FURTHER STUDIES OF CROSSING OVER IN ATTACHED-X CHROMOSOMES OF DROSOPHILA MELANOGASTER

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THE PROBLEM

For some years it has been known from genetic data that crossing over in the female of *Drosophila melanogaster* occurs at a stage of meiosis after the chromosomes have split equationally (BRIDGES 1916, L. V. MORGAN 1925, ANDERSON 1925, BRIDGES and ANDERSON 1925). More recently crossing over in several other organisms has been demonstrated, by genetic methods, to occur at such a stage of meiosis (RHOADES 1932—Zea; WHITING and GILMORE 1932—Habrobracon; LINDEGREN 1933—Neurospora; ALLEN 1930—Sphaerocaros; and others). The genetic evidence has been confirmed cytologically in Zea (MCCLINTOCK 1931). The uniformity, in essentials, of the meiotic mechanism as revealed by cytological studies of a large number of widely different organisms, considered in connection with the above mentioned genetic information justifies the generalization that in all organisms in which crossing over occurs at meiosis, it takes place after the equational division of the chromosomes. With the demonstration of this fact concerning the stage at which crossing over occurs, several questions arose relating to the process. Certain of these have received satisfactory answers; others have awaited the accumulation of additional data. Of the latter, the relation of the strands involved in a given crossover to those involves in other crossovers has not, as yet, been settled with the degree of certainty desired. Many problems of crossing over are inseparably associated with this question. For example, an understanding of interference in crossing over as expressed in single recovered strands is dependent on the relation of strands involved in adjacent crossovers (HALDANE 1931, WEINSTEIN 1932). Likewise the randomness or non-randomness of strands involved in different crossovers bears directly on the relation of crossover to recombination frequencies (EMERSON and RHOADES 1933). Finally, any theoretical consideration of the mechanism involved in the process of crossing over must take into account the relation of the individual events involved in multiple crossing over (SAX 1932, BELLING 1933).

MATERIALS AND METHODS

The ideal organism for a study of the problem defined above is one in which the four products of a single meiosis can be investigated. This con-

dition is realized in certain plants in which the products of meiosis remain together until maturity, for example in the flowering plant Epilobium (MICHAELIS 1931), in mosses (WETTSTEIN 1924), in liverworts (ALLEN 1924) and in many of the fungi. The ascomycete Neurospora has the advantages of the plants mentioned above and the additional convenience that the products of the two meiotic divisions can be differentiated (LINDEGREN 1933). However, in none of these organisms has the genetic analysis been carried sufficiently far to yield results which bear on the question under consideration. In Drosophila the use of attached-X females provides a practicable means of getting at the problem. Here only two of the four strands of a given tetrad can be recovered but an analysis of these should be sufficient for the purpose outlined.

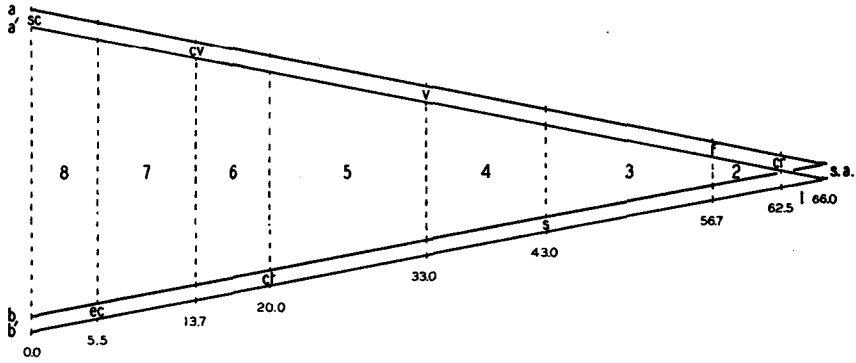

FIGURE 1.—Constitution of attached-X females used in the study reported in this paper. Crossover regions are indicated in larger numerals. Standard map positions are indicated by smaller numbers below. Spindle attachment is indicated by the letters "sa."

