REGIONAL ASSOCIATION OF CROSSING OVER IN NONHOMOLOGOUS CHROMOSOMES IN *DROSOPHILA MELANOGASTER* AND ITS VARIATION WITH AGE¹

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WHAT are the interrelationships of nonhomologous chromosomes in crossing over? This problem, important in a consideration of the basic activity of the nucleus as a whole, has sporadically occupied the attention of some *Drosophila* workers for many years. The most striking of the interchromosomal effects have come from mothers containing chromosome aberrations. For example, typical effects depend upon the presence of given heterozygous inversions in a chromosome pair or pairs, resulting in marked changes in recombination frequency in still another pair. These changes are largely increases in recombination and are influenced by regional position along the chromosome measured; they are rather easily followed, and are well established.

But in addition to these special effects depending upon gross structural heterozygosity, there have been further indications of the existence in structurally standard females, of interchromosomal correlations of crossing over within individual nuclei. These latter associations appear to be relatively small, and they are not very easily investigated. What evidence there is suggests that they vary in value, and possibly even in sign, depending upon the specific regions involved. Earlier statements concerning results of SCHULTZ and REDFIELD and of SCHULTZ and BRIDGES may be found in the Carnegie reports (MORGAN, BRIDGES, and SCHULTZ 1930, 1931); these and related results to 1951 have been reviewed by SCHULTZ and REDFIELD (1951). But the situation is not clear, and the conclusions are tentative. Definitive data are needed for the simple standard female. We must know more about the generalized interchromosomal relationships in the structural homozygote because these are basic to the problem as a whole, and because the general effects are not necessarily dependent upon precisely the same set of causal factors as those resulting in the marked effects found in the aberrant types. Thus a re-evaluation of the general situation in the structural homozygote now seems desirable, primarily because of its basic significance in biological terms—but also in the hope of a clearer understanding of the modifications taking place in the specialized types. This was the double purpose of the present experiments.

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Relevant details of earlier data: Previous results from structural homozygotes suggest that chromosomes II and III, and in particular the richly heterochromatic regions about the centromeres of these two large V-shaped autosomes, would be promising material for such study. Thus the 1931 experiment, only briefly reported, of SCHULTZ and BRIDGES involved females of the composition $b +/+ cn; D +/+ Sb$. (The loci of black and cinnabar span the spindle fibre of chromosome II and are at a standard distance of 9.0 units apart—Dichaete and Stubble occupy similar positions in III but are a considerably greater distance apart, 17.8 units. All standard loci mentioned in the present paper are taken from BRIDGES and BREHME 1944). In this experiment eggs were gathered daily from separate females for 32 days in order to detect effects dependent upon age. Viability relationships were good among F_1 classes. (The worst representation of the four mutants was that of Dichaete—49.0 percent, where expectation is 50.0 percent.) The totals ($N = 26,850$) gave evidence of a positive correlation in recombination between these two regions; “coincidence”, which provides a value for the ratio of actual to expected associated crossing over in the two regions, was significantly greater than 1.00. However there was some doubt as to the genetic homogeneity of the mothers used, for there had been no attempt to make them isogenic, and they apparently could be separated into several crossover types. Furthermore it is possible that there was appreciable undetected double crossing over in the relatively long region from Dichaete to Stubble, which could complicate the interpretation. But it is worth observing that when these data (kindly turned over to me by DR. JACK SCHULTZ) are arranged in age groups corresponding to the transfers of the more closely controlled crosses of the present paper they show considerable agreement with these subsequent crosses. The comparisons will be returned to later.

In 1956 KRAMER and LEWIS reported the use of a special technique permitting simultaneous detection of crossing over along all three major chromosomes of P_1 mothers. This was by means of separate testing of F_1 females, these F_1 individuals having been made heterozygous for extensive inversions (introduced from P_1 fathers) for each of the three chromosomes. This procedure was used to avoid viability complications, but marked viability effects on certain types were still found—particularly on those classes which included the mutants spanning the spindle fibre of II (black and purple showing in approximately half the expected proportions). Nevertheless it was held on statistical analysis that subsequent calculations were based on a representative sample, and so were not disturbed. A total of 854 gametes were tested from mothers of the composition $Xple/+; all/+; rucua/+$ involving 22 mutant loci. A questionable trend was observed towards positive association between the whole chromosomes II and III (the probability of association approaching the 10 percent level); it was concluded that “no association between the number of crossover points on one chromosome and the number of such points on other chromosomes could be demonstrated.” And “it would appear that age of female cannot be regarded as having an important effect on the relationships observed here.” However the numbers were too small to test regions separately.

Known relevant unpublished data for structurally standard females include odds and ends from various studies, a definite proportion of which suggest some degree of positive association for regions about the spindle fibres of II and III. Of special interest is the 1954–1955 experiment of DR. J. R. SINGLETON and DR. STANLEY ZIMMERING, which is mentioned with permission. This cross, in which a total of 26,174 flies were classified, attempted to cover the right limb of chromosome II along with its centromere region (black to cinnabar across the spindle fibre attachment, 9.0 standard units; cinnabar to curved, 18.0 units; and curved to brown, 29.0 units) together with the left limb of III and its centromere region (roughoid to hairy, 26.5 standard units; hairy to thread, 16.7 units; and thread to spineless across the spindle fibre, 15.3 units). Mothers were $b cn c bw/+; ru h th ss/+$. Totals from 12 days of egg laying gave a markedly positive correlation of crossing over between the proximal regions black to cinnabar in II, and thread to spineless in III. “Coincidence” here was 1.85 ± 0.18 . Furthermore a value of 1.20 ± 0.07 was obtained for the region next distal in III, hairy to thread, with the region black to cinnabar in II. These significantly high values they attributed to the effect of age-heterogeneity in crossover rate of the two chromosomes. They found, and were disturbed by, definite viability effects on various mutant combinations; moreover there were indications that the mothers were not a completely homo-

geneous lot. But in spite of the recognized difficulty of drawing conclusions about the interchromosomal effects from data involving such complications, the suggestive fact remains that in this cross a very high 85 percent more of associated crossovers for the two centromere regions emerged than was expected on chance. This may be taken to supply provisional support of the evidence from the cross of SCHULTZ and BRIDGES as reported in MORGAN, BRIDGES and SCHULTZ (1931), carried out under quite different circumstances, in which similar positively correlated crossing over was obtained for the regions about the centromeres of II and III. The possibility arises then that there is some basic influence at work on the centromere regions of these non-homologous chromosomes which makes crossing over within a nucleus more likely to occur in the one region when it occurs in the other.

One may recall that some time ago MATHER and LAMM (1935; MATHER 1936) gathered together the cytological evidence available from studies on a number of plants, concerning interdependence of chiasma formation in one bivalent on chiasma formation in other bivalents of the same nucleus. This study (like that of KRAMER and LEWIS 1956) involved consideration of chromosomes as a whole only, and did not deal with separate smaller regions. Cases of both positive and negative correlation were found—MATHER ascribed the occasional positive cases to environmental effects or to mixed material; the more frequent negative cases were taken to mean that the number of chiasmata in a nucleus tends to be limited and that there is competition between bivalents for such formation. Inconsistencies were attributed to different or changed competitive strengths. It may be noted as obvious that in these analyses in which whole chromosomes are the units of comparison, any regional effects which vary according to position in the chromosome will remain undetected or partially masked.

On the basis of the studies mentioned above, and of some others also, it seems that a really controlled attempt to investigate the interrelationships in *Drosophila* should begin with crosses in which, so far as possible, the following factors, not ordinarily so significant, are eliminated: (1) Environmental variations—thus in addition to standard care such precautions for example as raising mothers, as well as F_1 , at a controlled 25°C , and of making transfers at a given time of day, are taken; (2) Age change effects—this implies collection of data from frequent transfers of mothers, in the present case at two-day intervals; (3) Genetic variation in the mothers—they must therefore be derived from isogenic stocks, and be bred separately to detect any major discrepancies; (4) Viability imbalance in F_1 classes introduced by poor mutants, or by poor combinations of otherwise good mutants—it is preferable then to avoid all highly multiple mutant stocks; (5) Any other markedly increased mortality during development of the F_1 —such as that found when some inversions, or most translocations, are present in the mothers, or in many cases even in the fathers; and (6) Chromosome distances so long that under the conditions of the experiment they include appreciable undetected double crossing over within a region—this can with surprising ease mask certain interchromosomal relationships.

