THE GENETICS AND CYTOLOGY OF *DROSOPHILA SUBOBSCURA*

I. ELEMENT A. SEX-LINKED MUTANTS AND THEIR STANDARD ORDER

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(With One Text-figure)

Introduction

Christie (1939) published a linkage map of the sex chromosome of *Drosophila subobscura*. He obtained most of the mutants which he described from X-rayed material. He did not examine his material cytologically, and it may therefore have contained inversions.

Unfortunately, this paper is not an extension of Christie's work, as most of his mutants have been lost. It describes the linkage experiments made during the last few years, chiefly with mutants that have arisen spontaneously during that time. It includes data obtained from cultures which formed part of experiments on lethals and secondary non-disjunction, if the lethal or the maternal *Y*-chromosome have not been found in them after adequate tests.

Cytological examination of crosses between the multiple stocks made from the large experiments which form the great body of these data show that they are free from inversions on the *X*-chromosome. A few small, genetically unlocalized inversions have been found in the sex chromosomes of some stocks of sex-linked mutants. Data from a few crosses containing these may possibly be included.

While most of the observations here published, and all the calculations, were made by the author, this paper is to some extent a joint production of the Department of Biometry. In particular I wish to thank Dr U. Philip, who is responsible for all the cytological examination of the material; Dr J. M. Rendel for allowing me to include data from his experiments; Miss J. E. Jermyn for scoring many of the cultures; Prof. J. B. S. Haldane for devising new statistical methods, details of which will be published elsewhere; the Rockfeller Foundation for grants; and Rothamsted Experimental Station for hospitality. I am also grateful to Dr Philip and Miss Jermyn for allowing me to bring the chromosome map published in this paper up to date by including the loci of *dried wing*, *cocoa* and *bobbed*, which are based on their unpublished work.

Experimental procedure

Drosophila subobscura is fed on a maize meal-agar-molasses food. It is mated for 5–8 days in vials, and then transferred to half-pint milk bottles. During the early experiments it was kept at 18°C., as it was believed to become sterile at 20°C (Christie, 1939). We can now keep the species at temperatures up to 24°C., both stocks that have been established for 10 years, and freshly caught wild flies. The temperature fluctuates, being that of the laboratory; no incubators are used. The first flies emerge about 21–24 days after transferring the parents to the bottles. No controlled experiments on the effect of

temperature have been made since Rendel (1945) discovered that *D. subobscura* does not mate in the dark.

Cultures may be raised from several females mated to several males, from one female mated to several males, or from one female mated to one male. A female or group of females may be transferred from culture bottle to culture bottle, laying eggs in each. Groups of cultures obtained in this way and thus having one or more mothers in common are grouped into a 'family'. As some of the females may have died in a bottle before the group was transferred to the next, genetical homogeneity cannot always be expected between the sister cultures of a family that has more than one mother.

The data presented in this paper are from experiments in which three or more loci were usually segregating in the males at least. The constitution of these crosses is rarely given, but all data that give information on the point at issue, for instance, the viability of a given mutant or the recombination between two mutants when they enter a cross in coupling, are considered together. Most estimates are based on a fraction only of the total counts. The reasons for rejecting certain data are given wherever this was done.

DESCRIPTION OF MUTANTS

The square brackets after the name of each mutant include a statement of when, where and by whom it was discovered, or a reference to an earlier account. Autosomal mutants here designated by symbols are described in *D.I.S.* vols. **11–17.**

bg$_1$, *bulge*$_1$. [Nov. 1939. Several males in extraction of *sh wt*. S.] Surface of the eye enlarged. Expression variable, more extreme in males, where the eye surface may be $1\frac{1}{2}$ times its normal area and folded like the cerebral hemispheres of the lower mammals. Viability poor, and therefore penetrance never tested. Allelomorph lost.

bg$_2$, *bulge*$_2$. [March 1942. Several males among the offspring of two sib pairs from a culture heterozygous for *ct sn cp cd* and *l*(1)40*j*. S.] Similar to *bg*$_1$, but less extreme. Cannot be scored on females, and male penetrance may be incomplete. Has not been tested with *bg*$_1$ for allelomorphism. The recombination data shown not to be heterogeneous on p. 280 contain crosses made with both *bg*$_1$ and *bg*$_2$. Therefore the two mutants are allelomorphic or a gene doublet (Grüneberg, 1937).

cd, *cardinal*. [29 Feb. 1940. One male in F_1 from *hk* ♀♀ and *r* ♂♂. S.] Transparent scarlet eye colour. Orange testis sheath as in the wild type. Penetrance complete. It has been found to be non-autonomous in two gynandromorphs and autonomous in one. It is therefore probably homologous with *vermilion* in *melanogaster* and *pseudoobscura*, but the name will not be changed until this has been confirmed by conclusive transplantation or feeding experiments.

cp, *copper*. [May 1939. Many males in F_1 from *ct* ♀♀ × *wi* ♂♂. Recognized by the pale testis sheath. Not present in the stocks of *ct* or *wi* S.] Transparent eye colour browner than wild type, more like wild-type eye of *pseudoobscura*. Testis sheath very pale. Penetrance complete. Difficult to score on females, especially when more than 2 days old. The *cp cd* double recessive, a bright orange, is more different from *cp*$^+$ *cd* than *cp cd*$^+$ is from *cp*$^+$ *cd*$^+$.

ct, *cut*. [Christie (1939)]. Small pieces cut out from the wing margin. Penetrance complete. Expression variable, usually greater in males than homozygous females, where it may be reduced to a single 'bite' on the inner margin of one wing, or even a disarrangement of the marginal hairs. The lower joints of the arista are occasionally thickened.

ct^{fr}, fringed. [17 Nov. 1937. One male in F_1 from *r* ♀♀ × ♂♂ carrying a short fourth longitudinal vein segregating in F_2 from female named Studland 70 (Gordon, Spurway & Street, 1939). S.] Numerous small bites out of the wing margin throughout its length. Occasional flies with thickened aristae have been found. The expression is greater than that of *ct*, and the two allelomorphs can be scored separately when segregating in the same experiment. This phenotype is only seen in the male, where the penetrance is complete. Most females have entirely wild-type wings, not even the marginal hairs being affected. The few that do show any effect have one or a few large 'bites' removed from their wings.

ct^{an}, antennapedia. [Dec. 1939. Many males from a cross *ct^{fr} cv* + / *ct* + *y* ♀♀ × *ct^{fr} cv* + ♂♂ showed a marked antennapedia, which on outcrossing was found to be inseparable from *fringed*. S.] In extreme forms the antenna is overgrown and frequently branched, looking superficially like an antler or a crustacean maxillipede. Penetrance and expression dependent on age of culture, being complete in freshly hatching cultures and almost nil in old cultures. Wing character like *fringed* in phenotype, sex limitation and penetrance, and is always used for scoring. This allelomorph is called '*aristapedia ct^{ar}*' in *D.I.S.* 13.

