EGG MORTALITY AND INTERCHROMOSOMAL EFFECTS ON RECOMBINATION[1]

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IN *Drosophila melanogaster* appreciable increase in recombination may be produced for a given pair of chromosomes by the introduction of heterozygous inversions into other chromosome pairs. (A general review of the evidence has been published by SCHULTZ and REDFIELD 1951; details are given in papers mentioned there and in those quoted below; recent examples of similar effects in other Drosophila species may be found in the work of CARSON 1953, and LEVINE and LEVINE 1954.) The effects vary with the inversion set-up but they fall into a general pattern showing relatively large increments near the centromere and typically also at free ends of chromosome limbs; less marked increments are found in intermediate regions. These increases are striking and have been emphasized in the literature, but it must not be forgotten that decreases also are occasionally demonstrated. Compared with the increases, the decreases are quantitatively quite small, but they do appear to be real (MORGAN, REDFIELD and MORGAN 1943; STEINBERG and FRASER 1944; WELSHONS 1955.) But after recognizing these exceptions it is still desirable to focus attention primarily on the increases, since the positive effect produced by the latter is of a larger order of magnitude than is the negative effect of the relatively quite minor and definitely restricted decreases.

The basic cause of this influence of heterozygous inversions upon crossing over in "independent" chromosome pairs has remained obscure, although there is an obvious resemblance of the pattern of increase to the gross distribution pattern of heterochromatin along the chromosome limbs. Of probable relevance is the cytological study of SCHULTZ and HUNGERFORD (1953; their paper presented at the 1952 meeting of the Genetics Society of America was kindly made available) which shows that the pattern of somatic pairing between X chromosomes is significantly changed in the presence of heterozygous inversions in chromosomes II and III. This change accompanies the increases in crossing over demonstrated at the same time by REDFIELD (1953, 1955) for the X chromosomes of the same material.

Since the 1916 paper of BRIDGES it has been recognized that a curious relationship exists between crossing over and disjunction of the X chromosome, for in the exceptions resulting from secondary nondisjunction those X strands which emerge are noncrossovers. More recently STURTEVANT has briefly reported (MORGAN and STURTEVANT 1944) that heterozygous autosomal inversions which decrease secondary X nondisjunction, may increase primary X nondisjunction. Following this lead from primary nondisjunction COOPER, ZIMMERING and KRIVSHENKO (1955) suggest

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for complex structural heterozygotes an hypothesis involving pairing of non-homologues from two or more chromosome pairs, with subsequent nondisjunction of autosomes (as well as of X chromosomes), and with selective elimination in unhatched eggs of strands of lower rank crossover types. This they believe "will partially account for an interchromosomal effect on crossing over". Obviously such a series of assumed events might, if actually shown to be of sufficient frequency, be a factor of real significance in explaining the crossover increases with heterologous inversions. It seems advisable therefore to consider the expected consequences if elimination of noncrossover strands were to have a *major* role in the production of the observed increases—but, of course, COOPER, ZIMMERING and KRIVSHENKO are not to be held responsible for this extended use of their suggestion.

Increased death of eggs among offspring of inversion heterozygotes is a matter of common observation, and it has previously been explained (STURTEVANT and BEADLE 1936; CARSON 1946, 1953) as the result of the production of certain expected aberrant strands by exchanges between homologues, and within inverted sections. An alternative, or accessory, explanation of the egg mortality obviously exists if there is a marked amount of non-homologous pairing of chromosomes, for such would be expected to lead to considerable nondisjunction of major autosomes, and thus to appreciable production of lethal zygotes with an excess or a deficiency in the normal complement of these autosomes. This matter will be dealt with again later; but it may be briefly noted here that although such eggs might conceivably be assumed to carry with them to early destruction noncrossover strands of the heterozygous inversion pairs, it is not immediately clear that noncrossover strands of remaining chromosome pairs, that is those without inversions, would be selectively eliminated. And of course just this latter situation, in which heterologous inversions affect recombination in remaining structurally like pairs, is the critical test of the interchromosomal effect of inversions.

But the main concern of the present paper is to determine whether the amount of egg mortality actually produced by selected heterologous inversions under optimal experimental conditions can, in terms of elimination of noncrossovers, account statistically for the observed recombination increases given by the inversions. Recombination effects, shown by X chromosomes structurally alike, will be compared with egg mortality results. For this the relatively simple Curly and Payne inversions of chromosomes II and III, respectively, were used. These inversions were chosen, not only because they have figured prominently in establishing the interchromosomal effects on recombination, but primarily because as inversions go they have little effect on viability—and incidentally because they do not, unlike some inversions, include translocations which definitely complicate matters through independent production of autosomal nondisjunction.

RESULTS

The simple egg counts

The preliminary egg counts came from females of the "p-26" stock of this laboratory; the stock contains the left and right Curly inversions of II, and the left and right Payne inversions of III; the particular Payne chromosome used includes the identifying dominant Deformed eye (*Dfd*). (For a description of these inversions see BRIDGES and BREHME 1944.) To guard against loss of the inversions by crossing over within the stock, the Curly and Payne chromosomes are continued in each generation through a single male parent, the single female parent being introduced as a wild type virgin from "p-1 Oregon-R". (At the time, this latter stock had been inbred some 135 generations by individual brother-sister matings.) Continued introduction of inversions from the father is generally advisable for the stable maintenance of a number of inversions in a single stock; this has become evident through occasional experimental fiascoes shown to be dependent on loss of inverted limbs through the increased crossing over taking place, even in the inverted chromosomes, in females with multiple inversions. A final check of the proper presence of the inversions was provided by cytological examination of the salivary gland chromosomes by DR. JACK SCHULTZ. There remains the question of variation in the noninverted chromosomes; but since all chromosomes of p-26, other than inversion chromosomes, are from the highly inbred p-1, the noninverted chromosomes of the different types are within all reasonable limits the same and isogenic with those of p-1.