Meeting so far as is experimentally feasible these special conditions, the following simple crosses were carried out. They tested exclusively the sensitive spindle fibre regions of chromosomes II and III in presumably isogenic stocks. They dealt first with the structurally homozygous female, thus establishing the standard interchromosomal association in crossing over for these regions, and the variation in this association with age. Secondly they dealt with the corresponding relationship within the structurally heterozygous female resulting from substitution of one inverted scute⁸ X chromosome for a standard X. It was hoped that the use of

such a technique would facilitate the separation of the causal factors involved in the production of the interchromosomal effects on crossing over.

RESULTS FROM THE STRUCTURAL HOMOZYGOTE

The data for the first experiment were from 11 mothers of the composition *b cn/+ +; ri Sb/+ +*, these females having been derived from a cross of stocks of recent isogenic origin. Egg counts to measure early mortality were not made from these particular mothers since it was considered desirable to avoid the disturbance involved both to mothers and to eggs. However egg counts from similar mothers (in a previous experiment using the same stocks for another purpose) had given 11,692 eggs collected over the same age period of 16 days; of these eggs 436, that is 3.7 percent, did not hatch. This may be regarded as a low egg mortality, indicating the absence of undetected aberrancies of the common types leading to early death.

TABLE 1

Association of recombination for b-cn and ri-Sb in structurally homozygous mothers of the composition b cn/+ +; ri Sb/+ + and b +/+ cn; ri +/+ Sb. Transfers at 2-day intervals. (Rec.= recombination)

	Transfer								All transfers
	I	II	III	IV	V	VI	VII	VIII	
<i>b cn/+ +; ri Sb/+ +</i>									
N	345	1280	1779	1691	1284	1351	894	798	9422
Total <i>b-cn</i> rec.	7.8	4.8	4.5	4.1	5.4	5.4	4.8	5.0	4.9
<i>b-cn</i> recombination:									
Without <i>ri-Sb</i> rec.	7.7	4.6	4.4	4.0	5.2	5.3	4.4	5.0	4.8
With <i>ri-Sb</i> rec.	8.2	6.4	5.6	5.9	7.4	6.7	9.2	4.7	6.6
Percent increase	6	39	27	47	42	26	109	-6	37
Total <i>ri-Sb</i> rec.	17.7	11.0	10.1	8.0	8.4	7.7	8.5	8.0	9.2
<i>ri-Sb</i> recombination:									
Without <i>b-cn</i> rec.	17.6	10.8	9.9	7.8	8.2	7.6	8.1	8.0	9.0
With <i>b-cn</i> rec.	18.5	14.5	12.5	11.4	11.6	9.6	16.3	7.5	11.9
Percent increase	5	34	26	46	41	26	101	-6	32
Coincidence	1.05	1.32	1.24	1.43	1.38	1.25	1.91	0.93	1.33 ± 0.16
<i>b +/+ cn; ri +/+ Sb</i>									
N	186	666	898	843	744	902	799	563	5601
Total <i>b-cn</i> rec.	3.8	4.8	6.2	3.9	3.4	3.3	4.5	3.2	4.2
<i>b-cn</i> recombination:									
Without <i>ri-Sb</i> rec.	3.7	4.8	5.8	3.8	3.0	3.0	4.6	3.2	4.1
With <i>ri-Sb</i> rec.	4.5	5.1	9.5	5.4	7.3	7.8	3.0	3.2	6.0
Percent increase	24	8	62	43	138	162	-35	-1	48
Total <i>ri-Sb</i> rec.	11.8	11.7	10.6	6.5	7.4	7.1	8.3	11.2	8.9
<i>ri-Sb</i> recombination:									
Without <i>b-cn</i> rec.	11.7	11.7	10.2	6.4	7.1	6.8	8.4	11.2	8.7
With <i>b-cn</i> rec.	14.3	12.5	16.1	9.1	16.0	16.7	4.5	11.1	12.7
Percent increase	22	7	57	42	126	146	-46	-1	45
Coincidence	1.21	1.07	1.52	1.39	2.16	2.35	0.67	0.99	1.42 ± 0.23

The 11 mothers used were placed individually with tester *b cn; ri* males in shell vials to insure immediate mating. They remained in the vials two days, and were subsequently transferred at two-day intervals to fresh quarter-pint culture bottles, and provided with fresh males, up to age 17 days. Then they were discarded, for their fertility had by this time declined. The results are shown in the first section of Table 1. Since the computed values could be affected by differential viability it should be pointed out that the mutants used were highly viable: black was found in 50.4 percent of the 9,422 offspring, cinnabar in 50.5 percent, radius-incompletus in 49.5 percent, and Stubble in 49.1 percent—where of course expectation for each is 50.0 percent. Furthermore the viability of classes in which the combined mutants appeared was good: thus the class containing all four mutants (*b cn; ri Sb*) was 24.2 percent of the total noncrossovers (8,147), whereas the class containing no mutant (+; +) was 24.4 percent of that total. (The two other noncrossover classes, *b cn; +* and *+; ri Sb*, were represented by 26.1 percent and 25.2 percent respectively.) It can be stated also that apparently no marked imbalance of any class was present. Classification difficulties such as those sometimes encountered in the use of black (presumably dependent upon modifiers present in the stocks) did not appear here. Although the numbers of offspring obtained per female per transfer were necessarily small, there was no untoward deviation from mother to mother in the recombination values found—that is the mothers seemed a homogeneous lot. And from these separate mothers there was no apparent clustering of special crossover types.

The total data for the first experiment show a recombination value of 4.9 for *b-cn* and a corresponding value of 9.2 for *ri-Sb*. These values are lower than those given by the standard map (which are 9.0 for *b-cn* and 11.1 for *ri-Sb*) but the latter, of course, are based on combined data from many experiments representing varying genetic and environmental conditions, and most likely involving a quite different mean age range than does the present experiment. The data show for both regions the early rapid decrease in crossover value with age of mother long known to be characteristic of these centromere regions of II and III (BRIDGES 1927, 1929).

Now, in the total data for the age period used, there is for these particular sections higher recombination in either autosomal pair when recombination is found in the same egg cell in the other pair. Thus the recombination for *b-cn* is 4.8 computed from those individuals which are not recombinants for the region *ri-Sb*. However, among *ri-Sb* recombinants the recombination for *b-cn* is 6.6; this represents an increase of some 37.5 percent of the former value. Furthermore *ri-Sb* recombination without *b-cn* recombination is 9.0, but with *b-cn* recombination it is 11.9; the increase here is 32.2 percent of the former value. Also it is readily seen that such percentage increases, but increases which vary in absolute value, are found in all of the first seven transfers considered separately. The eighth and last transfer is empirically an exception in that it shows a small decrease with recombination in the nonhomologous counterpart. Thus recombination here is 5.0 for *b-cn* in the *ri-Sb* nonrecombinants, and in recombinants for *ri-Sb* is 4.7; for *ri-Sb* it is 8.0 in *b-cn* nonrecombinants, and is 7.5 in *b-cn* re-

combinants. This represents an "increase" of -6.0 percent for $b-cn$, and of -6.2 percent for $ri-Sb$. Obviously this reversal of sign at the oldest stage tested is in itself not significant, and may depend upon the small numbers of F_1 obtained from this last transfer. However both first and last transfers do give values very close to expectation on the basis of chance, whereas the percentage increases reach relatively high maxima between these two transfers.