An attached-X stock of the desired constitution was built up from triploids with two attached chromosomes. By this method genes can be introduced into the attached chromosomes by crossing over. The stock obtained in this way had the genes scute (*sc*), crossveinless (*cv*), vermilion (*v*), forked (*f*), and carnation (*cr*) in one chromosome and echinus (*ec*), cut-6 (*ct*), and sable-2 (*s*) in the attached homolog. This constitution together with the standard map positions of the genes is shown in figure 1. The alternated position of the genes has the advantage of reducing viability complications. In such an experiment viability differences are of relatively great importance and to reduce them still further the number of offspring per culture bottle was kept low. Daughters of females of the proper constitution were mated to Bar males (for the purpose of identifying detachments) and allowed to lay eggs for a 3 day period, transferred to a second bottle and allowed to lay for a 2 day interval, then discarded. Since attached-X females produce fewer offspring than free-X females

(YY individuals lethal and XXX superfemales practically so), this procedure resulted in cultures with near the optimum number of individuals. A temperature of 25°C and a fairly humid atmosphere were maintained. On hatching, the progeny were classified; those from parents of other than the desired constitution (because of crossing over) were discarded. Wild-type females only (about 50 percent) were of course set up as parents. Of these approximately 56 percent were of the proper constitution.

For the purposes of the study it was necessary to determine the genotypic constitution of a considerable number of the offspring of females of the constitution given above. Certain cultures were selected and all of the females hatching in these were mated for constitution tests. In all, tests were obtained on 1478 females. Since the genotype of a given female is deduced from the types of equationals appearing among her daughters and since the frequency of equationals for a given gene is a function of the distance of that gene from the spindle attachment, the position of genes in the proximal region of the chromosome in relation to those more distally located is often difficult. It was found to be impossible to obtain tests for carnation in all of the females. Tests for forked were not always obtained in single cultures. Since it was highly desirable to have tests for forked for all of the females, those from which forked daughters were not

TABLE 1

Phenotypic frequencies among daughters of attached-X mothers of the constitution $\frac{sc\ cv\ v\ f\ cr}{ec\ ct\ s}$.

PHENOTYPE	FREQUENCY	PERCENT	S.E.	PHENOTYPE	FREQUENCY	PERCENT	S.E.
wild-type	4093	51.07	0.56	*v*	166	2.07	0.16
sc cv v f cr	26	0.32	0.06	*f cr*	12	0.15	0.04
sc cv v f	47	0.59	0.09	*f*	18	0.22	0.05
sc cv v	456	5.69	0.26	*ct s*	118	1.47	0.14
sc cv	654	8.16	0.31	*ct*	93	1.16	0.12
sc	432	5.39	0.25	*s*	102	1.27	0.13
ec ct s	330	4.12	0.23	*sc cv f*	1	0.01	0.01
ec ct	645	8.06	0.31	*sc f*	2	0.02	0.02
ec	496	6.20	0.27	*sc f cr*	3	0.04	0.02
cv v f cr	11	0.14	0.04	*sc s*	5	0.06	0.03
cv v f	29	0.36	0.07	*ec f cr*	2	0.02	0.02
cv v	160	2.00	0.16	*ec v*	2	0.02	0.02
cv	58	0.72	0.10	*ec v f*	1	0.01	0.01
v f cr	9	0.11	0.04	*ec s*	3	0.04	0.02
v f	40	0.50	0.08				
				Total	8014		

obtained were tested further by raising mass cultures of the daughters. In this way definite tests were obtained for practically all of the females. Although there is a possibility of error in using tests obtained in the second generation, the magnitude of this error for the gene forked can be shown to be negligibly small. Females whose second generation tests showed no forked flies were tested in a third mass culture generation. The absence of forked in this generation was considered as definite evidence that the original tested female was homozygous for the normal allele of forked.

TABLE 2

Genotypic constitutions of all tested females with carnation disregarded. Single letter symbols are used for brevity. Equivalents are: scute, u; echinus, e; crossveinless, c; cut, t; vermilion, v; sable, s; forked, f. Phenotypes are indicated in bold face type followed by the total number of the given phenotype tested. Non-italicized symbols indicate that the genotype might equally probably be other than that given, for example, ucvf/ecv can be either ucvf/ecv or ucv/ecvf.

