THE MULTIPLE STOCK "XPLE" AND ITS USE

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Both in the construction and in the use of a map that shows the relative location of the genes contained in a given chromosome, it is necessary to have a table of corrections which gives for each two loci the amount by which the number of units of map-distance is greater than the percentage number of recombinations that is directly observed in an experiment. A few examples will make this clear. The percentage of recombinations for two linked characters is usually found by a "backcross" experiment, in which a female heterozygous for the two pairs of characters is mated to a male homozygous for the recessives of both pairs. Thus, if the recessive scute (lack of certain bristles from the scutellum) is crossed to the recessive echinus (a larger eye with coarser hairs on it) the F_1 female will be wild-type, for each parent contributes the recessive of one pair and the dominant wild-type allelomorph of the other pair $\left(\dfrac{s_c \quad +^{e_c}}{+^{s_c} \quad e_c}\right)$. This F_1 female is then mated to a male homozygous for both scute and echinus. The offspring are found to be of four classes. Two, scute and echinus, respectively, represent the original combinations of the four characters dealt with (scute with not-echinus, and not-scute with echinus). Two other classes are scute echinus and wild-type. These represent new combinations of the two pairs of characters that entered the cross (scute with echinus and not-scute with not-echinus). The percentage of recombination is found by dividing the sum of the two recombination classes by the total number in all classes, and multiplying by a hundred. A representative scute echinus backcross experiment (data from table 5) gave: $s_c = 9805$, $e_c = 9575$, $s_c\ e_c = 686$, $+ = 720$. The total recombinations were 1406, which is 6.8 percent of the total of 20,786 flies. With the crossveinless wing-character, scute gave 16.3 percent of recombination. In a given linkage group each two pairs of characters have a characteristic recombination percent.

The recombinations of linked characters is explained by "crossing over" of the chromosomes. In the cells that are about to give rise to eggs, the chromosome carrying scute comes to lie closely side-by-side with the homologous chromosome carrying vermilion. At some point or points

along the synapsed chromosomes the two chromosomes break across, and then rejoin in such a way that the part of each that lies to the left of the break is united with the part of the other that lies to the right of the break. Such points of crossing over may occur anywhere along the chromosome, and whenever one falls between the loci occupied by scute and echinus it gives rise to crossover chromosomes, one of which carries both mutant genes and the other of which carries neither. The individuals that come from the crossover chromosomes show recombinations of characters. The further apart the loci of two characters are, the more frequently will crossovers fall between them, and the higher will be the percentage of recombinations.

But if the loci of two characters are far enough apart (which is true for scute and crossveinless) two crossovers sometimes fall between them. In such an event, the genes that were together originally remain together in spite of the double crossing over between them. A section of chromosome lying between two genes is replaced by a section from the other homologue. No recombination of the characters is produced, and for each such case the total number of crossovers is increased by two, while the total number of recombinations of the linked characters is not increased at all. Thus the number of recombinations corresponds to the full number of crossovers only in case the two loci considered are so close together that no double crossing over occurs between them. In constructing the maps it is necessary to know the full number of crossovers (doubles counting as two, triples as three crossovers) that occur between the loci that are to be mapped. A unit of map distance corresponds to a section of chromosome of such length that one crossover falls within it for each average hundred gametes. In constructing the map of the first chromosome which is given as the base line in figure 1, scute and echinus were placed 6.8 units apart, which means that in the data on which that map was based (table 5) 6.8 crossovers between scute and echinus occurred for each hundred gametes. None of these were doubles, since scute and echinus are too close together to allow double crossing over between them. Scute and forked are there mapped as 62.0 units apart. Scute and forked are so far apart that not only double, but triple and quadruple crossing over has been found to occur between them. In constructing such maps it is first necessary to determine experimentally the amounts by which the various crossing over values exceed the directly observed recombination percents.

The experimental determination of the amount by which crossing over exceeds recombination is made through use of loci lying between the two

loci in question, and which are so close together that no double crossing over occurs between succeeding ones. Thus, the number of double cross-overs occurring between scute and crossveinless can be determined through use of the intermediate locus echinus, the scute-echinus interval and the echinus-crossveinless interval being free of double crossing over. An F_1 female from the cross between scute crossveinless and echinus $\left(\dfrac{s_c \ +^{e_c} c_v}{+^{s_c} e_c \ +^{c_v}} \right)$ is backcrossed to a scute echinus crossveinless male. All double crossovers that occur between scute and crossveinless are easily detectable, since they result in $s_c \ e_c \ c_v$ and in wild-type flies, the number of which can be counted. In the data of table 5 there were 0.03 percent such doubles, which gives a difference of 0.06 between crossing over and recombination. This difference is very slight, and it is partly because it is so slight (and partly from direct tests) that it is certain that no doubles occur within either of the two smaller sections. To determine the difference for loci as far apart as scute and forked requires the simultaneous use of several intermediate loci.