Females of the constitution *ct^{fr}/ct* usually have a pronounced *cut* phenotype. This was thought to be 100% penetrant and has been used in some linkage crosses. However, overlaps with *ct*$^+$ have been found in a few cultures. *ct^{an}/ct* females have a similar phenotype which seems 100% penetrant, but has not been tested extensively. *ct^{fr}/ct^{an}* females usually have normal wings. They occasionally have slightly thickened antennae.

The interaction in the heterozygotes *ct^{fr}/ct* and *ct^{an}/ct* resembles that of the autosomal allelomorphs *spineless* and *aristapedia* in *melanogaster*. *ss/ss* has normal antennae but *ss^a/ss^a* and *ss^a/ss* have an aristapedia. Though mutations at the two loci produce very different phenotypes it is curious that the antennae should be altered by both.

In this investigation allelomorphism is assumed if the double heterozygote shows the abnormal phenotype and similar linkage relations are obtained. Therefore gene doublets which interact with one another in the heterozygote may have been considered allelomorphic. I have made no extensive search of the progeny of such heterozygotes for the segregation of wild-type flies representing a cross-over between the loci. The appearance of two phenotypically *ct*$^+$ flies among the sons of *ct^{fr}/ct XXY* females (Spurway, unpublished) may be an example of this. It will be discussed in a later paper.

cv, cross-veinless. [Christie (1939).] Both cross-veins absent. Body colour browner than wild type. Frequently a small spot of vein tissue or a slight thickening of the costal vein between the junctions of the second and third longitudinal veins. Penetrance complete.

Lethal mutations. Many segregations showing or suggesting the presence of lethal mutations have been found. Only two of these will be considered.

l(1)40*j*. [Sex-linked lethal found Oct. 1940. S.] The offspring of a female (E 11/9) of the constitution + *cd y wi* / *sn* + + + were:

	♂♂								
Total ♀♀	*cd y*	*sn*	*sn cd y*	+ + +	*sn y*	*cd*	*sn cd*	*y*	Total
147	21	0	8	0	0	22	7	3	61

A lethal mutation close to the locus of *cd* was present on the chromosome carrying *sn* which had entered the cross from the father of E 11/9. It had presumably arisen by

mutation in him. *l*(1)40*j* has been used extensively in linkage work, and provides crucial evidence for map order (p. 277). No imago has been found that has been thought to be hemizygous for this lethal.

l(1)41*k*. [Sex-linked lethal found Nov. 1941. S.] The F_2 from the cross

$$sn\ cd\ y + wi\ ♀♀ \times + + + scl\ wi\ ♂♂$$

was reared as single female cultures after mass matings. Seven cultures were examined, and lethals segregated in two of them. Considering only the segregation of *cd* and *y* in males the counts were:

		♂♂				
Culture	Total ♀♀	*cd y*	+ +	*cd*	*y*	Total
2	83	28	0	16	5	49
6	71	22	2	10	4	38

Only one F_3 culture was obtained. The mother of this culture, A, was from 2 and of the constitution $+ + y\ wi\ /\ sn\ cd\ y\ wi$.

		♂♂		
Culture	Total ♀♀	*cd*	+	Total
A	39	22	2	24

Eight F_4 cultures were reared from this, but none contained the lethal, which was thus lost.

Both the lethals entered the cross on a paternal chromosome, and therefore must have arisen as mutations in the *scl wi* males.

Considering the ratio of *cd* to cd^+ cultures 2 and A are obviously homogeneous. The homogeneity χ^2 between the total of 2 + A and 6 is 0·426, $n = 1$ and P is just above 0·5. The ratios of *y* to y^+ in 2 and 6 are also homogeneous. Therefore the two lethals almost certainly arose from the same mutation, and were inherited from the same grandfather. This mutation must have occurred early enough in the development of the germ line to have segregated into at least two spermatozoa.

scl, short costal. [April 1939. Widely distributed in stock of pp^{cn}. S.] Wing margin defective from the end of the third longitudinal vein to a little way beyond the end of the fourth. Penetrance incomplete, expression variable. In most flies some marginal hairs are absent. After selection for stronger expression a small sliver seems cut from the wing, the costal vein being completely absent. As the hairs on the surface of the wing are unaffected they hang over, making the margin look complete. Thus the flies no longer have one of the characters of the genus which Kikkawa & Peng (1938) attribute to Fallén (1823), '*Wing* costa twice broken, reaches apex of fourth vein...'.* The relation of the costal vein to the tips of the third and fourth longitudinal veins is used as a systematic character in the Drosophilidae (Curran, 1934), and it is therefore interesting to find it altering as the result of a single gene substitution.

sin_1, *singed* 1. [Christie (1939).] All bristles and hairs twisted and coiled as though singed. Penetrance thought to be complete, but female sterile. *Singed* 1 and *singed* 2 (Christie, 1939) were allelomorphic. Both have been lost.

* I have been unable to discover in Fallén much of what Kikkawa & Peng imply to be a translation or close paraphrase of his description of the genus. Remembering the controversies concerning the homologies and correct naming of these veins I assume that the above quotation is a rendering of 'Alarum nervus auxiliaris simplex, brevis, tertiam costae partem fere occupans'.

sn, singed. [Summer 1936. Found on two separate occasions, circumstances not recorded. Called sin_3 and sin_4 in *D.I.S.* A. L. M. Christie. Aug. 1940. One male in + stock, called sin_5. J. M. Rendel.] All bristles and hairs shortened, thickened, bent, gnarled and frequently branched. Penetrance complete in sin_5 and believed so in sin_3 and sin_4, but these two latter female sterile. sin_3 and sin_4 shown to be allelomorphic by the phenotype of the heterozygous female, then latter discarded. sin_3 and sin_5 shown allelomorphic in the same way and former discarded. The sin_5 allelomorph is fertile in both sexes. Its symbol has been changed to *sn*, and the suffix has been dropped.

white and probable allelomorph. Three white-eyed males have been found. (1) Summer 1936. Dead. A. L. M. Christie. (2) Summer 1936. Frances Gordon. (3) May 1938. Stock *pp fs*. S. The latter being a double recessive with *poppy*, a scarlet eye colour, may have been a mutation to a pale but not white eye colour. I think it had white testes. (2) and (3) were mated to various virgin females. They both lived for a considerable time but were sterile.