The trend of these effects may be dealt with more compactly by use of an index of association identical with the original formula of WEINSTEIN (1918) for coincidence within a single chromosome—that is, in terms of the actual numbers, it is given by the number of associated crossovers in the two regions multiplied by N and divided by the product of the total numbers of crossovers in the two regions. The deviation of this coincidence from the value 1.00 will provide under present circumstances a measure of the amount and type of association in crossing over of the two regions under consideration. From the computations indicated in Table 1 it is seen that for regions $b-cn$ and $ri-Sb$ total coincidence has reached a value of 1.33; that is in this structural homozygote, associated recombination is for the first 16 days of age 33.0 percent greater than would be expected on the basis of chance assuming independent behavior of the regions. This coincidence value, 1.33, has a standard error of 0.16 (using Formula 2a of MULLER and JACOBS-MULLER 1925).

When one deals with the transfers separately it is found that for the first two days of age of the mothers, coincidence is 1.05, a value which may be considered very close to 1.00; and it should be noted that this is the age period of maximum total crossing over for both regions. For the next 12 days, as the table shows, crossing over is lower for both regions and coincidence is much higher—however, neither minimum for crossing over (at the fourth transfer for $b-cn$, and at the sixth for $ri-Sb$) is associated with the maximum value of 1.91 attained by coincidence (at the seventh transfer). This latter very high coincidence means that associated recombinants are almost twice as great during the age-period 12 to 14 days as the crossover values for that same period would lead one to expect. The eighth transfer exhibits a precipitous decline in the value for coincidence to 0.93—that is, for the first time in these data associated recombinants are fewer than expected—but there is no particular corresponding change in the total crossover values. Whether the relationships for separate transfers are all significant is of course questionable since for some transfers the totals are lower than is desirable. Still there is evident a definite trend of variation in the coincidence values, and the fact remains that with one exception the values actually obtained are all greater than 1.00. The variation in coincidence is shown graphically in Figure 1.

A second experiment seemed necessary, preferably one testing the repulsion cross, since this allows a check with the same mutants of possible hidden tendencies towards biased classification. For this purpose new stocks, in which the mutants for each chromosome were separated, were derived from crossovers from the first experiment; these stocks are basically isogenic with those of the first experiment. This precaution of mutual isogenicity was taken to permit legitimate combination of the data of the two experiments. There are, by the way, indica-

tions in preliminary crosses that the particular mutants used can in stocks not made homogeneous give rather erratic results—and the erratic tendencies are compounded if age change is not properly taken into account.

The question, ordinarily ignored, of the possible effect of the sequence of classification of characters upon the probability of finding certain crossover types may be briefly considered here in relation to the use of the repulsion cross. In the present experiments the order of classification remained constant in all crosses. Specifically, it involved first a separation as to sex; and then, in order, a separation for eye color, bristle type, body color, and vein type. Thus the sequence of detection of crossover types for the first cross was: 0-2-1-1, 2-2-0-1, 2-1-1-1, 2-0-2-1, 2-1-2-0. For the repulsion cross it became: 1,2-1-2-0-1-1,2-0-2-2-0-1, 2-1-0-2-1-1,2. (For the cross involving scute⁸, described in the next section, the sequence was that of the first cross.) It seems very unlikely that this system of classification could favor the selection of associated recombinants—especially since it must work in such a way that the coincidence values would change consistently with age, and since the proportion of doubles is so low that its relationship to the proportions of singles is not detectable as counts are made. And if there were somehow unconscious positive selection of the double types in the first cross, it would presumably be detected as negative selection in the second cross. Such a reversal, as will be seen, did not appear.

The test in the second experiment was then of *b* +/+ *cn*; *ri* +/+ *Sb* mothers by *b* *cn*; *ri* males. The number of mothers here was lower than in the first experiment, and the totals (grand total, 5,601) were lower also. Differential viability effects apparently were still negligible; thus *b* was found in 50.0 percent of the total offspring, *cn* in 49.9 percent, *ri* in 49.1 percent, and *Sb* in 51.0 percent.

Totals for the age period 0 to 16 days give a value of 4.2 for *b*-*cn* recombination, and of 8.9 for *ri*-*Sb*. When the recombinants for either region are classified according to recombination in the other region it is found, as it was in the previous cross, that associated recombination is higher than is expected. Thus *b*-*cn* recombination among *ri*-*Sb* recombinants is 6.0, but among *ri*-*Sb* nonrecombinants it is 4.1—the increase over the latter value amounting to some 48.3 percent. Similarly *ri*-*Sb* recombination among *b*-*cn* recombinants is 12.7 percent, but among *b*-*cn* nonrecombinants it is 8.7; here the increase is some 45.2 percent of the latter value. Total coincidence for this cross is 1.42 with a standard error of 0.23; this represents an even higher general association of recombination than was observed in the first experiment—in the present case the actual association of recombination in these regions is 42 percent in excess of what the recombination values for this age period (up to 17 days of age) would produce, assuming chromosomal independence. These results are shown in the second section of Table 1.

The table includes the results for the two-day transfers considered separately. It is immediately apparent that the general trend with age shown by the percentage increases for associated recombination and by coincidence is the same in the two experiments. Thus values are obtained for the association, as measured by coincidence, not very markedly different from chance expectation for either end of the age cycle; and a high maximum is reached between the ends indicating

intermediate association approaching, and in the present case even exceeding, twice that expected on chance. However the two experiments do show differences and these should be emphasized, since any difference may be regarded as potential (but not necessarily actual) evidence of heterogeneity. The second experiment, as could easily be expected from its definitely lower totals, shows more extreme variation in both directions than the first, and gives generally less consistent results. The maximum value for coincidence here is reached earlier than in the first experiment—and relatively speaking an extremely low coincidence value is produced at the seventh transfer. It seems likely that this very low value (0.67) represents an exceptionally early and precipitous decline, which may well be largely due to chance deviation of small numbers. These observed differences may be followed in the graphs of variation of coincidence in Figure 1.

But perhaps these differences should not be overemphasized, for the similarities are marked, as may be seen in the curves. And when the data for the two presumably comparable experiments are dealt with simultaneously the variation in coincidence with age becomes quite smooth. Table 2 gives results for these crosses when combined; the raw data upon which the computations are based are included here in somewhat condensed form. For the complete age range, 0 to 16 days inclusive, N is 15,023. Total $b-cn$ recombination is 4.7 ± 0.17 , and total $ri-Sb$ recombination is 9.1 ± 0.23 ; coincidence is 1.36 ± 0.13 . The percentage increase for $b-cn$ recombination in $ri-Sb$ recombinants compared with $ri-Sb$ non-recombinants is 41.9; and the comparable increase in $ri-Sb$ recombination with $b-cn$ recombination is 39.0 percent. The age change in crossing over shows the customary early rapid decline, with the lowest value reached at the fourth transfer for $b-cn$, and at the fourth and sixth transfers for $ri-Sb$ —and apparently with

FIGURE 1.—Relationship between age of mother and interchromosomal coincidence for (A) $b\ cn/+ +$; $ri\ Sb/+ +$ and (B) $b\ +/+ \ cn$; $ri\ +/+ \ Sb$.