The selection of these intermediate loci is a problem of no little difficulty, and it is only recently that mutant loci of suitable characteristics have become available. First, only mutant characters that are separable from the wild-type without error may be used. This requires a character that does not fluctuate greatly and for which the gap between the wild-type and the fluctuant closest to the wild-type is sharp. Second, the mutants must have practically normal viability, fertility and productivity. Some mutants that themselves have normal viability show great mortality if they are combined with certain other mutants. Thus, sable has been found to greatly decrease the viability of multiple stocks. Third, those used together must be sharply separable from one another, and every class possible from combination of the constituent characters must be sharply separable from every other class. Thus, ruby and garnet eye colors cannot be used together, nor singed and forked bristles. From the suitable mutants as small a number should be selected as possible and still have the number of doubles between successive loci either zero, or negligibly small (less than one double per 2000 flies), or small and of an amount known from other experiments.

In 1916 the best selection for the scute to forked interval was echinus, cut, vermilion and garnet. To carry out backcrosses a multiple stock containing these recessives together with scute and forked was necessary. The $s_c \ e_c \ c_t \ v \ g \ f$ multiple recessive was made by BRIDGES and called "Xple" (X-chromosome multiple). The Xple stock was used in the COLUMBIA

laboratory in finding the locus of several sex-linked mutants, and was sent to various workers elsewhere, who used it in studying the effect upon crossing over of several environmental and genetic agencies.   Thus, PLOUGH (1921) used Xple in experiments that confirmed the finding of BRIDGES (1915) that the age of the mother made little or no difference in the crossing over frequencies for the X chromosome. By use of the same stock PLOUGH showed that heat treatment was likewise without any certain effect.   GOWEN and GOWEN (1923) used Xple to show that a third-chromosome recessive gene suppressed all crossing over in the X chromosome.   MOHR (1923b) used Xple in testing the length of the deficient section due to notch 8, and in showing that crossing over outside of the deficient section itself is not affected. Mrs. WARD (1923) used Xple to test the effect upon crossing over in the X chromosome of curly, which suppressses nearly all crossing over in the second chromosome. The data indicated that curly influences crossing over in the X to a slight extent.   Mohr (1923a) used Xple in finding the locus of a semilethal character chlorotic, whose locus is slightly to the left of scute.

In table 1 is given a summary of the data involving Xple. In table 2 are given the data secured by MOHR in the cross of notch 8 by Xple. The

TABLE 1

Summary of data involving $\dfrac{s_c\ (1)\ e_c\ (2)\ c\ \ (3)\ v\ (4)\ g\ (5)f}{+\ \ \ \ +\ \ \ \ +\ \ \ \ +\ \ \ \ +\ \ \ +}$.

SOURCE OF DATA	CROSSOVER REGIONS										
	0	1	2	3	4	5	1,2	1,3	1,4	1,5	2,3
PLOUGH '21	2,216	204	575	521	393	424	17	44	56	60	25
MOHR '23b; ♂♂	434	56	131	135	57	55	..	8	6	4	4
MOHR '23b; ♀♀*	659	73	193	175	105	111	..	5	11	7	5
Total	3,309	333	899	831	555	590	17	57	73	71	34

TABLE 1 (continued)

SOURCE OF DATA	CROSSOVER REGIONS											
	2,4	2,5	3,4	3,5	4,5	1,2,3	1,2,5	1,3,5	1,4,5	2,3,5	2,4,5	3,4,5
PLOUGH '21	63	77	13	39	10	1	1	1	1	1	1	3
MOHR '23b; ♂♂	7	16	1	7	1	..	..	..	..	..	..	..
MOHR '23b; ♀♀*	8	22	3	9	..	..	..	..	..	..	..	..
Total	78	115	17	55	11	1	1	1	1	1	1	3

* Data unpublished, kindly supplied by MOHR.

TABLE 2

Data from MOHR (1923 a) involving $\dfrac{s_c \ +\ e_c\ (2)\ c_t\ (3)\ v\ (4)\ g\ (5)\ f}{+\ N_8 +\ \ +_t\ \ +\ \ +\ \ +}$.

							CROSSOVER REGIONS					
0	2	3	4	5	2,3	2,4	2,5	3,4	3,5	4,5	2,3,4	2,3,5
897	243	251	162	155	4	25	36	11	28	7	1	2

data of table 2 and the other data involving notch 8 show that the crossing-over relations to the right of echinus are probably not affected by the action of notch 8, and accordingly these data can be used in connection with those of table 1. The combined data give the following percentagesof recombination for the successive sections: $s_c\text{-}e_c = 7.9$, $e_c\text{-}c_t = 16.4$, $c_t\text{-}v = 14.6$, $v\text{-}g = 10.6$, $g\text{-}f = 12.0$. The map based on these data would be about 62.0 units long from scute to forked, which is slightly longer than the old standard map of 56.5 (BRIDGES 1921). The greatest difference is in section 1 (7.9 instead of 5.5) and in section 2 (16.4 instead of 14.5).

