THE EFFECT OF AGE ON THE RELATIONSHIP BETWEEN COINCIDENCE AND CROSSING OVER IN DROSOPHILA MELANOGASTER

J. M. RENDEL

Animal Genetics Section, Commonwealth Scientific & Industrial Research Organization, Zoology Department, Sydney University, NSW, Australia

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BRIDGES (1915), working on a two-strand model, noticed that recombination in general decreases as the age of the female increases to about 16 days and then increases again following a wave-like pattern. Further, he produced evidence suggesting that interference does not always bear the relationship to recombination which one would expect and suggested a model according to which crossing over was brought about by twisting of the chromosomes round one another. The twist would produce nodes at which the chromosomes would eventually be broken and a rejoin of A to B would result in a crossover. The distance between twists he called internode length; the chance that crossing over takes place at a node which is not necessarily unity, he called the coefficient of crossing over. He points out that a study of interference and crossing over will show whether changes in the frequency of crossing over are due to changes in internode length or the coefficient of crossing over. If the former is responsible, there will be a change in intereference; if the latter, there will be none. In his first paper his data were not adequate to show definitely what changes in coincidence, the measure of interference used, had in fact taken place. In a later paper (1927) working with the third chromosome he unfortunately chose a length which straddles the centromere and once more his data are not conclusive. If it can be established that changes in recombination and coincidence can be independent of one another, it will support BRIDGES' suggestion that there are nodes of crossing over on any particular pair of chromosomes, not necessarily localized at the same point in all pairs, at which crossing over may take place but does not necessarily do so.

In a previous paper (RENDEL 1957) a comparison of recombination and coincidence on the X chromosome with and without the *Cy* inversion was made. It was shown that on the probit scale the relationship between coincidence and recombination follows a straight line as standard map length is increased. That is, as more nodes are included in the region marked, coincidence increases in a regular way. This relationship takes account of the fact that as distance increases doubles will be missed. When an environmental agent, such as age or an inversion in another chromosome, is used to increase recombination in a particular section, if an increase in the number of nodes present is responsible, the same relationship between recombination and coincidence should hold. If it does not hold, a two stage process of some kind is indicated as suggested by BRIDGES. When the *Cy* inversion

was used to increase recombination on the X, internode length decreased, i.e. number of nodes increased but not to the extent expected. That is to say, coincidence did not rise to the point indicated by the graph. Some increase in the coefficient of crossing over must be assumed. In this paper the effect of age has been examined. BRIDGES found this to be zero for the X chromosome in his stocks but the frequency of crossing over in later hatched flies in the crosses with the *Cy* inversion suggested that the effect of age might be worth re-examining in the stocks used here.

METHODS AND RESULTS

F_1 cultures in which the female progeny were of the constitution

$$\frac{sc\ ct\ v,}{+\ +\ +}\quad \frac{sc\ +\ +,}{+\ ct\ v}\quad \frac{+\ ct\ +}{sc\ +\ v}\quad \text{and}\quad \frac{+\ +\ v}{sc\ ct\ +}$$

were set up in halfpint milk bottles containing standard medium. These cultures were segregating for the *Cy* inversion, and as they emerged, normal and *Cy* sisters from the same culture were set up one female to a bottle. These females were mated to *sc ct v* males and transferred every four days to a fresh bottle for four transfers. Any female not having progeny in all four bottles was discarded. The F_2 progeny were scored and the results are shown in the tables.

