THE EFFECT OF AUTOSOMAL INVERSIONS ON CROSSING OVER IN THE X-CHROMOSOME OF DROSOPHILA MELANOGASTER

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INTRODUCTION

THE first evidence of a crossover suppressor was found in 1913 by STURTEVANT. In 1921 STURTEVANT found the first definite evidence of an inversion when he showed that several third chromosome genes in *Drosophila melanogaster* were arranged in an inverted order with respect to similar genes in the third chromosome of *D. simulans*. At this time STURTEVANT predicted that a heterozygous inversion would suppress crossing over and suggested that the Nova Scotia stock (STURTEVANT 1917 and 1919) had an inversion. It was not until 1926, however, that it was actually demonstrated that in at least one case crossover suppression was due to an inversion (STURTEVANT 1926).

The sole effect on crossing over ascribed to inversions until recently is that the number of recovered crossovers is reduced in the chromosome in which the inversion is located. But as long ago as 1919, STURTEVANT suspected that crossover suppressors (inversions) might have an interchromosomal effect. He reported that a high value for crossing over between black and purple was due, in part at least, to a dominant gene (really an inversion) in the third chromosome, which when heterozygous reduced crossing over in this chromosome.

WARD (1923) tested the effect of the curly inversion on crossing over in the first and third chromosomes; her observations on crossing over were of no value because of the small number of flies used, but she did notice an increase in multiple crossovers. PAYNE (1924) tested the effect of the Payne inversion on crossing over in the first and second chromosomes. While he counted large numbers of flies, the chromosomes were poorly marked ($w^e m$ on the first chromosome and $b\ pr\ c\ sp$ on the second) and he ran no controls; he therefore noticed no interchromosomal effect on crossing over. PAYNE made no statement in regard to the number of multiple crossovers recovered.

The problem rested here until 1932, when SCHULTZ and REDFIELD (MORGAN, BRIDGES and SCHULTZ 1932) stated that inversions in the first and second chromosomes increased crossing over in the third chromosome. In the following year they published a short account (MORGAN, BRIDGES

and SCHULTZ 1933) in which they stated that inversions in the first and third chromosomes increased crossing over in the second. The increased crossing over in both cases was due to an increase in multiple crossing over.

The experiments reported in this paper are concerned with the effects of inversions in the second and third chromosomes on crossing over in the first chromosome.

MATERIALS AND TECHNIQUE

The autosomal inversions used in the experiments were the Payne inversions (C3LP *Dfd* C3RP *ca*) in the third chromosome and the Cy inversions (Cy C2L.C2R$_{Cy}$) in the second chromosome. The Payne inversions (PAYNE 1924) reduce crossing over almost completely throughout the length of the third chromosome. The Curly inversions (WARD 1923) gave no crossover flies in a total of 2487 from a cross of heterozygous Cy/2ple females by 2ple/2ple males. Crossing over in the first chromosome was detected by means of the alternated X^7 stock (*y tv v f/ec ct^6 g^2*). This is identical with the alternated X-ple stock (BRIDGES and OLBRYCHT 1926) except for the substitution of *y* for *sc*.

The crosses were as follows:

1. +/+; +/+;   *y cv v f/ec ct^6 g^2* ♀ ×Oregon-R ♂ (controls)
2. +/+; +/Cy;   " /   "   ♀×   "   ♂
3. +/Payne; +/+;   " /   "   ♀×   "   ♂
4. +/Payne; +/Cy; " /   "   ♀×   "   ♂

