CONSEQUENCES OF CROSSING OVER IN OÖGONIAL CELLS

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IN TESTCROSSES it is commonly supposed that each recombination offspring owes its phenotype to chiasmata in a single primary oöcyte which gives rise to no other offspring. This assumption should be questioned in view of the demonstration that crossing over may be induced by X rays in oögonial cells of *Drosophila melanogaster* (WHITTINGHILL 1938). Irradiation is known to increase the percentage of recombinations in females in some manner, and in males the same agent is able to induce crossovers which originate largely or exclusively in spermatogonial cells. It is therefore appropriate to consider the various possible effects of crossing over in oögonial cells upon the whole picture of recombinations as found in testcrosses. Any oögonial crossing over would make the basic assumption of a linear relationship between chiasma frequencies and recombination partially incorrect.[1] Hence conclusions regarding the quantitative effects of various external and interchromosomal agents upon the process of crossing over may be subject to large errors usually unsuspected.
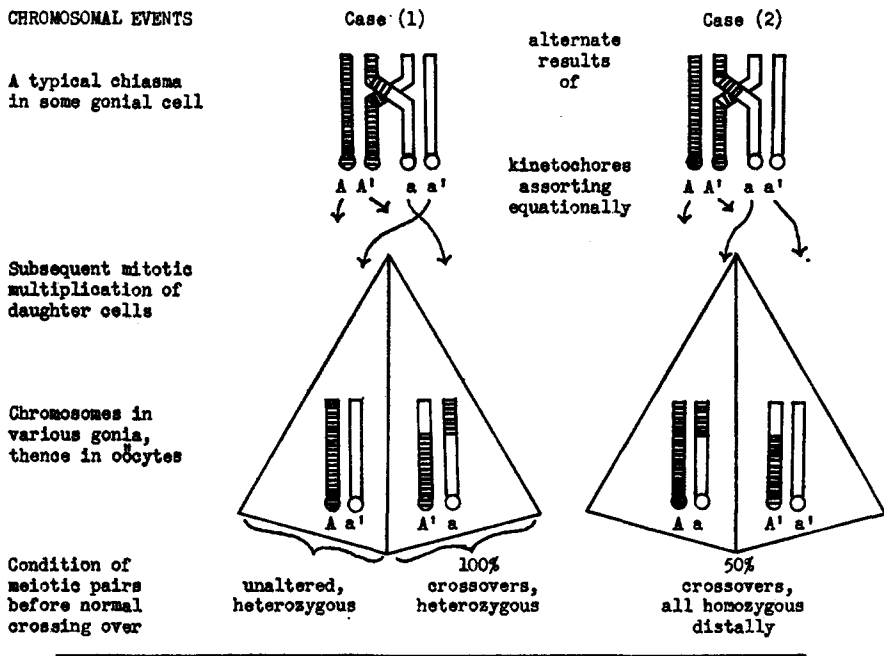
Large changes in the frequency of certain classes of progeny will result from one crossing over in an early gonial cell, and smaller effects will follow an exchange in a later cell division. This lack of a constant proportionality between crossover offspring and inferred chiasmata, so clearly demonstrated by this author in males (1937) and revealed in *c3G* females (1938), may be reasonably applied also to ordinary heterozygous females. This variable relationship may be visualized in figure 1 by imagining that the pyramids shown there may be of various sizes representing the different numbers of cells formed by mitosis after any gonial exchange and before the initiation of meiosis in the descendant cells. The methods of detecting oögonial crossing over through the smoke screen of more numerous meiotic exchanges will vary depending on whether the oögonial exchanges have occurred early or late.

The essential ideas of this paper have been incorporated into the diagrams and tabular material of figure 1. It shows the kinds of effects produced in a testcross by a single gonial crossing over depending on the alternate ways in which the four strands of the tetrad may be distributed to daughter cells. STERN (1936) in an extensive investigation of segregation in somatic tissues has shown that anaphase separations of the two kinds figured may be expected following crossing over during mitosis. Those of type (2) would produce segre-

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[1] Very recently COOPER (1949) has challenged this classic tenet in another way by showing that chiasmata may occur without genetic crossing over. For the sake of brevity this paper will retain the older usage, *i.e.*, "chiasma" here means "one occurrence of the process of crossing over" regardless of how many crossover offspring may be produced by it.

gation (qualitative reduction) of the formerly heterozygous loci distal to the inferred chiasma resulting in somatic spots of recessive phenotype, but anaphase separations of type (1) would produce no somatic segregation because both daughter cells would remain heterozygous and of dominant phenotype. In the gonial line, however, either type of segregation of chromatids would have genetic consequences as indicated in figure 1.