The F_1 females were made up so that the recessive genes at the three heterozygous loci were in coupling and repulsion in all possible combinations. This was done to discount the effect of differential viability between the crossover and non-crossover classes, which, being of different genotype, will have different mortalities. It is possible that having the recessive genes differently distributed between the two X chromosomes of the heterozygous female may alter the crossover

TABLE 1a

Crossing over in X chromosome in females normal for second chromosome

F_1 Female	Recombination classes ++				Proportions				
	I	II	I & II	TOTAL	I	II	I & II		
$\frac{sc\ ct\ +}{+\ +\ v}$	85	95	3	739	.1150	.1286	.0041		
$\frac{sc\ +\ v}{+\ ct\ +}$	115	64	2	695	.1655	.0921	.0029	First laying period	
$\frac{sc\ ct\ v}{+\ +\ +}$	266	172	9	1156	.2301	.1488	.0078		
$\frac{sc\ +\ +}{+\ ct\ v}$	63	64	1	429	.1469	.1492	.0023		
					.1644	.1297	.0043	Recom 1	16.87%
								Recom 2	13.40%
								Coincidence	0.1902

TABLE 1a—Continued

$\frac{sc\ ct\ +}{+\ +\ v}$	80	79	4	709	.1128	.1114	.0056		
$\frac{sc\ +\ v}{+\ ct\ +}$	132	90	4	973	.1357	.0925	.0041	Second laying period	
$\frac{sc\ ct\ v}{+\ +\ +}$	237	164	19	1376	.1722	.1192	.0138		
$\frac{sc\ +\ +}{+\ ct\ v}$	109	70	8	594	.1835	.1178	.0135		
					.1511	.1102	.0093	Recom 1 Recom 2 Coincidence	16.04% 11.95% 0.4852
$\frac{sc\ ct\ +}{+\ +\ v}$	38	57	2	543	.0700	.1050	.0036		
$\frac{sc\ +\ v}{+\ ct\ +}$	72	60	9	687	.1048	.0873	.0131	Third laying period	
$\frac{sc\ ct\ v}{+\ +\ +}$	163	165	9	1412	.1154	.1169	.0064		
$\frac{sc\ +\ +}{+\ ct\ v}$	89	80	6	544	.1636	.1471	.0110		
					.1135	.1141	.0085	Recom 1 Recom 2 Coincidence	12.20% 12.26% 0.5683
$\frac{sc\ ct\ +}{+\ +\ v}$	17	45	0	390	.0436	.1154	....		
$\frac{sc\ +\ v}{+\ ct\ +}$	67	59	7	929	.0721	.0636	.0075	Fourth laying period	
$\frac{sc\ ct\ v}{+\ +\ +}$	67	54	4	581	.1153	.0929	.0069		
$\frac{sc\ +\ +}{+\ ct\ v}$	85	63	4	454	.1872	.1388	.0088		
					.1046	.1027	.0058	Recom 1 Recom 2 Coincidence	11.04% 10.85% 0.4842

TABLE 1b

Crossing over in X chromosome in females having the second chromosome Cy *inversion*

Female	Recombination classes I	II	I & II	TOTAL	Cy Proportions I	II	I & II		
sc ct + / + + *v*	171	112	10	732	.2336	.1530	.0137		
sc + *v* / + *ct* +	91	67	7	409	.2225	.1638	.0171	First laying period	
sc ct v / + + +	308	210	18	1195	.2577	.1757	.0151		
sc v + / + + *ct*	124	89	4	685	.1810	.1299	.0058		
					.2237	.1556	.0129	Recom 1 Recom 2 Coincidence	23.66% 16.85% 0.3236
sc ct + / + + *v*	200	134	14	941	.2125	.1424	.0149		
sc + *v* / + *ct* +	111	77	7	538	.2063	.1431	.0130	Second laying period	
sc ct v / + + +	325	229	24	1331	.2442	.1721	.0180		
sc v + / + + *ct*	123	85	3	680	.1809	.1250	.0044		
					.2110	.1457	.0126	Recom 1 Recom 2 Coincidence	21.36% 15.83% 0.3680
sc ct + / + + *v*	132	115	5	908	.1454	.1267	.0055		
sc + *v* / + *ct* +	98	56	8	464	.2112	.1207	.0172	Third laying period	
sc ct v / + + +	283	211	22	1433	.1975	.1472	.0154		
sc v + / + + *ct*	95	64	6	513	.1852	.1248	.0117		
					.1844	.1299	.0125	Recom 1 Recom 2 Coincidence	19.69% 14.24% 0.4458

