SALIVARY CHROMOSOME BANDS AND THE FREQUENCY OF CROSSING OVER IN *DROSOPHILA MELANOGASTER*[1]

G. LEFEVRE, JR.

Department of Biology, San Fernando Valley State College, Northridge, California 91324

Received September 11, 1970

WITH the discovery of linkage and crossing over, geneticists acquired a technique for estimating the physical distances between genes on the chromosome. From the first, however, the accuracy of linkage maps has been viewed with reservation. Too many extraneous factors, including temperature (PLOUGH 1917) and maternal age (BRIDGES 1927), were shown to modify linkage values. Furthermore, both crossover suppressors and enhancers were identified. Nevertheless, until independent procedures were developed, recombination values, of necessity, remained the only kind of measure that could be applied to the estimation of gene distances.

The detailed cytogenetic analysis of rearranged chromosomes later provided a new kind of map, the chromosome or cytological map (DOBZHANSKY 1929, 1930a, 1930b, 1932a, 1932b). At once, a serious discrepancy was noted between the old linkage maps and even the first crude cytological maps: although the linear order of genes was the same in both, the relative distances between them were quite different, especially in the neighborhood of the centromere. Later, with the use of salivary gland chromosomes, cytological maps were greatly refined (PAINTER 1934; BRIDGES 1935, 1938). Genes could be localized in particular bands; gene intervals could be measured in microns. BRIDGES (1937) noted the poor correlation between gene distances as seen in the salivary chromosome and in linkage maps of the right end of chromosome *2R*. As a result of his studies, he proposed "coefficients of crossing over" to reflect localized variations in crossing over per unit of chromosome length.

Recently, RUDKIN (1965) produced another type of map. By measuring the UV absorption of salivary chromosome bands, he determined the relative DNA content of successive intervals along the *X* chromosome, each interval being delimited by genes whose cytological locations were accurately known. He showed that the distribution of induced mutations among the intervals correlates closely with the proportion of DNA in each. Further, when the distribution of induced chromosome breaks (data from KAUFMANN 1946) is plotted against DNA content, the correlation is even better than that for induced mutations (LEFEVRE 1969). This clearly implies that the amount of DNA available for breakage and mutation in a given region of the gametic *X* chromosome is in direct proportion

[1] This investigation was aided by a research grant from the U.S. Public Health Service (GM 13631).

to the amount of DNA in the corresponding region of the salivary gland *X* chromosome.

By contrast, as RUDKIN's Table 2 shows, the correlation between DNA content and recombination values appears rather poor. Close inspection, however, reveals that discrepancies are pronounced only at the ends of the chromosomes, that is, in the neighborhood of the telomere and centromere. Through most of the long central portion of the *X* chromosome, from white (*w*, *1*–1.5) to forked (*f*, *1*–56.7), DNA content correlates nicely with crossing over. Only in the short interval between split (*spl*, *1*–3.0) and echinus (*ec*, *1*–5.5) is the agreement less than good. These relationships, as well as the variability of crossover per band values, are shown in Table 1.

If the assumption is made that, like mutations and breaks, crossovers are randomly distributed along the *X* chromosome (except where they are specifically affected by overriding influences) in direct proportion to DNA content, it then follows that regions represented by DNA-rich, heavily compacted, prominent bands in the salivary chromsome should exhibit significantly more crossing over than do regions characterized by similar numbers of faint, thin bands. This assumption has been put to a direct test by determining the cytological and linkage positions of a number of loci which, on cytogenetic investigation, were found to be located close to the vermilion (*v*, *1*–33.0) locus. This locus not only occupies a position dead center on the standard linkage map of the *X* chromosome, but is also very near the center of the euchromatic portion of the salivary chromosome in band 10A1–2 (GREEN 1954). This band stands out in strong contrast to its immediate neighbors, which are bands of the faintest variety.

The detailed results, a preliminary statement of which appeared recently (LEFEVRE 1969), are reported below.

TABLE 1

The relationship between crossing over, DNA content, and band number in the region from white* (w) *to forked* (f)

Interval†	Crossing over		DNA content*		Bands	
	Map length of interval	Percent of *w–f*	Percent DNA in interval	Percent of *w–f*	No. of bands in interval	Percent co./band
w–spl	1.5	2.72	1.8	2.60	5	0.300
spl–ec	2.5	4.53	3.8	5.48	20	0.125
ec–ct	14.5	26.27	17.2	24.82	173	0.084
ct–v	13.0	23.55	18.0	25.97	175	0.074
v–g	11.4	20.65	13.0	18.76	146	0.078
g–f	12.3	22.28	15.5	22.37	189	0.065
w–f	55.2	100.00	69.3	100.00	708	0.078

* Data from RUDKIN (1965).

† Markers: *w*, white *1*–1.5; 3C2
spl, split *1*–3.0; 3C7
ec, echinus *1*–5.5; 3F1–2
ct, cut *1*–20.0; 7B3–4
v, vermilion *1*–33.0; 10A1
g, garnet *1*–44.4; 12B7–8
f, forked *1*–56.7; 15F1–2

MATERIALS AND METHODS

Described below are the various mutants and chromosomal rearrangements that were employed in the cytogenetic analysis of the region surrounding the *v* locus. The region considered in detail extends, on the standard linkage map, from the locus of raspberry (*ras*) at 32.8 through *v* at 33.0 and miniature (*m*) at 36.1 to the locus of furrowed (*fw*) at 38.3 (these markers are fully described by LINDSLEY and GRELL 1968), and on the cytological map, from 9E to 11A (BRIDGES' 1938 revised map). This region is photographically illustrated in Figure 1, in which the approximate cytological locations of the four loci are indicated.

Lethal mutants: X-ray-induced lethals were produced by 2000 r exposures of wild-type males. Of 320 sex-linked recessive lethals exhibiting no mutant phenotype when heterozygous for *v* and *m*, 14 were found subsequently to be cytologically normal and located in the interval between *ras* and *fw*. (These lethals were described by LEFEVRE 1969). One of these lethals was lost before analysis could be completed, leaving 13 which were suitable for this investigation. These were augmented by 3 more provided by A. CHOVNICK, two of which, *l(1)Q54* and *l(1)Q66*, are described by LINDSLEY and GRELL (1968). Of these 16 available cytologically normal lethal loci between *ras* and *fw*, 2 were found by crossover analysis to be between *ras* and *v*, 9 between *v* and *m*, and 5 between *m* and *fw*.