Wild-type (705)		**ucv** (54)	3	*uetvf/ets*	3	*ucvf/evf*	8	*utf/ets*	5
ucvf/ets	398	*ucvf/ucv*	31	*etvf/uets*	2	*uetvf/cvf*	1	*etf/uts*	1
etsf/ucv	39	*ucvf/ucvs*	22	*etv/ets*	1	*uevf/cvf*	1	*uctvf/ets*	3
etf/ucvs	46	*ucvsf/ucv*	1	**e** (109)		**v**(45)		*etvf/ucts*	1
etvf/ucs	82	**uc** (90)		*evf/ets*	38	*ucvf/etv*	4	*utvf/ets*	2
evf/ucts	29	*ucvf/ucs*	51	*ecvf/ets*	52	*etvf/ucv*	3	*etvf/uts*	2
ecvf/uts	38	*ucvf/ucts*	33	*etsf/ev*	1	*ucvf/etv*	7	*utf/etvs*	1
cvf/ets	18	*ucsf/ucv*	2	*etsf/ecv*	5	*ucvf/ev*	3	**s** (28)	
cvf/uets	18	*uctsf/ucv*	2	*etf/evs*	3	*evf/ucv*	7	*ucvs/ets*	4
ucv/ets	2	*uctf/ucvs*	1	*etf/ecvs*	3	*ucvf/ev*	5	*ucs/ets*	6
ucvsf/et	1	*uctvf/ucs*	1	*etvf/ecs*	1	*ucvf/etvs*	3	*ucvsf/ets*	1
ucsf/etv	1	**u** (94)		*uevf/ets*	2	*etvf/ucvs*	1	*etsf/ucvs*	1
uctsf/ev	1	*ucvf/uts*	42	*evf/uets*	3	*ucvf/evs*	7	*ucvsf/ets*	1
utsf/ecv	2	*ucvf/uets*	34	*ecv/ets*	1	*evf/ucvs*	3	*ucsf/ets*	2
uetsf/cv	2	*utsf/ucv*	4	**cvf** (19)		*uctvf/evs*	1	*etsf/ucs*	8
etsf/cv	1	*uetsf/ucv*	2	*ucvf/ecvf*	12	*cvf/etvs*	1	*ucsf/ets*	4
ucf/ets	2	*utf/ucvs*	3	*ucvf/cvf*	7	**f** (22)		*ucts/es*	1
etf/ucs	1	*uetf/ucvs*	2	**cv** (27)		*ucvf/etsf*	8	**uf** (3)	
ucf/etvs	1	*utvf/ucs*	2	*ucvf/ecv*	5	*ucvf/etf*	10	*ucvf/utsf*	1
uctf/evs	2	*uetvf/ucs*	4	*ucvf/ecv*	2	*etvf/ucsf*	1	*ucvf/uetf*	1
uetf/cvs	1	*uevf/ucts*	1	*ucvf/cv*	1	*evf/uctsf*	1	*ucf/uetvf*	1
etf/cvs	1	**ets** (48)		*cvf/ucv*	3	*ecvf/utsf*	1	**us** (2)	
ucvf/es	5	*ets/ets*	15	*ucvf/cv*	4	*cvf/uetf*	1	*uets/ucs*	1
uctvf/es	2	*etsf/ets*	27	*ucvf/ecvs*	5	**ts** (22)		*ucsf/uts*	1
utvf/ecs	2	*uetsf/ets*	1	*ecvf/ucvs*		*ucts/ets*	4	**ef** (1)	
uetvf/cs	3	*uetsf/ets*	5	*ucvf/cvs*	3	*uts/ets*	1	*ecvf/etf*	1
etvf/cs	3	**et** (123)		*cvf/ucvs*	1	*uctsf/ets*	1	**ev** (1)	
ucvf/ts	1	*etf/ets*	37	**c** (10)		*etsf/ucts*	2	*ecvf/etv*	1
cvf/uts	1	*etvf/ets*	69	*ucvf/ecs*	2	*utsf/ets*	1	**evf** (1)	
ecv/uts	1	*etsf/et*	1	*ucvf/cs*	5	*etsf/uts*	2	*ecvf/etvf*	1
tsf/ecv	1	*etsf/etv*	4	*cvf/ucs*	2	*utsf/ets*	11	TOTAL	1478
ucvf (17)		*etf/etvs*	1	*ucvf/ets*	1	**t** (20)			
ucvf/ucvf	17	*uetf/ets*	3	**vf** (37)		*uctf/ets*	2		
		etf/uets	2	*ucvf/etvf*	27	*etf/ucts*	3		

EXPERIMENTAL DATA

Phenotypic types and their frequencies among daughters of females of the correct constitution (figure 1) are given in table 1.

