMANN, 1937 The gene. Carnegie Inst. Wash. Yearbook **36**: 44-51.
- DOBZHANSKY, TH., 1932 Cytological map of the X-chromosome of *Drosophila melanogaster*. Biol. Zentr. **52**: 493-509.
- EDSTRÖM, J. E., 1964 Chromosomal RNA and other nuclear RNA fractions. pp. 137-152. *The Role of Chromosomes in Development*. Edited by M. LOCKE. Academic Press, New York.
- HARRIS, B. B., 1929 The effects of aging of X-rayed males upon mutation frequency in *Drosophila*. J. Heredity **20**: 299-302.
- HEITZ, E., 1933 Die somatische Heteropyknose bei *Drosophila melanogaster* und ihre genetische Bedeutung. (Cytologische Untersuchungen an Dipteren. III) Z. Zellforsch. Mikroskop. Anat. **20**: 237-288.
- IVES, P. T., 1959 Chromosomal distribution of mutator- and radiation-induced mutations in *D. melanogaster*. Evolution **13**: 526-531.
- KAPLAN, W. D., and J. E. SISKEN, 1960 Genetic and autoradiographic studies of tritiated thymidine in testes of *Drosophila melanogaster*. Experientia **16**: 67-69.
- KAPLAN, W. D., H. D. GUGLER, K. K. KIDD, and V. E. TINDERHOLT, 1964 Nonrandom distribution of lethals induced by tritiated thymidine in *Drosophila melanogaster*. Genetics **49**: 701-714.
- MULLER, H. J., 1927 The problem of genic modification. Verhandl. V Intern. Kongr. Vererb. 234-260. — 1951 The development of the gene theory. pp. 77-99. *Genetics in the 20th Century*. Edited by L. C. DUNN. Macmillan, New York. — 1954a The nature of the genetic effects produced by radiation. pp. 351-473. *Radiation Biology*, Vol. 1. Edited by A. Hollaender, McGraw-Hill, New York. — 1954b The manner of production of mutations by radiation. pp. 475-626. *Radiation Biology*, Vol. 1. Edited by A. HOLLÄENDER. McGraw-Hill, New York.
- MULLER, H. J., and T. S. PAINTER, 1932 The differentiation of the sex chromosomes of *Drosophila* into genetically active and inert regions. Z. Ind. Abst. Vererb. **62**: 316-365.
- OLIVER, C. P., 1932 An analysis of the effect of varying the duration of X-ray treatment upon the frequency of mutations. Z. Ind. Abst. Vererb. **61**: 447-488.
- RUDKIN, G. T., 1960 The application of ultraviolet absorbance measurements to problems in cell biology. IRE Trans. Med. Electron. **ME-7**: 122-129. — 1961 Cytochemistry in the ultraviolet. Microchem. J., Symp. Ser. **1**: 261-276. — 1964 The structure and function of heterochromatin, Proc. 11th Intern. Congr. Genet. **2**: 359-374.
- RUDKIN, G. T., J. F. ARONSON, D. A. HUNGERFORD, and J. SCHULTZ, 1955 A comparison of the ultraviolet absorption of haploid and diploid salivary gland chromosomes. Exptl. Cell Res. **9**: 193-211.

- RUDKIN, G. T., S. L. CORLETTE, and J. SCHULTZ, 1956 The relations of the nucleic acid content in salivary gland chromosome bands. (Abstr.) *Genetics* **41**: 657-658.
- RUDKIN, G. T., and J. SCHULTZ, 1961 Disproportionate synthesis of DNA in polytene chromosome regions in *Drosophila melanogaster*. (Abstr.) *Genetics* **46**: 893-894.
- SCHULTZ, J., and G. T. RUDKIN, 1960a DNA content in relation to the genetic unit in *Drosophila melanogaster*. *Proc. 10th Inter. Congr. Cell Biol.* 71. — 1960b Direct measurement of deoxyribonucleic acid content of genetic loci in *Drosophila*. *Science* **132**: 1499-1500.
- SPENCER, W. P., and C. STERN, 1948 Experiments to test the validity of the linear r-dose/mutation frequency relation in *Drosophila* at low dosage. *Genetics* **33**: 43-74.
- STADLER, L. J., 1951 Spontaneous mutation in maize. *Cold Spring Harbor Symp. Quant. Biol.* **16**: 49-63.
- STERN, C., 1957 A note on the detection of differential effects of mutagens. *J. Genet.* **55**: 276-279.
- SWIFT, H., 1962 Nucleic acids and cell morphology in Dipteran salivary glands, pp. 73-125. *The Molecular Control of Cellular Activity*. Edited by J. M. ALLEN. McGraw-Hill, New York.
- WALEN, K. H., 1962 Cytogenetic studies of X-ray and ingested P³² induced sex-linked recessive lethals in *Drosophila melanogaster*. *Hereditas* **48**: 123-131.
- WELCH, R. M., 1957 A developmental analysis of the lethal mutant *l(2)gl* of *Drosophila melanogaster* based on cytophotometric determination of nuclear desoxyribonucleic acid (DNA) content. *Genetics* **42**: 544-559.