Because of a significant deviation of the crossover value in region 1 of the controls from the standard value (to be discussed below) it is important that the relation of the chromosomes of the test stocks to those of the controls be explained. The derivation of the stocks was such that one set of autosomes and one X chromosome in each stock came from the original *ec ct^6 g^2* stock. The second X chromosome in each case came from the original *y cv v f* stock. The origin of the remaining chromosomes was as follows: in the controls they all came from the *y cv v f* stock. In the +/+; +/Cy; *y cv v f/ec ct^6 g^2* stock the remaining third chromosome had an equal chance of coming from either the *y cv v f* or the Curly stock; the same holds true for the fourth chromosome. In the +/Payne; +/+; *y cv v f/ec ct^6 g^2* stock the remaining second and fourth chromosomes had an equal chance of coming from either the Payne or the *y cv v f* stock. Since twenty cultures with from two to three females in each were counted for each cross, it is likely that the origins of these chromosomes were very nearly equally divided between their possible sources. In the +/Payne; +/Cy; *y cv v f/ec ct^6 g^2* stock only the origin of the fourth chromosome is in doubt. It had an equal chance of coming from either the Payne, the Curly or the *y cv v f* stock.

In all the crosses the male offspring only were classified for each of the seven first chromosome characters involved. The crosses were run at $25 \pm 1°C$ on the usual cornmeal-agar-molasses food medium.
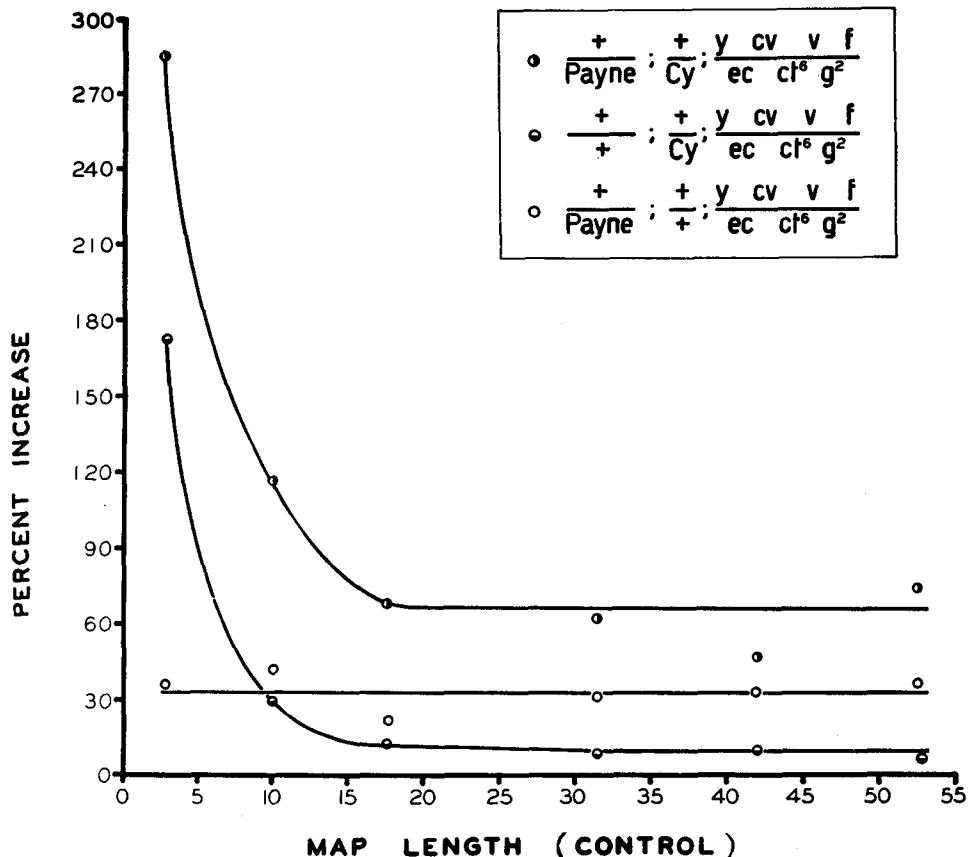

FIGURE 1.—Plot of percent increase in crossing over in the test crosses over that of the controls against the control map length. (Data from table 1.)

THE DATA

The crossover values in the controls for all regions except region 1 compare favorably with the standard map distances (table 1). Region 1, however, gives a value which is but half that of the standard value. Since a large number of flies was counted (3937) and classification was certain, this difference must be considered significant. The construction of the stocks used in the test crosses makes it unlikely that this low value is due to any autosomal modifiers. Since the source of the X chromosomes is constant throughout the experiment it is almost certain that the cause of this low value in region 1 of the controls had no effect on the relative

values of the results, although the possibility remains that there might have been some effect on the absolute values.