The results of constitution tests are presented in tables 2 and 3. Because of the large numbers of classes the constitutions are given in abbreviated form as explained in the heading of the table. Partly because of differences

TABLE 3

Genotypic constitutions of females for which carnation tests were obtained. Only those showing crossovers in region 1 or 2 are included here since other regions can be treated from the data in table 2. These data are all included in table 2 with carnation disregarded. The symbols are the same as in table 2 with the addition of r for carnation. Phenotypes are given in bold face type followed by the total number of females of the given phenotype for which carnation tests were obtained. Non-italicized symbols are used as in table 2.

Wild-type (505)		**ets (19)**		*ucvfr/ecvf*	3	**v (25)**		**ts (12)**	
etsr/ucvf	7	*etsr/ets*	4	*ucvfr/cvf*	1	*evsr/ucvf*	1	*uctsr/ets*	1
ucvr/ets	1	**et (53)**		*ucvfr/evf*	2	*ucvsr/evf*	1	**s (15)**	
etsr/cvf	1	*etsr/etvf*	2	**vfr (5)**		**fr (9)**		*ucvsr/ets*	1
esr/ucvf	1	*etsr/uetvf*	1	*ucvfr/etvfr*	4	*ucvfr/etsfr*	4	*etsr/ucs*	1
ucvfr (7)		**e (71)**		*uetvfr/cvfr*	1	*ucvfr/utfr*	4	*u sr/ets*	2
ucvfr/ucvfr	7	*etsr/ecvf*	2	**vf (7)**		*ecvfr/utsfr*	1	*uctsr/es*	1
ucvf (6)		*ecvr/etsf*	1	*ucvfr/etvf*	3	**f (4)**		**ufr (1)**	
ucvfr/ucvf	6	**cvfr (6)**		*ucvfr/etvf*	2	*etsfr/ucvf*	1	*ucvfr/uetfr*	1
u (63)		*ucvfr/ecvfr*	6	*ucvfr/evf*	1	*etvfr/ucsf*	1	**efr (1)**	
ucsr/uetvf	2	**cvf (8)**		*evfr/ucvf*	1	*ucvfr/etf*	1	*ecvfr/etfr*	1
		ecvfr/ucvf	2			*etfr/ucvf*	1		

in fertility and partly intentionally the different phenotypes were not tested in numbers exactly proportional to those in which they occurred. For this reason, in certain of the analyses, proportional frequencies must be used. These are obtained by multiplying the frequencies of the genotypes of a given phenotype by the frequency with which that phenotype occurred in the total of the counts of phenotypic constitution.

RIGHTMOST DETECTED CROSSOVERS

ANDERSON'S studies on crossing over in attached-X females of Drosophila (1925) indicated that the rightmost crossovers (those nearest the spindle attachment) are equally likely to involve two attached strands (reciprocal) or two strands, one from each pair of attached strands (equational)—(see EMERSON and BEADLE 1933 for diagrams). This is the expectation if the rightmost crossover involves any two non-sister strands at random. EMERSON and BEADLE (1933) summarized the data of ANDERSON (1925) and of STURTEVANT (1931) together with new data for the regions from vermilion (33.0) to Bar (56.7). All of the data are in substantial agreement. The results from the present study are given for in-

dividual regions in table 4. The three types aa/ab, ba/bb, and ba/ab are, assuming random crossing over between non-sister strands, expected in equal frequencies. The observed values agree very well with those expected. The totals of the proportional percentages for all regions are 24.56, 21.90 and 24.77 for the types as listed. These data therefore confirm the conclusions arrived at from previous data, namely that the crossover nearest the spindle attachment involves non-sister strands at random.

RELATION OF STRANDS INVOLVED IN DOUBLE CROSSOVERS

Excluding crossovers between sister strands, there are three types of double exchanges possible. These are designated two strands, three strand, and four strand. These types can be described by designating the strands a, a′, b, and b′. Two strand double exchanges involve the

TABLE 4

Proportional frequencies of different types of single crossovers. The numbers in italics represent the rightmost crossovers in crossovers of higher rank.