On the basis of the data of table 1 alone the scute-forked map distance is 60.9, while the scute-forked recombination percentage is 45.7. That is, each hundred flies involved 15.2 cases of crossing over that did not give rise to recombinations for scute and forked. Discussion of the other points brought out by these data will be deferred until further data have been presented.

In 1919 the excellent sex-linked recessive mutant "crossveinless" was discovered (BRIDGES 1920), and its locus was found to be about half way between those for echinus and cut. By use of crossveinless the distance of about 15 units between echinus and cut could be broken into two sections of about 8 and 7 units. Each of these sections would be so short that no double crossing over would occur within it, and the number of the doubles occurring between echinus and cut could be accurately determined by direct experiment. An X-multiple that includes crossveinless in addition to the others already in use would be much more effective. Also, two other mutants had been discovered by BRIDGES that offered improvements over those in the old Xple. The mutant cut in the old Xple was the original cut found by BRIDGES in 1915. An allelomorph of cut, namely cut[6], was found that was more viable than the original cut and that did not affect the eyes, antennae, and abdomen to such an extent as the other allelomorphs. Classification was perfect on the basis of the wing character, the wing being "cut" to a sharp point. Likewise a new allelomorph of garnet, namely, garnet[2], was found, that had a lighter eye

TABLE 3

Backcross data s_e (1) e_c (2) c_v (3) c^s (4) v (5) g^2 (6) f

CROSSOVERS IN REGION

Culture Number	0		1		2		3		4		5		6		1,2		1,3		1,4		1,5		1,6		2,3	
	s_e e_c c_v c_t v g f	+	s_e	e_c c_v c_t v g f	s_e e_c	c_v c_t v g f	s_e e_c c_v	c_t v g f	s_e e_c c_v c_t	v g f	s_e e_c c_v v	g f	s_e e_c c_v c_t v g	f	s_e	e_c	s_e	e_c c_v	s_e	e_c c_v c_t	s_e	e_c c_v c_t v	s_e	e_c c_v c_t v g	s_e e_c	c_v c_t
2	46	55	6	6	7	7	9	15	16	19	12	15	5	10	1	.	.	.	1	1	.	2	1	1	.	.
4	65	58	16	3	11	9	9	9	16	13	12	6	11	9	.	.	.	1	.	2	2	.	.	.	.	.
6	42	54	16	7	10	9	12	6	17	14	13	13	9	8	.	.	1	.	2	3	.	5	1	1	.	.
7	73	87	11	7	24	12	15	13	21	18	12	10	9	13	.	.	.	.	2	.	.	.	1	.	1	.
9	71	109	21	13	23	18	20	26	26	34	19	21	9	13	.	.	.	.	1	.	4	.	4	5	.	.
11	28	92	17	6	15	14	13	13	17	28	19	19	4	16	.	.	.	.	.	2	.	4	1	1	.	.
12	86	133	23	13	20	22	19	18	37	25	14	17	13	17	.	.	1	.	.	2	1	1	.	2	.	.
13	61	78	9	9	15	4	16	7	24	23	11	19	15	15	.	.	.	.	2	4	3	.	1	.	.	.
14	19	29	7	4	5	7	6	3	2	10	7	12	5	8	.	.	.	.	.	.	1	.	1	.	.	.
16	70	104	11	14	10	11	11	16	23	25	12	14	14	17	.	1	.	.	1	2	2	3	.	2	.	.
Totals	561	799	127	82	140	113	130	126	199	209	121	146	94	126	1	1	2	1	9	16	13	15	9	15	1	0
	1360		209		253		256		408		267		220		2		3		25		28		24		1	

TABLE 3 (continued)

CROSSOVERS IN REGION

Each region column records the two reciprocal crossover classes (genes: *sc, ec, cv, ct, v, g, f*). Cell entries are given as *class 1, class 2*; a dash (–) indicates none.