Table 1 shows the crossover values for each of the four different crosses as well as a comparison of the crossover values of the test crosses with those of the controls. These values are calculated from the raw data shown in table 5. There is an increase in crossing over in each of the six regions of the test crosses as compared with the controls. This increase is greatest

TABLE 1

Comparison of crossover values.

REGION	1		2		3		4		5		6		MAP LENGTH		TOTAL NUMBER OF FLIES
	% CROSS-OVER	% IN-CREASE	% CROSS-OVER	% IN-CREASE	% CROSS-OVER	% IN-CREASE	% CROSS-OVER	% IN-CREASE	% CROSS-OVER	% IN-CREASE	% CROSS-OVER	% IN-CREASE	MAP LENGTH	% IN-CREASE	
$\dfrac{+\ +\ y\ cv\ v\ f}{+\ +\ ec\ ct^6\ g^2}$ —;—;——	2.7	—	7.4	—	7.7	—	13.6	—	10.7	—	10.7	—	52.8	—	3937
$\dfrac{+\ +\ y\ cv\ v\ f}{+\ Cy\ ec\ ct^6\ g^2}$ —;—;——	7.4	171.1	9.8	32.4	8.7	13.0	14.8	8.8	11.9	11.2	11.5	7.5	64.1	21.4	2733
$\dfrac{+\ +\ y\ cv\ v\ f}{Payne\ +\ ec\ ct^6\ g^2}$ —;—;——	3.7	37.0	10.6	43.2	9.2	19.5	17.8	30.9	14.3	33.6	14.8	38.3	70.4	33.3	3612
$\dfrac{+\ +\ y\ cv\ v\ f}{Payne\ Cy\ ec\ ct^6\ g^2}$ —;—;——	10.4	285.2	16.1	117.6	12.9	67.5	22.2	63.2	16.0	49.5	19.4	75.4	97.0	83.7	1554

in the cross involving both sets of inversions, next greatest in the cross with the Payne inversions and least in the cross with the Curly inversions (table 1, fig. 1). This is due to a large increase in multiple crossovers which is again greatest in the cross involving both sets of inversions, and least in the cross involving the Curly inversions.

The Curly inversions have their greatest effect on the left end of the X chromosome, causing an increase in crossing over of 171.1 percent between y and ec (region 1, table 1). The effect drops rapidly as the right end of the chromosome is approached so that between cv and ct^6 (region 3, table 1) the increase is only 13.0 percent. From this point on the effect on crossing over is approximately constant (table 1, fig. 1).

The Payne inversions, unlike the Curly inversions, have an approximately uniform effect on crossing over throughout the portion of the X chromosome under observation in this experiment (table 1 and fig. 1). This effect is to cause an increase of approximately 33 percent in each of the six regions. According to SCHULTZ and REDFIELD (MORGAN, BRIDGES and SCHULTZ, 1933), the Payne inversions did not have a uniform effect throughout the length of the second chromosome but affected crossing

over in this chromosome in much the same manner as does triploidy. Why the effect of the Payne inversions on one chromosome is different from their effect on another cannot be explained at present.

The Curly and Payne inversions when combined affect crossing over in the first chromosome in somewhat the same manner as do the Curly inversions alone (table 1 and fig. 1). Here again, the increase is greatest in region 1 (285.2 percent) and decreases rapidly to 65.7 percent in region 3 and remains practically constant thereafter (table 1 and fig. 1). The effect of the combined inversions is greater in every region than is the effect of the inversions taken singly. Furthermore, the effect of the combined inversions exceeds in every region the sum of the effects of each of the two pairs of inversions used separately (table 1).

The increase in crossing over noted in the crosses involving the inversions singly or together is due to an increase in multiple crossover strands and a decrease in non-crossover strands (tables 2, 3 and 4). In the cross

TABLE 2

Classification of strands into crossover classes.