CROSSOVER REGION	aa / ab	ba / bb	ba / ab
1	0.32		
	0.49		
x*	0.91	1.29	
	1.63	*1.18*	
2	0.59	0.87	0.71
	1.10	*0.63*	*1.25*
3	3.26	2.32	2.82
	2.47	*2.46*	*2.11*
4	2.32	2.42	3.33
	1.63	*1.18*	*1.19*
5	4.63	4.52	5.94
	1.00	*0.80*	*1.21*
6	2.99	2.16	2.10
	0.07	*0.28*	*0.06*
7	2.41	2.96	2.75
	0.14		
8	1.95	1.30	1.30
Total (2 to 8)	24.56	21.90	24.77
Calculated	23.74	23.74	23.74

* Region x is used for regions 1 and 2 combined in cases where they cannot be differentiated.

same two strands at the two levels, for example, a and b at level 1 and a and b at level 2. Three strand doubles involve three different strands at the two levels, for example, a and b at level 1 and a and b′ or a′ and b at level 2. Four strand doubles involve two strands at one level and the two remaining strands at the second level, for example, a and b at level 1 and a′ and b′ at level 2. With random crossing over between non-sister strands, these types are expected to occur in the ratio 1:2:1.

From the two strands recovered in the daughters of attached-X females certain genotypes can be used as a measure of the frequency of different types of double exchanges. First, consider the frequencies of two and four strand doubles. The type aaa/bab or aba/bbb can come from either two or three strand doubles. The type baa/aab or baa/abb results from either

TABLE 5

Frequencies of genotypes which measure the relative frequencies of two and four strand double exchanges.

CROSSOVER REGIONS	aaa / bab	baa / aab	aba / bbb	bba / abb
2–3	1	1	1	0
2–4	1	1	0	0
2–5	3	0	0	1
2–6	1	1	0	0
2–7	0	2	0	0
2–8	1	0	0	0
3–4	0	0	1	1
3–5	4	3	2	8
3–6	3	7	1	2
3–7	5	0	1	2
3–8	1	3	1	0
4–5	4	2	2	1
4–6	7	3	2	3
4–7	5	3	5	1
4–8	3	1	0	2
5–6	5	0	3	1
5–7	2	0	2	2
5–8	5	2	3	2
6–8	1	0	2	3
7–8	1	1	0	0
Totals	53	30	26	29

three or four strand double exchanges. Since three strand doubles contribute equally to the two classes (complementary products of one double exchange) it follows that if the two types are equal in frequency, two and four strand double exchanges occur equally often (see EMERSON and BEADLE 1933, for diagrams.) EMERSON and BEADLE (1933) give a summary of the available data which show that the two types occur with frequencies which show no statistically significant difference. The

relative frequencies of these types for different pairs of regions are given in table 5. The totals in absolute numbers are 53:30 for aaa/bab and baa/aab; 26:29 for aba/bbb and bba/abb. The deviation from a 1:1 ratio in the first comparison is about 3.8 times its probable error. The second difference is in the opposite direction but is not significant The sum of the two pairs, 79:59, shows a deviation from a 1:1 ratio which is about 2.5 times its probable error. These data combined with the totals (corrected) given by EMERSON and BEADLE (1933) give totals of 152:121 or percentages of 55.6 to 44.4. The deviation from equality is 2.8 times its probable error. There is an excess of two over four strand types but it is of doubtful statistical significance. In such a determination as the above there are certain sources of error which should be considered in evaluating the observations. Viability differences should not exist since the types compared are of identical phenotypes. Mutation is a possible source of error that should be taken into account. Lethal mutations (at single loci or associated with chromosome aberrations) would eliminate individuals homozygous for the lethal gene and would give apparent two strand doubles in regions adjoining a marker gene close to the lethal. Numerous published control experiments in induced mutation studies show that about 2.5 lethal mutations per thousand X chromosomes can be expected per generation. Thus among the total of the females tested in this and previous studies (3308), 17 lethal mutations might reasonably be assumed to have been present. Granting the legitimacy of such a correction, the deviation from equality of two and four strand types would be reduced to a value not much larger than its probable error. Unfortunately in most of the tests, counts were not made so there is no way of directly checking possible errors due to mutation. However we can gain some idea of the reasonableness of the assumption that a few such errors do exist by considering the ratio of the two types in doubles in adjacent regions. Doing this for the present study (table 5), we find a ratio of 18:7. If the excess of two strand doubles is eliminated on the assumption that it represents mutation rather than crossing over, we obtain totals of 46:30 and 22:29. Alone or combined with previous data these totals show no significant deviations from equality of the two types of doubles.