TABLE 2

Summary of data from structurally homozygous mothers, with computations on associated recombination for *b-cn* (region 1) and *si-Sb* (region 2). Transfers at 2-day intervals. (Rec. = recombination)

	Transfer								All transfers
	I	II	III	IV	V	VI	VII	VIII	
Crossover type									
0	420	1646	2286	2252	1783	1994	1481	1181	13043
1	28	81	117	92	82	91	70	53	614
2	77	206	255	179	151	156	133	122	1279
1,2	6	13	19	11	12	12	9	5	87
N	531	1946	2677	2534	2028	2253	1693	1361	15023
Total <i>b-cn</i> rec.	6.4	4.8	5.1	4.1	4.6	4.6	4.7	4.3	4.7
<i>b-cn</i> recombination:									
Without <i>ri-Sb</i> rec.	6.2	4.7	4.9	3.9	4.4	4.4	4.5	4.3	4.5
With <i>ri-Sb</i> rec.	7.2	5.9	6.9	5.8	7.4	7.1	6.3	3.9	6.4
Percent increase	16	27	42	48	67	64	41	-9	42
Total <i>ri-Sb</i> rec.	15.6	11.2	10.2	7.5	8.0	7.5	8.4	9.3	9.1
<i>ri-Sb</i> recombination:									
Without <i>b-cn</i> rec.	15.5	11.1	10.0	7.4	7.8	7.2	8.2	9.4	8.9
With <i>b-cn</i> rec.	17.6	13.8	14.0	10.7	12.8	11.6	11.4	8.6	12.4
Percent increase	14	24	39	45	63	61	38	-9	39
Percent doubles	1.13	0.67	0.71	0.43	0.59	0.53	0.53	0.37	0.58
Coincidence	1.13	1.23	1.36	1.42	1.59	1.56	1.36	0.92	1.36 ± 0.13

a subsequent minor rise. For each transfer the percentage increase in recombination for one region, in the presence of recombination in the other region, is somewhat more extreme in the case of *b-cn* than it is for *ri-Sb*. These percentage increases show orderly variation with age of mother from a quite low value for the first transfer to a maximum relatively high value for the fifth transfer; then there is again a subsiding to the lowest increases found, which are indeed negative in sign, and are reached at the eighth transfer. It may be noted that the observed percentage of associated crossovers (the "doubles") does not necessarily follow this effect, for the value for doubles is highest for the first transfer (1.13), lowest for the last transfer (0.37), and in intermediate transfers shows no detectable trend. Nevertheless the variation in coincidence is even—from a low 1.13, close to the value expected with chance association of crossovers for the regions; then increasing gradually to a high 1.59 at the fifth transfer, indicating definite regional association; and decreasing, again gradually, to 0.92 which is the lowest value found and is again not far from expectation with independent behavior. This trend of variation in coincidence for the combined data is readily seen in one of the graphs of Figure 2.

RESULTS FROM THE STRUCTURAL HETEROZYGOTE WITH SCUTE⁸

It is of interest to determine the relationship of these variations in inter-chromosomal dependence for the centromere regions of II and III found in the

structurally standard female, to the comparable variations produced in the female whose crossover values for these regions are modified by the presence of an inversion in one X chromosome. For this experiment, carried out a few months after the crosses with the structural homozygote, the scute^s inversion was chosen. Although this inversion does not have so marked an effect on crossing over in these regions as do some other chromosome aberrations, still it does have the great advantages for present purposes of introducing an experimental tool which is relatively simple, and of producing very little developmental mortality—hence the side effects with which one has frequently to contend, and which are apt to confuse analysis, are largely eliminated.

Conditions under which the 27 *sc^s, w^a/w^a; b cn/+ +; ri Sb/+ +* mothers (XX without a Y) were tested (by separate matings to *b cn; ri* males) are quite like those described for the first experiment, except in two respects. The mothers here were made homozygous for the X-chromosome mutant apricot in order that exceptions for the X, produced in appreciable numbers in the presence of the inversion, might be classified by themselves. In the second place, some possibility exists of the introduction of miscellaneous modifiers of crossing over. Considering previous tests of the stocks, such modifiers giving any marked effects would presumably be recessive. But the chance of the same recessive modifier, at least beyond the extreme left end, being present in these two X chromosomes is low—the mothers of the two experiments are most likely comparable. However admittedly some remote possibility remains of a modifying effect of the apricot gene itself, or of the presence of some undetected modifier near the apricot locus.

As before, egg counts were not made from the particular mothers yielding the F₁ counts; but egg counts made previously from similar mothers heterozygous for scute^s had given 215 unhatched eggs in a total of 3,145 eggs laid. This egg mortality of 6.8 percent is characteristic of *sc^s/+* in XX mothers within the age range customarily used. The excess thus provided of 3.1 percent over the mortality, 3.7 percent, found for the *b cn/+ +; ri Sb/+ +* mothers without *sc^s*, is largely accounted for by the lethal effects of four-strand double crossing over within the inversion itself (STURTEVANT and BEADLE 1936). In the present data among the 21,961 total offspring classified, 11,346 were females of which seven were exceptional apricot, and 10,597 were males of which 425 were exceptional nonapricot. The percentage of primary exceptions among females is then 0.06, and among males is 4.01; these values are characteristic of *sc^s/+* mothers in other data. The marked difference exhibited by the sexes is expected, for the eggs lacking an X are, as shown by STURTEVANT and BEADLE, produced largely as a result of the four-strand double crossing over within the inverted section of the X, presumably immobilizing strands thus provided with two centromeres or with none. However, it is recognized that some increment is supplied to the proportion of male exceptions by typical nondisjunctional chromosome loss, either meiotic or nonmeiotic. Incidentally the use of a paracentric inversion ending appreciably further from the centromere than does scute^s, would have provided still another small increment depending upon the three-strand double crossing over within the X tetrad with one exchange inside and one outside the inversion (PTASHNE 1960).

TABLE 3

Data from $sc^s, w^a/w^a; b\ cn/+ +; ri\ Sb/+ +$ mothers, with computations on association of recombination for $b-cn$ (region 1) and $ri-Sb$ (region 2). Transfers at 2-day intervals. (Rec. = recombination)

Crossover type	Regular F ₁									Male exceptions All transfers
	Transfer									
	I	II	III	IV	V	VI	VII	VIII		
0	806	3149	3172	1842	2648	2814	1898	1415	17744	367
1	70	190	160	102	165	150	104	95	1036	19
2	174	501	448	244	348	376	265	186	2542	36
1,2	20	36	31	18	33	27	23	19	207	3
N	1070	3876	3811	2206	3194	3367	2290	1715	21529	425
Total <i>b-cn</i> rec.	8.4	5.8	5.0	5.4	6.2	5.2	5.5	6.6	5.8	5.2
<i>b-cn</i> recombination: without <i>ri-Sb</i> rec.	8.0	5.7	4.8	5.2	5.9	5.1	5.2	6.3	5.5	4.9
with <i>ri-Sb</i> rec.	10.3	6.7	6.5	6.9	8.7	6.7	8.0	9.3	7.5	7.7
Percent increase	29	18	35	33	47	31	54	48	36	57
Total <i>ri-Sb</i> rec.	18.1	13.8	12.6	11.9	11.9	12.0	12.6	11.9	12.8	9.2
<i>ri-Sb</i> recombination: without <i>b-cn</i> rec.	17.7	13.7	12.4	11.7	11.6	11.8	12.2	11.6	12.5	8.9
with <i>b-cn</i> rec.	22.2	15.9	16.2	15.0	16.7	15.2	18.1	16.7	16.6	13.6
Percent increase	25	16	31	28	44	29	48	44	33	53
Percent doubles	1.87	0.93	0.81	0.81	1.03	0.80	1.00	1.11	0.96	0.70
Coincidence	1.22	1.15	1.29	1.26	1.40	1.27	1.44	1.39	1.30±0.08	1.49±0.76
Percent male exceptions	1.00	2.35	4.01	4.74	4.87	4.55	4.79	4.98	4.01	

Among the 21,529 regular offspring produced viability effects are negligible: black is represented in 49.5 percent, cinnabar in 49.3 percent, radius-incompletus in 49.6 percent, and Stubble in 49.4 percent. Among the much smaller number, 425, of male exceptions with which we are additionally concerned, there are (not surprisingly) mild discrepancies from the 50.0 percent expectation: the values become 49.9 percent for black, 50.3 percent for cinnabar, 46.8 percent for radius-incompletus, and 47.0 percent for Stubble.

The raw data in condensed form, as well as the crossover results derived from them, are found in Table 3. The seven female exceptions are omitted from this table—these include five noncrossovers, in addition to one crossover in *b-cn* alone, and one crossover in *ri-Sb* alone. Since their derivation in terms of X chromosome mechanics differs from that of the male exceptions it is necessary to exclude them from the computations with the latter.