	CONTROLS $\frac{+ + y\ cv\ v\ f}{-;-;}$ $+ +\quad ec\ ct^6g^2$		$\frac{+ + y\ cv\ v\ f}{-;-;}$ $+ Cy\quad ec\ ct^1\ g^2$		$\frac{+ + y\ cv\ v\ f}{-;-;}$ Payne $+\quad ec\ ct^6\ g^2$		$\frac{+ + y\ cv\ v\ f}{-;-;}$ Payne Cy $\ ec\ ct^6\ g^2$	
	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
non-crossovers	2052	52.1	1213	44.4	1532	42.4	470	30.2
singles	1697	43.1	1292	47.3	1632	45.2	702	45.2
doubles	181	4.6	224	8.2	430	11.9	344	22.1
triples	7	0.2	4	0.1	16	0.4	37	2.4
quadruples	—	—	—	—	2	0.1	—	—
quintuples	—	—	—	—	—	—	1	0.1
totals	3937		2733		3612		1554	

TABLE 3

Showing comparison of tetrads having a crossover in a given region with those having a crossover in the given region and in one other region. All values are in percent.

REGIONS	1 SINGLES	1 DOUBLES	2 SINGLES	2 DOUBLES	3 SINGLES	3 DOUBLES	4 SINGLES	4 DOUBLES	5 SINGLES	5 DOUBLES	6 SINGLES	6 DOUBLES
$\frac{+ + y\ cv\ v\ f}{-;-;}$ $+ +\quad ec\ ct^6\ g^2$	2.8	2.3	9.9	4.4	9.8	5.0	19.9	2.8	16.1	5.0	10.4	6.5
$\frac{+ + y\ cv\ v\ f}{-;-;}$ $+ Cy\quad ec\ ct^6\ g^2$	6.7	7.7	13.8	8.7	9.2	7.9	17.9	11.1	11.3	11.8	7.3	15.1
$\frac{+ + y\ cv\ v\ f}{-;-;}$ Payne $+\ ec\ ct^6\ g^2$	2.3	4.6	7.0	11.9	6.8	11.6	14.0	20.1	11.0	15.3	4.4	23.0
$\frac{+ + y\ cv\ v\ f}{-;-;}$ Payne Cy $\ ec\ ct^6\ g^2$	1.4	11.4	−1.3	26.0	5.9	13.4	6.6	21.0	2.8	21.3	1.0	27.6

with the Curly inversions the double crossover strands are increased by 78 percent (table 2). The non-crossover strands are decreased from 52.1 percent to 44.4 percent, a reduction of 14.8 percent. There is an apparent reduction in the number of triple crossovers but the numbers involved are small and therefore the reduction is probably not significant. The increase

TABLE 4

Distribution of crossovers in tetrads. All values are in percent. Symbols are explained in the text. (The 5 point crossover is not included in the calculations of $T._i$, $T._{ij}$, etc.; the values of the T_{ijk} tetrads are omitted.)

CLASS	+ + y cv v f —;—;———— + + ec ct⁶ g²	+ + y cv v f —;——;— + Cy ec ct⁶ g²	+ + y cv v f —;—;——— Payne + ec ct⁶ g²	+ + y cv v f —;—;— Payne Cy ec ct⁶ g²
T_0	13.4	5.2	8.8	4.7
$T._i$	68.9	62.4	44.4	16.4
$T._{ij}$	16.2	31.6	45.2	59.6
$T._{ijk}$	1.4	0.8	0.0	19.2
$T._{ijkl}$	—	—	1.6	—
REGION		**BY REGIONS**		
1	2.8	6.7	2.3	1.4
2	9.9	13.8	7.0	−1.3
3	9.8	9.2	6.8	5.9
4	19.9	17.9	14.0	6.6
5	16.1	11.3	11.0	2.8
6	10.4	7.3	4.4	1.0
1, 2	−0.1	−0.1	0.0	0.0
1, 3	0.0	0.4	0.0	1.3
1, 4	0.5	2.3	1.9	3.1
1, 5	0.7	2.3	1.5	2.1
1, 6	1.2	2.8	1.2	4.9
2, 3	−0.1	—	0.2	0.3
2, 4	0.7	1.5	2.9	9.0
2, 5	1.2	4.2	3.2	8.2
2, 6	2.6	3.1	5.6	8.5
3, 4	0.7	0.6	2.0	1.8
3, 5	1.5	3.1	4.1	5.1
3, 6	2.8	3.8	5.2	4.9
4, 5	1.3	1.6	4.4	1.8
4, 6	−0.4	4.8	8.9	5.2
5, 6	0.2	0.6	2.1	4.1