CULTURE NUMBER	2,4	2,5	2,6	3,4	3,5	3,6	4,5	4,6	5,6	1,4,5	1,4,6	2,4,6	3,5,6
2	2, –	2, 1	2, 1	1, 1	–, –	1, –	2, 2	1, 2	–, –				
4	–, 1	1, 1	4, 1	–, –	–, –	–, 1	3, –	2, 1	–, –				
6	2, –	2, 1	1, 3	1, –	–, 1	–, –	1, –	–, 2	1, –				1
7	1, 1	3, 1	5, 1	–, –	–, –	–, 1	1, 1	5, 1	–, 1				
9	–, 2	2, 2	3, 1	1, 1	1, 1	–, –	–, –	1, 3	1, –	1			
11	1, –	1, 2	–, 2	–, 1	3, 2	2, 1	1, –	2, 2	–, –		–, 1		
12	–, 3	2, 4	4, 1	–, 1	2, 1	3, 1	–, 2	4, 5	–, –		2, –		
13	2, 2	2, –	3, –	–, –	–, 1	5, 2	–, –	3, –	–, 1				
14	–, –	–, –	2, –	–, –	–, –	1, 2	–, 1	1, 3	1, 1				
16	1, –	1, 1	1, 3	–, –	–, –	–, –	–, 2	–, 1	1, –			1	
Totals	9	16, 13	25, 13	3, 4	6, 6	12, 8	8, 8	19, 20	4, 3	1, 0	2, 1	1, 0	1, 0
	18	29	38	7	12	20	16	39	7	1	3	1	1

color than the original garnet and that could be separated from the wild-type with greater speed and sureness. A second advantage of garnet^2 over garnet is that the vermilion garnet^2 double recessive is a light yellow-orange color easily separated from garnet^2, while the vermilion garnet double recessive is a darker red-orange that is not strikingly different, especially in older flies, from garnet itself. Accordingly, a new Xple was constructed in 1920 with the formula $s_c \ e_c \ c_v \ c_t{}^6 \ v \ g^2 f$. This Xple has replaced the old both at the COLUMBIA laboratory and at the other laboratories where the most active work is being carried on.

The new Xple had come from various sources, and included only parts of the old Xple. There had been opportunity for a new set of modifiers of the amount of crossing over to be brought together. It was not anticipated that any of these modifiers would be of striking effect, for the stocks used were known to give at least approximately standard values. To test the condition of the new Xple a backcross was made by OLBRYCHT, whose results are given in table 3.

According to the data of table 3 the recombination percentages for the various sections of the Xple were: s_c-$e_c = 9.0$; e_c-$c_v = 10.5$; c_v-$c_t = 9.2$; c_t-$v = 15.9$; v-$g = 11.2$; g-$f = 10.9$. The length of the map from scute to forked is 66.7 units, which is 10.2 units longer than the standard map. Again the increase was largest at the extreme left end and gradually decreased toward the right until the g-f value was slightly below the old standard. The s_c-f recombination percent was 49.8, which is 16.9 lower than the crossover value.

An examination of the details of table 3 shows a striking characteristic of the pairs of complementary classes that constitute the crossover classes. In a given crossover class the two complementary classes are expected to be equal, since the two classes of gametes from which they come are equal. But if the zygotes of one class have a heavier mortality than the zygotes of the complementary class their inequality appears in the ratio of zygotes. In crossover classes 3 and 4 the complementary classes are very nearly equal (130 : 126 and 199 : 209), but in crossover classes 1 and 6 they are quite unequal (127 : 82 and 94 : 126). In crossover class 1 the heavy mortality of the $e_c \ c_v \ c_t{}^6 \ v \ g^2 f$ class is due to the summation and interaction of six mutant characters, while the relatively slight mortality of the complementary class is due to the single mutant s_c. In crossover class 4 the mortality of the combination of four mutants $s_c \ e_c \ c_v \ c_t{}^6$ is only slightly greater than that of three mutants $v \ g^2 f$; and the same is true in class 3 for $c_t{}^6 \ v \ g^2 f$ and $s_c \ e_c \ c_v$. In crossover classes 2 and 5 the complementary classes have 2 and 5 mutant characters, and the inequality

is intermediate between that due to a 3-4 division and to a 1-6 division. It is to be expected that the 7-0 division, namely, Xple and +, as it exists in the non-crossover class, would give the greatest departure from equality of complementary classes. This is undoubtedly the case, but the above data have been selected in such a way as to exclude cultures in which the inequality of Xple and wild-type was strikingly great. The selection was made in the following manner: About 15 cultures were started originally and after they had been hatching a few days the five cultures in which the inequality between Xple and wild-type was greatest were discarded and the counts were completed only for the 10 cultures in which the classes seemed fairly equal. The justification for this selection lies in the fact that those cultures in which the greatest inequalities exist are those in which the culture conditions are most adverse to normal viability, and in which the disturbing effect of differential viability upon the various crossover classes is greatest. The best single index of the normality of viability conditions is the Xple to wild-type ratio, because the greatest effect is expected there, and because in the above experiment those classes were the non-crossover classes and hence the largest classes present. The correlations between the mortality disturbances in the various classes is large, but not so large that a selection based primarily on the Xple and wild-type classes would give equally normal ratios in the other classes. Hence, in the above data the ratio in the 7-0 division (41.2 : 58.8) departs from equality more than one of the 6-1 divisions ($s_c\, e_c\, c_t{}^6\, v\, g^2 : f = 42.7 : 57.3$), but not more than the other ($e_c\, c_t{}^6\, v\, g^2 f : s_c = 39.2 : 60.8$). The difference is slight, and for the primary purpose of the experiment, the determination of recombination percentages and crossover values, the data are more valid than they would be if no selection had been made.