In January 1940 J. E. Jermyn found fourteen males in stock bl_{22} with a pale pinkish yellow eye colour, and white testis sheaths. This might have been a pale allelomorph at the *white* locus. Mated to virgin sisters, *yellow* and *withered*. All sterile. A few more males, which were found in the progeny of their sisters were similarly tested, and found also to be sterile. Kalmus (1943) showed that white and very pale-eyed *D. melanogaster* flies do not react to moving contours, and Rendel (1945) found that *subobscura* does not mate in the dark. This may explain the sterility of the *white* and pinkish yellow-eyed males.

wi, withered. [Sept. 1936. Found in F_2 from wild female Slough 42 (Gordon *et al.* 1939). Out of eight paired cultures examined *wi* segregated in four. In three of these and one other a mild plexus phenotype segregated. This was not found again in the F_3. It may have been a form of *wi*. P. A. R. Street.] Wings defective. They may have all the *dumpy* shapes found in *melanogaster*. They frequently contain bubbles, and may be reduced to a small bladder. Sometimes the shape is normal but the venation is defective or plexuses are present. The penetrance is incomplete, but can be improved to 100% by selection. The two wings of a fly seldom have the same form. This phenotype is only found in females. The males cannot be distinguished from wi^+ males. Occasional phenotypically *wi* males have been found, usually in poor cultures raised at high temperatures. When tested genetically these have either not transmitted their phenotype to males or it has been shown to be due to an autosomal mutation. Of three tested cytologically one was found to be *XO*, one *XY* but trisomic for an autosome and the other apparently normal (Philip & Rendel, unpublished). Since the phenotype of $X^{wi}X^{wi}Y$ females is usually *withered* (Philip & Rendel, unpublished) there is no *wi* allelomorph on the *Y*-chromosome.

y, yellow. [June 1938. Four males in stock of Notch 2 (Christie, 1939). J. M. Rendel.] Body, hairs, and bristles yellow. Rendel (1945) found that non-yellow females are reluctant to allow *y* males to mate with them.

Of the above eleven loci of visible mutants, four (*ct, cv, white* and *y*) are probably homologous with their namesakes in other species, though ct^{fr} resembles in phenotype and sex limitation *scalloped* in *melanogaster*: *cardinal* is probably the *vermilion* of other species. Of the two *singed* loci one may be *sn* and one *f* in *melanogaster*. These are all loci of element A (Muller, 1940) which forms the whole or one arm of the sex chromo-

some of all the species so far described (Sturtevant & Novitski, 1941). The remaining four loci (*bg*, *cp*, *scl* and *wi*) have not yet been reported from other species.

SINGLE-FACTOR RATIOS

The viabilities of nine mutants which are completely penetrant are given in Table 1. All cultures are the progeny of heterozygous females, and those cultures where the females segregated had fathers carrying the recessive in question. The totals of flies

Table 1. *Viability of fully penetrant mutants*

	1	2	3	4	5	6
Sex of sample and mutant	Total showing mutant A	Total showing wild-type allelomorph B	No. of cultures n	Test of homogeneity χ^2	Test of homogeneity Probability	Viability
Males						
ct	3724	4051	132	184·875	0·0014	0·919 ± 0·026
ct^{pr}	1220	1240	43	52·576	0·13	0·984 ± 0·040
ct^{an}	2796	2897	89	93·106	0·33	0·965 ± 0·026
sn	3346	3873	112	161·512	0·0013	0·864 ± 0·026
sn$_3$	280	379	16	14·980	0·45	0·739 ± 0·058
cp	1560	1652	41	39·715	0·48	0·944 ± 0·033
cd	5982	6141	196	211·057	0·21	0·974 ± 0·018
cv	2166	2295	59	78·526	0·038	0·944 ± 0·034
y	7451	7854	244	283·422	0·038	0·949 ± 0·017
Females						
ct	636	749	22	24·666	0·26	0·849 ± 0·046
sn	2031	2175	65	75·554	0·15	0·934 ± 0·029
cd	3426	3546	120	135·600	0·14	0·966 ± 0·023
cv	1921	2054	67	81·431	0·095	0·935 ± 0·030
y	2461	2625	90	100·386	0·19	0·938 ± 0·026

showing the mutant gene and those showing its wild-type allelomorph are given in columns 1 and 2. These would be equal in both sexes on Mendelian expectation. Column 3 gives the number of cultures added together to make up these totals. These were selected from the total of cultures segregating for the mutant in question on the following basis. No other mutant within 30 units was segregating, except *wi* in male data involving *y*. All samples contained at least ten flies. Samples with less than ten flies were discarded or added to samples of the same sex if these existed in the same family. In male samples from single female cultures no sex-linked lethal was present anywhere on the chromosome. In female samples all visible and lethal mutants were assumed to be recessive. On these data the χ^2 given in column 4 was calculated as a test for homogeneity and the probability of so large a value of χ^2 is given in column 5.

If A is the total of flies showing the mutant gene, B the total showing the wild-type allelomorph, S is the sum of $A+B$, n the number of samples and s_r the number of flies in the rth sample, the estimate of viability given in column 6 is

$$\frac{A}{B} \pm \sqrt{\frac{AS}{B^3}},$$

except where the sample has been shown to be heterogeneous ($P < 0{\cdot}05$ or $0{\cdot}049$ in Table 2), when the following estimate of the standard error is used:

$$\left[\frac{AS}{B^3}\left\{1+\frac{(\chi^2-n+1)\,\Sigma s_r^2}{S\,(S-n)}\right\}\right]^{\frac{1}{2}} \quad \text{(Haldane, 1944}b\text{)}.$$

If the different ratios in different cultures segregating for the same gene pair were only due to chance, half the probabilities would be expected to exceed 0·5. No probability exceeds 0·5 and only five exceed 0·2. It is clear therefore that there is some other source of heterogeneity in many of the samples. Similarly, if the different viabilities differed from those of their respective wild allelomorphs only by chance, half of them would be expected to be greater than unity. None of them is greater than unity, though half of them do not differ from it significantly.

All the five mutants tested in both sexes seem to be equally viable in males and females. Only one mutant, the female-sterile sin_4, has a viability significantly less than 0·9.

It is unexpected that the two more extreme allelomorphs at the *cut* locus should have more regular segregations and also perhaps be more viable than the less extreme *ct* allelomorph.