The main thing about oögonial exchange is that it prevents all cells from being identical when meiosis begins. Four different starting conditions are possible, as diagrammed, and these will be further altered, detectably or not,

FIGURE 1.—Effects of crossing over in an early gonial cell upon recombination phenotypes.

by the usual meiotic exchanges. The genetic consequences of random meiotic crossing over acting uniformly upon the different synaptic mates are tabulated under each kind of pairing situation. It is of special interest that most of the changes deduced in the figure have already been separately reported by several experimenters to be cited in turn. The explanation of these independent observations may be partly or wholly the consequences of relatively few oögonial chiasmata. The figure considers one typical chiasma, but it should be remembered that in other parallel germ lines effective chiasmata may occur in various regions along the chromosome and also that they may produce cumulative results. Both individual and mass effects will be elaborated.

The accurate detection of gonial crossing over in either sex requires that the data of the various families be scrutinized for inconsistencies before the totals for a whole experiment are made. In fact within the family irregularities may appear if egg-laying is prolonged through many successive cultures. Six ways in which families may be inhomogeneous will now be described. The first three could indicate any individual early gonial crossing over with many progeny derived from it. The last three could reveal the combined action of later gonial exchanges, particularly if they were numerous in occurrence. The six phenomena exhibit some interdependence.

First, an excess of crossovers from one region in one family or subfamily may point beyond meiotic exchanges to a single crossing over in that region in an early gonial cell. An excess of crossovers could result after either chance of segregation, (1) or (2). It is rather well established that spotty distributions of crossovers do actually exist particularly near spindle attachments.

Second, an imbalance of complementary crossover classes independent of viability differences may result following segregation (2), provided that the segregants pass into gonial cells which divide at unequal rates. This would be a departure from the more usual multiplication shown in figure 1, where equal sides of a pyramid symbolize equal reproduction of the two kinds of daughter cells. Gross inequalities of this kind have been found by the writer and others following high temperature treatment and after irradiation of male heterozygotes. Hence they may be occurring also in females. If the complementary crossovers are unequally propagated, it is a corollary that a certain non-crossover will likewise be unequally reproduced, namely, the one with the homologous, non-identical kinetochore. Any difference of this sort would usually be diluted by the large numbers of non-crossovers from all other sources; therefore, the favoring of one non-crossover would show only in extreme cases. FRIESEN (1936) has reported some families from X-rayed males with total recombinations of 25 percent, 27 percent and 31 percent which had the complementary crossover classes in ratios of about 2:1, 5:1 and 8:1, respectively. His published data did not show separately the two classes of non-crossovers, which would be of particular interest in these three families.

Third, a reduction of recombination values in regions distal to some region showing an increase would indicate that partial homozygosis had been produced by segregation of type (2). This homozygosis would cause a certain proportion of the subsequent meiotic crossing over to produce no detectable

recombinants of distal genes. Such a depression of the distal recombination frequency would be less than the elevation of the proximal region of exchange, because many of the meiotic tetrads would have given rise to non-crossover strands anyway. Although the effect of distal homozygosis caused by one gonial exchange will be rather small, the effect of several should add up to a conspicuous phenomenon, if crossing over has occurred in many oögonial cells. This and other cumulative effects comprise the remaining three ways of detecting whether oögonial crossing over has taken place under one set of conditions more than under another.

Fourth, progressive distal homozygosis may be expected to result in the masking of some of the subsequent meiotic (and rarely other gonial) exchanges. Homozygosis of a locus before meiosis will be produced by some 50 percent of the gonial exchanges which form between it and the spindle attachment. Loci which are farther from the spindle attachment will become homozygous more often than those which are closer. A similar rise in homozygosis of the more distal genes has been described by BEADLE and EMERSON (1935) in attached-X chromosomes, where, as in mitosis, two chromatids from a tetrad may be recovered. Hence the strands which physically cross over in the region of the spindle attachment, whether during mitotic or meiotic divisions, have the best chance of being represented as crossover offspring. It is a fact that the greatest increases in recombination are at the spindle attachment region following the use of any of the presently known inducing agents except partial starvation of larvae. The regions more removed from the spindle attachment will be less likely to show their usual number of meiotic crossovers, and these losses of normal recombinations should be most pronounced in the most distal region. Hence the induced recombinations formed distally in oögonial cells may not raise crossover values above normal because they will be added to classes already diminished.