For the total regular F_1 of this cross recombination is for *b-cn* 5.8 ± 0.16 , and for *ri-Sb* 12.8 ± 0.23 . Among the total male exceptions *b-cn* recombination is 5.2 ± 1.08 , and *ri-Sb* recombination is 9.2 ± 1.40 . The difference between regulars and male exceptions is not significant in the case of *b-cn*, but for *ri-Sb* it reaches a value of 3.6 ± 1.42 ; this latter presumably represents a real difference. But for a proper comparison here, one needs age change data for recombination among the male exceptions.

Before dealing with the details of the results from the regular offspring, which are our main concern, we may consider briefly the effects exhibited by these primary male exceptions. The apparently lower *ri-Sb* recombination for male exceptions, compared with *ri-Sb* recombination from regular F_1 , is potentially interesting since such exceptions from XX mothers are largely crossovers for the eliminated X—indeed double crossovers. This result is in contrast with that obtained for autosomal crossing over as computed from exceptional XX eggs from mothers without inversions. For in such exceptions, there is on the one hand no crossing over in the X chromosomes (BRIDGES 1916)—at the same time, at least under certain experimental conditions, an increase in autosomal recombination about the centromere has been reported (SCHULTZ, in MORGAN, BRIDGES, and SCHULTZ 1935; and SCHULTZ and REDFIELD 1951). A second, possibly related, result emerges on dealing with the male exceptions in the separate transfers of the present experiment. According to the values shown in Table 3 there is an effect of age of the mother upon the production of exceptions, the percentage of male exceptions being comparatively quite low for the first two transfers. The extreme limits of these values are found in the first (1.00 percent) and last (4.98 percent) transfers. Very likely there is here a real increase with age, and this would seem to be dependent upon changing production of four-strand double crossovers within the scute^s inversion.

The regular offspring of the mothers heterozygous for scute^s may now be further considered. Among these offspring, as previously among offspring from the structural homozygotes, there is no marked imbalance between contrary classes, nor any apparent clustering of crossovers (or of exceptions) from particular mothers. When comparisons are made between recombination values

from these regular F_1 of the mothers containing scute⁸, with the recombination values previously obtained from the mothers without scute⁸ (where $b-cn$ is 4.7 ± 0.17 and $ri-Sb$ is 9.1 ± 0.23 as seen in Table 2) a difference is detected, significant for both regions, due to the presence of the inversion. This difference amounts to 1.1 ± 0.23 for $b-cn$, and to 3.7 ± 0.33 for $ri-Sb$. Thus the inversion is responsible for an overall increase of 23.4 percent in $b-cn$ recombination, and of 40.6 percent in $ri-Sb$ recombination. The separate transfers also show, for each transfer, higher values for these regions for the inversion mothers, when they are compared with the noninversion mothers; that is with one exception—the third transfer gives a value of 5.1 for $b-cn$ from noninversion mothers and of 5.0 from inversion mothers. Obviously this does not represent a significantly exceptional case.

Now in the inversion mothers—as previously in the noninversion mothers, excepting the last transfer—recombination is higher for either region among recombinants for the other region, than it is among nonrecombinants for this other region. Here this is true for all transfers without exception. And, as in the previous crosses, these particular percentage increases are somewhat higher for $b-cn$ than for $ri-Sb$. This persistent higher sensitivity shown by $b-cn$ might conceivably depend upon a basic difference between chromosomes II and III, or upon the relative shortness of the region dealt with in II, or of course upon both. Also for these inversion mothers, as before for the noninversion mothers, the total coincidence is significantly greater than 1.00. It reaches a value here of 1.30 ± 0.08 , which is to be compared with the very similar value of 1.36 ± 0.13 obtained with noninversion mothers.

However, examination of the coincidence values for the separate transfers shows in the present experiment a somewhat different trend with age than that found (Tables 1 and 2) for noninversion mothers. The structurally heterozygous mothers give less variation with age than did the structurally homozygous mothers. Thus there is in Table 3 no coincidence value for regular F_1 falling below 1.15, nor any value rising above 1.39, whereas for the combined data of

FIGURE 2.—Relationship between age of mother and interchromosomal coincidence for: (A) combined $b\ cn/+ +$; $ri\ Sb/+ +$ and $b\ +/+ \ cn$; $ri\ +/+ \ Sb$; (B) sc^8 , w^a/w^a ; $b\ cn/+ +$; $ri\ Sb/+ +$; and (C) $b\ +/+ \ cn$; $D\ +/+ \ Sb$ (data of SCHULTZ and BRIDGES).

Table 2 the lowest observed value is 0.92, and the highest is 1.59. This situation is illustrated by the simple graphs of coincidence of Figure 2. A second difference is obvious in these curves, although its significance remains hidden. While the present coincidence values suggest chance variation about a straight line with a mild positive slope, it seems fairly clear that coincidence values from the crosses lacking the inversion fall along a different type of curve which exhibits definite rise and fall within the age range tested.

Figure 2 includes also a graph of coincidence values for corresponding age periods, computed from the previously discussed data of SCHULTZ and BRIDGES, for structural homozygotes of the composition $b +/+ cn; D +/+ Sb$. Values derived from these females, compared with those from the structural homozygotes which utilize the region $ri-Sb$ in II (rather than $D-Sb$), give a curve which in general behaves similarly with age, but which is as might be expected rather different in detail. The differences depend, perhaps, upon a number of factors—such as the greater relative length of the $D-Sb$ region (giving rise to complicating doubles), the alteration in position of the centromere with respect to the extremities of the region measured, a different genetic background of modifiers, and so on.

The question arises whether one can further analyze the differing interchromosomal relationships observed in mothers which are on the one hand structural homozygotes, and on the other hand structural heterozygotes, for chromosome I. For this purpose, simple computations were made whose results show the effect produced on the various crossover types at the different transfers by addition of the scute⁸ chromosome. From mothers without the inversion, non-crossovers, that is offspring which are associated noncrossovers for the two regions amount to 86.8 percent of the total offspring for the eight transfers combined. From mothers with the inversion the value is 82.4 percent. The presence of the inversion produces a decrease of only 5.1 percent of the value reached

FIGURE 3.—Effect of addition of sc^8 , w^u/w^u , in terms of percentage increase, on production of: (A) Singles in $b-cn$ only, without recombination in $ri-Sb$; (B) Singles in $ri-Sb$ only, without recombination in $b-cn$; and (C) Doubles, that is recombinants in both $b-cn$ and $ri-Sb$.

without the inversion; and there is practically negligible variation with age.

But if we consider the single crossovers in *b-cn*, we find an increase of 17.1 percent produced, for all transfers combined, by the presence of scute^s. There is here much more variation with age percentage-wise than in the values for the noncrossovers—as seen in Figure 3 the percentage increases for these crossovers in *b-cn* alone give a W-shaped curve with minimal increases at the third and seventh transfers, and with the relatively larger increases coming at Transfers I, IV, V, and VIII. There is a slight decrease, presumably insignificantly a decrease, found at the third transfer. The single crossovers in *ri-Sb* show for all transfers combined an increase of 38.8 percent due to the inversion, a considerably higher increase than was produced for *b-cn*. As a matter of simple observation the *ri-Sb* curve differs not only in degree of variation but in shape also from that for *b-cn*. The two extreme transfers show lower values than those for *b-cn*, and all intermediate transfers give higher values. The lowest values for *ri-Sb* within the tested age range are found at Transfers I and VIII; the maximum value falls at transfer VI. Obviously the associated crossovers for the two regions, the doubles, are of special interest. These doubles exhibit the greatest percentage increases found; but the values for doubles are of less significance than values for other types, for of course they depend upon much smaller numbers. For the eight transfers combined, the percentage increase in doubles due to scute^s is 65.5. For separate transfers, the values are highest at the first, fourth, and eighth transfers; minima occur at the third and sixth transfers where in fact the values fall below corresponding values for *ri-Sb* singles. In general form, the curve for doubles resembles that for the singles in *b-cn*—however, the doubles curve is placed higher, that is shows a consistently greater effect, and there is also much more extreme variation. It may be noted that in the present crosses total recombination for either region is determined largely by the singles in that region. Hence curves showing the effect of the presence of scute^s on *b-cn* and *ri-Sb* recombination would not differ materially from those given in Figure 3 for the “singles.”