TABLE 1b—Continued

Crossing over in X chromosome in females having the second chromosome Cy *inversion*

Female	Recombination classes			*Cy* TOTAL	Proportions				
	I	II	I & II		I	II	I & II		
sc ct + / + + *v*	103	100	4	782	.1317	.1279	.0051		
sc + *v* / + *ct* +	86	75	3	668	.1287	.1123	.0045	Fourth laying period	
sc ct v / + + +	230	131	13	946	.2431	.1385	.0137		
sc v + / + + *ct*	105	74	2	567	.1852	.1305	.0035		
					.1722	.1278	.0067	Recom 1	17.89%
								Recom 2	13.40%
								Coincidence	0.2794

frequency, quite apart from effects of genotype on viability. If this is true it will not be possible to distinguish such an effect from differential viability. The best way of allowing for both effects seems, therefore, to calculate the fraction of the population belonging to each crossover class separately for all four types of crosses and then to take an unweighted average of these fractions. By this method undue weight may be given to the cross which has the smallest number of flies but any error should be unbiased.

The tables show that the highest rate of crossing over takes place in the eggs laid during the first four days. Crossing over declines steadily from then on till the 12th–16th day, as found by BRIDGES (1927). Coincidence on the other hand is least in the eggs laid early and increases to the twelfth day after which it drops again. The same trends are followed in the progeny of both *Cy*/+ and +/+ but the effect of age on coincidence is more noticeable in the +/+ cultures. Although the crossing over frequency in the *Cy*/+ cultures is higher than in the +/+ the coincidence is much lower except during the first laying period. Thus age reduces crossover frequency and increases coincidence whereas the *Cy* inversion does the reverse. It may be noted that whereas in the *Cy* cultures the actual proportion of double crossovers is very similar in all but the last laying period, the proportion of double crossovers in the +/+ cultures is very much higher in the second and third laying period. An analysis of variance and covariance based on the percent recombination and the coincidence values shows that the covariance term is always strongly negative. In the *Cy* cultures the effect of age and cross on coincidence and recombination are highly significant. In the +/+ cultures neither the effect of age or cross on coincidence is significant, but the covariance term is always strongly negative, and the effect of age and cross on recombination highly significant.

DISCUSSION

Before discussing the bearing of these findings on theories of crossing over at meiosis the possibility that observed changes in coincidence are due to crossing over at mitosis must be considered. WHITTINGHILL (1955) has shown that gonial crossing over during mitosis could theoretically have an effect on coincidence. He has, however, shown that the presence of an inversion did not affect the frequency of somatic crossing over brought about by radiation (WHITTINGHILL 1947) so that it is unlikely that the effects of the *Cy* inversion reported here were brought about during gonial mitosis. SCHWARTZ (1954) found that twin spotting resulting from somatic crossing over in attached-X individuals increased with age and put this down to increased sister strand crossing over, which would not be detectable in the experiment reported here. BROWN and HANNAH (1952) also suggested that age increases sister strand crossing over. They found aged females mated to males carrying a ring X chromosome had progeny with a greater number of mosaics than young females. These mosaics almost all appeared to owe their origin to the loss of the ring chromosome. The elimination of the ring was put down to sister strand crossing over as the authors did not believe it could be due to crossing over between the ring and its normal partner, since there is little if any pairing between them at the time the loss occurred, and further because such an interchange should give rise to more than one type of mosaic and only one was found.

Thus, although these authors have demonstrated that age affects events taking place at mitosis they do not believe that it increases the over-all frequency of mitotic crossing over. But let us suppose for the sake of argument that it does, and that the tenfold increase with age in twin spotting found by SCHWARTZ in $\overline{XX}$ females was due to an increase in frequency of mitotic crossing over. Then if the number of crossovers in male Drosophila can be used as a guide to the frequency of gonial crossing over in females we may expect 1 in 10,000 offspring to be the result of gonial crossing over. As in the experiment reported here where only half the X chromosome is marked, this might show up in 1 in 20,000 flies. If age increases the rate tenfold the frequency becomes 1 in 2,000. Of these, only 15 to 20 percent will appear as double crossovers, depending on the rate of meiotic interchange, and so only very few extra doubles, the result of somatic crossing over, are expected throughout the whole experiment. This would not noticeably alter the results and we may safely assume that any effects observed are due to events happening at meiosis.