Table 2. *Subdivision of heterogeneous totals in Table* 1

	1	2	3	4	5	6
	Total showing mutant	Total showing wild-type allelomorph	No. of cultures	Test of homogeneity		
Mutant	A	B	n	χ^2	Probability	Viability
			Single ♀ cultures			
ct	2816	2962	103	139·782	0·0077	0·951 ± 0·030
sn	2230	2447	83	110·644	0·019	0·911 ± 0·032
cv	261	287	11	7·110	0·72	0·909 ± 0·078
y	3070	3306	125	112·787	0·76	0·929 ± 0·023
			Mass cultures			
ct	908	1089	29	38·833	0·084	0·834 ± 0·037
sn	1116	1426	29	41·461	0·049	0·783 ± 0·031
cv	1905	2008	48	71·201	0·013	0·949 ± 0·039
y	4381	4548	119	169·405	0·0014	0·963 ± 0·026

The four totals whose probability was less than 0·05 were divided according to whether they were obtained from single female or mass cultures. The estimates are given in Table 2. They are all samples of males. Male samples are expected to be more heterogeneous than female samples because more mutants were usually segregating in them, semi-lethals were expected, and mass cultures were not examined for the presence of full lethals. Some mutants may also be more variable when hemizygous than when homozygous.

The smaller probabilities for *ct* and *sn* in the single female cultures are merely due to the larger numbers of cultures tested. The ratio of χ^2 to its expected value $n-1$ is almost the same in both sets of cultures. For *cv* and above all for *y* the mass cultures are definitely more heterogeneous. But the mutants are more viable, though insignificantly so. This must mean that in a culture where overcrowding and the secondary effects of it on the medium are expected to have occurred, the ratio of mutant flies to wild type may be increased as well as decreased. On the other hand, *ct* and *sn* in mass cultures seem equally homogeneous but significantly less viable. The expected results of overcrowding are therefore not demonstrated by these data.

However, the expected reduction in viability as compared with the wild-type allelomorph is demonstrated. As the majority of these flies were scored before they were 4 hr. old and almost all before they were 40, this relative viability is only that of the

egg, larval and pupal stages and the emergence from the pupa. The reduced viability of the abnormal fly, e.g. the increased proneness to desiccation of *y* (Kalmus, 1941) and loss of fertility for any cause, e.g. the repugnance shown for *y* males by y^+ females (Rendel, 1945), are not shown by this estimate. Therefore the estimate of viability cannot be regarded as an estimate of fitness in a Darwinian sense, even under laboratory conditions.

Table 3 gives the single-factor ratios for the remaining mutants and compounds. Scoring of all of them is difficult, and in all cases except the two *bg* allelomorphs, stocks

Table 3. *Single factor ratios of mutants and compounds not shown in Table* 1

Mutant	Sex	Total showing mutant	Total showing wild type	Estimate corresponding to viability in Table 1
ct^{fr}	♀	136	482	0·282
ct^{an}	♀	501	793	0·632
ct^{fr}/ct	♀	142	186	0·763
ct^{an}/ct	♀	110	126	0·873
bg_1 and bg_2	♀	116	157	0·739
scl	♀	877	991	0·885
scl	♂	340	345	0·986
wi	♀	2966	4491	0·660

genetically pure for the recessives have produced some wild-type flies. Further, in all the *bg* cultures *ct* and *sn* were present in coupling, in most of the *wi* cultures *y* was present in coupling, thus presumably reducing the numbers of mutants, while among the males in most of the *scl* cultures, *y* was in repulsion, presumably increasing their number. In the remainder no closely linked genes were segregating. Thus the figure in the last column is not an estimate of viability, but of the joint effects of viability, penetrance, and, in some cases, of linked genes.

Probably overlap, i.e. low penetrance, is responsible for most of the deficiency of ct^{fr}, ct^{an} and *scl*, low viability for most of the deficiency of ct^{fr}/ct, ct^{an}/ct and *bg*, while both reduce the number of *wi*. In later counts where its expression was poor, it was hard to score *scl* impartially owing to its close linkage with *y*. For these reasons I have not applied statistical methods to the results summarized in Table 3, though some of them were obviously heterogeneous.

CHROMOSOME MAP

A map of the *X*-chromosome is shown in Fig. 1. Three loci not described are included. These are *dw* (*dried wing*); *co* (*cocoa*), an orange-brown opaque eye colour; and *bb* (*bobbed*),

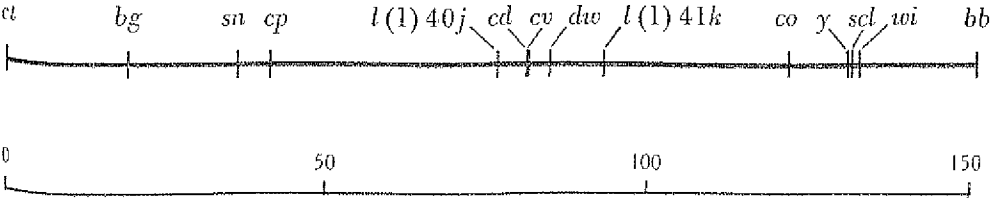

Fig. 1.

an extreme semi-lethal allelomorph, limited to females as in other species. They were located while this paper was in preparation by my colleagues Dr U. Philip and Miss J. E. Jermyn who will publish the data shortly.

Since in other species *bb* is near the spindle attachment it is provisionally assumed to be so in this, and is thus made the most extreme right-hand locus. As Christie (1939) showed that *ct* was nowhere near the end of the chromosome and this has been confirmed by the study of inversions (Philip & Spurway, unpublished), the loci have not been numbered, but a scale has been given instead.

The reasons for the location of *l*(1)41*k* have already been given (p. 271). The map has been deduced almost entirely from the consideration of the multiple recombination classes of crosses in which three and usually more loci were marked. Therefore these crosses will be described before the recombination percentages, which have provided relatively little information about the order of the loci on the chromosome.

Evidence for order

Order ct(1) *sn*(2) *cp*(3) *cd or cv*(4) *y*

The counts from all five-point experiments made with these five loci have been added together irrespective of the constitution of the actual crosses. The only omissions are the male flies of cultures in which a sex-linked lethal was segregating.

Non-cross-overs	Recombination							
	in 1	in 2	in 3	in 4	in 1 and 2	in 1 and 3	in 1 and 4	in 2 and 3
357	182	34	222	288	10	124	135	9

Recombination							
in 2 and 4	in 3 and 4	in 1, 2, 3	in 1, 2, 4	in 1, 3, 4	in 2, 3, 4	in 1, 2, 3, 4	Total
23	151	1	3	70	9	1	1619

Similar counts from all four-point experiments are given in Table 4. Thus the full evidence as to the linkage relations of any four of these five genes may be obtained by adding the additional data, if any, of Table 4 to the five-point data.