TABLE 4

Ratios of complementary classes from table 3.

DIVISIONS	COMPLEMENTARY CLASSES	RATIO
7–0	$s_c\, e_c\, c_v\, c_t\, v\, g\, f$: wild-type	41.2 : 58.8
6–1	$s_c\, e_c\, c_v\, c_t\, v\, g$: f	42.7 : 57.3
5–2	$s_c\, e_c\, c_v\, c_t\, v$: $g\, f$	45.3 : 54.7
4–3	$s_c\, e_c\, c_v\, c_t$: $v\, g\, f$	48.8 : 51.2
3–4	$s_c\, e_c\, c_v$: $c_t\, v\, g\, f$	50.8 : 49.2
2–5	$s_c\, e_c$: $c_v\, c_t\, v\, g\, f$	55.3 : 44.7
1–6	s_c : $e_c\, c_v\, c_t\, v\, g\, f$	60.8 : 39.2
0–7	Wild-type: $s_c\, e_c\, c_v\, c_t\, v\, g\, f$	58.8 : 41.2

TABLE 5

Alternated backcross of Xple $\dfrac{s_c \qquad c_v \qquad v}{(1)\ e_c\ (2) \quad (3)\ c^6\ (4) \quad (5)\ g^2\ (6)}\ f$.

Each crossover class (0, 1, 2, 3, 4, 5, 6, 1,2, 1,3, 1,4) is shown as two reciprocal phenotype sub‑columns (a / b). Gene labels for the sub‑columns: 0 = $s_c\,c_v\,v\,f$ / $e_c\,c_t\,g$; 1 = $s_c\,e_c\,c_t\,g$ / $c_v\,v\,f$; 2 = $s_c\,c_t\,g$ / $e_c\,c_v\,v\,f$; 3 = $s_c\,c_v\,c_t\,g$ / $e_c\,v\,f$; 4 = $s_c\,c_v\,g$ / $e_r\,c_t\,v\,f$; 5 = $s_c\,c_v\,v\,g$ / $e_c\,c_t\,f$; 6 = $s_c\,c_v\,v$ / $e_c\,c_t\,g\,f$; 1,2 = $s_c\,e_c\,c_v\,c_t\,v\,g\,f$; 1,3 = $s_c\,e_c\,v$ / $c_v\,c_t\,g\,f$; 1,4 = $s_c\,c_c\,c_t\,v\,g\,f$ / $c_v\,g$.

Culture numbers	0a	0b	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	1,2a	1,2b	1,3a	1,3b	1,4a	1,4b
1	61	57	6	5	6	11	5	9	10	13	14	9	11	13	.	.	1	.	1	.
2	81	77	3	2	16	13	14	11	26	22	16	16	7	11	.	.	.	.	.	.
3	128	101	13	15	16	15	22	23	27	31	32	23	16	29	.	.	.	.	.	1
4	110	106	4	8	11	14	10	9	25	15	18	16	21	8	.	.	.	.	1	.
5	122	127	12	12	24	12	28	15	32	25	29	21	24	19	.	.	.	.	.	.
6	69	103	8	9	12	24	7	16	19	19	29	12	19	17	.	.	.	.	2	1
7	115	107	13	15	16	19	12	19	31	24	9	16	16	6	.	.	.	.	.	.
8	58	56	4	6	7	9	15	6	12	22	12	18	10	8	.	.	.	.	.	.
9	78	77	8	12	12	12	20	13	20	22	26	24	28	16	.	.	.	2	1	2
10	77	69	4	9	11	8	10	3	22	20	17	12	11	13	.	.	.	.	1	.
11	75	61	8	3	11	12	9	10	24	17	9	14	10	9	.	.	.	.	1	1
13	85	90	11	14	18	21	8	15	29	20	16	16	17	10	.	.	.	.	1	1
15	86	76	9	6	18	10	16	10	21	22	21	11	14	9	.	.	.	.	1	1
16	52	80	16	14	7	8	4	8	19	15	12	13	13	21	.	.	.	.	1	1
Totals	1197	1187	119	130	185	188	179	167	317	287	251	221	217	189	.	.	1	2	9	7
(A) xx	980	922	97	94	144	134	112	160	239	237	140	175	169	158	1	.	1	.	6	9
(B) xxy	767	748	61	66	117	114	101	107	177	205	135	130	114	119	1	.	.	3	5	5
(C) xx	421	443	49	65	73	69	69	60	129	119	82	88	92	70	.	.	.	.	2	5
(D) xxy	888	808	74	105	108	152	139	149	240	227	142	135	102	120	1	.	1	1	9	9
(E) xxy	635	558	72	62	96	85	85	97	157	178	113	105	101	74	.	.	1	.	15	6
Grand total	4888	4666	472	522	723	742	685	740	1259	1253	863	854	795	730	3	0	4	6	46	41
	9554		994		1465		1425		2512		1717		1525		3		10		87	