From the results of the studies reported here and those previously reported (l.c.) we have a considerable body of data bearing on the relative frequencies of two and four strand double exchanges. There is no sound basis for assuming that they occur in any other than the 1:1 ratio expected on the assumption of random crossing over between non-sister strands.

Detected double crossovers in which the rightmost crossover is reciprocal (ba/ab) provide a means of comparing the frequencies of two and three strand double exchanges. Data presented by EMERSON and BEADLE (1933) suggest that three strand doubles occur in the proportions expected

with random crossing over but the data are too limited to establish this conclusion with any degree of certainty. Likewise studies of L. V. MORGAN (1933) on females heterozygous for a closed X chromosome indicate such a relation; here the argument is not as direct as might be desired. The data presented in this paper are the most nearly adequate of any available for testing this point. Following a detected reciprocal rightmost crossover, three classes of double crossover are detected. The type aba/bab comes from a two strand double exchange. The classes bba/bab and aba/aab come from three strand doubles. Four strand doubles are not recovered as double crossovers (see EMERSON and BEADLE for diagrams showing this and the foregoing relations).

TABLE 6

Proportional percentages of genotypes which measure the relative frequencies of two and three strand double exchanges.

CROSSOVER REGIONS	aba / bab	aba / aab	bba / bab
2–3	0.09		
2–4		0.16	
2–5	0.17	0.10	0.45
2–7			0.18
2–8			0.10
3–4	0.07	0.11	0.07
3–5	0.07	0.18	0.26
3–6	0.07	0.18	0.06
3–7	0.21	0.23	0.28
3–8	0.14	0.11	0.07
4–5	0.07		0.13
4–6	0.14	0.09	0.17
4–7		0.17	0.17
4–8	0.07	0.11	0.07
5–6	0.14	0.09	
5–7	0.14	0.11	0.06
5–8	0.22	0.23	0.22
6–8		0.06	
Total	1.60±0.4	1.93±0.4	2.29±0.4

Since the types indicated above all differ phenotypically, it is necessary in comparing their frequencies to use proportional frequencies. These are listed for the three types, for separate regions, in table 6. The totals are 1.60, 1.93, and 2.29 percent for the types as listed. The expected ratio from random crossing over between non-sister strands is 1:1:1. The deviation from this ratio is not statistically significant.

The available evidence from recovered types of double crossovers in attached-X chromosomes can be summarized by saying that the attachment of the strands at the spindle attachment ends does not prejudice the

strands taking part in the crossover nearest to it and that the strands involved in one exchange do not influence those concerned in any other. This is equivalent to saying that if two strands (a and b) cross over at one level then an adjacent crossover between the remaining two strands (a′ and b′) is interfered with to the same extent as is a crossover between the same two strands (a and b); the same relation applies to the other two possibilities at the second level (a and b′ or a′ and b). In other words, the evidence supports the thesis that non-sister strands are involved in crossovers at random.

CROSSOVER VALUES

From the proportional frequencies of the different genotypes as determined in the tests, the crossover values for the different regions can be computed. If this is done by treating the two recovered strands separately, the values obtained are comparable to the standard map values. The number of strands on which these values are based is of course equal to twice the number of tested individuals, that is, 2956. The values obtained in this way together with standard values are given in table 7. It can be

TABLE 7

Crossover frequencies in the eight regions of the attached-X chromosomes. Regions 1 and 2 are computed as twice the observed homozygosis occurring in those regions; other regions are computed from genotypic tests (table 2) treating the two recovered strands separately.

REGIONS	*s.f.-cr* 1	*cr-f* 2	*f-s* 3	*s-v* 4	*v-ct* 5	*ct-cv* 6	*cv-ec* 7	*ec-sc* 8
Attached-X	1.6	3.4	10.5	8.7	14.5	7.0	8.2	5.5
Standard	3.5	5.7	13.8	10.0	13.0	6.3	8.2	5.5
Difference	−1.9	−2.3	−3.3	−1.3	+1.5	+0.7	0	0

seen that in all except regions near the spindle attachment the two sets of values show good agreement. The spindle attachment-carnation and carnation-forked values are obtained directly from the homozygosis values for the carnation and forked genes. This is legitimate only on the two assumptions, (1) that crossovers in these regions involve non-sister strands at random with respect to the attachment and that (2) there is no double crossing over within the spindle attachment-forked intervals. The first of these has been shown to be justified; the second is directly tested by the data and is valid. The homozygosis value for a gene, for example, forked, is, in the absence of double crossing over, equal to one-fourth of the total exchange frequency between the spindle attachment and that gene. Standard crossover values represent one-half of the total exchange frequency. Therefore to get a value corresponding to standard crossover values, the homozygosis values are multiplied by two.