Three crosses were involved in this first experiment; for each of these, virgins were isolated and mated in vials in small numbers (ordinarily five per vial) to three times their number of males. After a period of two to three days in the vials to insure proper mating, the flies of each cross were put into a single bottle, with 20 to 30 females per bottle. Twice daily, metal trays were provided with a layer of standard molasses-agar-Moldex medium the surface of which was scored by a needle into conveniently small rectangles, and painted with a solution of fresh yeast. Thus the first count of the eggs was made either early in the morning or late in the afternoon; then allowing from this count an interval of 24 hours for hatching, a second count was made of the eggs which did not hatch. Actual counts took place at room temperature—otherwise development of both parents and offspring was at a controlled 25°C. No eggs were collected after the females were 7 days old. The chances are believed good that with this technique all females are properly mated, and that all eggs are fertilized.

Data from the three crosses are shown in table 1; the results of the counts are clear-cut. Since the genetic constitution is controlled, the differences can be attributed to the presence of inversions in the mothers. Both the mating of wild type females by wild type males, and that of sister wild type females by brother *Cy/+; Payne/+* males, give quite the same result—namely an 88 percent hatch of the eggs laid. Thus there is in each case a 12 percent loss by death in the egg stage, which may be regarded as a measure of the innate genetic weakness of the eggs of this particular stock. There is in these crosses no loss attributable to the weakening of the egg stage by any combination of the inversion chromosomes. A significant point for our purposes is that no nondisjunction of major autosomes has here been produced in the fathers by the presence of the inversions, since such would have given additions or losses transmitted by the sperm and would be detected as elimination of eggs in the one cross but not in the other.

Now the data show that the third cross, from the same culture bottles of *Cy/+; Payne/+* females by wild type males gives a much higher egg loss: 78.6 percent of the eggs hatch, that is 21.4 percent die—this death rate is then approximately ten

TABLE 1

Egg counts from highly inbred stock. Inversions were introduced as indicated

Inversions			
Mothers	None	None	*Cy; Payne, Dfd*
Fathers	None	*Cy; Payne, Dfd*	None
Eggs laid	7450	5043	5093
Eggs unhatched	11.7%	11.8%	21.4%

percent greater than that shown by the other two crosses. There seems to be no immediate reason for attributing this observed increase of ten percent to nondisjunction of II and III since, as explained in the paragraph above, it does not occur in the reciprocal cross where the inversions are introduced from the fathers. It is well known (from work of MULLER, DOBZHANSKY, and of others) that nondisjunction of II and III, in those cases where it does with certainty occur, as in the presence of translocations, is found in both sexes. There is no justification for assuming that inversions would limit any nondisjunction they might cause to one sex only. However even if we were completely to ignore the significance of the demonstrated lack of autosomal nondisjunction in the inversion male, and were to assume all this ten percent difference really to be the product of nondisjunction of autosomes in the inversion female, this actual value is still far from sufficient statistically to account for observed recombination increases produced by these inversions in other crosses. This matter will be dealt with later, as will also the matter of the more probable explanation of the difference as the result of four strand double crossing over within inversions.

Egg counts accompanied by crossover counts

Evidence of a direct nature concerning a possible relationship between egg mortality and the recombination increases might be expected if crossover counts for the X chromosome were made for both *Cy*+; *Payne*/+ mothers and for mothers without inversions, of the F_1 adults actually developing from survivors of egg counts. But in such an experiment one must, as will become evident, guard against complications resulting from additional lethal effects at the egg stage due, for example, to the particular mutants introduced to measure the crossing over.

A relatively simple cross was planned making use of the left portion of the X, for which region marked recombination increases are known to be produced by the Curly and Payne inversions of stock p-26 (REDFIELD 1955). Three additional stocks were utilized here which had been derived about a year previously by crosses such that all chromosomes were necessarily from p-1 with the possible exception of chromosome IV, and excepting in the X the yellow-split region and also small regions surrounding the mutant loci crossveinless and vermilion. These stocks had been carried as small mass cultures since their origin, and consequently cannot be regarded as genetically pure in the sense that p-26 is pure. Females of the composition y^2w^a *spl v*/*cv v* were obtained, with and without the inversions, and were crossed to males from the tester stock with all the recessive mutant genes except vermilion, and with the additional recessive suppressor of apricot. These genes have the following loci on the standard map: y^2, yellow^2, 0.0; w^a, apricot, 1.5; *spl*, split, 3.0; *cv*, crossveinless, 13.7;

v, vermilion, 33.0 (BRIDGES and BREHME 1944). *Su-w^a*, suppressor of apricot, is between the loci for *y* and *w* according to information supplied by DR. JACK SCHULTZ. It was hoped to obtain some incidental measure of primary nondisjunction of the X; this may be detected in female offspring of this cross by the presence of vermilion, and in male offspring by the presence of the suppressor of apricot which unmistakably darkens the apricot eye. The technique for egg collection did not differ from that described in the section above except that the number of males was four times the number of females instead of three times that number. For the crossover counts sample blocks of the medium from the egg collecting trays, each block containing approximately 100 larvae, were after the second egg count, placed separately in ordinary culture bottles where they developed into adults. It was not considered feasible to allow all surviving eggs to develop, and excess blocks were discarded. No accurate count was attempted of larvae or of pupae, and it is not known precisely what the mortality was at these stages; nevertheless dead larvae of late stages and dead pupae were certainly not present in large numbers.