Chi-square tests of significance: The statistical significance of the conclusions reached can so far be judged only by standard errors of differences between values for recombination and coincidence. Chi-square computations seem desirable to test the significance of the association of recombinants in the two nonhomologous chromosomes, and to test the degree of homogeneity of the recombination data from separate mothers.

The tests whose results are shown in Table 4 compare: (A) The actual numbers obtained for structural homozygotes with those expected had there been chance association between recombinants for the centromere regions of II and III; (B) The data derived from X structural heterozygotes with the numbers expected, similarly assuming chance association of the recombinants; and finally of somewhat incidental interest for present purposes; (C) The data from the structural heterozygotes with those from the structural homozygotes. There is a numerical advantage in these computations in combining the data for each set of two succeeding transfers; and since the main concern is with the general trend of the effect there would seem to be no real objection to this procedure.

For the structural homozygotes (A) the values for chi-square, as would be expected from the previous discussion of the direct observations, increase to a peak for the combined Transfers V and VI (5.11) corresponding (with 1 degree of freedom) to a value of P between 0.01 and 0.02—

TABLE 4

Chi-square tests

	Age in days (inclusive)				
	1-4	5-8	9-12	13-16	1-16
(A) Structural homozygote <i>vs.</i> chance*					
Chi-square	0.65	3.65	5.11	0.19	$\Sigma\chi^2=9.60$
Degrees of freedom	1	1	1	1	4
P	.50>P>.40	.10>P>.05	.02>P>.01	.70>P>.60	.05>P>.02
(B) Structural heterozygote <i>vs.</i> chance					
Chi-square	2.07	3.46	5.81	5.73	$\Sigma\chi^2=17.07$
Degrees of freedom	1	1	1	1	4
P	.20>P>.10	.10>P>.05	.02>P>.01	.02>P>.01	.01>P>.001
(C) Structural heterozygote <i>vs.</i> structural homozygote					
Chi-square	45.30	90.21	179.18	88.98	$\Sigma\chi^2=403.67$
Degrees of freedom	3	3	3	3	12
P	<.001	<.001	<.001	<.001	<.001

Comparisons are: (A) Observed results from structurally standard females, with results expected assuming chance association between recombinants for *b-cn* and *ri-Sb*; (B) Observed results from females heterozygous for scute⁸, with results expected assuming chance association between recombinants for *b-cn* and *ri-Sb*; and (C) The results from the scute⁸ heterozygotes with the results from the structural homozygotes.

* It should be noted that the first transfer for these structural homozygotes (age to two days inclusive) gives a value of 0.042 for χ^2 corresponding to .90>P>.80; and the last transfer (15 to 16 days inclusive) gives a value of 0.0019 for χ^2 corresponding to .97>P>.95. Such values, which definitely indicate chance association, are not obtained for the comparable first and last transfers of the structural heterozygotes. In fact the sc⁸/+ mothers give for the first transfer 0.83 for χ^2 with .40>P>.30; and for the last transfer 3.00 for χ^2 with .10>P>.05.

this is clearly out of the range of the assumption of chance association which is under consideration. Thus, there is definite positive association for the mothers of intermediate age.

It should be pointed out however that combining the transfers does obscure the relationship in the first and last transfers, which transfers as will be recalled showed apparent chance association of the recombinants. And indeed for Transfer I alone (age 1 and 2 days) chi-square has a value of 0.042, corresponding to a probability of fit between the 80 and 90 percent levels. Transfer VIII (age 15 and 16 days) is even more striking in this respect, for here χ^2 is 0.0019, and $0.97 > P > 0.95$. Thus there is little question that the association of *b-cn* and *ri-Sb* recombinants for the first and last transfers is a chance association, and that a peak of positive association is reached between these transfers. This conclusion is in complete agreement with the impression given by inspection of the coincidence values as seen for example in Figure 2.

For the structural heterozygotes, comparison among the age groups of the values for χ^2 and P also shows agreement with the trend previously exhibited by the coincidence values (most readily followed in the proper curve of Figure 2). It may be noted particularly that the early transfers of scute⁸ heterozygotes give values of χ^2 and P which indicate less fit with the assumption of chance association for the first four days of age (where $0.20 > P > 0.10$) than did the homozygotes for the same age range (where $0.50 > P > 0.40$). Or put in positive terms, the heterozygotes show greater probability of early association of recombinants than do the homozygotes. Furthermore, the closeness of the fit to the assumption of chance association decreases in the case of the heterozygotes in a relatively regular manner to the end of the age range (where

$0.02 > P > 0.01$). This latter fact, then, contrasts with the situation for the homozygotes where there is a secondary decline for coincidence and a corresponding marked secondary increase for P , the probability of chance association.

The chi-square test shown in (C) is somewhat more complex than those in (A) and (B), for the comparison here involves all observed differences in crossover types produced by structural homozygotes *versus* structural heterozygotes. For each age group in (C) P is less than 0.001. This very small value of P indicates the high improbability of the specific assumption under test, in this case that the scute⁸ heterozygote belongs to the same crossing over system as the structural homozygote. The conclusion to be drawn that the systems are different is of course already clear from the accumulated general mass of information on recombination effects of heterologous inversions.

Another set of chi-square tests can be used to test the heterogeneity of the mothers within separate groups with respect to recombination in the two regions. For these tests early transfers were excluded; mothers for the three crosses were tabulated individually for Transfers V to VIII inclusive. Details need not be given; the results will be only briefly described. Completing these four transfers were five mothers of the composition $b\ cn/++$; $ri\ Sb/++$. Coincidence for these mothers for these transfers combined is 1.68. The χ^2 value for these mothers with respect to $b\text{-}cn$ recombination is 3.833 which corresponds to a value of P between 0.50 and 0.60 (the degrees of freedom, here 5, depending upon the number of mothers available). For $ri\text{-}Sb$ recombination χ^2 is 1.727 placing P between 0.80 and 0.90. Similarly for six $b\ +/+ \ cn$; $ri\ +/+ \ Sb$ mothers used here, C is 1.59, and P for $b\text{-}cn$ recombination is between 0.97 and 0.99, while P for $ri\text{-}Sb$ is between 0.80 and 0.90. For eight usable mothers of the composition $sc^8, w^a/w^a$; $b\ cn/++$; $ri\ Sb/++$, C is 1.65; P for $b\text{-}cn$ lies between 0.95 and 0.97, and for $ri\text{-}Sb$ between 0.60 and 0.70. These values on the whole indicate moderately good homogeneity of the mothers within the separate groups. However, these particular tests are not regarded as completely satisfactory because usable mothers include only those completing all the transfers involved. Since occasional mothers died before reaching the eighth transfer, or were lost on transfer, and since a few cultures could not be counted due to illness; the mothers eligible in each cross for such testing are to some degree a selected group, although so far as can be judged they represent a random sample of the maternal population.