Deficiencies and duplications: Seven deficiencies and 4 duplications were used in the cytological analysis in order to seriate and localize, as well as possible, the 16 lethals. Figure 2 shows the cytological extents of these deficiencies and duplications, nearly all of which were described by

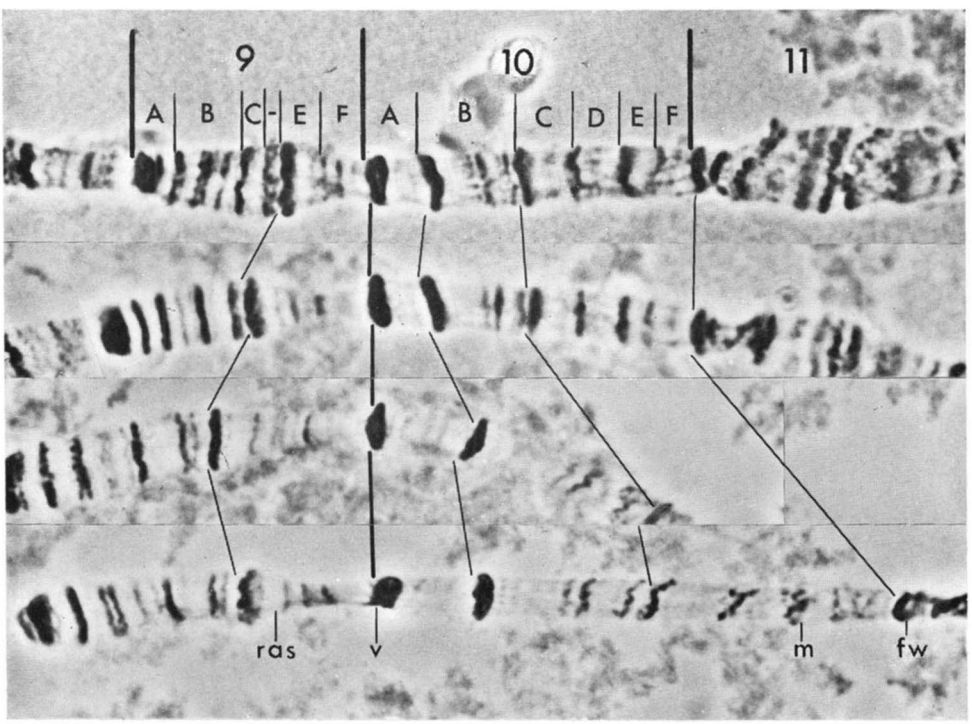

FIGURE 1.—Four chromosomes, exhibiting different degrees of stretch, illustrate the normal banding patterns found in sections 9 and 10. The cytological locations of *ras*, *v*, *m*, and *fw* are indicated. Note the extreme contrast between the large, dense bands, 10A1–2 and 10B1–2, and the faint bands between them.

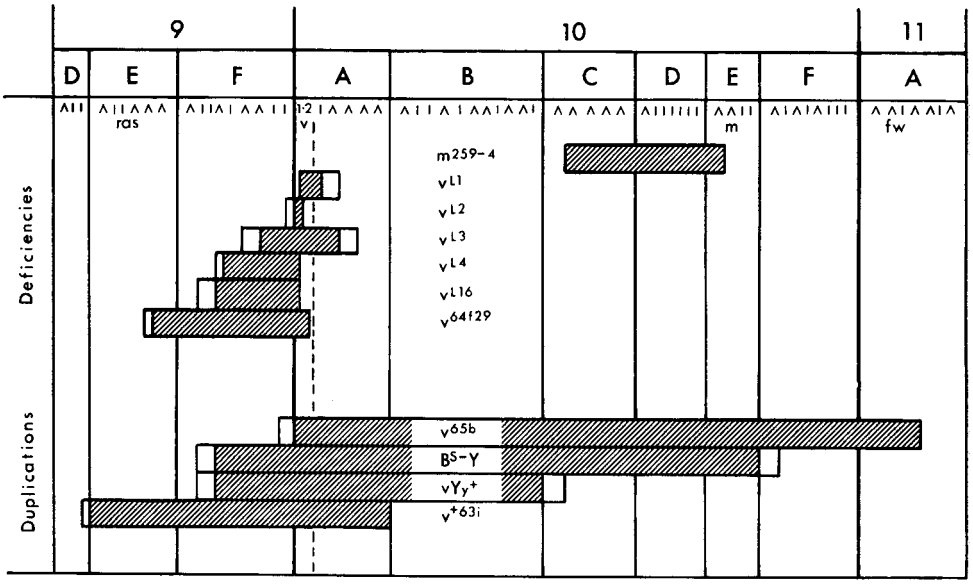

FIGURE 2.—Cytological extents of the 7 deficiencies and 4 duplications used in the cytogenetic analysis of the *ras-v-m-fw* region. Unshaded areas represent uncertainty in the exact position of the breakpoints.

LEFEVRE (1969). One previously undescribed duplication is an altered *Y* chromosome containing *X*-chromosome material extending from 9F through 10E and covering both *v* and *m*. It is a spontaneous derivative of $B^{S}v+Y\ y+\#3$, described by SCHALET in Drosophila Information Service **44**: 123 (1969), and kindly provided by him. For convenience, this chromosome will be symbolized simply as B^{S}-*Y*. A new *v* deficiency, $Df(1)v^{L16}$, extends from 9F3 through 10A1. Finally, $Df(1)v^{64f29}$ has been corrected; it extends farther to the left than previously realized. On the basis of its interactions with this set of duplications and deficiencies, any gene between 9D4 and 11A7 can readily be localized to one of the following intervals: 1. to the left of 10A, 2. in 10A, 3. in 10B, 4. between 10C2 and 10E3, and 5. to the right of 10E2. More precise localization in the 9F–10A region is possible.

Procedures for linkage crosses: All linkage crosses were carried out following a uniform procedure. Female heterozygotes were collected as virgins, aged 3 days, and placed two to an 8-dram vial containing standard cornmeal-molasses-agar-yeast food medium. Appropriately marked males were then added to each vial, and 24 hr later, the parents were transferred to fresh vials (Brood A). The mating vials were discarded. Vials were subcultured twice at 2-day intervals, followed by two further subcultures at 3-day intervals, providing a total of five broods, A–E. The progeny from each vial were counted and classified two or three times between the 12th and 18th day after the culture was started. All cultures were maintained at 25 ± 1°C.

Control crosses: Control crosses were carried out in order to confirm the linkage relationships of the marker loci, *ras*, *v*, *m*, and *fw*. In the subsequent crossover analyses, control rather than standard map values were used. The procedures outlined above were followed in two series of crosses: $ras^{2}\ v\ m/+ \times lz^{50e}\ ras^{2}\ v\ m$ (lz^{50e} is a female-fertile allele of lozenge at *1*–27.7) and $ras^{2}\ v\ m\ fw/+ \times lz^{50e}\ ras^{2}\ v\ m\ fw$.