Table 2 gives the data for egg counts and for crossover counts. The picture presented by the egg counts is somewhat different from that shown by the results of the preceding section, for there is here a much more marked difference between the percentages of unhatched eggs obtained from the two types of mother. Mothers without inversions (table 2) show a lower percentage of egg lethals, 3.2 percent, than do comparable mothers of the p-26 stock shown in table 1 which gave 11.7 percent. On the other hand inversions mothers (table 2) show 26 percent of egg lethals, whereas those in table 1 show 21.4 percent. Thus the percentage of so-called "dominant lethals" due to the presence in the mothers of the inversions is approximately ten percent (21.4 percent minus 11.7 percent) for p-26 stock, but it is approximately 23 percent

TABLE 2

Egg counts and recombination counts from y^2 w^a spl + v/+++ cv v ♀♀ × y^2 su-w^a w^a spl cv ♂♂. *The inversions are introduced as indicated*

Inversions		
Mothers	None	*Cy; Payne, Dfd*
Fathers	None	None
Eggs laid	8824	8317
Eggs unhatched	3.2%	26.0%
Crossover type		
0	2218 + 2178	1238 + 831
1	21 + 23	67 + 83
2	17 + 17	34 + 42
3	187 + 171	290 + 347
1,3	1 + 0	3 + 4
2,3	0 + 0	1 + 0
N	4833	2940
X nondisjunction	0.06%	0.17%
Crossover values		
y^2 – w^a	0.93 ± 0.14	5.34 ± 0.41
w^a – *spl*	0.70 ± 0.12	2.62 ± 0.29
spl – *cv*	7.43 ± 0.38	21.94 ± 0.76

For each pair of contrary classes the class with y^2 is given first.

(26.0 percent minus 3.2 percent) for the present crosses in which the crossover counts were made.

As expected, table 2 shows that increases in crossover values are produced by the presence of the inversions: $y^2 - w^a$ recombination increases to a value some six times its value without inversions, this factor for $w^a - spl$ is about 4, and for $spl - cv$ about 3. The differences for the values between inversion and noninversion mothers are clearly significant. The actual values reached are within the range of those customarily obtained for this region both with and without these inversions (although the effect produced here is relatively somewhat more marked in $y^2 - w^a$ compared with that in $w^a - spl$ than is the case for some other slightly different, and better controlled, crosses for this region—see REDFIELD 1955.)

Now on their face value and without further analysis these data from table 2 might tempt one to conclude that they are consistent with the hypothesis of death of eggs through some such process as autosomal nondisjunction, and that false increase of crossover values might here be produced by elimination of noncrossovers in the dead eggs. That this conclusion is not justified in this experiment is easily demonstrated by exposing a complicating cause of death. A definite clue is supplied by the facts that for mothers without inversions the hatch in the present cross was some 10 percent greater than from comparable mothers of table 1, whereas for inversion mothers it was some five percent less. The first fact shows that the intrinsic genetic viability of the eggs from noninversion mothers is markedly higher in the present cross than in the relatively more pure p-26 strain, and the second fact shows that addition of the inversions nevertheless reacts in some way to give less viable eggs than it did in the previous experiment. A concrete reason for this reversal emerges on examination of the contrary classes, which exhibit a deficiency of crossveinless adults for inversion mothers, but not for noninversion mothers. The ratio of the total of all *cv* adults to the total of all non-*cv* adults is for females without inversions 0.99; for *Cy*/+; *Payne*/+ females it is only 0.72. And for all major crossover types considered separately the same general relationship holds as a glance at the actual numbers in table 2 shows. Thus although *cv*/*cv* and *cv*/+ classes are practically equal for noninversion mothers, among the offspring of inversion mothers a large proportion of *cv* homozygotes die, and it is obvious that they die in both crossover and noncrossover classes. Whether this semilethal effect is due to the *cv* gene itself or to some mutant closely associated with that gene is not known, but it is immaterial. The effect seems not to be dependent upon a direct combination of homozygous crossveinless with any permutation of the inversion chromosomes; the classes +, *Cy*, *Dfd*, and *Cy Dfd* (not recorded) were apparently equal. If verified this suggests the additional involvement of some maternal influence on *cv*/*cv* eggs of the inversion chromosomes, but this possibility was not dealt with further—it does however fit in well with the high egg mortality, since maternally inherited effects are very apt to appear early in development.

It is interesting that the peculiar specificity of the relationship here is somewhat similar to that shown by a case recently reported by STURTEVANT (1956), in which a dominant in III causes the death of all prune (X chromosomal) flies which carry it; this latter situation involves no maternal effect.

That the semilethality of the crossveinless homozygotes is operative primarily at the egg stage seems fairly certain, not only because there is an observed high death rate of eggs, but also because the ordinary appearance of the inversion culture bottles gave no gross indication of increased numbers of dead larvae or pupae. In view of the results it is regretted that no counts were made of pupae, or of the exact number of eggs from which the counted adults came. Such egg counts are available, however, for the next experiment reported below.