The values for attached-X females for the spindle attachment-forked

regions are lower than the standard values for these regions. The value for forked equationals obtained here is 2.51. This agrees fairly well with the value, 2.85, obtained by EMERSON and BEADLE (1933) using the same original stock of attached-X. A third experiment with this stock (BEADLE, unpublished) gave a value of 2.9. Using three stocks of attached-X females (different original occurrences), including the one used here, L. V. MORGAN (1925), ANDERSON (1925), STURTEVANT (1931), and RHOADES (1931) obtained values for forked homozygosis of 5.1, 5.2, 5.1 and 4.9. The explanation of the difference between the two series, which is certainly significant, is not known. Apparently some change which reduces crossing over near the spindle attachment has taken place in the particular stock used here. The higher values obtained by previous workers show that the attachment of the chromosomes does not, in itself, result in any material change in crossing over.

HOMOZYGOSIS

It is apparent that homozygosis for any locus in attached-X chromosomes is a function of crossing over between the spindle attachment and that locus. The observed values for the study reported here are given in table 8. Their relation to map distance from the spindle attachment is given in figure 2.

TABLE 8

Observed and calculated homozygosis values in percentages for genes in attached-X chromosomes.

GENE	OBSERVED	CALCULATED
sc	20.3	18.9
ec	18.4	18.8
cv	18.0	17.8
ct	14.8	16.5
v	11.8	11.1
s	7.0	7.4
f	2.5	2.5
cr	0.8	0.8

From the crossing over detected in recovered strands (genotypic data), the expected homozygosis curve can be calculated. Specific assumptions as to the occurrence of sister strand crossovers and the relation of strands involved in the crossover nearest the spindle attachment and in successive crossovers must be made in order to do this. Such a calculation should serve as a check on the validity of the assumptions that are made. Evidence is available which suggests that sister strand crossovers either do not occur or are not equivalent in interference to non-sister strand crossovers (WEINSTEIN 1932, L. V. MORGAN 1933). The assumption that they do not

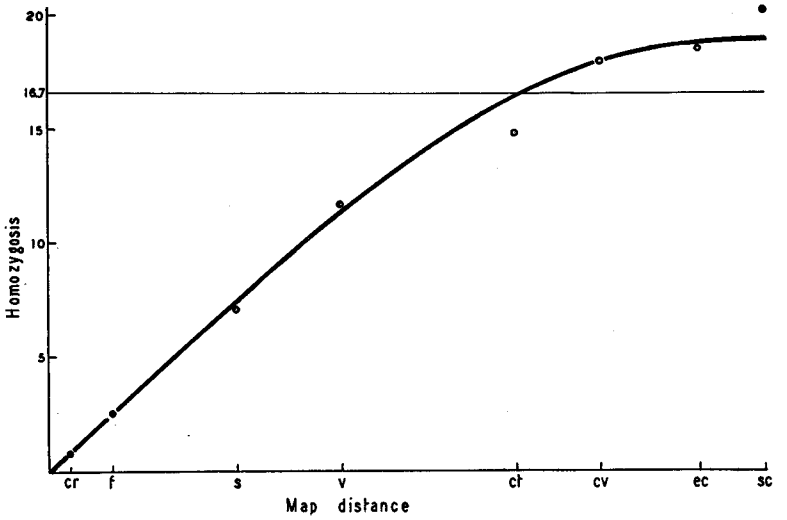

FIGURE 2.—The relation of percentage homozygosis to map distance. Distances on the base line are taken from the crossover values obtained in this experiment (see text). The curve is drawn through the points calculated from the crossover data. The circles represent observed homozygosis values.

occur will be made. The evidence concerning strands involved in multiple exchanges has already been considered and justifies the assumption that the strands involved in crossovers at different levels are independent. From the genotypic data, treating the recovered strands separately, the relative frequencies of strands with 0, 1, 2, 3, and 4 detected crossovers for an interval from the spindle attachment to any marked locus can be calculated. To do this it is necessary to correct for undetectable reciprocal crossovers in regions 1 and 2. The necessary corrected proportional per-

TABLE 9

Proportional percentages of single strands of different crossover types. Corrected for undetected reciprocals in region x.