Many observed facts fit this scheme. The first known effect of X rays upon crossing over was a non-inherited decrease in recombination in the distal part of the sex chromosomes of *D. melanogaster*, reported by MAVOR (1923), although he soon found that irradiation increased recombination near the spindle region of the second chromosome pair. SCHULTZ and REDFIELD (1932, 1933) observed that interchromosomal effects upon recombination were greatest in the spindle region, least distally. Sometimes the enhancing agent acts negatively in the farthest region, and KIKKAWA (1934) has pointed out the rather frequent finding of what he has termed "proximal increases and compensatory distal decreases." In experiments using a nitrogen mustard, the present writer (1948b) has found similar results. Treated rucuca heterozygotes of *D. melanogaster* had fewer recombinations in the two ends of the chromosome than untreated sisters had, and elsewhere they gave above normal values which increased regularly toward the spindle attachment to a pronounced peak there, $4\frac{1}{2}$ times the control value. Less extreme but similarly graded departures from controls appeared in rucuca heterozygotes as an interchromosomal effect of 7 out of 8 X-chromosome inversions tested by STEINBERG and FRASER (1944) and showing any increases. An explanation of the tendency to compensatory

distal decreases is inherent in the present hypothesis. If numerous chiasmata have formed crossovers in gonial cells at any time, then there may be a net scarcity of the more distal recombinations without recognizable clusters of the various proximal crossovers responsible. Although each crossover adds to the recombination in its own region, half of them subtract from two, three or all distal regions.

In addition to the better transmission of recombinations formed in the spindle attachment region, an actual concentration of gonial crossing over in that vicinity may further increase the departure of testcross data from normal. Although it does not seem feasible to study directly the distribution of gonial crossing over in females, a spermatogonial map of chromosome 3 has been made by the writer (1948a) using data from irradiated males of *D. melanogaster*. Forty-nine percent of the third chromosome exchanges occurred in the short scarlet-curled region of males, which is 6 units in length on the female map, whereas 51 percent were elsewhere in 94.7 standard units of length. If the spermatogonial crossing over map applies also to the gonial cells of females, that distribution combined with distal homozygosis would account for the great peak of relative increases in recombination at the spindle attachment region of this chromosome.

Fifth, reduced interference should also result from gonial crossing over. Since gonial and meiotic chiasmata occur in different cell generations, there can be no mechanical interference between them in chiasma formation. Hence double and multiple recombinations will increase. Interference will appear to be reduced between the spindle attachment and the locus of the oögonial crossing over. Distal to any gonial exchange interference will appear to be affected in opposite ways depending upon the kind of mitotic segregation which has occurred. When the two crossover chromosomes go together, (1) in figure 1, any meiotic crossing over distally can be detected when it occurs, so such data will point to lessened interference. SCHULTZ and REDFIELD (1933) have observed a general reduction. STEINBERG and FRASER found it in some experiments but not in others. When segregation of kind (2) occurs in mitosis fewer meiotic crossovers for distal regions will be found. Thus when heterozygosis is retained until meiosis, coincidence will rise toward 1, and when heterozygosis is lost coincidence values will be depressed toward 0 for certain distal regions. It may be concluded from the above reasoning that coincidence even in massed data may appear to be increased proximally more than distally by the action of oögonial crossing over.

Sixth, an excess of any of the rarely occurring classes of crossovers may point to oögonial crossing over. Rare crossovers may be multiple, double or single crossovers, and they should be defined within each family in terms of the recombination measured there. Non-random distribution of rare crossovers has been found by SEREBROVSKY (1927), BRIDGES (personal communication), and others including the writer. Their interpretation in terms of the gonial hypothesis or some other theory now awaits the accumulation of additional data. At present it may be said that rare crossovers are often of different kinds in different families and that they emerge very unevenly within families.

SUMMARY

Certain results of oögonial crossing over should be detectable in spite of their partial masking by the large amount of meiotic crossing over in Drosophila females. In individual families and subfamilies a single oögonial crossing over may be inferred from (1) an excess of total recombination in any one region, (2) complementary crossover classes which are unequal after allowing for viability differences, (3) decreased recombination in one or all regions distal to a region of increase.

In the combined data of whole experiments, indications of many exchanges in various gonial cells during early or late mitoses could be (4) increases in recombination above expected values which are greatest at the spindle attachment and progressively less, or even negative, in distal regions, (5) altered interference values and, finally, (6) non-random distribution of rare crossover offspring. Each of the above expectations, derived chiefly from figure 1, has already been reported independently as an observed fact. No single experiment is known to the author which shows all of the above indications of gonial crossing over in unmistakable relationship, but a promising one has been planned. Since good examples may occur anywhere in a wide variety of experiments, the writer would appreciate it if other research workers would be on the look-out for such signs of gonial crossing over as have been discussed above and for any others which may come to mind.

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