TABLE 5 (continued)

Culture numbers	1,5 a	1,5 b	1,6 a	1,6 b	2,3 a	2,3 b	2,4 a	2,4 b	2,5 a	2,5 b	2,6 a	2,6 b	3,4 a	3,4 b	3,5 a	3,5 b	3,6 a	3,6 b	4,5 a	4,5 b	4,6 a	4,6 b
1	1	1	·	2	·	·	1	·	1	·	1	1	·	·	2	1	1	2	·	·	2	·
2	1	·	1	·	·	1	·	·	1	1	3	2	·	·	·	2	1	2	4	·	3	5
3	·	1	1	1	·	1	2	·	4	2	2	2	·	·	3	1	3	3	1	1	7	3
4	2	·	2	3	·	·	2	2	5	3	5	1	·	·	·	1	1	1	1	1	3	4
5	3	4	·	1	·	1	·	3	4	3	4	4	1	·	2	2	6	3	2	2	2	3
6	·	1	·	3	·	·	3	2	3	3	·	1	·	·	·	1	6	·	4	2	3	4
7	1	1	1	4	·	·	2	5	1	6	3	6	1	·	3	·	5	3	·	1	2	2
8	1	1	1	1	·	·	1	·	·	1	·	2	·	·	·	·	·	·	1	1	1	2
9	1	4	·	1	·	·	·	1	1	1	2	4	·	·	2	1	3	1	·	·	5	5
10	1	·	3	1	·	·	2	·	5	1	2	2	·	·	·	2	2	2	·	4	2	4
11	1	·	·	2	·	·	·	·	·	5	3	3	·	·	·	2	·	1	·	·	2	4
13	3	1	3	2	·	·	·	·	2	1	5	1	·	1	2	2	1	3	2	1	2	4
15	·	1	·	3	·	1	·	1	3	3	5	·	·	·	1	2	·	·	·	1	1	·
16	·	·	1	1	·	·	1	1	1	1	1	5	·	·	·	·	1	1	·	1	2	2
Totals	15	15	13	25	·	6	14	15	31	31	36	34	2	2	15	17	30	22	15	13	37	42
(A)xx	12	12	18	13	·	·	5	4	19	15	18	24	2	3	10	7	12	15	5	8	19	16
(B)xxy	13	8	6	11	·	·	6	11	10	11	17	14	2	1	10	12	10	13	4	5	20	12
(C)xx	4	9	8	7	·	·	4	7	7	8	8	15	2	1	3	6	10	5	6	2	14	10
(D)xxy	13	18	10	12	2	·	8	5	21	15	21	14	4	4	10	12	14	11	6	8	15	15
(E)xxy	12	9	14	10	·	·	6	10	8	15	12	17	2	1	3	5	15	4	1	3	12	13
Column total	69	71	69	78	2	6	43	52	96	95	112	118	14	12	51	59	91	70	37	39	117	108
Grand total	140		147		8		95		191		230		26		110		161		76		225	

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TABLE 5 (continued)

CULTURE NUMBERS	1,2,3,6 s_c e_c c_v ct g f	1,2,3,4 g	4,5,6 s_c c_v	3,5,6 s_c e_c c_v ct v g f	3,4,6 s_c e_c c_v ct v g f	2,5,6 e_c c_v v g f	2,4,6 s_c e_c c_v ct v g f	2,4,5 e_c c_v f	2,3,4 s_c g	1,5,6 c_v v g f	1,4,6 s_c e_c c_v ct v g f	1,4,5 s_c c_v ct v g f	1,3,6 e_c c_v ct g f	1,3,5 s_c e_c v g	1,2,6 s_c e_c c_v v	5,6 s_c e_r c_v ct v g f
0																
2				1												3
3																3
4	1	1													1	1
5												1				1
6			1					1								1
7							1									
8																
9												1				
10												1	1	1		1
11						1					1	1	1			1
13				1						1						1
15					1		2				1	2				2
16					1						1					
Totals	1	1	1	1 1	1 1		2	1	1		2 1 1 2	4 1 2	2 1	1 1	1	7 12 5 2 2 4 1
(A)xx																
(B)xxy																
(C)xx																
(D)xxy																
(E)xxy									1	1	1					
Grand total	1	1 1	1 1	1 2	3 4	1 1	2 4	1 1	1	1	5 9	5 7	3 3	2 2	1 1	20 26 46

With the exception just noted, there is a regular seriation in the ratios of complementary classes in the above data (see table 4). It is further to be noted that the number of characters has far greater weight than the particular kind. This is due to the fact that in making Xple only such characters were included as were known to have excellent viability.

