* This investigation was supported in part by a research grant from the National Institute of Health, U. S. Public Health Service. From the Department of Zoology and Entomology and the Department of Botany and Plant Pathology (No. 519), Ohio State University.

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THE NATURE OF LINKAGE VARIATION WITH AGE IN INVERSION HETEROZYGOTES OF DROSOPHILA MELANOGASTER

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Communicated by J. N. Couch, August 17, 1950

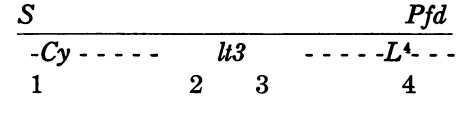
Two kinds of variation in linkage values in Drosophila experiments are well known but poorly understood. One is the change in crossover values with increasing age of the female as shown by Bridges[1]; the other is excessive variation from female to female as pointed out by Gowen.[2] Studies reported in the present paper on an age effect in a special situation may contribute to the interpretation of both problems. They also serve as controls for the irradiation paper which follows.[3]

The chief result of aging is a rapid decrease in recombination values during the first six days of egg-laying, particularly at or near the spindle attachment of the chromosome. After that period smaller changes consist, with variations, of a slight rise and second fall. In recognition of this Bridges[4] has defined as a condition for chromosome mapping the use of

data from young females. From such data inferences are made as to the amount of actual crossing over which has occurred between genes which are assumed to be heterozygous at the time when the germ cells enter meiosis. That this procedure may overestimate the amount of crossing over has been elaborated by the senior author both by reasoning back from observable clustering of the data[5] and by deducing certain consequences of crossing over premeiotically, in gonial cells.[6] The present experiment shows an age effect and clustering attributable to oögonial crossing over in the same body of data, where meiotic crossovers have been greatly reduced or eliminated by inversions.

The age effect in this stock was encountered by accident. In the course of stockmaking by the senior author it was noticed that the infrequent crossovers from *al*[2] *Cy lt*[3] *L*[4] *sp*[2]/*S Pfd* females appeared in early cultures but not in later transfers. Of 14 crossovers in the *Cy-L* interval, 12 were found in the first two cultures and none at all in the last two of a series of five transfers. It was not clear whether this was an accidental result until a larger experiment had been carried out by the junior author and a genuine age effect found.

The experiment was planned with several improvements on the original mass matings. By testcrossing to light males use was made of the *lt* locus, which differentiated the crossovers into two regional kinds. The map order of the loci, the number designations of the crossover regions and the extent of the inversions may be diagrammed as follows:



The spindle attachment lies to the right of *lt*. In the experiment, females were mated individually to these light males, so that the contribution of each family to the totals would be known. Furthermore, the data for each family came separately from five consecutive coded cultures which were not decoded and summed as to families until the experiment was over.

Results and Discussion.—This experiment confirmed the age effect which had been encountered in mass mating. Hence data from both tests have been combined in table 1. In the first two cultures crossovers comprised about 1% of the offspring, in the third they were 0.25% and in the last two transfers practically zero. Almost all of the crossovers came from eggs laid during the first five days of adult life. A chi-square test showed that this was a highly significant departure from the mean of all 11 days. We were therefore dealing with an age effect exhibited by these crossovers.

The data were next examined family by family to see whether the crossovers had appeared at random or in clusters. Random distribution would be in accord with the usual assumption that crossing-over occurs at meiosis,

meaning that no more than one crossover chromatid from each tetrad is recoverable by breeding. Clustering would indicate that there was some gonial influence[7] either (*a*) completed crossing-over in a gonial cell, or (*b*) weakening of a certain place in a chromosome followed later by crossing-over in identical regions in numerous related primary oöcytes. Table 2 shows agglutination of the data, as mathematicians call it, after adding up the cases of crossovers in the two regions of the eighteen families. Although a majority of the 36 cases had no crossovers, paradoxically three-fourths of the crossovers appeared in groups of two or more, as if from the same event of exchange. Furthermore the cases of solitary crossovers, 8, seem too low for a Poisson distribution. Some probabilities will be presented in later paragraphs.

TABLE 1

SUMMARY OF SINGLE CROSSOVERS RECOVERED FROM *Star Pufdi/Curly light Lobe*[4] INVERSION HETEROZYGOTES IN BOTH EXPERIMENTS ACCORDING TO AGE OF FEMALES IN DAYS

Cultures	1	2	3	4	5
Age of female	0–3	3–5	5–7	7–9	9–11
Crossovers	17	20	4	1	1
Total offspring	1747	2062	1677	1752	1886

TABLE 2

AGGLUTINATION IN THE FINDING OF SINGLE CROSSOVER FLIES, FOR EITHER REGION, AMONG 18 FAMILIES OF *S Pfd/Cy lt L*[4] INVERSION HETEROZYGOTES

CROSSOVERS/FAMILY	CASES	TOTAL CROSSOVERS
0	19	0
1	8	8
2	7	14
3	1	3
4	1	4
Totals	36	29

NOTE: Inclusion of seven multiple crossovers would increase the numbers of pairs and especially of triplets.

More detailed familial data appear in table 3. The smallest family, No, 1, contained 4 crossovers in region 2, and these were not all from the same culture, so that they tend to confirm each other. The next smallest family had 3 crossovers in region 3, all found in the same culture. In seven other cases 2 crossovers of a kind were found, and more often than not these were in separate cultures rather than together. Many families, representative in size, failed to have any crossovers in one or both regions. Because of this clustering the crossover values showed great variability among the different families, from about 5 per cent to zero.