The most precise test of homogeneity of mothers of a group would be expected as the result of analysis of individual transfers. Here some difficulty arises due to the low numbers of double crossovers which are produced by a single female in a two-day laying period; these may fall below the number required for the chi-square test. For this analysis then those mothers were necessarily selected which gave in the transfer used six or more individuals of this smallest crossover type. There is obviously in such selection an accompanying selection for mothers giving the somewhat higher recombination values, especially for the black-cinnabar region. Actually, however, this apparent difficulty is to be considered a distinct advantage for purposes of the present tests, because the basic question here is whether homogeneous mothers give coincidence values greater than 1.00. When in each cross that transfer is used which yields the largest number of offspring, the following results are found. Transfer IV for $b\ cn/++$; $ri\ Sb/++$ mothers gives a coincidence value of 1.51; while P for $b\text{-}cn$ recombination is between 0.80 and 0.90, and for $ri\text{-}Sb$ is also between 0.80 and 0.90. For Transfer III of the cross with $b\ +/+ \ cn$; $ri\ +/+ \ Sb$ mothers, very similar values are found. Coincidence is 1.45; P for $b\text{-}cn$ is between 0.80 and 0.90, and for $ri\text{-}Sb$ is between 0.70 and 0.80. For Transfer IV in the $sc^8, w^a/w^a$; $b\ cn/++$; $ri\ Sb/++$ cross, where coincidence is 1.53, P for $b\text{-}cn$ is between 0.95 and 0.97 while P for $ri\text{-}Sb$ is between 0.40 and 0.50. Thus, so far as these tests go, it seems reasonably clear that the high values for coincidence are not dependent upon heterogeneity of the mothers with respect to the recombination values they produce.

DISCUSSION

The precautions observed in the present experiments are believed to preclude an explanation of the results in terms of heterogeneity of the genetic material,

or of its treatment. But if the positive associations are not to be accounted for by such heterogeneity, it becomes desirable to formulate an hypothesis which will to some degree explain the observed facts, which may be summarized as follows. These crosses give for the age range of 0 to 16 days an overall positive association of recombinants for centromere regions of chromosomes II and III. This is shown on the one hand for standard females lacking gross chromosome aberrations, and on the other for females heterozygous structurally for their X chromosomes, that is for females containing one *scute*^s X chromosome, and in which crossing over has consequently been increased for both of these autosomal centromere regions. However this interchromosomal relationship involves considerable variation with the age of the mothers. Thus for the structural homozygotes, the first transfer, which measures the effect during the first two days, and the last transfer, which measures the effect during the 15th and 16th days, both show an association very close to that expected on the basis of chance. But between these extreme transfers the homozygote gives a marked degree of association of recombinants, and the variation in this degree is apparently regular between these age limits. It reaches a peak, where the double recombinants are over 1.5 times as great as expected on chance, and then declines. The variation with age for the structural heterozygote, as observed in females with the *scute*^s inversion present in one X, is different. In this case, there is no certainty of very early chance association of recombinants—and the terminal association is quite definitely not chance. In these *scute*^s heterozygotes the degree of positive association increases rather regularly and relatively slowly, and in what appears (measured by coincidence) to be a linear manner. And here the double recombinants are always fewer than 1.5 times those expected—which is a value somewhat lower than that reached by maximum coincidence for the structural homozygotes. It is of special interest that for both structural types the highest coincidence values are reached, not early in the reproductive life of the mother when both recombination values are decreasing rapidly, but at a considerably later stage when crossing over has become comparatively stabilized.

These relationships may now be considered from the standpoint of the behavior of the chromosomes within separate nuclei. Other workers have previously pointed out that altered coincidence, that is altered association of recombinants, can result statistically from the lumping of heterogeneous recombination data obtained from differently behaving mothers. This was clearly indicated for the single chromosome pair in *Drosophila* by WEINSTEIN as early as 1918. It has been discussed more recently by STURTEVANT (1955) who suggested that a heterogeneity of eggs might explain, at least formally, the unexpectedly high proportions of double crossovers obtained from the extremely short distances concerned in fourth chromosome crosses of diplo-IV triploids (STURTEVANT 1951).

Although the present experiments are believed largely to eliminate both genetic and environmental heterogeneity, still it might be held that there is a possible type of heterogeneity which cannot be controlled with the precautions observed.

For example, changes in identical mothers could conceivably introduce an excess of noncrossover strands from cell lines in which crossing over has for some unknown reason not taken place. The proportion of these special oöcytes which are capable of producing only noncrossover gametes, may then be assumed to be dependent upon the age of the mother. Thus crossover values and values for expected associated crossing over, as they are ordinarily computed, would change with age. We may then ask whether change in recombination, and the appearance of positive association of recombinants, might in fact be causally related through this transformation in the crossing-over behavior of selected oöcytes. The immediate question is whether the consequences of such a situation would be consistent with present results, and with well established results of previous work.

It is obvious that any change in a crossover value can be expressed in simple mathematical terms by a given increment (positive or negative) in the proportion of adults from noncrossover oöcytes. Consider a population, N , consisting of say n' adults from oöcytes in which crossing over can take place, and $N-n'$ adults from oöcytes in which crossing over does not occur at all in any region. The group n' consists say of w' associated noncrossovers for two regions a and b , and of x' single crossovers in region a , y' single crossovers in region b , and z' associated crossovers for regions a and b . In this case crossing over for region a may be expressed as $a_1 = (x' + z')/N$, and for region b as $b_1 = (y' + z')/N$. Coincidence here, C_1 , is $z'N/(x' + z')(y' + z')$. If in the population, N , there is a change to a new number, n'' , of adults derived from oöcytes able to cross over, we then have n'' consisting of the new values w'' , x'' , y'' , and z'' . Adults from oöcytes in which crossing over can not occur are now $N-n''$. Crossover values in this second case become $a_2 = (x'' + z'')/N$ and $b_2 = (y'' + z'')/N$. Coincidence in this second case is $C_2 = z''N/(x'' + z'')(y'' + z'')$. By assumption $x'/n' = x''/n''$; $y'/n' = y''/n''$; and $z'/n' = z''/n''$. It follows by simple substitution that

$$a_1/a_2 = n'/n'' = b_1/b_2 = C_2/C_1.$$

Hence, if change in recombination with age, or indeed with any other agent such as the presence of heterologous inversions, were due predominantly to change in the proportion of oöcytes which are incapable of crossing over, it would follow that the new recombination value for a region is directly proportional to the old value, and coincidence would vary inversely as recombination values. The constant of proportionality would be the same irrespective of the regions measured. This should hold no matter what the positional relationship of any two measured regions to each other—when they are in the same chromosome pair, whether or not they are contiguous; and also even when they are located in separate nonhomologous chromosomes. But it is quite well known that such a constant relationship is not exhibited by recombination values when they are affected by age (BRIDGES 1927, 1929). Also the relationship definitely does not hold when the agent causing recombination change is the presence of heterologous inversions (SCHULTZ and REDFIELD 1951). Both types of recombination change are characterized above all else by position within the chromosome arm. Thus if such a heterogeneity of oöcytes were the major factor concerned in situations involving

recombination change, it would lead to recombination effects with age, and to recombination effects with heterologous inversions, which are at complete variance with established experience.

Furthermore judging by the general trend of results, it may be stated that the hypothesis is not consistent with the data for coincidence of the present experiments. It is clear (see Figures 1 and 2) that for both structural homozygotes and structural heterozygotes the highest coincidence values appear after the crossover values have become relatively stabilized. And a consideration of the slope of the curves for coincidence values shows that there is just as great rate of change in coincidence after the period of initial extreme decrease in crossing over, as occurred while this decrease was taking place. The relationships expressed by the simple equations $a_1/a_2 = b_1/b_2 = C_2/C_1$ can be numerically tested with the data of separate transfers of the crosses. Unfortunately these tests are not completely satisfactory because larger populations are desirable for some transfers. However, computations do show a tendency in a given transfer for the ratios a_1/a_2 and b_1/b_2 to be like one another, and for these two ratios to be unlike C_2/C_1 . This would suggest in the first place that, as expected, recombination change with age in these two centromere regions is produced largely by some common causal agent. For the reasons cited above, this apparently does not involve a duality of oöcytes depending upon ability versus inability to cross over. In the second place, the high coincidence values which are the result of positive associations of recombinants, apparently depend upon the additional activity of still another agent. As will be seen presently, this second agent may well be the direct consequence of known positional relationships of nonhomologous chromosomes.