The inviability effects will be equalized and neutralized to the greatest extent if the number of mutants in complementary classes is equalized as much as possible. This is accomplished by the "alternated backcross" (BRIDGES 1919, 1921), in which half the characters enter from one parent and half from the other, and in which characters consecutive in the map alternate in the homologous chromosomes of the heterozygous female. Thus, the seven characters of the Xple would be divided into 4 and 3 and the parental combinations would be $s_c c_v vf$ and $e_c c_t^6 g^2$. The heterozygous female would be $\dfrac{s_c \;\; +^{sc} \;\; c_v \;\; +^c \;\; v \;\; +^{g2} \;\; f}{+^{sc} \; e_c \;\; +^{cv} \;\; c_t^6 \;\; +^v \;\; g^2 \;\; +^f}$, or more simply formulated, $\dfrac{s_c \qquad c_v \qquad v \qquad f}{(1) \;\; e_c \;\; (2) \;\; (3) \;\; c_t^6 \;\; (4) \;\; (5) \;\; g^2 \;\; (6)}$. The non-crossover complementary classes contain 4 and 3 mutants, and so also do each of the pairs of complementary classes due to crossing over in the six crossover sections. Also, a majority of the pairs of complementary classes due to multiple crossing over are divisions into 4 and 3. (Thus, 1, 3 double crossing over gives the two classes $s_c e_c vf$ and $e_c c_t^6 g^2$). Only a small proportion of the flies would be in 2-5 divisions, while almost no flies would be in 1-6 or 0-7 divisions.

The stocks of $s_c c_v vf$ and $e_c c_t^6 g^2$ were prepared and crossed by BRIDGES and the F_1 wild-type females were backcrossed to Xple males. About 5000 flies of the alternated backcross were raised by OLBRYCHT (table 5, upper section) for direct comparison with the non-alternated results of table 3, and BRIDGES raised some 16,000 others in the course of studying the effect of the Y chromosome on crossing over in the XXY female (table 5, A, B, C, D and E).

In the total of 20,786 flies none was in the 7-0 division, and only 2, or 0.01 percent, were in 6-1 divisions. In the classes in which the division was 5-2, the total flies were 480, or only 2.2 percent. The ratio was 235 : 245, which is practically equality. All of the remaining flies, 20,304 or 97.8 percent, were in 4-3 divisions, and the ratio in the totals was 10,205 : 10,099, which is very close to equality.

In the non-crossover class and each of the single-crossover classes the number of flies was large, and in each case the complementary classes

were practically equal. It is thus seen that the alternated-backcross method was successful in distributing the mortality uniformly throughout the population.

The recombination percents for the various sections are $s_c\text{-}e_c = 6.8$, $e_c\text{-}c_v = 9.6$, $c_v\text{-}c_t = 8.4$, $c_t\text{-}v = 14.7$, $v\text{-}g = 11.0$, $g\text{-}f = 11.4$. Only one of these sections, namely, $c_t\text{-}v$, is long enough for double crossing over to occur within it. Experiments in which tan or lozenge was present between cut and vermilion have shown that there is roughly one double crossover between cut and vermilion for each 2000 flies. The map value for $c_t\text{-}v$ is therefore 0.1 larger than the observed recombinations percent (14.7 +0.1 = 14.8). The maps of the X chromosome corresponding to the alter-
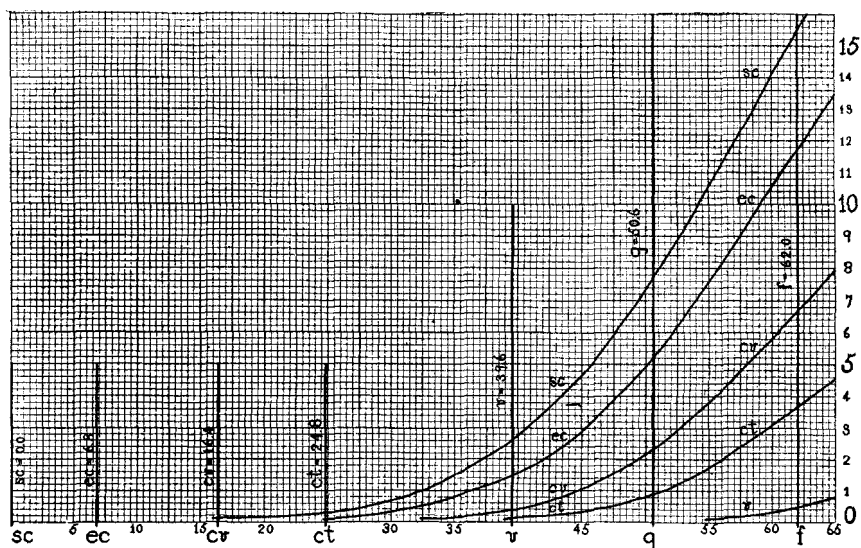
FIGURE 1.—Curves showing difference between map-distance and percentage of recombination for the characters of the alternated-Xple backcross.