Genetic localization of lethal mutants: In order to localize the 16 recessive lethal mutants, the same procedures as described above for the control crosses were followed. Females heterozygous for one of the lethals and the $ras^{2}\ v\ m\ fw$ marker chromosome were testcrossed with $lz^{50e}\ ras^{2}\ v\ m\ fw$ males. From nearly 2,100 to more than 24,000 progeny were raised in the individual testcrosses. The map position of each lethal was determined not only from the proportion of male

offspring that exhibited recombination with the nearest markers, but also from information gained by progeny-testing all females that were recombinant for flanking markers.

Seriation of lethal mutants: In some cases, the map positions of two or more of the lethals were so similar that the correct sequence of the loci was unsure. In these cases, and some others, crosses involving double lethal heterozygotes were carried out. From the localization crosses, stocks carrying each of the lethals with one or more of the marker loci were obtained. The availability of the duplication chromosomes made possible the production of females carrying two different marked lethals. In order to test lethals in the *v–m* interval, for example, *ras² v l¹*++/++*l² m fw* heterozygotes were crossed to *lz⁵⁰ᵉ ras² v m fw* males. According to the markers exhibited by male progeny, which occur only following a crossover between the lethals, the proper sequence of the two lethals can be determined. In addition, twice the ratio of male to female progeny, expressed as a percent, equals the map distance between the lethals. Further linkage information was gained, again, by progeny-testing all females that exhibited recombination between the flanking markers. In all but one case, an unequivocal seriation of the recessive lethals was obtained.

RESULTS

Control crosses: A large-scale control experiment was undertaken to establish the linkage relationships between *ras*, *v*, *m*, and *fw*. This was required not only because the present testcross procedures differ noticeably from those recommended by BRIDGES (BRIDGES and BREHME 1944), who was primarily responsible for the standard linkage map, but also because serious discrepancies soon became evident between the position of the markers as calculated from the control data and their standard positions. Therefore, the control crosses were repeated several times.

As shown in Table 2, only in the case of the *v–m* interval was good agreement

TABLE 2

Recombination frequencies in the ras–v, v–m, *and* m–fw *intervals in control crosses and in crosses involving recessive lethal mutants*

Interval	Broods A (4–6 d.)	B (6–8 d.)	C (8–11 d.)	D (11–14 d.)	E (14–17 d.)	Total A–E
Control crosses:						
ras–v	97/14,599 = 0.66%	107/15,226 = 0.70%	113/16,365 = 0.69%	108/18,039 = 0.60%	114/17,207 = 0.66%	539/81,436 = 0.66%
v–m	447/14,599 = 3.06%	499/15,226 = 3.28%	504/16,365 = 3.08%	547/18,039 = 3.03%	514/17,207 = 2.99%	2,511/81,436 = 3.08%
m–fw	35/6,984 = 0.50%	51/7,397 = 0.69%	62/7,846 = 0.79%	83/9,782 = 0.85%	74/9,062 = 0.82%	305/41,035 = 0.74%
Crosses involving lethals:						
ras–v	174/27,660 = 0.63%	263/39,317 = 0.67%	287/50,985 = 0.56%	274/49,173 = 0.56%	264/48,819 = 0.54%	1,262/215,953 = 0.58%
v–m	779/25,348 = 3.07%	1,154/36,981 = 3.12%	1,195/48,111 = 3.11%	1,380/46,156 = 2.99%	1,372/46,474 = 2.95%	6,180/203,070 = 3.04%
m–fw	82/12,342 = 0.66%	101/17,354 = 0.58%	167/22,210 = 0.75%	138/21,195 = 0.65%	151/20,229 = 0.75%	639/93,330 = 0.68%

Females were brooded 5 times between the age of 4 and 17 days.

found between the standard recombination value, 3.1%, and the control value, 3.08%. For the *ras–v* interval, the standard value is 0.2%; the control data showed 0.66%. For the *m–fw* interval, the standard value is 2.2%; the controls gave only 0.74%. Because one of the control values is 3 times greater, one exactly equal, and one one-third the corresponding standard value, the control results can hardly be accounted for either by chromosome abnormalities in the test stocks or by the differences in test procedures. Rather, the original determination of the linkage relationships of *ras* and *fw* may well have been incorrect.

The map positions of *ras* and *fw* as determined from the control crosses, different though they may be from their standard positions, are supported by supplementary information, also shown in Table 2, derived from crosses carried out to localize the various recessive lethals in the *ras–fw* region. Although none of the lethals is associated with any detectable cytological aberration and, individually, none significantly reduces crossing over in its vicinity, collectively their presence in testcrosses appears to result in a small reduction in recombination frequencies, as compared with control values.

For the purpose of the present study, the standard map positions of *v* at 33.0 and *m* at 36.1 are accepted, but *ras* is placed at 32.35 (not 32.8) and *fw* at 36.85 (not 38.3). The total interval from *ras* to *fw* is taken as 4.5 map units. All of the recessive lethals were located with respect to these marker positions.

Genetic localization of the recessive lethals: Altogether, 15 of the 16 recessive lethals were located by means of extensive testcrosses. The unlocated lethal, *l(1)Q66*, was obtained late in the program and turned out to be so close to one of the localized lethals that an independent localization was not carried out.

To illustrate the method of lethal localization, the data relating to one of the lethals, *l(1)L12*, are given in detail. In the testcross generation following a cross of *l(1)L12 / ras² v m fw* × *lz⁵⁰ᵉ ras² v m fw*, 42 ++ *m fw* and 223 *ras v* ++ sons appeared. Thus, 42 of 265 recombinants between *v* and *m* were between *v* and *l(1)L12*. Additionally, all daughters exhibiting recombination between *v* and *m* (++ *m fw* and *ras v* ++) were saved for progeny tests. A total of 501 such recombinants were fertile, and 78 of them proved to carry a crossover between *v* and *l(1)L12*. The combined data from both male and female progeny, then, show that 120 of 766 (or 15.67%) were *v-l(1)L12* crossovers. From these figures, a map position for *l(1)L12* at 33.49 can be obtained ($33.0 + 0.1567 \times 3.1$).

In a similar fashion, all of the lethals except *l(1)Q66* were located. In Table 3, the results are recorded. (It may be noted that *l(1)Q66* is approximately 0.02 map units to the right of *l(1)L8*.)