Presumably the role of oögonial or other premeiotic crossing over, as described by WHITTINGHILL (1950, 1955) for special conditions, need not be seriously considered in these effects. It has already been noted that under the circumstances of the present experiments there was no observed clustering of crossover types among the offspring of particular mothers. Of interest in this connection is the conclusion of RASMUSON (1957) that there is no significantly demonstrable gonial crossing over in Bar heterozygotes which are, aside from the negligible complication introduced by the Bar locus, structurally standard. RAMEL (1962) has further demonstrated statistically that the amount of premeiotic crossing over in females containing heterologous inversions cannot be higher than that in controls without inversions.

Why then do given regions of "independent" chromosome pairs, particularly in the structural homozygote, show correlations in their crossing over behavior? Perhaps one should note immediately that in keeping with the concept that any character may be affected by the genes, genic effects on crossing over which might be termed interchromosomal are not unknown. Thus the modification by a gene, or by a constellation of genes, in one chromosome pair of recombination in another pair, is a common phenomenon (LEVINE and LEVINE 1954)—indeed such modifiers frequently are a complicating nuisance in the investigation of the more clearly defined types of interchromosomal effects. A striking case which seems to depend upon a gene difference is that of GOWEN and GOWEN (1922) who reported a third chromosome mutant, *c3G*, which when homozygous results

in the almost exclusive recovery of noncrossover strands for all chromosome pairs. It is of interest that HINTON (1962) has briefly reported an effect of heterozygous *c3G* on heterologous chromosome pairs, apparently similar to that produced by inversions; the salivary third chromosomes are said to be normal. However the possibility remains that chromosomes of females with *c3G* may behave aberrantly with respect to some process not immediately related to crossing over per se. Conceivably, for example, *c3G* might inhibit meiotic synapsis.

It seems more likely that the crossing over process itself is at the center of the problem in the present crosses, particularly those involving the simple structural homozygotes. If the association of recombinants in these standard females for the centromere regions of nonhomologous chromosome pairs reflects a phenomenon within the individual cell, then this could in a sense be considered the consequence of something related to cell heterogeneity. For the observations indicate that within a given oöcyte the likelihood of crossing over in the centromere region of II (or III) is greater when it occurs also in the centromere region of III (or II). The tendency of the recombinants to emerge together in one egg means that previously they tended to be produced together in the same oöcyte.

We may now enquire what influences might be at work in nonhomologous centromere regions making them behave similarly within the environment provided by a single oöcyte. Significantly in this connection the centromere regions of the two V-shaped autosomes are known to be alike in a number of respects: they both contain a relatively great deal of heterochromatin; they are both regions in which mutant loci are highly concentrated; they are peculiarly sensitive to a number of agents affecting crossing over and breakage; and so on. The most outstanding similarity is that they are at the center of coordination of position of the nonhomologous chromosome pairs. For every since the work of HEITZ (1933, 1935) it has been clear that throughout those stages which are favorable to the cytological study of continuous chromosomes, the centromere regions of nonhomologous pairs of this form tend persistently to remain in close proximity. The familiar figures are formed with the centromere regions gathered together in a common chromocenter. Although, as is also well known, *Drosophila* chromosomes prove elusive at the presumed stage of crossing over—still whenever the chromosomes are properly visible as chromosomes, centromere regions tend to be spatially associated over and above the fact that they are in the same cell. A persistence, or partial persistence, of this pattern seems likely therefore during the process of crossing over itself. If this is true any localized influence at this stage, as of a specific chemical or ionization, which increased crossing over in the centromere region of one pair would be expected to increase crossing over in a spatially associated region—as at the centromere region of a nonhomologous pair with similar properties. And presumably most likely to satisfy these conditions of spatial proximity of chemical (*e.g.*, heterochromatic) and other similarity are the two centromere regions of the major V-shaped autosomes.

Various tentative predictions might be made as to effects on the basis of such an hypothesis. Thus if one of the regions lies farther away from its centromere,

and hence from the chromocenter, less association of recombinants might be expected. Also the influence of interference within a single chromosome might further decrease the degree of association as one region is chosen progressively away from the centromere. The association of recombinants for the ends of nonhomologous chromosomes is of particular interest for several reasons; for example there might be an influence of the selective terminal adhesions which are observed in salivary gland chromosomes of this species, and which have been analyzed especially by HINTON (1945). Some preliminary recombination evidence for such interrelationships is on hand, but more data are needed under properly controlled conditions. The hypothesis outlined in the previous paragraph will not now be further elaborated, and is to be regarded as probationary. It bears some resemblance to a more specialized hypothesis of OKSALA (1958) which assumes a complete bouquet stage in *Drosophila*, and which was devised to explain certain interchromosomal, and other, effects on crossing over and disjunction associated with various chromosome aberrations.

There is reason for believing that the distribution and concentration of heterochromatin as observed along the chromosome (SCHULTZ 1947; HANNAH 1951) is correlated with interchromosomal effects dependent upon the presence of heterologous inversions (SCHULTZ and REDFIELD 1951; REDFIELD 1957). The possible influence of such distribution on interchromosomal relationships shown by the structural homozygote is also a matter of considerable interest. It may be that distribution of heterochromatin is a factor of differential value in the contrasting results from structural homozygote and structural heterozygote, as seen in the present experiments.

It is increasingly clear that studies of interchromosomal effects must take into proper account those changes which depend upon maternal age, either when one compares structural homozygotes with structural heterozygotes, or even when either type is dealt with separately. If a given relationship persists throughout the transfers of a considerable age range, or if there is consistent change in the relationship within this age range, the probability of deriving valid conclusions from the recombination data becomes appreciably enhanced. This is particularly true when those regions of nonhomologous chromosomes are involved which show the more marked and therefore more usable interchromosomal crossing-over effects, since such regions frequently are quite sensitive to age.

If interchromosomal effects are shown to persist after elimination of the known possible sources of heterogeneity, these effects are presumably not, so far as the evidence goes, to be attributed to pooling of heterogeneous data. The effects which cannot be further reduced are then, at least temporarily, usable as a foundation for causal analysis. Such a procedure should eventually provide considerable insight into the interrelationships in crossing over of nonhomologous chromosomes.

SUMMARY

On the basis of indications from previous work, crosses were made with *Drosophila melanogaster* to test interchromosomal relationships in crossing over for the sensitive centromere regions of the two major autosomes, both in the

standard structural homozygote and in the structural heterozygote with one scute^s X chromosome. Special precautions were taken concerning isogenicity, age change, viability imbalance, length of regions under consideration, stability of the environment, and so on. Chi-square tests corroborated the significance of the following observations.

For the overall age range from 0 to 16 days there is a positive association from structurally homozygous mothers of the recombinants for the region from black to cinnabar in chromosome II with the recombinants for the region from radius-incompletus to Stubble in chromosome III. But for these structural homozygotes there is variation with age within this period, from association close to that expected on chance for the youngest mothers, then with regular increase to a peak near the middle of the range, and with subsequent decrease in the degree of association again to that determined by chance.

To compare this basic situation in the standard structural homozygote with that in the structural heterozygote in which crossing over in these same regions is altered by the presence of a heterologous inversion, one scute^s X chromosome was introduced into mothers. The scute^s/+ mothers also give an overall positive association for recombination in *b-cn* and *ri-Sb*. In contrast to the data from the structural homozygotes, these latter data arranged according to age of mother show a general linear increase in coincidence up to the end of the age period studied. For these mothers with the X inversion there is an apparent increase with age in the proportion of male exceptions produced.

The tendency of recombinants to emerge together is presumably a phenomenon of the individual oöcyte. The high coincidence values appear at an age when recombination values have become relatively stabilized.

An hypothesis is suggested to explain positive association of recombinants at these centromere regions of nonhomologous chromosome pairs which is based upon the assumed persistence, or partial persistence, through the critical crossing over stage, of the pattern of chromocentral association observed in cytological preparations in *Drosophila*.

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