One can easily estimate what proportion of crossveinless homozygotes have died as eggs among the offspring of inversion mothers shown in table 2. Assuming the same ratio of *cv/cv* to *cv/+* adults expected for the two types of mother (that is 0.99), then the expected number of *cv/cv* adults from *Cy/+; Payne/+* mothers is 1695. The loss of *cv/cv* adults is then 469, or 14 percent of the expected total adults. This results (assuming larval and pupal death completely negligible in the crossveinless effect, which may be too extreme) in approximately 17 percent as the total expected death of eggs, which in turn gives about 14 percent for the expected value for "dominant lethals"—that is, expected if the secondary semilethal effect of crossveinless were not operating. This estimated value is not far from the ten percent demonstrated "dominant lethals" of table 1 (especially if one considers that the two separate experiments are not strictly comparable), and it would necessarily more closely approach ten percent if one could take into account whatever larval and pupal death may actually be involved in the crossveinless effect. That the larval and pupal component is, however, very small (or is even nonexistent) is indicated by the close agreement of this value of 14 percent as it stands with that obtained in the clearly more comparable experiment of table 3 which gives a value of 12 to 16 percent.

The importance of limiting the egg mortality in such studies to that irreducible minimum which is a necessary concomitant of the presence of the inversions in the mothers becomes obvious. An even simpler experiment than that just described was therefore next carried out and is reported in table 3. Mothers were used which were heterozygous for yellow^2, apricot, and split and which were at the same time of two types containing or not containing the Curly and Payne inversions.

Females without inversions were crossed to tester males (*y*^{2} *su-w*^{a} *w*^{a} *spl*) both with and without the inversions; inversion females were crossed only to noninversion tester males. Thus there were three crosses of *y*^{2} *w*^{a} *spl/+* females, but at the time it was impossible to carry out all three simultaneously; one of these, namely the cross of noninversion females by inversion males, was begun some six weeks later than the other two. For this reason this cross is not as strictly comparable with the other two as these two are with each other; this is true particularly in that the mothers derived later are from a different set of cultures. In general the technique was similar to that described for the preceding experiment. However it differed in that blocks of medium containing approximately 150 eggs were removed from the egg collecting trays and were placed separately on rounds of toweling paper in small Petri dishes (diameter 2 inches)—here the eggs were first counted, and a fresh supply of yeast in the form of a thin paste was then added to the paper. After the second egg count each block of medium with its paper and larvae was put into a standard culture bottle where the adults developed. Thus the counts of adults gave the desired re-

TABLE 3

Egg counts and recombination counts from y^2 w^a spl/+++ ♀♀ × y^2 su-w^a w^a spl ♂♂.
The inversions are introduced as indicated

Inversions			
Mothers	None	None	*Cy; Payne, Dfd*
Fathers	None	*Cy; Payne, Dfd*	None
Eggs laid	10683	10216	8106
Eggs unhatched	2.2%	5.5%	18.0%
Larval and pupal deaths	6.9%	9.6%	10.9%
Crossover type			
0	4808 + 4762	4393 + 4090	2733 + 2602
1	42 + 35	48 + 47	137 + 136
2	31 + 26	33 + 37	85 + 66
1,2	0 + 0	0 + 0	1 + 0
N	9704	8648	5760
♂ X nondisjunction	Undetermined	0.07%	0.10%
Crossover values			
$y^2 - w^a$	0.79 ± 0.09	1.10 ± 0.11	4.76 ± 0.28
$w^a - spl$	0.59 ± 0.08	0.81 ± 0.10	2.64 ± 0.21

For each pair of contrary classes the class with y^2 is given first.

combination values from a known number of surviving eggs; also they gave incidentally the combined larval and pupal mortality.

The results in table 3 from parents lacking inversions are, as expected, like those shown in table 2, with 2.2 percent egg mortality (compared with the 3.2 percent of table 2), and with recombination values for $y^2 - w^a$ and $w^a - spl$ within the ordinary range. For inversion females mated to males without inversions the computations give almost identical recombination values with those shown in table 2; the egg mortality on the other hand is definitely lower (that is 18 percent instead of 26 percent); this is expected in view of the large loss of eggs in the preceding experiment among homozygous crossveinless zygotes. "Dominant lethals" here amount to 15.8 percent of the eggs laid; this compares quite well with the estimated value (eliminating the *cv* effect) of 14 percent from the earlier data.

It is true that the third cross shown in table 3, of noninversion females by inversion males, did not give precisely the results which the first experiment led one to expect (see table 1), that is identically the same egg mortality as when both parents lack inversions. However the difference as seen in the present data amounts to only 3.3 percent (5.5 percent minus 2.2 percent). Possibly the six weeks' time elapsing between the two sections of this third experiment is somehow implicated in this small difference. The particular cultures from which the parents came were of course not the same, and the environmental conditions might have differed by some unknown minor factor. This discrepancy in fact may be taken as typical of the variation exhibited by inversion crosses, depending no doubt upon extreme sensitivity to experimental conditions.