Seriation of the lethal mutants: Particularly in the case of the lethals just to the right of *v*, *l(1)L12*, *l(1)L8*, and *l(1)L1*, and 4 of the 5 lethals between *m* and *fw*, the map positions are so close together that their proper seriation could not be assured from the crossover data alone. However, the correct seriation of the first 3 lethals can be determined from cytological information, as shown below. For the lethals in the *m–fw* intervals, tests of doubly heterozygous females were carried out. For example, *l(1)L6* and *l(1)L17* were tested by crossing both ++ *l(1)L6 fw / v m l(1)L17* + and *v m l(1)L6* + / ++ *l(1)L17 fw* females, separately,

TABLE 3

Map positions of 15 recessive lethal mutants located with respect to ras *at 32.35,* v *at 33.00,* m *at 36.10, and* fw *at 36.85*

Interval	Mutant	Number of progeny	Recombinants identified			Map position
			(A)		(B)	$\frac{(A)}{(B)} \times 0.65 + 32.35$
ras–v			*ras–l*	*l–v*	Total	
	*l(1)S12**	11,351	15	39	54	32.53
	*l(1)Q54**	9,952	50	20	70	32.81
			(A)		(B)	$\frac{(A)}{(B)} \times 3.10 + 33.00$
v–m			*v–l*	*l–m*	Total	
	l(1)L12	24,392	120	646	766	33.49
	l(1)L8	12,604	56	260	316	33.55
	l(1)L1	7,357	33	126	159	33.64
	l(1)L4	4,712	65	64	129	34.56
	l(1)L11	5,574	85	66	151	34.75
	l(1)L9	6,689	126	53	179	35.18
	l(1)L5	3,588	63	10	73	35.68
	l(1)L16	2,065	56	4	60	35.89
			(A)		(B)	$\frac{(A)}{(B)} \times 0.75 + 36.10$
m–fw			*m–l*	*l–fw*	Total	
	l(1)L6	7,633	16	21	37	36.42
	l(1)L10	5,375	17	22	39	36.43
	l(1)L17	9,644	23	24	47	36.47
	l(1)L18	5,425	18	14	32	36.52
	l(1)L13	10,511	50	7	57	36.76

* Provided by A. CHOVNICK.

with ras^2 *v m fw* males. In the first case, 2 *v m fw* sons appeared among 3,493 daughters; in the second, 2 males without markers occurred among 3,588 females. These results place the two lethals 0.11 map units apart ($2 \times 4/7{,}081 \times 100$), with *l(1)L6* to the left of *l(1)L17*. Females exhibiting recombination between *m* and *fw* and carrying the appropriately recombined markers were saved for progeny testing. Of 24 such fertile females, 3 were not heterozygous for a lethal. From these figures, the lethals appear to be separated by 0.09 map units ($3/24 \times 0.75$). Thus, *l(1)L6* can be placed about 0.1 map units to the left of *l(1)L17*. The genetic localization tests placed them only 0.05 units apart (Table 3). A much larger experiment would be required in order to provide a more accurate positioning of genes so close together, but the true answer probably lies somewhere between the values obtained by the two different methods.

In Table 4, the results of all the lethal/lethal crosses undertaken are summarized. In no case was it necessary to modify the seriation of any lethals from that originally determined from the results of the localization crosses. However, in the case of *l(1)L17 / l(1)L18*, no sons appeared among 7,092 daughters, nor were any *m–fw* recombinant daughters heterozygous for a lethal-free chromosome. Thus, a confirmation of their sequence is not available.

TABLE 4

Crosses involving lethal/lethal heterozygotes

Cross†	Markers (interval)	Total ♀ progeny (A)	Viable males (B)	No. of fertile recombinant females: Lethal-free (C)	No. of fertile recombinant females: Total (D)	Distance between lethals*: 2(B)/(A) . 100	Distance between lethals*: (C)/(D) . marker interval
l(1)S12 / *l(1)Q54*	*ras–v* (0.65%)	3,601	3	1	9	0.17	0.07
l(1)Q54 / *l(1)L8*	*ras–m* (3.75%)	7,180	27	19	110	0.75	0.65
l(1)L12 / *l(1)L1*	*v–m* (3.10%)	6,432	5	2	109	0.16	0.06
l(1)L8 / *l(1)Q66*	*v–m*	8,339	1	0	81	0.02	...
l(1)L8 / *l(1)L1*	*v–m*	6,761	2	5	113	0.06	0.14
l(1)L6 / *l(1)L10*	*m–fw* (0.75%)	2,905	1	2	12	0.07	0.12
l(1)L6 / *l(1)L17*	*m–fw*	7,081	4	3	24	0.11	0.09
l(1)L6 / *l(1)L18*	*m–fw*	12,049	5	7	42	0.08	0.12
l(1)L10 / *l(1)L18*	*m–fw*	7,644	4	4	26	0.10	0.12
l(1)L10 / *l(1)L17*	*m–fw*	3,988	0	1	5	...	0.15
l(1)L17‡ / *l(1)L18*	*m–fw*	7,092	0	0	43	...	...

* Map distances between lethals are calculated both from the ratio of males to females and from the fraction of appropriately recombinant females that proved to be lethal free.
† In each cross, the lethal written above the line is located to the left of the one written below.
‡ Correct sequence not confirmed.

Cytological localization of lethal and marker loci: The results of crosses with the various duplication and deficiency chromosomes allow fairly close estimates of the cytological locations of *ras*, *v*, *m*, and the lethal mutants that lie to the left of 10B1–2. The positions of *fw* and the remaining lethals cannot be gauged so accurately. All of the available information regarding the cytological positions of the various mutant and marker loci is recorded in Table 5. Some uncertainty is introduced by the fact that faint banding, especially in 9F and 10A immediately around band 10A1–2, makes precise identification of breakpoints impossible.

A *ras* deficiency, *Df(1)ras–v*^{17Cc8}, has been described by VALENCIA (1966) to lack bands 9E4 through 10A4. With this additional information, the position of *ras* can be restricted to the short interval between 9E3 and 9E7. The *v* locus must be in 10A1, not only because *Df v*^{L2} is not deficient for 10A2, but also because

TABLE 5

Cytological localizations of the recessive lethal mutants and the ras, v, m, *and* fw *loci*

Mutants	Covered by *Dp*	"Allelic" with *Df*	Not "allelic" with *Df*	Cytological limits
ras	v^{+63i}	none	all	9E1–9E6
l(1)S12	v^{+63i}	v^{64f29}	v^{L16}	9E7–9F2
l(1)Q54	all but v^{65b}	v^{L3}, v^{L4}, v^{L16}	v^{L2}	9F8–9F13
v	all	all but m^{259-4}	m^{259-4}	10A1
l(1)L12	all	v^{L1}, v^{L3}	v^{64f29}	10A3–10A5
l(1)L8, Q66	all	v^{L3}	v^{L1}	10A6–10A7
l(1)L1	all	none	all	10A8–10A11
l(1)L4, L11, L9	all but v^{+63i}	none	all	10B1–10C2
l(1)L5, L16	v^{65b}, B^{S}-*Y*	m^{259-4}	all *v Df*'s	10C3–10E2
m	v^{65b}, B^{S}-*Y*	m^{259-4}	all *v Df*'s	10E3*
l(1)L6, L10, L17, L18, L13	v^{65b}	none	all	10F1–11A7
fw	v^{65b}	none	all	10F1–11A7

* The *m* locus lies immediately to the right of *Df* m^{259-4} (DORN and BURDICK 1962).