Table 4. *Totals of experiments in which only four fully penetrant mutants were segregating*

Loci and regions in cross	Region of breaks								
	—	+—	−+−	—+	++−	+−+	−++	+++	Total
ct sn cp cd	1886	1142	182	1354	29	731	51	15	5390
ct sn cp y	56	33	3	45	1	39	6	2	185
ct sn cd y	561	286	402	420	191	281	309	132	2582
ct cp cd y	127	84	93	115	67	81	86	39	672

Order ct bg sn

The counts including *bg* were sons of females of the constitution + + +/*ct bg sn*. The totals are:

ct bg sn	+ + +	*ct*	*bg sn*	*bg ct*	*sn*	*ct sn*	*bg*
76	105	28	19	16	32	2	5

The order is therefore fairly certainly as given.

Order sn l(1)40*j cd cv*

Adding together all counts in which *sn l*(1)40*j* and *cd* and/or *cv* were segregating the total numbers of male flies are:

No recombination	Recombination between *sn* and *l* only	Recombination between *l* and *cd* or *cv* only	Recombination in both regions
992	562	74	15

On this evidence the order is *sn l*(1)40*j cd* or *cv*.

The three-point experiments containing *l*(1)40*j*, *cd* and *cv* are given below. The two flies showing recombination between *cd* and *cv* could only have been produced by single cross-overs if the order is as shown:

	Non-cross-overs	Cross-overs in 1	Cross-overs in 2	Double cross-overs
	cd +	+ *cv*	*cd cv*	+ +
l + *cv*/+ *cd* +	35	1	1	0
	+ *cv*	*cd* +	+ +	*cd cv*
l cd + /+ + *cv*	669	31	1	0

Order cd y scl wi

Considering only the phenotypically *scl* or *wi* flies, the offspring from a cross of the constitution *y* + *wi*/+ *scl* + ♀ × *y* + *wi* ♂♂ were:

♂♂		♀♀	
scl	*y scl*	*y wi*	*wi*
340	1	211	2

The *y scl* male when mated to *y wi* females from stock produced no *wi* daughters. He produced *scl* grandsons as expected. I made no counts, but the numbers were great enough to exclude any danger of *wi* being missed through its incomplete penetrance. His constitution was therefore *y scl wi*$^+$. One of the *y*$^+$ *wi* females was dead when found. The other produced *y scl*$^+$ and *y*$^+$ *scl* sons. Therefore her constitution was + *scl wi*/*y* + *wi*. Flies of these constitutions could only have been produced by single cross-overs if the locus of *scl* was between those of *y* and *wi*.

Considering only the *wi* females, the counts from crosses containing *cd y* and *wi* are:

Cross	No recombinations	Recombination between *cd* and *y* only	Recombination between *y* and *wi* only	Recombination in both regions
cd y wi/+ + +	621	453	13	9
+ *y wi*/*cd* + +	73	50	1	0

On this evidence the order is assumed in this paper to be *cd y wi*, but experiments with loci nearer to *y*, e.g. *co*, are needed.

EVIDENCE FOR DISTANCE

Table 5 gives the recombination percentages on which the map distances are based. All recombinations between *ct sn cp cd cv* and *y* are given, but only the recombination with the adjacent loci or the nearest loci of which large counts exist are given for sim_1, *bg*, *l*(1)40*j*, *l*(1)41*k*, *scl* and *wi*. Except in the actual estimate of crossing-over between them *ct* and *cv* are assumed to be one locus. Where both were segregating *cd* is considered, since this mutant is less likely to have been mis-scored than *cv*. The *l*(1)41*k* data have already been discussed. Only the *scl* and *wi* flies have been used to calculate the recombination between these two loci and *y*. The *y scl* distance is extremely doubtful. The slight phenotype and obvious nearness to *y* makes unbiased scoring impossible, and many flies in the crosses were tested. It was found that *scl*$^+$ flies had been classified as *scl* as well as the reverse. In the totals in Table 5 only two of the *y scl* flies had been tested, and the other two showed a slight doubtful phenotype. Therefore I can only say that *scl* is very close to *y*. It has been shown to be between *y* and *wi* above.

The offspring of mass and single female cultures have been added together for the estimate given in Table 5. The selection of the single female cultures is described in the

Table 5. *Recombination percentages using all data—see text*

Region		*a b* / + + or coupling					*a* + / + *b* or repulsion					Combined recombination
a	*b*	*ab*	+ +	*a* +	+ *b*	Recombination %	*a* +	+ *b*	*ab*	+ +	Recombination %	%
ct	*sin*$_1$	—	—	—	—	—	191	176	7	3	2·65±0·83	—
ct	*bg*	† 92	127	30	24	*19·8 ±2·4	—	—	—	—	—	—
ct	*sn*	† 397	452	221	193	32·8 ±1·3	3601	3620	1838	2153	35·60±0·45	34·17 ±0·71
ct	*cp*	1873	2015	1244	1252	39·10±0·61	546	616	311	376	37·2 ±1·1	38·12 ±0·64
ct	*cd* or *cv*	1241	1339	1304	1221	49·46±0·70	2311	2384	2096	2348	48·63±0·52	49·04 ±0·44
ct	*y*	1409	1760	1508	1587	50·20±0·63	746	794	688	764	48·53±0·91	49·37 ±0·55
bg	*sn*	† 95	133	21	24	*16·5 ±2·2	—	—	—	—	—	—
sn	*cp*	†1080	1166	52	61	4·79±0·44	2865	2830	140	165	5·08±0·28	*4·935±0·084
sn	*l*(1)40*j*	† —	781	386	—	33·1 ±1·4	† 411	—	—	283	40·8 ±1·9	36·8 ±1·2
sn	*cd* or *cv*	2723	2800	1974	1975	41·69±0·51	1676	1786	1145	1326	41·65±0·64	41·67 ±0·41
sn	*y*	921	975	903	915	48·95±0·82	1210	1346	1113	1412	49·69±0·70	49·32 ±0·54
cp	*cd* or *cv*	423	465	310	300	40·7 ±1·3	2762	2793	1714	1847	39·06±0·51	*39·89 ±0·68
cp	*y*	157	185	177	167	50·1 ±1·9	1044	1028	990	1102	50·24±0·77	50·2 ±1·0
l(1)40*j*	*cd* or *cv*	† —	741	—	35	4·51±0·74	† —	1568	—	88	5·31±0·55	*4·90 ±0·48
cd	*cv*	1229	1348	4	3	0·27	1068	1014	1	1	0·096	†0·161±0·065
cd	*l*(1)41*k*	—	—	—	—	—	† 98	—	—	13	*11·7 ±3·1	—
cd or *cv*	*y*	3974	4214	3141	3047	43·04±0·41	825	853	629	778	45·61±0·90	44·32 ±0·49
l(1)41*k*	*y*	—	—	—	—	—	† —	59	—	28	32·2 ±5·0	—
y	*scl*	—	—	—	—	—	806	732	4	48	*†0·5	—
y	*wi*	2230	3262	399	57	§2·49±0·33	608	527	8	24	§1·50±0·52	* §1·93 ±0·36

* Map distance. † Actual data examined for heterogeneity. ‡ Calculated on *scl* flies only. § Calculated on *wi* flies only.

section on the homogeneity of recombination (pp. 280–81 below). The mass cultures were selected thus: for distances under 10 units all families were included; for the other distances only families with 10 or more offspring relevant to the estimate were used. For several of the distances no mass families exist.