The production from the inversion mothers of a 1,2 double crossover may be noted in passing. This was a y^2 *spl* male; it was tested, and from it a y^2 *spl* stock was derived. It could not have been the result of contamination, since no y^2 *spl* flies were then present (or had been present) in the laboratory. Such double crossovers within this small region (from the yellow locus to that of split) have previously been found in three tested instances from comparable inversion mothers (see REDFIELD 1955); none has been found among the much larger numbers of examined offspring from mothers lacking inversions. The significance of such cases is obvious in connection with our interest in the actual mechanics of pairing and the crossing over process; the X chromosomes are definitely more free to exchange very small segments when heterozygous inversions are present in II and III. These multiple crossovers within genetically quite small distances show also that it is necessary to deal with an extremely well marked chromosome if one is to pick up all crossing over produced in it by inversions in other chromosome pairs.

The simplest of calculations will give the amount of loss required from the cross without inversions to increase the recombination on the basis of elimination to the value observed in the presence of the inversions. Thus for the region y^2 — w^a the number of observed crossovers is 77. If y^2 — w^a crossing over is to reach a value of 4.76 (the value observed with inversions) without disturbing the crossover class, then the expected total number of adults would be 77/.0476, or 1618. The expected loss of noncrossovers is then 9704 (the observed N) minus 1618, or 8086; this is 83 percent of the observed adults, or 76 percent of the total number (10,683) of eggs laid. This results, added to the 2.2 percent of observed egg loss, in an expected total loss of over 78 percent of all eggs laid. Such an extreme egg mortality is arithmetically necessary to account for the observed increase of the crossover value in terms of elimination of noncrossovers. If we make a similar computation for the region w^a — *spl*, we get a corresponding value of 73 percent.

A comparable treatment of those two crosses given in table 3 which are reciprocal with respect to the inversion chromosomes is in one sense a better choice for such computations since the viability relationships are more alike. Here again we shall consider only the amount of elimination which would be necessary to give the increased recombination, ignoring for the moment the secondary question of the presumed means of elimination. The result is that we still find the extremely large discrepancies between the observed egg mortality and the expected egg mortality, for the latter in this case must reach 60 to 70 percent. Thus when the regions dealt with at this left end of the X are small enough that one can be certain of detecting all crossing over, the enormous values of 60 to 80 percent for egg mortality are found necessary to explain, in terms of elimination of noncrossovers, the recombination increases which are actually produced. And such extreme egg mortality as this is not exhibited by these *Cy/+; Payne/+* mothers under the experimental conditions in operation here, for the observed value for total egg mortality reaches only 18 percent.

It is desirable to indicate briefly the results which can be derived from the data when they are calculated separately according to the day of age of the mothers. Unfortunately the daily totals for an age change study of crossing over in the two small regions must of necessity be very high; the data will not be presented in detail here because it is not certain the results are significant in their entirety. However

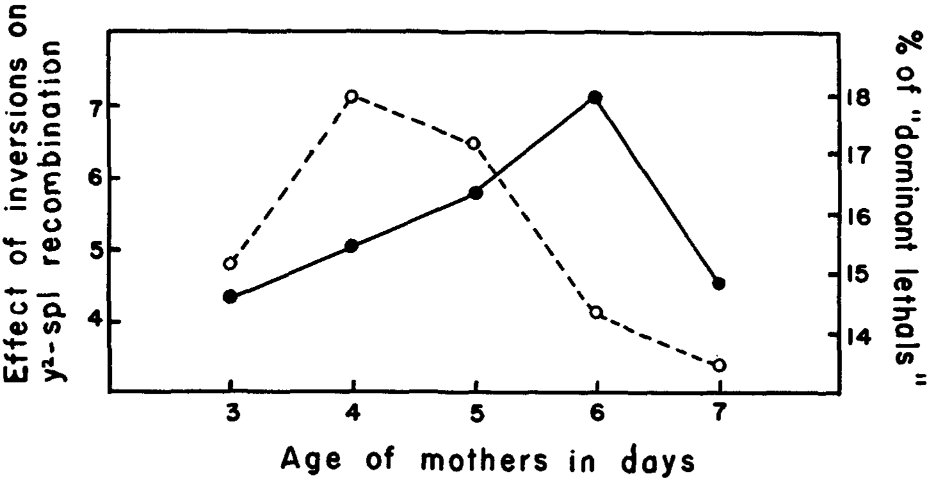

FIGURE 1.—Data the same as those for table 3, but arranged for the days taken separately. The graphs show: (1) Age change of the effect of combined Curly and Payne inversions on recombination in the region $y^2 - spl$ (solid curve—ordinates at left); this is measured by the ratio of the value for recombination from *Cy*/+; *Payne*/+ mothers, to the value from the cross without inversions. (2) Age change of the excess egg mortality given by *Cy*/+; *Payne*/+ mothers over that from mothers of the cross without inversions (broken curve—ordinates at right).

graphs of the gross results, for ages 3 to 7 days, appear in figure 1. The graph for production of "dominant lethals" (broken line) suggests a maximum of about 18 percent reached on the fourth day of life of the mothers, with a gradual decrease to about 13.5 percent on the seventh day. The graph for the recombination effect (solid line, and calculated for the sake of significance for the combined regions) shows a more gradual rise with an apparent maximum on the sixth day; and since there was no detected age change without inversions, practically all the recombination age effect as shown (expressed as the ratio of $y^2 - spl$ crossing over with and without inversions, and varying from 4.3 to 7.1) would seem to be attributable to the inversion mothers. These age change relationships should not be stressed, particularly since the possibility exists that they may be influenced by complications such as waning fertility of one sex, or of both. Still it can be stated that the maximum effect of the inversions on recombination does not, so far as the data now go, fall at the same time as the maximum for the differences for egg mortality; on the other hand the lowest values attained within this time range correspond fairly well in this respect for the two separate phenomena. This matter cannot be discussed further without additional experiments.