in single crossover strands is probably significant. ($D/\sigma_d = 3.5$.) The Payne inversions cause an increase in double crossover strands of 154 percent and a decrease of 18.4 percent in the non-crossover strands. The single crossover strands do not change (43.1 percent in the controls and 45.2 percent in the Payne cross). Here again the numbers of three point crossover strands are too small to give decisive evidence as to the effect on triple crossovers. The combined inversions have a great effect on both the mul-

tiple crossover strands and the non-crossover strands. Double crossover strands are increased by 369 percent and triple crossovers by 1100 percent. The single crossover strands remain constant. The non-crossover strands are decreased by 41.7 percent (52.1 percent in the controls and 30.2 percent when both the Curly and Payne inversions are present).

The behavior of the tetrads in all four crosses was deduced by means of the following formulae for the distribution of crossovers in tetrads. (These have been derived by D. R. CHARLES [unpublished] from WEINSTEIN'S generalized tetrad formulae [WEINSTEIN 1932 and 1936].)

$$T._{ijkl} = 16s._{ijkl}$$
$$T._{ijk} = 8(s._{ijk} - 4s._{ijkl})$$
$$T._{ij} = 4(s._{ij} - 3s._{ijk} + 6s._{ijkl})$$
$$T._{i} = 2(s._{i} - 2s._{ij} + 3s._{ijk} - 4s._{ijkl})$$
$$T._{o} = s._{o} - s._{i} + s._{ij} - s._{ijk} + s._{ijkl}$$

Where $T._{ijkl}$ = all tetrads with four crossovers regardless of the regions involved; $T._{ijk}$ = all tetrads with three crossovers regardless of the regions involved etc. and where $s._{ijkl}$ = all strands with four crossovers regardless of the regions involved etc.

Distribution of tetrads with crossovers in specific regions:

$$T_{ijkl} = 16s_{ijkl}.$$
$$T_{ijk.} = 8(s_{ijk.} - s_{ijk.l})$$
$$T_{ij.} = 4(s_{ij.} - s_{ij.k} + s_{ij.kl})$$
$$T_{i.} = 2(s_{i.} - s_{i.j} + 3s_{i.jk} - s_{i.jkl})$$

Where $T_{ijkl.}$ = all tetrads which are crossovers in the specific regions ijkl only and $T_{ijk.}$ = all tetrads which are crossovers in the specific regions ijk only, etc., and $T_{ijk.l}$ = all tetrads which are crossovers in the specific regions ijk and one other region, l, and where $s_{ijkl.}$, $s_{ijk.}$, etc., have the same meaning for strands as $T_{ijkl.}$, etc., has for tetrads.

The tetrad computations of the data from the Curly cross indicate that those tetrads which show no crossovers in the portion of the X chromosome under observation (T_o in table 4) are greatly reduced in number and those tetrads which show one crossover ($T._i$ in table 4) are slightly reduced in number as compared with the controls. The numbers of tetrads showing two crossovers is greatly increased. In the cross with the Payne inversions, the decrease in non-crossover tetrads is not as great as that in the cross with the Curly inversions, but the effect on the single crossover and double crossover tetrads, while in the same direction as that caused by the Curly inversions, is far greater. As is to be expected, the combined inversions have a much greater effect than either of the inversions alone. They reduce the non-crossover tetrads to 4.7 percent of the total and single crossover

tetrads to 16.4 percent while they increase the double crossover tetrads to 59.6 percent and the triple crossover tetrads to 19.2 percent.