The estimate of recombination based on both the coupling and repulsion figures has been calculated from the following formula of Fisher (1936). If $100p$ is the recombination percentage between two mutants a and b and we represent the various classes thus:

	$++$ plus ab	$a+$ plus $+b$
Coupling	C	c
Repulsion	r	R

$$\frac{p}{1-p} = \sqrt{\frac{cr}{CR}},$$

and the variance of p is

$$\tfrac{1}{4}p^2(1-p)^2\left(\frac{1}{C}+\frac{1}{c}+\frac{1}{R}+\frac{1}{r}\right).$$

The differences between the estimates of recombination obtained from coupling and repulsion data are, with two exceptions, less than twice their respective standard errors. The two exceptions are *sn-l*(1)40*j* and *cd-y*.

When considering the recombination between a lethal and a visible mutation one of the non-recombination and one of the recombination classes are not obtained. Therefore the viability of the visible mutant has a much greater effect on the estimate of recombination than have the viabilities of two visible mutants when all four classes are counted. If, in a sample of n flies, a (the non-recombination class) have a viability estimated from other data as $1-c\pm\alpha$, and b (the recombination class) have a viability 1, then the corrected recombination value is

$$\frac{b}{n}\left(1-\frac{ac}{n}\right) \text{ approximately,}$$

and its standard error is

$$\sqrt{\frac{ab}{n^3}}\sqrt{\left(1+\frac{ab\alpha^2}{n}\right)} \quad \text{(Haldane, unpublished).}$$

The relative viability of *sn* males in single female cultures has been found to be 0·911 ±0·032 (Table 2), and the new estimates are 35·2 ± 1·8 on coupling data and 38·6 ± 2·5 on repulsion data. The difference between these estimates is not significant, and the combined estimate in Table 5 which allows the viabilities in the two kinds of experiments to balance one another is probably near the true value.

I can offer no explanation for the discrepancy of the estimates of the recombination between *cd* or *cv* and *y*. The data obtained from single female cultures are given in Table 6. Both these samples are homogeneous among themselves, and the estimates from their totals do not show this significant difference. Therefore some mass cultures in which the two mutants were in repulsion must have produced the significant change. These were the first counts made, and the curious raising of the estimate from its probable true value in the region of 43% may be due to environmental changes. As two loci (*dw* and *co*) have been found between *cv* and *y* this does not affect the map given on p. 275.

Homogeneity of recombination

Homogeneity χ^2 between adjacent loci (and a few others) are shown in Table 6.

The following subtractions and adjustments were made to the total data showing the relevant recombination. The numbers refer to the indices in column 3 (the number of samples) of Table 6.

1. Any culture having more than one mother was discarded. No such culture existed for the *bg* and *l*(1)40*j* data. All cultures from the same mother were considered separately to detect differences due to the culture conditions and the age of mother. As only a few counts containing *bg* had been made, the data were conserved by including cultures which contained lethals. The counts were made entirely on males, and two mutations not tested for allelomorphism are considered. As the χ^2 for *y-wi* was calculated entirely on *wi* females the discarding of male samples containing a lethal was irrelevant.

Table 6. *Homogeneity of recombination between adjacent loci in single female cultures*

Each culture from the same female and males and females within a culture considered separately. The indices to the number of samples are explained in the text.

Interval		Type of experiment	No. of samples $n+1$	Homogeneity χ^2	P	ab	$++$	$a+$	$+b$	Recombination % on these data
a	*b*									
ct	*bg*	Coupling	10[1]	9·231	0·48	92	127	30	24	19·8
ct	*sn*	Coupling	20[3]	24·836	0·17	397	452	221	193	32·78
		Repulsion	166[3]	212·984	0·0070	1644	1789	3144	3198	35·12
bg	*sn*	Coupling	10[1]	6·523	0·25	95	133	21	24	16·5
sn	*cp*	Coupling	40[2]	39·959	0·46	1080	1166	52	61	4·79
		Repulsion	103[2]	121·970	0·09	136	164	2664	2653	5·34
sn	*l*(1)40*j*	Coupling	39[1]	47·259	0·14	—	781	386	—	33·1
		Repulsion	22[1]	20·410	0·54	—	283	411	—	40·8
cp	*cd* or *cv*	Coupling	17[3]	14·618	0·55	324	359	237	243	41·27
		Repulsion	117[3]	157·865	0·0059	1264	1372	2063	2051	39·05
l(1)40*j*	*cd* or *cv*	Coupling	25[1]	24·653	0·47	—	741	—	35	4·51
		Repulsion	66[1]	64·662	0·51	—	88	—	1568	5·31
cd or *cv*	*y*	Coupling	191[3]	209·019	0·16	2927	3164	2310	2210	42·60
		Repulsion	24[3]	13·813	0·93	213	248	298	291	43·90
y	*wi*	Coupling	63[1]	83·245	0·10	1324	—	—	18	1·34

2. In addition to the above any male sample in which a lethal mutant anywhere on the chromosome was segregating was discarded. This was done because the removal of two of the relevant classes makes the estimate of recombination more subject to disturbance by the viabilities of the visible mutants segregating, as is shown by the different estimates of the recombination between *sn* and *l*(1)40*j*.

Because of the effects of viability on an estimate and because the sons and daughters of a female usually segregated for different mutants they have been considered separately in these tests for homogeneity.

χ^2 is usually regarded as an inaccurate method of testing for heterogeneity if the expectation in the smallest class is less than 5. In the data considered in 1 and 2 in many or all the samples the expectation is below this, either because the recombination is small as in the *sn-cp* distance, or because the total number of flies in a sample is small as for *sn-l*(1)40*j*, or both as where the recombination of *y-wi* must be calculated on *wi* females only and *wi* is only partially penetrant. Therefore the usual method of estimating the probability could not be used.