Effects of the Curly and Payne inversions on the entire X

In a study of the significance of the proportions of crossover types produced, it is clear that one is at an advantage if the entire X chromosome can be dealt with simultaneously. This is because the noncrossovers in a short region (such as the inclusive region from yellow to split which reaches a genetic value of less than two units in the controls of the present experiments) are in a large part undetected crossovers in other regions of the chromosome. Although this does not alter the computations which have been given, the noncrossover strands presumably eliminated are strictly speaking among those only which are noncrossovers for the whole X. It is advisable to reach some approximation of the proportion of the emergent strands which are completely noncrossover, since the question obviously exists whether this proportion can be large enough to account for the increases in recombination by means of partial or complete elimination of these lowest rank strands. Technically it is practically impossible, of course, simultaneously to cover the complete X in as small sections as have been used here for the left end of the X. Any feasible cross will, expecially when the inversions are present, result in a value for noncrossovers which is too large by virtue of undetected double crossing over in some, if not all, regions. However we can to a degree deal with the rough estimate obtained, even though it is admittedly too large. Some years ago an experiment by REDFIELD was reported briefly which made an attempt to approach the desired complete coverage of the X chromosome (MORGAN, REDFIELD and MORGAN 1943); it was concerned with the effects of the Curly and Payne inversions on crossing over in *y^2 cv v f/ec car bb* females (echinus, carnation, and bobbed not previously mentioned, have the standard loci 5.5, 62.5, and 66.0 respectively—BRIDGES and BREHME 1944). Results obtained from these crosses are given in table 4; the recombination values produced are shown, also the proportions of crossover types among the observed emerging strands. These latter proportions were derived from that portion only of the total data in which all six regions could be followed at the same time. Calculations from percentages of observed strands give directly the percentages of types of tetrads (in parentheses) from which the strands arose; the equations used were obtained from WEINSTEIN'S analysis (1932, 1936). It is interesting that the proportions of observed strands from the restricted control data of table 4 agree within reasonable limits with those from data combined by WEINSTEIN (1936) from three different sources (BRIDGES and OLBRYCHT 1926; ANDERSON 1925; and WEINSTEIN) involving a total of 28,239 individuals from mothers from an entirely different combination of six X chromosome regions (*sc ec cv ct v g f*).

Table 4 shows clearly that typical recombination differences are produced by the inversions; and there is incidental evidence of the usual pattern effect along the X, namely relatively greater increases at either end of this single limbed chromosome and less marked increases in intermediate regions. The results agree, region for region, with those from experiments with less complete coverage of the X. In connection with the possibility of autosomal nondisjunction it is desirable to observe that increases are found when Curly and Payne act alone, as well as when they act in combination; the implications of this fact will be seen presently.

Of special interest are the relationships obvious from the proportions of observed emerging strands, or (if one prefers to use the data in another form) in an even more striking degree from the computed proportions of tetrads producing these strands. A simple comparison of the percentages of types of strands emerging from mothers without inversions with those from *Cy/+; Payne/+* mothers immediately makes it certain that no amount of elimination of low rank strands from the former mothers will result in the observed changed balance between the higher rank strands from the latter mothers. There seems to be no doubt that the inversion mothers have a

TABLE 4

Recombination values, percentages of crossover types of emerging strands, and computed percentages (in parentheses) of tetrads with the designated number of crossovers, from y^2 + cv v f ++/+ ec +++ car bb ♀♀ × In(1)HR, sc ec cv ct^6 v f car bb^1 ♂♂ *or* y^2 ec cv v f car; Y^{-bb} ♂♂

Inversions in mothers	None	*Cy*	*Payne*	*Cy; Payne*
Crossover values				
y^2 – ec	5.9	10.7	9.0	18.0
ec – cv	8.0	11.1	11.8	17.2
cv – v	22.2	24.7	27.3	33.8
v – f	20.4	22.8	29.0	35.2
f – car	5.9	8.0	10.6	12.6
car – bb	3.4	3.5	5.8	10.4
Total	65.8	80.8	93.5	127.2
N	4343	4403	3878	3349
Percent observed strands (and computed tetrads)				
Noncrossovers	42.4 (7.3)	39.0 (6.9)	33.6 (5.6)	20.3 (3.7)
Singles	45.9 (49.6)	45.6 (38.5)	45.3 (25.2)	39.5 (0.7)
Doubles	11.3 (39.2)	14.5 (47.9)	19.2 (54.7)	30.5 (43.0)
Triples	0.5 (4.0)	1.0 (5.6)	2.0 (13.4)	8.7 (35.7)
Quadruples	0 (0)	0.07 (1.1)	0.08 (1.3)	1.06 (17.0)
N	997	1539	1198	1037

The Curly and Payne inversions were present in the mothers as indicated. The data for the strands are based on F_1 males of the cross involving Y^{-bb}, for in these offspring only were all six regions simultaneously observed.

greater tendency to produce the higher rank types, and the highest ranks show the greatest difference between the two types of mother. In other words there is, in a real mechanical sense, a facilitation of exchange produced by the heterologous inversions.