Df v^{L1}/*Df* v^{L4}/*v Y* y^{+} females exhibit a typical *v* phenotype. (These females are lethal in the absence of a duplication for the *v* locus.) Finally, the *m* locus must be in 10E3, immediately to the right of *Df* m^{259-4} since m^{+} recombinant sons can be obtained from *Df* m^{259-4}/*m* females (DORN and BURDICK 1962).

DISCUSSION

Salivary chromosome band–crossover relationships: Some of the mutants utilized in this study lend themselves to an analysis of the relationships between band structure and the distribution of crossovers. Best for this purpose are *l(1)L12* and *l(1)L8*, both confined to the faintly banded region between 10A1–2 and 10B1–2. These, together with *ras* and *v*, have been quite accurately positioned on both the linkage and the cytological map. The "anchor" locus is *v*, located genetically at 33.0 and cytologically in band 10A1. It might be noted that the *v* locus (more accurately, the mutant site of the v^{1} allele) cannot be literally at the left edge of 10A1, for in that case *Df* v^{L1} (as well as *Df* v^{166}; see LEFEVRE 1969) would not exhibit a *v* phenotype unless the *v* locus were mutant, rather than being included in the deficiency. If that were the case, then *Df* v^{L1}/*Df* v^{L4} heterozygotes should live as *v* females. Since they, in fact, die, the *v* locus must be deleted in both deficiencies and thus physically located within the substance of band 10A1–2.

The recessive lethal mutants, *l(1)L12* and *l(1)L8*, lie close together on the linkage map, about 0.5 units to the right of *v*. Neither of them is associated with 10A2 as both are viable in combination with *Df* v^{64f29}. The lethal closest to *v*, *l(1)L12*, must be in the region from 10A3 to 10A5, only 2 to 4 bands from *v*. Thus, the intensity of crossing over between *v* and *l(1)L12* may be characterized as being between 0.12 and 0.25 map units per band. The most probable value, on the assumption that *l(1)L12* is, in fact, associated with band 10A4, would be 0.17. The other lethal, *l(1)L8*, is confined to the 10A6–7 region, 2 or 3 bands to the

right of *l(1)L12*. Since these two lethals exhibit about 0.06% recombination, crossing over in the *l(1)L12–l(1)L8* interval amounts to some 0.02–0.03 map units per band, a level no more than a sixth that characteristic of the *v–l(1)L12* interval, which is approximately the same length in band number.

If *ras* is placed at 9E5, with an error of perhaps one band in either direction, it is separated from *v* by 19 ± 1 bands and shows 0.65% recombination with *v*. These values suggest that, in the *ras–v* interval, there is about 0.035% crossing over per band. In successive intervals, then, crossing over per band values change from 0.035% (*ras–v*) to 0.17% (*v–l(1)L12*) to 0.02–0.03% (*l(1)L12–l(1)L8*).

The entire *X* chromosome has a map length of 66.0 units and BRIDGES' (1938) map shows 1,012 bands. Thus, on the average, there is 0.065% crossing over per band. From this information, following BRIDGES' (1937) approach, we may calculate that the 3 intervals exhibit coefficients of crossing over as follows: *ras–v*, 0.54; *v–l(1)L12*, 2.6; and *l(1)L12–l(1)L8*, 0.31–0.46.

The question immediately arises as to what special properties of the middle interval favor the much greater apparent concentration of crossovers there in comparison with that exhibited by the two surrounding intervals. After all, salivary chromosomes do not cross over; their morphology does not necessarily bear any correlation with that of meiotic chromosomes. Yet, only in the middle interval is there a prominent, dark-staining band; the adjacent intervals are populated primarily by faint, thin, difficult-to-resolve bands. Of the 19 or so bands in the *ras–v* interval, only 9F5–6 is typically seen as darkly staining, but even it is thin and it occurs in a narrow, "goose-neck" region. The bands in the *l(1)L12–l(1)L8* interval are especially faint. However, if the density of stain in a band is proportional to its DNA content, as it should be, then because of the presence of 10A1–2, the *v–l(1)L12* interval should not be considered as short; in DNA content it would be "long." If crossing over is distributed from interval to interval in proportion to DNA length, as it may well be, then the *v–l(1)L12* interval would be expected to show an appreciable amount of crossing over, much more than shown by the *l(1)L12–l(1)L8* interval, an amount perhaps approaching that evidenced by the apparently longer *ras–v* interval. Since this is, in point of fact, precisely what is observed, serious consideration must be given to the hypothesis that the quality, in terms of DNA content, of salivary chromosome bands, not their number, correctly reflects the DNA "length" of corresponding meiotic chromosome regions and that crossing over occurs randomly along the length of meiotic chromosomes in proportion to DNA content. That is to say, there is no reality to BRIDGES' (1937) coefficients of crossing over; rather, the physical length of chromosomes is distorted by the nonhomogeneous manner in which DNA is compacted. However, for the hypothesis to be valid, the concentration of DNA associated with a given band must represent a tightly folded configuration of the main genetic axis (see DUPRAW and RAE 1966); bands cannot be composed of accessory DNA extending laterally from an axis that passes straight through the band.

It is not necessary to conclude that, at the time of crossing over, the DNA of meiotic chromosomes is nonhomogeneously compacted in direct mimicry of the

salivary chromosome condition. It is sufficient to conclude that distances, in terms of DNA lengths, between successive loci along the axis of the meiotic chromosome are in exactly the same proportion as they are in the salivary chromosome.

DNA content and crossover frequencies: RUDKIN (1965) measured the relative DNA content of various intervals along the entire length of the salivary *X* chromosome. When the genetic extents of the intervals are compared with their respective DNA contents, a serious disproportion is seen at the left end of the chromosome. The region from yellow (*y*, *1*–0.0) to zeste (*z*, *1*–1.0) contributes only 1.5% of the total map length, despite containing 12.1% of the total *X* chromosome DNA. However, crossing over is inhibited in the vicinity of the telomere; similarly, it is inhibited in the vicinity of the centromere. (It might be noted that, compared with the *X* chromosome, the major autosomes are characterized by a much more pervasive centromeric inhibition of crossing over, extending at least half the length of each arm. In partial compensation, perhaps, telomeric inhibition appears to be much less pronounced.) Throughout the long internal portion of the *X* chromosome from *w* to *f*, as shown in Table 1, crossovers are, in fact, distributed in direct proportion to DNA content. Even the short *w–spl* interval, composed primarily of highly compacted bands and long considered to be an outstanding example of a region showing a high coefficient of crossing over (REDFIELD 1955), exhibits a level of recombination entirely consistent with the proportion of DNA that it contains. The concept of the random distribution of crossovers in proportion to DNA content may safely be applied, therefore, to regions of the chromosome that are removed from the overriding influences of the telomere and centromere.