A special method has been developed by Haldane (1944*a*) for such cases. In addition to the figures given in Table 6, the sum of the reciprocals of the numbers of flies in each culture, and the sum of the squares of these reciprocals are required. Given these, the distribution of χ^2 in the absence of heterogeneity may be calculated, and hence the probability of the observed deviation from expectation. In most cases this is less than on the classical theory.

3. For the larger distances further adjustments were made so that the usual use of χ^2 was legitimate. These were as follows. No sample containing less than ten flies was included. Any such sample was discarded or added to the next smallest sample from the same mother, preferably of the same sex, these two being considered as one sample. More than two groups of sibs might be added together if they were all individually less than ten. From the totals thus obtained a provisional recombination percentage was calculated. From this the minimum size of culture in which the expectation of the recombination class was more than five was calculated. Any samples smaller than this minimum were discarded or added to their sibs as before. Cultures thus discarded in the sample tested for homogeneity are not included in what is called 'total data' in Table 5.

The value of P for the homogeneity of these data is obtained by the following transformation (Haldane, 1938). Where $n+1$ is the number of samples,

$$\xi = \left(\frac{9n}{2}\right)^{\frac{1}{2}} \left[\left(\frac{\chi^2}{n}\right)^{\frac{1}{3}} + \frac{2}{9n} - 1\right],$$

and if the data are drawn from a homogeneous population ξ is almost normally distributed with mean = 0 and variance = 1. Hence given ξ the value of P can be read off from a table of the normal probability integral.

It will be seen that two out of the fifteen values of χ^2 correspond to probabilities below 0·01, and are significantly high. None of the others are significant. We should expect one value of P in thirteen taken from homogeneous populations to lie below 0·1; however the fact that nine values of P out of thirteen are below 0·5 suggests that very large counts would reveal heterogeneity. This is, of course, to be expected, as temperature and other external variables were not carefully controlled.

The two clearly heterogeneous sets of data were recalculated after grouping together all the progeny of each single female (Table 7). The values of P were increased, but

Table 7. *Recalculation of samples shown heterogeneous in Table 6 considering the total offspring of one female as a sample, i.e. number of mothers = number of samples*

Interval		Type of	No. of	Homogeneity	
a	*b*	experiment	females	χ^2	P
ct	*sn*	Repulsion	116	148·514	0·019
cp	*ct* or *cv*	Repulsion	86	107·961	0·047

remained significant. This difference between mothers might have been due to the presence in some of them of a small inversion. If so the distribution of the apparent cross-over values should have been bimodal. The *ct-sn* data were plotted according to the graphical method of Fisher & Mather (1943) and showed no trace of bimodality. It may be that crossing-over in this region of the chromosome is particularly sensitive to environmental influences, but there is no evidence suggesting the presence of an inversion.

Coincidence

The coincidence values given in Table 8 are calculated from the data given in the section on evidence for order (p. 276) plus those from three-point experiments made with adjacent loci (*ct sn cp*; *sn cp cd* or *cv*; *cp cd* or *cv y*).

Table 8. *Coincidence*

Regions considered		No recombination in p or q	Recombination in p but not in q	Recombination in q but not in p	Recombination in both p and q	Coincidence
p	q	z	y	x	w	
ct-bg	*bg-sn*	181	47	38	7	0·79 ±0·25
ct-sn 1	*sn-cp* 2	4598	2595	321	63	0·468±0·053
sn-cp 2	*cp-cd* or *cv* 3	4327	296	2907	101	0·645±0·054
sn-l(1)40j	*l(1)40j-cd* or *cv*	992	562	74	15	0·48 ±0·11
cp-cd or *cv* 3	*cd* or *cv-y* 4	1535	1012	1252	698	0·941±0·022
cd-l(1)41k	*l(1)41k-y*	50	9	26	2	0·56 ±0·35
cd-y	*y-wi*	694	503	14	9	0·93 ±0·24
ct-sn 1	*cp-cd* or *cv* 3	2770	1501	1796	942	0·987±0·020
ct-sn 1	*cd* or *cv-y* 4	1585	794	1200	622	1·013±0·025
sn-cp 2	*cd* or *cv-y* 4	885	54	644	36	0·95 ±0·12

The formula used is

$$\frac{wn}{(w+x)(w+y)} \pm \left\{\frac{wn\,[w^2z+xy\,(x+y+z)]}{(w+x)^3\,(w+y)^3}\right\}^{\frac{1}{2}},$$

where w = number of individuals showing recombination in segments p and q and nowhere or anywhere else; x = number of individuals showing recombination in segment q, no recombination in segment p and recombination nowhere or anywhere else; y = number of individuals showing recombination in segment p, no recombination in segment q and recombination nowhere or anywhere else; z = number of individuals showing no recombination, or showing recombination anywhere except in p and q, and $n = w+x+y+z$ (Muller & Jacobs-Muller, 1925; and Stevens, 1936).

Considering only those coincidences that have standard errors of less than 0·1 it is seen that there is significant interference between adjacent regions but none between non-adjacent regions even though region 2 (*sn-cp*) is only 5 units long.

Triple crossing-over

Although Stevens's treatment is perfectly correct, the notion of interference between two non-adjacent segments is somewhat artificial because we might expect crossing-over in segment 1 to have a different influence on the probability of crossing-over in segment 3, according as crossing-over does or does not occur in the intervening segment 2. If crossing-over relieves a tension in the coiled chromatids, we should expect more interference if there is no intervening cross-over than if there is one.

Consider the four genes *ct*, *sn*, *cp*, *cd*. The total counts are:

— — —	2531	—+—	239
+— —	1459	++—	42
—++	1727	—++	69
+++	925	+++	17

The coincidence between *ct sn* and *cp cd* where no cross-over has been recorded between *sn* and *cp* is $\frac{925 \times 6642}{2384 \times 2652}$, or $0{\cdot}972 \pm 0{\cdot}020$. The coincidence when there has been a cross-over recorded between *sn* and *cp* is $1{\cdot}23 \pm 0{\cdot}23$. In neither case is the difference from unity quite significant, but it is in the expected direction. In Table 9 all the coincidences between non-adjacent segments are thus treated. The data are given on p. 276 and in Table 4. For calculation of the coincidences of *ct sn* and *cd y* the five-point and four-point experiments must be considered separately, as double crossing-over in the intervening region is observed in the former but is scored as no crossing-over in the intervening region in the latter.