A variety of manipulations of the data of this table could be carried through, but they must all on the basis of simple elimination ultimately result in a clash with observed values. If one assumes tentatively according to the computations from table 3 that about 83 percent of the adults are lost through elimination, it is evident that many more than all of the noncrossovers have disappeared, for table 4 shows only 42.4 percent of noncrossovers from noninversion mothers. It is possible to use the data of table 4 without reference to those of table 3 (in this process, of course, losing the advantage of closer though more limited marking). Thus the region from yellow to echinus which is partially comparable to the regions dealt with in the experiment of table 3, shows a recombination increase from 5.9 to 18.0; for this increase 72 percent of the adults should disappear. If the region of lowest increase is considered, namely that from crossveinless to vermilion, the necessary loss is still some 40 percent of all adults. And even this lowest value of 40 percent of adults would require an egg mortality much higher than that actually observed in the crosses where egg counts were made. Computations based upon the chromosome considered as a whole, that is in which the pattern effect is averaged, are equally futile. The crossover classes of the noninversion cross are, as table 4 shows, 57.6 percent of the total; this amounts to 575 individuals. The total crossing over observed in the X in the presence of the combined inversions is roughly twice that shown in the absence of the inversions. Assuming this is due to simple elimination of noncrossovers, the relationships between all crossover classes remain undisturbed. The expected total with inversions is then 575/1.152, or approximately 500; this estimated total is less even than the actual number of crossovers alone. Such a *reductio ad absurdum* means, as suggested above, that there are not enough noncrossovers to account for the increase. It is necessary then to assume the disappearance also of a considerable proportion of crossover strands; these would presumably be of the next low ranking type, the single crossovers. But the addition of this further assumption proves useless also since the observed discrepancies among the remaining crossover types, namely those of higher rank, still remain unexplained on the basis of any possible elimination of lower types. Crossing over is basically different in the X chromosomes of the mothers with Curly and Payne inversions, and the difference must rest primarily upon an actual increased exchange in one pair of chromosomes, dependent upon the presence of heterozygous inversions in other chromosome pairs.

DISCUSSION

The present paper is concerned with the possibility that the interchromosomal effects of inversions on crossing over might be the direct result of selective elimination of low rank strands in those zygotes which die as eggs. Conceivably the means by which this elimination takes place might involve autosomal nondisjunction following the pairing of those non-homologous chromosomes which are members of pairs of structural heterozygotes. However on second thought one realizes that if autosomal nondisjunction were a factor of major consequence one should expect no interchromosomal effects on recombination when bivalent formation and segregation proceed normally, as is believed (see, for example, COOPER, ZIMMERING and KRIVSHENKO 1955) to be essentially the case when one chromosome pair only is structurally heterozygous. Nevertheless it is clear not only from the results provided by table 4, but from a number of other experiments also that recombination increases are produced by heterologous inversions present in just one pair of chromosomes (for examples see STURTEVANT 1919; WARD 1923; MORGAN, BRIDGES and SCHULTZ 1930, 1932, 1933; STEINBERG 1936; MORGAN, REDFIELD and MORGAN 1943; STEINBERG and FRASER 1944; and REDFIELD 1955—excepting the last, these papers are reviewed by SCHULTZ and REDFIELD 1951).

Other obvious difficulties prevent our accepting the basic implication of autosomal nondisjunction in any general explanation of interchromosomal effects of inversions on recombination. For apparently there is involved a series of uncertain events: the pairing of non-homologues of those chromosome pairs with inversions (incidentally not noticed in the salivary gland cells by SCHULTZ and HUNGERFORD); subsequent nondisjunction of the large autosomes of such pairs; and passage of noncrossovers for other chromosome pairs (of singles also?, since the noncrossovers have been shown to be too few) into zygotes which die as eggs. But such an hypothesis would still not be enough without the support of further assumptions to explain either the general pattern of increase along the chromosome limb, or the decreases shown by particular regions. It is also not consistent with accumulating evidence of the production in the presence of heterologous inversions, of multiple crossovers of an extremely high rank and (which amounts to the same thing) of double crossovers within an extremely short region. The most striking of such classes are not found at all in controls, and they cannot be due to a shift in the proportions of already existing classes; this point has been made in a previous paper (REDFIELD 1955).

The obscure problem of the relation of primary nondisjuction of the X chromosome to recombination should be mentioned, since conceivably it might act as a factor in isolating noncrossovers to be subsequently eliminated. STURTEVANT (MORGAN and STURTEVANT 1944) states briefly that the presence of heterozygous autosomal inversions increases primary nondisjunction of the X; he regards this as a general phenomenon and attributes it to true nondisjunction rather than to chromosome elimination. Such an influence of course represents an interchromosomal effect at meiosis—it is corroborated by the data of COOPER, ZIMMERING and KRIVSHENKO (1955) for the setup which includes heterozygous inversions in the X. Of especial interest in connection with the results of the present paper is their report of one mating of females with structurally like X chromosomes and with heterozygous autosomal inversions, but this cross showed no nondisjunction of the X ($N = 3,709$ adults). My own data for females without the complicating X inversions have long given a somewhat consistent increase (still resulting in an extremely small value) for the detected primary nondisjunction of the X, in the presence in II and III of viable heterozygous inversions producing marked recombination increases in the X. Within a single experiment the values obtained for nondisjunction are not statistically significant, and the differences between inversion and non-inversion females are uncertain. However in my crosses females with Curly and Payne inversions are apt to give primary nondisjunction of the X in the neighborhood of 0.15 percent; this is apt to be some two or three times the value obtained in the controls without the inversions. The values shown in tables 2 and 3 agree with these results. The role of primary nondisjunction of the X in isolating noncrossovers in these inversion crosses with structurally like X's must be considered negligible. It might perhaps be thought that one could assume a practically complete masking effect due to undetected X nondisjunction through elimination in the eggs—but this, if significant in the recombination increases, should then emerge in the extreme mortality rates necessary to explain the recombination effects. And the data have been demonstrated not to exhibit such rates.