One may compute the probability that these are merely chance deviations

from randomness. In region 2 the over-all frequency of crossovers is approximately 0.002, (15/7534 from table 3). In ten families having some 4700 offspring no such crossovers were found, and the probability of this as a random result is 0.998^{4700}, or 0.00008. On the same hypothesis, the probability of drawing a sample of 108 including as many as 4 similar crossovers (Family 1) is 0.0008. Then the joint probability of occurrence of these two aspects of the experiment is 6.4×10^{-8}. Inclusion of the fact that pairs of crossovers were found more often than singles would make it even more improbable that the crossovers in region 2 were independently determined.

TABLE 3

SUMMARY OF TESTCROSS DATA FROM 84 FERTILE, CODED CULTURES AS REASSEMBLED INTO THE ORIGINAL 18 FAMILIES AFTER CLASSIFICATION. FAMILIES LISTED IN ORDER OF INCREASING SIZE

FAMILY NO.	TOTAL PROGENY	SINGLE CROSSOVERS REGION 2	SINGLE CROSSOVERS REGION 3	MULTIPLE CROSSOVERS
1	108	4[a]	1	
2	135	1	3	
3	268	1	0	
4	387	0	1	
5	408	1	2[a]	
6	417	2	0	
7–11	5 × 439.4	0	0	
12	445	2[a]	2	
13	481	0	0	S Cy;[a] lt, wild.
14	490	2[a]	1	
15	520	0	2	Cy L
16	533	2[a]	1	
17	533	0	1	S lt Pfd
18	612	0	0	S lt Pfd L;[a] L.
Totals	7534	15	14	7

[a] Denotes crossovers obtained from two different coded cultures.

Similar calculations for region 3 show that events there are very far from random. The probability that families containing a total of 3975 offspring would have no crossovers, if the latter were randomly distributed at a frequency of 0.183%, is 0.99817^{3975}, which is less than 0.0006. Turning to the other end of the distribution we find that the probability of getting as many as 3 crossovers in a family of only 135 offspring is 0.0157 on the same assumption. The joint probability for the two extremes is more remote, and the further joint probability for region 2 crossovers and region 3 crossovers is 6×10^{-15}.

Up to this point the negative aspects of data of table 3 have been discussed. The kind of non-randomness pointed out may be explained by a

limited amount of gonial crossing over plus gonial multiplication. While this is perhaps the better explanation, there is another interpretation which could reserve crossing over until meiosis. Thus a tendency to produce crossovers near the spindle attachment regardless of region may be possessed by some heterozygotes more than by others. In table 3 one may see that pairs and larger clusters in one region are almost always accompanied by at least one crossover in the adjacent region. This is true in 6 out of 8 families; only females No. 6 and No. 15 had 2 crossovers in one region without any single exchanges in the other. Yet a double crossover, a Curly Lobe fly, appeared in family No. 15 in the same culture with 2 Star Lobe single crossovers. This makes 7 cases of crossing over adjacent to the regions represented by the 8 clusters.

Further evidence for this alternative may be obtained from the multiple crossovers as a whole, which have therefore been included in table 3, even though they are not fully understood. The most plausible multiple crossovers are the Curly Lobe fly just mentioned and the Star light Pufdi of family No. 17. These could be region 2–3 double crossovers, and each was formed in a female which also had one or two single crossovers in region 3. The expectation for these double crossovers in an entire experiment of this size is less than one fly even if figured on the basis of the initial high point of recombination values, 1%, and with no interference. The other multiple crossovers are far less likely than the preceding. The finding of a wild and a light fly, the latter verified by breeding, in the same culture of family No. 13 might be explained as contamination; or these might be triple crossovers, or even quadruples, in some way related to a Star Curly quadruple crossover independently classified in a previous culture. In the subsequent experiment in which similar heterozygotes were irradiated (Hinton and Whittinghill[3]), two more light phenotypes were found in different cultures, so it remains a possibility that this *lt* fly was a 1-2-4 triple crossover. It is indeed strange that there were no single crossover offspring in this family which had two different triples and a quadruple crossover. In one other family, 18, single crossovers were lacking. The only crossovers in this the largest family of all, were a *S lt Pfd* L^4 2–3–4 triple and a verified L^4 1–3 double. Neither kind was detected in the next experiment. However the sporadic nature of the multiple crossovers may be viewed as an extension of the non-randomness already demonstrated among the single crossover types of regions 2 and 3. No single crossovers were recovered from regions 1 or 4 due to the inversions there.

Summary.—There is a decrease in the frequency of crossover offspring from *S Pfd/Cy lt* L^4 females as they age. Crossover values drop practically to zero in seven days, in agreement with the previously known decrease from normal females. Variability in crossover values from family to family was greater than one would expect by chance if each crossover

had been formed separately and independently of every other crossover, the usual assumption about meiosis.[8] Two better explanations have been considered. An entire cluster of crossovers may represent only one actual crossing over in an oögonial cell which was the common ancestor of several eggs. Such an hypothesis is preferred, but an alternate explanation may fit the data of this one experiment without recourse to gonial crossing over. The clustering may begin with a gonial conditioning, such as a permanent weakening of a chromosome, which may influence the location of several later meiotic crossings over. Because identical crossovers and adjacent crossovers tended to be found in the same families in this favorable stock of flies, either of the new hypotheses is appropriate for the present data. Great variability of spontaneous crossover values near spindle attachments would seem to be inescapable in experiments based on the early broods prescribed by Bridges. Hence the collection of data for maps might well be done from a later egg-laying period and, particularly, from large numbers of females to get a measure of the variability.

Acknowledgments.—These experiments were substantially aided by a grant from the Carnegie Research Fund of the University of North Carolina during the winter of 1949, for which we are very grateful. For aid in making certain calculations we wish to thank Dr. A. S. Householder, Head of the Mathematics Panel, Oak Ridge National Laboratory. We thank Miss Margaret Stewart of this laboratory for coding the 90 cultures to hide their familial identity.

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