nated Xple is given as the base line of figure 1. In comparison with the standard map (BRIDGES 1921) the alternated-Xple map is longer in the sections to the left of vermilion and shorter to the right of garnet. It is important to have a map based solely on alternated-Xple data, since the probability is that for several years the use of alternated Xple will be the standard method for testing crossing over in the X chromosome.

The crossover value for scute and forked on the basis of the data of table 5, is 62.0 units. With respect to scute and forked each average hundred gametes was found to consist of 45.91 non-crossovers, 46.37 single crossovers, 7.53 doubles, 0.18 triples and 0.01 quadruples. The total number of $s_c\text{-}f$ crossovers is thus $46.37 + 2 \times 7.53 + 3 \times 0.18 + 4 \times 0.01$,

which totals 62.01. The percentage of recombinations for scute and forked was 46.55 (the sum of the singles and triples). The number of crossovers per hundred gametes thus exceeded the number of recombinations by 15.46. This difference is also directly obtained as $2 \times 7.53 + 2 \times 0.18 + 4 \times .01 = 15.46$.

In figure 1 this difference of 15.5 is represented by the height of an ordinate at f. Likewise the s_c-g map distance has been calculated and found to be 50.6, and the corresponding recombination percent is 42.9, the difference of 7.7 being represented by the height of an ordinate at g. For s_c-v the difference is 2.6; for s_c-c_t it is 0.3; for s_c c_v it is 0.1. The smooth curve drawn through these experimentally determined points gives a means of predicting the difference between crossing over and recombinations for scute and any other locus. Thus, to find the expectation for the difference for scute and miniature, the locus of miniature is intercalated in the alternated-Xple map between vermilion and garnet with the same ratio between the v-m and m-g intervals as exists in the standard map, (3.1 : 8.3 : : 3.0 : 7.0). The proportionate locus of miniature is thus 3.0 units to the right of vermilion, or at 42.6, and the ordinate at 42.6 cuts the curve from scute at a height of 3.3 units. Similar smooth curves can be drawn through the other points determined by the above experiment, and predictions can thus be made for echinus and any other locus, for crossveinless and any other locus and for cut, vermilion and garnet and other loci.

Curves originating at other points can be intercalated between the curves originating at the points of Xple. Thus, a curve originating at ruby can be intercalated between those originating at echinus and at crossveinless, with the distance from the intercalated curve to the other two curves proportional to the e_c-r_b and the r_b-c_v map distances (2.0 : 6.2).

By extrapolation the curves can be continued beyond the locus of forked until they intersect the ordinates from fused and bobbed. There is also some direct evidence at hand as to the course of the curves beyond forked, so that the extensions need not be pure extrapolation.

The knowledge of the amount of corrections, gained principally from the above data, has been applied in the construction of a standard map with correction curves. This map covers the region to the right of forked, and has intercalated curves originating from the other principal loci besides those included in Xple. It has been published as part of the summary and review of the Drosophila work that appeared in Vol. II of Bibliographia Genetica under the editorship of LOTSY. A map with curves similar to that of figure 1, but based on smaller numbers in the

alternated backcross, has appeared in the CARNEGIE INSTITUTION OF WASHINGTON Year Book for 1924.

At present, experiments are under way to extend the information on the course of the curves in the region to the right of forked. These experiments make use of the dominant mutation minute-n, situated at about six units to the right of forked, and the recessive bobbed, situated about 13 units to the right of forked.

In constructing the old standard map (MORGAN and BRIDGES 1916) use was made of experiments that covered the length of the X chromosome with only a few intermediate loci, or that covered particular parts fairly closely. MULLER (1916) devised a method for covering the length of the X chromosome satisfactorily even with mutants that were poorly viable and hard to classify; but the method was so laborious, involving a genetic test of each individual recorded, that he secured only 712 individuals. For some unknown reason the amount of crossing over was abnormally low in MULLER'S data. Most of the later work suggests that a new standard map should be made, and that it would correspond rather closely to the alternated-Xple map presented here.

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