There is no doubt that females containing inverted chromosomes produce appreciable percentages of eggs which do not hatch, and that under some conditions these percentages become considerable. Now a number of quite simple minor factors exist which operate to cause the production of lethal zygotes in early stages. Ordinary semilethal relationships of a type not peculiar to females with inversions (and which may act on the egg) apparently are rather common from parents in which the inversion setup is elaborate. They may remain undetected in the absence of suitable tests, as is clear from the analysis of the second experiment of the present series. There are also other possible factors; for example unfertilized eggs are sometimes laid, but of course in *D. melanogaster* do not hatch.

But a major factor in the loss of eggs from inversion females is generally supposed to be dependent upon four strand double crossing over within the inversion, or the two (or more) inversions together, of a given pair of chromosomes (STURTEVANT and BEADLE 1936; CARSON 1946, 1953). One would expect such a tangle to produce aberrant strands with two centromeres or with none, as the direct consequence of unravelling. This exchange would automatically eliminate a certain number of the strands deriving from the inverted pairs themselves, presumably at the egg stage. There is of course no direct evidence from recombination concerning the frequency attained by these strands in Curly-Payne females. However that single crossing over is considerable within one of the inverted sections is suggested, for example, by the data of GRAUBARD (1932) who detected inside the right inversion of Curly a frequency of surviving doubles which would indicate an expectation within this inversion of some 3.5 percent of singles—and this under conditions of structural homozygosis in all other limbs of the chromosome complement. To extrapolate from such a datum to the production in the Curly-Payne females of four strand double crossovers, perhaps mainly with one crossover in each of two limbs, and in which crossing over is increased by the simultaneous presence of inversions in four chromosome limbs involves a number of assumptions which cannot at present be made with assurance. Significantly in this connection we are interested here particularly in higher rank crossing over. Nevertheless it seems within reason to suppose that the total production of the four strand double crossovers within inversions will in Cury-Payne females reach appreciable proportions, and may very well closely approach the ten percent shown under the optimal conditions of the experiment reported in table 1 to represent the total production of "dominant lethals".

It should be clear however that the above discussion by no means excludes the possibility of the production of lethal eggs from some inversion females through autosomal nondisjunction. Indeed the data of COOPER, ZIMMERING and KRIVSHENKO (1955) for at least some inversion combinations apparently show equal numbers of male and female exceptions, and this may demonstrate that for these combinations lethal eggs are produced in considerable numbers as the result of autosomal nondisjunction. The consequences of such a situation would no doubt prove complex and far reaching.

The experiments reported in the present paper were planned as a simple test of the possibility of explaining the recombination increases for the X chromosome of Curly-Payne females as depending statistically upon the elimination in eggs of noncrossovers for the X. The real issue is whether under proper experimental conditions the increase in egg mortality produced in these inversion heterozygotes is sufficient to account for the boost in crossing over shown by the structurally like X chromosome pair. The results show that the egg mortality is much too low.

The critical third experiment involving both egg counts and counts of adults from the surviving eggs, showed that the value for the observed total egg loss in the presence of the Curly and Payne inversions was 18 percent. But the recombination increases for this experiment, those actually produced by the inversions for the yellow-split region of the X, require a total egg loss of 60 to 70 percent on the basis of elimination of noncrossovers. The situation is essentially the same when data are analyzed from adult counts only from a previous experiment in which the entire X was covered.

Simple comparison here of the proportions of crossover types demonstrates that no elimination of noncrossovers can produce the changed balance given by the inversion mothers among remaining crossover types. To put the matter in its simplest terms there are numerically not enough noncrossovers to account for the increases when all of these lowest rank strands are assumed lost. The additional disappearance of a considerable proportion of crossover strands of rank 1, the singles, would still leave unexplained the observed discrepancies in balance between the higher ranking types. And the higher the types which are compared, the greater is the difference between inversion and control mothers. There is a persistent tendency in the inversion mothers, no matter how many low ranking strands are assumed eliminated, to show in the remaining types a proportionally greater exchange between short chromosome segments. This cannot be explained as a statistical illusion depending upon simple low rank elimination; it seems to demonstrate a real facilitation of crossing over by heterologous inversions.

SUMMARY

A series of experiments is described for *Drosophila melanogaster* which tests the possibility of explaining the interchromosomal recombination effects produced by heterozygous Curly and Payne inversions, in terms of major elimination at the egg stage of low ranking strands for the X. It was found that egg mortality under proper experimental conditions is much lower than that which would be necessary to produce the observed recombination increases in the X. Simple comparison of the proportions of higher ranking crossover strands produced by the inversion females with those from control noninversion females shows further that no amount of elimination of low ranking strands can account for the changed balance exhibited among higher ranking strands. Thus these heterologous inversions so influence exchange between the two members of a structurally like pair of chromosomes that there is a much greater tendency for production of higher multiple types.

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