Table 3 shows a comparison of tetrads having a crossover in a given region with those having a crossover in the given region plus one other region. In the controls we find that most of the crossing over in all six regions comes from single crossover tetrads (tetrads having only one crossover, that crossover being in the given region). In the cross with the Curly inversions, in regions one and six most of the crossing over occurred in $T_{i.j}$ tetrads (tetrads with two crossovers, one of which is in the given region i). In region 5 about the same amount of crossing over occurred in $T_{i.}$ tetrads as in $T_{i.j}$ tetrads. In the remaining regions most of the crossing over took place in $T_{i.}$ tetrads. In the cross involving the Payne inversions, most of the crossing over took place in the $T_{i.j}$ tetrads. This is the reverse of what took place in the controls. Finally, in the cross with the combined inversions, not only did most of the crossing over take place in $T_{i.j}$ tetrads, but these tetrads exceeded the $T_{i.}$ tetrads by at least 100 percent in every region. In regions 1, 2, and 6 the excess of $T_{i.j}$ tetrads over $T_{i.}$ tetrads is tremendous (table 3). Note the great reduction in the number of tetrads which crossed over in region 2 only.

TABLE 5

Showing the numbers and types of ♂ offspring of each of the four different classes of ♀ ♀ listed below.

REGIONS	$\dfrac{+\ +\ y\ cv\ v\ f}{+\ +\ \ ec\ ct^8\ g^2}$		$\dfrac{+\ +\ y\ cv\ v\ f}{+\ Cy\ \ ec\ ct^8\ g^2}$		$\dfrac{+\ +\ y\ cv\ v\ f}{Payne\ +\ \ ec\ ct^8\ g^2}$		$\dfrac{+\ +\ y\ cv\ v\ f}{Payne\ Cy\ \ ec\ ct^8\ g^2}$	
	y	*non-y*	*y*	*non-y*	*y*	*non-y*	*y*	*non-y*
0	1033	1019	640	573	861	671	254	216
1	44	35	59	87	39	46	21	50
2	118	122	102	99	122	123	67	40
3	137	107	96	85	109	118	52	59
4	228	231	161	161	230	208	76	90
5	173	194	124	113	186	156	58	58
6	145	163	97	108	171	124	81	50
1, 2	0	0	0	0	0	1	1	1
1, 3	0	1	0	3	0	0	3	5
1, 4	2	3	8	9	6	12	10	12
1, 5	2	5	8	8	5	10	6	12
1, 6	6	6	10	9	6	6	10	15
2, 3	0	0	0	0	1	1	1	2
2, 4	4	4	8	5	15	14	24	22
2, 5	7	6	20	10	16	23	24	13
2, 6	13	15	16	6	26	29	23	18
3, 4	4	5	4	0	9	9	8	8
3, 5	10	5	13	9	22	16	11	13
3, 6	15	15	13	14	19	29	14	12
4, 5	7	7	6	6	22	22	13	8

TABLE 5 (*Continued*)

Showing the numbers and types of ♂ offspring of each of the four different classes of ♀ ♀ listed below.

4, 6	24	11	18	16	42	42	19	23
5, 6	2	2	1	4	10	17	4	9
1, 2, 3	0	1	0	0	0	0	0	1
1, 2, 4	0	0	0	1	0	0	0	0
1, 2, 5	0	0	0	0	0	0	1	0
1, 3, 5	0	0	0	0	0	0	0	2
1, 4, 5	0	0	0	0	0	0	1	4
1, 4, 6	0	0	0	0	0	2	1	3
1, 5, 6	0	0	0	0	1	0	1	0
2, 3, 4	0	0	0	0	0	0	1	0
2, 4, 5	0	0	1	0	· 2	1	1	2
2, 4, 6	1	0	1	0	2	0	2	5
2, 5, 6	0	2	0	0	3	2	1	0
3, 4, 5	0	0	0	0	0	0	0	1
3, 4, 6	0	2	0	0	0	0	4	2
3, 5, 6	0	0	1	0	1	0	0	0
4, 5, 6	0	1	0	0	2	0	2	2
1, 2, 4, 6	0	0	0	0	0	1	0	0
2, 4, 5, 6	0	0	0	0	1	0	0	0
1, 3, 4, 5, 6	0	0	0	0	0	0	1	0
Totals	1975	1962	1407	1326	1929	1683	796	758
Grand totals		3937		2733		3612		1554