Unfortunately, RUDKIN (1965) did not provide figures for the DNA content of short intervals near *v*. Thus, it is impossible to verify the implication that the *v–l(1)L12* interval has a DNA length nearly equivalent to that of the much longer (by band count) *ras–v* interval. Nonetheless, the principal assumption seems clearly justified that regions characterized by a concentration of dark-staining, highly compacted bands exhibit more crossing over than do regions containing equal numbers of faint, thin bands. This correlation between band quality and crossing over is not limited to the *X* chromosome. BRIDGES (1937) specifically identified the plexus–brown (*px*, *2*–100.5; *bw*, *2*–104.5) interval as having "a very high coefficient [of crossing over] of 2.4." As his map of *2R* shows, *px* and *bw* are located on either side of a series of dark bands found in sections 59A–D.

Crossover–nucleotide length correlations: RUDKIN (1965) estimated the total DNA content of the *X* chromosome to be 3×10^7 nucleotide pairs per haploid chromosome strand, and, since the entire chromosome has 1,012 bands, an average band is associated with approximately 3×10^4 nucleotide pairs per strand. The *w–f* interval, in which crossing over occurs freely, is 55.2 map units in length, contains 708 bands (from 3C2 to 15F1–2), and, according to RUDKIN, 69.3% of the total *X*-chromosome DNA. In this delimited interval, an average band also contains about 3×10^4 nucleotide pairs, but is associated with 0.078% crossing over. Obviously, the distribution of bands of different size classes is not different in the middle as compared with the ends of the chromosome, but crossing over

per band in the *w–f* interval is noticeably higher than 0.065%, the figure calculated above for the entire *X* chromosome. Considering the uncertainty accompanying these calculations, we may accept a value of 0.08% as representative of the amount of crossing over that is associated with an average band in the long internal portion of the *X* chromosome. Since an average band is simultaneously associated with 3×10^4 nucleotide pairs, we may infer that 0.01% crossing over occurs over a length of some 3,700–3,800 nucleotide pairs. If it were not for the interference with crossing over at its ends, the *X* chromosome would have a total map length of about 80 units.

Band–gene relationships: Information about the properties of an "average" band provides no useful clue to the constitution of a specific band such as 10A1–2. However, if the amount of crossing over attributable to a particular band can be determined, then it may be characterized by comparison with the average band. For example, about 0.7% recombination occurs between *l(1)Q54* and *l(1)L12;* they are separated by 6 to 10 bands. Except for 10A1–2 itself, however, the bands in this interval are extremely faint, some perhaps so faint that they would not have been resolved by RUDKIN's technique. (He noted that the smallest measurable band corresponded to 5×10^3 nucleotide pairs.) The *l(1)Q54–l(1)L12* interval, with 0.7% crossing over, should contain well over 2.5×10^5 nucleotide pairs; after subtracting the contribution of 4 to 8 bands of minimum size, we can estimate that 10A1–2 alone, containing the bulk of the DNA in the interval, includes at least 2×10^5 and possibly as many as 2.4×10^5 nucleotide pairs and by itself is responsible for 0.60–0.65% crossing over. This value may be taken as the maximum for individual *X*-chromosome bands, for none appears to be larger than 10A1–2.

Besides band 10A1–2, the only other prominent band in Section 10 is 10B1–2. Although the 3 lethals in 10B have not been cytologically localized, the amount of recombination in the interval between *l(1)L1* and *l(1)L4* which includes band 10B1–2 is about 50% greater than that between *l(1)L4* and *l(1)L9*, an interval that includes only relatively faint bands. This fact argues strongly that the association of prominent bands and large crossover values is not a unique peculiarity of band 10A1–2; rather, it is a general phenomenon applicable throughout the chromosome.

Despite the wealth of nucleotides associated with 10A1–2, no more than two genetic functions can be ascribed to it: "vermilionness" and lethality. In point of fact, in none of the available mutants affecting 10A1–2 has lethality been separated from an effect on the *v* locus; its closest nonallelic neighbors are not included in 10A1–2. Furthermore, this band may not actually be double. BEERMANN (1962) has argued persuasively that the doublet appearance of many bands so described by BRIDGES is a fixation artifact. BERENDES (1970), using the electron microscope, failed to resolve many long-accepted doublets. Band 10A1–2 may thus be a single large band containing the *v* locus. Although male-viable *v* mutants occur, deficiency of the *v* locus may be lethal; that is to say, there may be but one genetic function associated with 10A1–2.

It is virtually impossible to imagine a single gene, or even two, no matter how

complex, that could span a length of nearly a quarter of a million nucleotide pairs. Still, there is good reason to believe that genes and bands are related one-to-one (BEERMANN 1962, 1967; SHANNON, KAUFMAN and JUDD 1970), even though bands vary from large to small. What, then, can be the function of the "quiet" (in the sense of not showing detectable structural information) DNA associated with larger bands? It is tempting to suppose that this DNA is repetitive in nature, with larger bands representing loci that are more highly replicated than are loci associated with thinner bands. Alternatively, band DNA may have regulatory function, may be involved with the initiation of synthesis, or may be nongenetic "spacer" DNA. Whatever its function may be, it clearly belongs to the main genetic axis, participates normally in recombinational events, and serves to assure that many crossovers do, in effect, occur between genes.

Studies on recombination between various *v* alleles show them to cluster exceptionally close together (GREEN 1954; SCHALET, personal communication), exhibiting much less intragenic recombination than is characteristic of other loci, such as *w*, *spl*, and *lz*, that have been similarly analyzed (JUDD 1964; WELSHONS 1965; GREEN and GREEN 1956). By no means can the known mutant sites at the *v* locus be scattered throughout band 10A1–2. They are, in all probability, restricted to a short interval about 0.10–0.15 map units from the left edge but nearly 0.5 map units from the right edge of 10A1–2, as judged from the amount of recombination exhibited between *l(1)Q54* and *v* and between *v* and *l(1)L12*.