Table 9

Region	Data used	Coincidence when no crossing-over recorded between regions concerned	Coincidence when crossing-over is recorded between regions concerned
ct sn cp cd (1 3)	4- and 5-point	$0{\cdot}972 \pm 0{\cdot}020$	$1{\cdot}23 \pm 0{\cdot}23$
sn cp cd y (2 4)	4- and 5-point	$0{\cdot}85 \pm 0{\cdot}13$	$1{\cdot}27 \pm 0{\cdot}28$
ct sn cd y (1 4)	5-point	$0{\cdot}969 \pm 0{\cdot}052$	$0{\cdot}905 \pm 0{\cdot}071$
ct sn cd y (1 4)	4-point	$1{\cdot}094 \pm 0{\cdot}037$	$0{\cdot}958 \pm 0{\cdot}053$

Table 9 is inconclusive. But it suggests that interference may extend across region 2 or region 3 if there has been no crossing-over within this region, while it does not extend across the longer segment between the loci of *sn* and *cd*. However, this could only be proved if much larger counts were made. Also the probability that double cross-overs in some of these long regions have been scored as no cross-overs makes this data not very suitable for the examination of the effect, if any, of an intermediate chiasma on interference.

DISCUSSION

The general results are much like those found in other *Drosophila* species, but certain features are unusual in the genus, while a number of points have been investigated for the first time.

The expected 1 : 1 segregation was investigated in both sexes for five mutants, and in males only for four. There was always a shortage of mutants, presumably due to a higher death-rate in the early part of the life cycle. But this was only statistically significant in seven of the fourteen cases. In only one case was the viability of the mutant significantly below 90% of that of its normal sibs.

These viabilities may be compared with those found by Punnett (1932) for mutants in *Lathyrus*, by de Winton & Haldane (1933) for those in *Primula sinensis*, and by Timofeeff-Ressovsky (1934) for sex-linked mutants in *Drosophila funebris*. The viabilities of eighteen mutants in *Lathyrus* ranged from $1{\cdot}038 \pm 0{\cdot}004$ (C-white) to $0{\cdot}821 \pm 0{\cdot}005$ (marbled). Even the monstrous cretin had a viability of 0·886. The median value was 0·970. The viabilities of twenty-four mutants in *Primula* ranged from about 1·03 (peculiar eye) to 0·33 (feeble), but only six had viabilities of 95% or less. Thus the mutants with which I worked had viabilities comparable with those found in domestic plants, and cannot be regarded as exceptionally inviable. The main difference from the plants was

that some of my mutants were incompletely penetrant, whereas all those in the plants, except flake in *Lathyrus*, which only shows up in presence of a modifier, are fully penetrant.

Timofeeff-Ressovsky's five sex-linked recessives had viabilities in his standard environment ranging from 104 to 70%, four being below 90%. They were therefore on the whole less viable than my own. He worked on nearly isogenic stocks with no other visible genes segregating, and his results are in this respect more reliable than those of other workers. But as he made no tests for homogeneity, his standard errors may be too small. He also investigated the effects of environmental changes on viability, and estimated it directly from counts of oval, larval, and pupal mortality, getting figures similar to those obtained from segregation.

The only systematic work on variability of recombination is that of Gowen (1919) on *Drosophila melanogaster* and of de Winton & Haldane (1935) and Haldane (1936) on *Primula sinensis*. Gowen reached the startling conclusion 'that crossing-over is one of the most highly variable phenomena known'. This is not borne out by his published data. The cross-over values obtained from different cultures naturally vary greatly when the numbers in the cultures are small. So do single-factor ratios or sex ratios. As some of his cultures contained under forty flies he naturally obtained very great variation. Had his cultures been four times as large, he would have halved his coefficients of variation, which were in fact mainly due to sampling errors.

I have not recalculated his entire data, which are based on 240 cultures, segregating for five or seven genes. But for the sixteen cultures in his Table B, $\chi^2 = 38{\cdot}7$, 43·3, 16·2, and 11·2 for the four regions concerned. Thus in two regions there is strong evidence of heterogeneity, in the others none. De Winton & Haldane found no conclusive evidence of heterogeneity in any single region. But their pooled data show that it must exist. They found recombination values somewhat less variable than single-factor ratios.

The data of this paper show that four of the fourteen χ^2 values for the homogeneity of single-factor ratios of fully penetrant mutants (the sexes being considered separately) were significantly high, and none was less than its expected value, corresponding to $P = 0{\cdot}5$. But among the fifteen χ^2 values for the homogeneity of recombination values, only two were significantly high, and four fell below their expected values. This result agrees with those of de Winton & Haldane.

Bridges & Morgan (1923), in their instructions for constructing chromosome maps, stated that, apart from the effects of what are now known to be inversions, their recombination values for the third chromosome of *Drosophila melanogaster* fell into four 'systems', with the same gene order, but different recombination frequencies. The data from about three-fourths of the experiments, 'although still heterogeneous', were used for the standard system. The χ^2 test of homogeneity was found to be of assistance in assigning a segregation to one system or another. Unfortunately, neither the crude data nor the values of χ^2 were published; so it is not certain whether recombination in *D. melanogaster* is really more variable than in *D. subobscura*. A statistical treatment of crossing-over in this organism by modern methods would be of considerable interest.

The linkage map of the *X*-chromosome given in Fig. 1 is 150 units long. This is known to cover only part of the chromosome and to include one region of 40 units and one of 37 units. Therefore this genetic chromosome is likely to be the longest *X*-chromosome with a terminal spindle attachment recorded in the genus, as the current map of the

X-chromosome of *D. virilis virilis*, till now the longest, is 170 units long, contains thirty-five loci and the largest unbroken region is only 14 units long (Chino, 1936). A long map may mean that the chromosome is long, pliable, or fragile. The slight interference shown in Table 8 suggests that it is pliable. There also seems to be a clumping of loci along the chromosome. This may be due to their spatial position or to a localization of chiasmata.

The question of interference across a chiasma which the data were insufficient to decide upon may be important in these long chromosomes. In *D. melanogaster* it does not arise, Bridges & Olbrycht (1926) finding only forty-five triple or higher cross-overs in their total of 24,034 flies.

SUMMARY

Twenty-one visible mutants and two lethal mutants are described in *Drosophila subobscura*. Three have been described before. Preliminary mention only is made of three others. They are believed to mark sixteen loci on the sex chromosome.

The homogeneity of the divergence from expectation in the 1 : 1 segregation of nine fully penetrant mutants in different cultures has been investigated.

A linkage map containing fourteen loci and 150 units long is constructed. The homogeneity of the recombination between adjacent loci in different cultures is investigated.

Coincidence values have been discussed.

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