REGIONS		REGIONS		REGIONS		REGIONS	
0	48.213	x-3	0.135	4-5	0.300	x-3-5	0.075
x*	3.440	x-4	0.190	4-6	0.515	x-3-6	0.010
3	7.658	x-5	0.390	4-7	0.560	x-3-7	0.055
4	6.618	x-6	0.235	4-8	0.370	x-4-5	0.010
5	11.555	x-7	0.290	5-6	0.475	x-4-8	0.020
6	5.550	x-8	0.135	5-7	0.325	x-5-6	0.025
7	5.925	3-4	0.198	5-8	0.725	x-5-8	0.020
8	3.598	3-5	0.550	6-7	0.000	x-6-8	0.010
		3-6	0.352	6-8	0.120	3-7-8	0.035
		3-7	0.885	7-8	0.035		
		3-8	0.493				

* See footnote to table 4.

centages are given in table 9. The corrections are made on the assumption that reciprocal and equational crossovers are equal in frequency (random). From these data the proportions of 0, 1, 2, 3, and 4 exchange tetrads for the interval between the spindle attachment and any designated marked locus can be determined from the following relation between exchanges and detected crossovers:

	Crossovers detected in single strands				
Exchanges	0	1	2	3	4
0	1				
1	1/2	1/2			
2	1/4	1/2	1/4		
3	1/8	3/8	3/8	1/8	
4	1/16	4/16	6/16	4/16	1/16

Having done this, the expected homozygosis value is determined directly from the frequencies with which different numbers of exchanges give homozygosis. These are (for a single allele of a marked locus):

Exchange	Percent homozygosis
0	0
1	25
2	12.5
3	18.75
4	15.625

The two steps can be combined which gives the formula

$$y = 0.5\ S_x - 0.5\ D_x + 1.5\ T_x - 2.5\ Q_x$$

where y is percentage homozygosis for one allele of a gene at locus x and S_x, D_x, T_x, and Q_x are percentages of recovered single strands with 1, 2, 3, and 4 crossovers between the spindle attachment and the locus x. This is the same method of calculation as that used by SAX (1932).

The values calculated by the above method together with the observed values are given in table 8 and shown graphically in figure 2. It can be seen that with the exception of two values, cut and scute, the agreement between observed and calculated values is very good. The observed value for scute is high, that for cut low. The low value can reasonably be ascribed to a disproportionate depression of viability by the cut gene. The value for scute is significantly higher than that calculated from the crossover data. The most reasonable explanation is that the entire calculated curve is depressed somewhat by differential inviability. It is known that flies homozygous for recessive mutants are usually less viable than those of

wild-type. Equational crossovers in proximal regions give, if no other crossover occurs, individuals homozygous for the genes distal to the crossover. However if a second crossover occurs the number of genes homozygous will be reduced. Therefore double crossovers have a certain advantage over single crossovers and there will be a corresponding error in the data. This error will be in such a direction that the calculated curve will be somewhat too low. The high value of scute may then be due to the fact that its effect on viability is less than that of certain of the other genes used.

On the whole the agreement between observed and calculated homozygosis values is good. The agreement constitutes a verification of the assumptions on which the calculations are based, namely that crossing over does not occur between sister strands and is random between non-sister strands. Random crossing over between any two strands including sister strands would give homozygosis values not exceeding 16.7 percent. The observed values obtained by RHOADES (1931) and in the present study clearly exceed this value and therefore confirm the conclusion that sister strand crossovers either do not occur, or are not equivalent to non-sister strand crossovers.

SUMMARY AND CONCLUSIONS

Crossing over was studied over the entire length of the X chromosome in attached-X females of *Drosophila melanogaster*. The genotypic constitutions of 1478 individuals were determined.

The data are in agreement with the assumptions that sister strand crossovers do not occur or at least do not occur independently of non-sister strand crossovers, and that crossing over between non-sister strands involves such strands at random.

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