The *w* locus is related to band 3C2 (LEFEVRE and WILKINS 1966). This band (half of the 3C2–3 doublet) is associated with about 0.3% crossing over and should contain well over 10^5 nucleotide pairs. According to JUDD (1964), the total length of the *w* locus, from w^{Bwx} to w^{sp}, is approximately 0.025 map units. This represents a DNA length of less than 10^4 nucleotide pairs. Clearly, mutant sites at the *w* locus do not occur throughout the substance of 3C2, but can occupy no more than a tenth of its length.

By contrast, WELSHONS (1965) reported that mutant sites at the *spl* locus (including Notch alleles) span a distance of 0.14 map units. This represents a significant length, nearly twice that of an average band, of about 5×10^4 nucleotide pairs. Thus, alleles at this locus are separated by distances as great as those between the 4 nonallelic lethal mutants in the 10A region, *l(1)L12*, *l(1)L8*, *l(1)Q66*, and *l(1)L1*. Yet, *spl* and Notch mutants are all associated with band 3C7. From its appearance, 3C7 can be placed in the size range of bands somewhat, but not greatly, exceeding the average. Thus, mutant sites may be distributed throughout the substance of 3C7, unlike the relationship between *w* alleles and 3C2 or *v* alleles and 10A1. Although it may be possible for a polycistronic gene to extend for 5×10^4 nucleotides, there may be appreciable amounts of "quiet" DNA intercalated between the mutant sites in 3C7.

Alleles at the *lz* locus which has been localized to section 8D, are also separated by as much as 0.14 map units (GREEN and GREEN 1956; BENDER 1967). None of the bands in 8D appear to be much more prominent than 3C7, so that mutant sites at the *lz* locus may, like *spl* alleles, extend throughout the substance of the particular band with which *lz* is associated. In any event, loci such as *spl* and *lz* can-

not possibly be limited to the spaces between bands; the length of interband spaces has been estimated to be of the order of 10^3 nucleotide pairs (BEERMANN 1966). By contrast, the *w* locus might occupy a position that includes some or all of the region between band 3C1 and 3C2 (see GANS-DAVID 1953; ARCOS-TERÁN and BEERMANN 1968). This view, of course, implies that interband spaces are relatively uniform, that dark bands are not formed by packing thin bands close together without interband separation.

In sum, there can be no simple, uniform relationship between genes and bands throughout the genome. The most delicate bands, such as those in the region between 10A1–2 and 10B1–2, may be totally occupied by individual, vital genes that recombine as infrequently as do alleles of genes like *spl* and *lz*, which are contained within a single, larger band. By contrast, the *v* locus is associated with a particularly large band containing, by any reasonable criterion, what seems to be a remarkable excess of DNA.

The finding that crossover frequencies are correlated with band size has significance in connection with the "master-slave" hypothesis of CALLAN (1967). The band cannot consist of slave replicas of a master locus if recombination is restricted to the master locus. Further, the studies of LAIRD and MCCARTHY (1969) suggest that there is far too little repetitive DNA in Drosophila for all of the band DNA to be repetitive in nature. Even the somewhat greater proportion reported by ENTINGH (1970) is insufficient to support the proposition that bands represent tandem replicas of single loci. If evolutionary divergence of repeated units has occurred to such an extent that annealing procedures fail to identify the bulk of the once identical repeated units, then one would expect to find numerous nonallelic loci associated with single larger bands; but this does not seem to be the case. Thus, the genetic significance of the "quiet" DNA remains to be explained.

The consistency of crossover data: Without question, recombination values are subject to considerable variation; statistical, environmental, and genetic influences combine to frustrate crossover analysis. Yet, the present experiments demonstrate the uniformity that can be achieved, at least in the *ras–fw* interval, by the application of rigid standards of procedure. Most surprising is the absence of an effect of maternal age on the crossover values (see Table 2). Perhaps this simply reflects the exclusion of very young females, 0–4 days of age, from the group tested; perhaps the central portion of the *X* chromosome is less sensitive to disturbing influences than are regions near the telomere and centromere; perhaps the large scale of the present testcrosses effectively damped out the usually encountered variation.

With the extension of the present kind of analysis to the entire *X* chromosome, an internally consistent linkage map could be produced that would more accurately reflect genetic intervals than does the existing map. For the present, the principle that dark bands represent regions having more crossing over than do light bands, together with information about the specific amount of crossing over that occurs in particular regions, can be applied in order to estimate the cytological location of genes from linkage data, and *vice versa*.

Predictions: The concept that linkage maps can provide a more accurate assess-

ment of the relative distances between genes than do cytological maps is a sharp departure from commonly accepted views. Nonetheless, a few readily testable predictions, mostly stemming from a consideration of data recorded by LINDSLEY and GRELL (1968), together with an evaluation of the appropriate salivary chromosome regions, are presented in the hope that they will be checked by interested investigators who possess the necessary test stocks.

1. Either the map position or the cytological location, or both, of diminutive (*dm*, *1*–4.6; 3D1–2) is in error; *dm* must be located further to the right than 3D1–2, or else its map position must be considerably closer to *spl*.

2. The map position of echinus (*ec*, *1*–5.5; 3F1–2) is in error by at least 0.5 map units; its cytological location is essentially correct.

3. The interval from ruby (*rb*, *1*–7.5; 4C6–8) to rugose (*rg*, *1*–11.0; 4E1–3) cannot be correctly described; either the map positions (most likely that of *rg*) are badly in error, or else the cytological information is wrong.

4. The *fw* locus must be in 11A3–5.

A caveat: Only provisional acceptance of the specific nucleotide-pair values calculated above is warranted. That portion of the salivary gland *X* chromosome surveyed by RUDKIN (1965) may not contain 3×10^7 nucleotide pairs; the true value may be higher or lower, though surely not by an order of magnitude. The distribution of DNA from one region of the chromosome to the next is also subject to correction; RUDKIN measured only four chromosomes. Even the band number may be revised, as a consideration of the reality of double bands indicates (BERENDES 1970). The standard linkage map, as shown above, is not free from defects, nor is the cytological map. Future work will surely lead to refined values for the amount of crossing over that is associated with an average band, as well as its nucleotide content. It is unlikely, however, that future work will require abandonment of the proposition that a large band represents a region having an appreciable length of DNA in which proportionally more crossing over occurs than is found in a reigon represented by a thin band.

With pleasure, I acknowledge the excellent technical assistance provided by Mrs. BERIT N. BARNES, Miss MARY JEAN SMITH, and Mrs. KATHLEEN PETERSON.