SUMMARY

The effects of autosomal inversions on crossing over in the first chromosome were measured. Four different crosses were made: (1) controls (no inversions in the autosomes); (2) Curly inversions in the second chromosome, third chromosome normal; (3) Payne inversions in the third chromosome, no inversion in the second chromosome; (4) Curly inversions in the second chromosome and Payne inversions in the third chromosome simultaneously. In all four crosses crossing over was measured in the first chromosome by means of the alternated 7ple gene complex.

Autosomal inversions increase crossing over in the X chromosome. This increase is accompanied by a great increase in multiple crossing over. These results confirm those of SCHULTZ and REDFIELD on the negative correlation of the interchromosomal effect with the intrachromosomal effect of inversions on crossing over.

The Curly inversions have their greatest effect on the yellow end of the X chromosome. This effect decreases rapidly until the cv-ct^6 region is reached and thereafter it remains constant. The Payne inversions have an approximately uniform effect on crossing over throughout the portion of the X chromosome marked in this experiment.

The combined inversions have an effect similar to that of the Curly inversions, that is, greatest in the y-ec region, and constant to the right of the cv-ct⁶ region. This effect is greater in all regions than that of either the Curly or Payne inversions when used singly.

The total increase in map length of the y-f interval caused by the inversions was greatest in the cross with the inversions combined and least when the Curly inversions were used alone.

Analysis of the strand data shows that while there was a decrease in non-crossover strands and an increase in multiple crossover strands; the single crossover strands, with the probable exception of those in the cross involving the Cy chromosome alone, remained constant. Here again the order of magnitude of the effects of the inversions on the strand data was Cy; Payne > Payne > Cy.

Tetrad analysis showed that there was a reduction in non-crossover and single crossover tetrads and an increase in multiple crossover tetrads. As in the above two cases the order of magnitude of the effects of the inversions was Curly; Payne > Payne > Curly.

LITERATURE CITED

BRIDGES, C. B. and OLBRYCHT, T. M., 1926 The Multiple Stock "X-ple" and its Use. Genetics 11: 41–56.

MORGAN, T. H., BRIDGES, C. B. and SCHULTZ, J., 1932: The constitution of germinal material in relation to heredity. Yearb. Carnegie Instn. 31: 303–307.

———— 1933 The constitution of germinal material in relation to heredity. Yearb. Carnegie Instn. 32: 289–302.

PAYNE, F., 1923 Crossover modifiers in the third chromosome of Drosophila melanogaster. Genetics 9: 327–341.

STURTEVANT, A. H., 1913 A third group of linked genes in Drosophila ampelophila. Science, 37: 990.

———— 1917 Genetic factors affecting the strength of linkage in Drosophila. Proc. Nat. Acad. Sci. 3: 555–558.

———— 1919 Inherited linkage variations in the second chromosome. Pub. Carnegie Instn. 278: 305–341.

———— 1921 A case of rearrangement of genes in Drosophila. Proc. Nat. Acad. Sci 7: 235–237.

———— 1926 A crossover reducer in Drosophila melanogaster due to inversion of a section of the third chromosome. Biol. Zbl. 46: 679–702.

WARD, L., 1923 The genetics of the Curly wing in Drosophila. Another case of balanced lethal factors. Genetics 8: 276–300.

WEINSTEIN, A., 1932 A theoretical and experimental analysis of crossing over. Proc. Sixth Int. Congress Genetics 2: 206.

———— 1936 The theory of multiple-strand crossing over. Genetics 21: 155–199.