SUMMARY

A cytogenetic analysis of a series of recessive sex-linked lethal mutants located in the *ras–v–m–fw* region indicates that the frequency of recombination observed between neighboring loci is directly correlated with the salivary chromosome banding pattern. Regions containing dark-staining heavy bands, such as 10A1–2 with which the *v* locus is associated, exhibit more crossing over than do regions populated by equal numbers of thin, faint bands. Information provided by RUDKIN (1965) on the relative DNA content of successive intervals along the *X* chromosome can be used to calculate the length of DNA, in nucleotide pairs, that is associated with specific recombination frequencies in the long interval from *w* to *f*, which is free from interference by the telomere and centromere. Further, the nucleotide length of an "average" band can be calculated, together with the

amount of crossing over that can be attributed to it. These correlations make possible predictions of the cytological locations of genes from linkage data, and *vice versa*.—Even though bands vary in size from those barely resolvable and containing 5×10^3, or fewer, nucleotide pairs (per haploid strand) to those like 10A1–2, containing nearly 2.5×10^5 nucleotide pairs, there appears to be a one-to-one relationship between genes and bands. Thus, larger bands contain a great excess of "quiet" DNA whose significance is not immediately evident. A smaller band like 3C7, with which the *spl* locus is associated, appears to have mutant sites distributed throughout its substance, but even such a band might have a significant amount of "quiet" DNA intercalated between mutant sites. Only the thinnest bands, such as those between 10A1–2 and 10B1–2, may be totally composed of information-containing DNA associated with specific loci.

LITERATURE CITED

ARCOS-TERÁN, L. and W. BEERMANN, 1968 Changes of DNA replication behavior associated with intragenic changes of the white region in *Drosophila melanogaster*. Chromosoma **25**: 377–391.

BEERMANN, W., 1962 Riesenchromosomen. Protoplasmologia **6**: 1–160. ——, 1966 Differentation at the level of the chromosomes. pp. 24–54. In: *Cell Differentiation and Morphogenesis*. Edited by W. BEERMANN. Wiley, New York. ——, 1967 Gene action at the level of the chromosome. pp. 179–201. In: *Heritage from Mendel*. Edited by R. A. BRINK. Univ. Wisconsin Press, Madison.

BENDER, H. A., 1967 Radiation induced tandem duplications in *Drosophila melanogaster*. Genetics **55**: 249–254.

BERENDES, H. D., 1970 Polytene chromosome structure at the submicroscopic level. Chromosoma **29**: 118–130.

BRIDGES, C. B., 1927 The relation of the age of the female to crossing over in the third chromosome of *Drosophila melanogaster*. J. Gen. Physiol. **8**: 689–700. ——, 1935 Salivary chromosome maps. J. Heredity **26**: 60–64. ——, 1937 Correspondences between linkage maps and salivary chromosome structure, as illustrated in the tip of chromosome *2R* of *Drosophila melanogaster*. Cytologia, Fujii Jubilee Vol. pp. 745–755. ——, 1938 A revised map of the salivary gland *X* chromosome of *Drosophila melanogaster*. J. Heredity **29**: 11–13.

BRIDGES, C. B. and K. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. **552**.

CALLAN, H. G., 1967 The organization of genetic units in chromosomes. J. Cell Sci. **2**: 1–7.

DOBZHANSKY, TH., 1929 Genetical and cytological proof of translocations involving the third and fourth chromosomes of *Drosophila melanogaster*. Biol. Zentrbl. **49**: 408–419. ——, 1930a Cytological map of the second chromosome of *Drosophila melanogaster*. Biol. Zentrbl. **50**: 671–685. ——, 1930b Translocations involving the third and fourth chromosomes of *Drosophila melanogaster*. Genetics **15**: 347–399. ——, 1932a Cytological map of the *X* chromosome of *Drosophila melanogaster*. Biol. Zentrbl. **52**: 493–509. ——, 1932b Studies on chromosome conjugation. I: Translocations involving the second and the *Y* chromosomes of *Drosophila melanogaster*. Z. Ind. Abst. Vererbl. **60**: 235–286.

DORN, G. L. and A. B. BURDICK, 1962 On the recombinational structure and complementation relationships in the *m–dy* complex of *Drosophila melanogaster*. Genetics **47**: 503–518.

DUPRAW, E. J. and P. M. M. RAE, 1966 Polytene chromosome structure in relation to the "folded fibre" concept. Nature **212**: 598–600.

ENTINGH, T. D., 1970 DNA hybridization in the genus Drosophila. Genetics **66**: 55–68.

GANS-DAVID, M., 1953 Étude génétique et physiologique du mutant *z* de *Drosophila melanogaster*. Bull. Biol. (France Belg.) (Suppl.) **38**: 1–90.

GREEN, M. M., 1954 Pseudoallelism at the vermilion locus in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. **40**: 92–99.

GREEN, M. M. and K. C. GREEN, 1956 A cytogenetic analysis of the lozenge pseudoalleles in *Drosophila melanogaster*. Z. Ind. Abst. Vererbl. **87**: 708–721.

JUDD, B. H., 1964 The structure of intralocus duplication and deficiency chromosomes produced by recombination in *Drosophila melanogaster*, with evidence for polarized pairing. Genetics **49**: 253–265.

KAUFMANN, B. P., 1946 Organization of the chromosome. I: Break distribution and chromosome recombination in *Drosophila melanogaster*. J. Exptl. Zool. **102**: 293–320.

LAIRD, C. D. and B. J. MCCARTHY, 1969 Molecular characterization of the Drosophila genome. Genetics **63**: 865–882.

LEFEVRE, JR., G., 1969 The eccentricity of vermilion deficiencies in *Drosophila melanogaster*. Genetics **63**: 589–600.

LEFEVRE, JR., G. and M. D. WILKINS, 1966 Cytogenetic studies on the white locus in *Drosophila melanogaster*. Genetics **53**: 175–187.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. **627**.

PAINTER, T. S., 1934 The morphology of the *X* chromosome in salivary glands of *Drosophila melanogaster* and a new type of chromosome map for this element. Genetics **19**:448–469.

PLOUGH, H. H., 1917 The effect of temperature on crossing over. J. Exptl. Zool. **24**: 147–209.

REDFIELD, H., 1955 Recombination increase due to heterologous inversions and the relation to cytological length. Proc. Natl. Acad. Sci. U. S. **41**: 1084–1091.

RUDKIN, G. T., 1965 The relative mutabilities of DNA in regions of the *X* chromosome of *Drosophila melanogaster*. Genetics **52**: 665–681.

SHANNON, M. P., T. C. KAUFMAN and B. H. JUDD, 1970 Lethal patterns of mutation in the zeste–white region of *Drosophila melanogaster*. (Abstr.) Genetics (Suppl.) **64**: s58.

VALENCIA, R. M., 1966 Report of new mutants. Drosophila Inform. Serv. **41**: 58.

WELSHONS, W. J., 1965 Analysis of a gene in Drosophila. Science **150**: 1122–1129.