This being so, we have to explain how coincidence can become greater as crossover frequency becomes less with age, and how the reverse happens when inversions are present in another chromosome. The effect of age on recombination was quite large. In crosses without *Cy*, recombination dropped from 30 to 22 percent, and in the stocks with *Cy*, from 41 to 31 percent. This might be expected to reduce coincidence from about .24 to about .07 and from about .5 to about .24 respectively. In fact it rose from .2 to .5 in the cross without *Cy* and from .32 to .44 before falling to .27 in the crosses with *Cy*. It has been suggested that such events

would be possible if crossing over were determined in two stages. First, points down the length of each bivalent are determined at which an interchange may take place but at which it does not necessarily do so, and second, the frequency with which interchange takes place at these points may be higher or lower according to circumstances. It would then be possible to vary coincidence and crossing over frequency to some extent independently. Coincidence would depend on the average distance between potential points of crossing over, and crossing over frequency would, in addition, be affected by the frequency of interchange at each such point.

Three main mechanisms have been suggested as responsible for crossing over. BELLING (1933) suggested that interchange took place between new strands during reduplication as a result of new genes becoming joined after formation to the gene string of the opposite chromosome. BRIDGES (1915) and DARLINGTON (1935) have suggested that torsion due to coiling will break the chromatids, the broken ends of which will then join up with the chromatid of the other chromosome. HALDANE (1954) has suggested that during reduplication at meiosis the gene string may become discontinuous, being held in place by a skeletal structure, possibly a template formed on the original gene string, which remains continuous. Four discontinuous gene strings are then formed. These will then join into four continuous threads and the way they do so will determine the amount of crossing over; eventually the skeletal structure becomes discontinuous and the gene threads can separate.

Clearly, on BELLING's and HALDANE's hypotheses, the position of one strand relative to a homologue will make a great difference to the chance of a join being formed which results in a crossover and it could well be that the degree of internal and relational coiling of the chromatids will determine the number of points where homologous genes on different chromatids are close enough to permit them to join up with the neighbouring genes of their partner chromatid. This would be the distance between nodes of BRIDGES and, as on his model, dependent on relational coiling. If this picture is basically correct, it should be possible to relate the distance between nodes of relational coils with the minimum distance between two crossovers. Thus the least distance between double crossovers on the X chromosome of *D. melanogaster* appears to be about ten units, and the coiling seen in the Xs in salivary chromosomes appears from published pictures to have between six and seven nodes along the chromosome which is thus divided into units between the nodes of about the right length. However, as the relationship between coiling in salivary chromosomes and coiling at meiosis is not known this observation cannot be taken very seriously.

BRIDGES' idea that the twisting of the chromosomes actually broke them assumes that two breaks will always occur at exactly the same place. BELLING's theory only fits four strand crossing over if sister strand crossing over also takes place.

Evidence that sister strand crossing over does take place is not conclusive, though recently (e.g. SCHWARTZ *loc. cit.*) some very suggestive arguments have

been put forward in its favour. These, however, do not rule out the possibility that even if sister strand crossing over does take place, four strand crossing over may take place directly. There seems no reason to suppose that a "new" chromatid can join with an "old" one if it is a sister strand but not if it is a homologous non-sister strand unless it can be conclusively proved to be so.

On the whole HALDANE's theory, which is in essence the same as BELLING's but permitting four strand crossing over, seems to fit all the factors so far brought forward better than any other.

SUMMARY

The effect of age on crossing over is the reverse of its effect on coincidence.

Where age is taken into account the effect of the *Cy* inversion on crossing over is also the reverse of its effect on coincidence.

The bearing of this on the theories of crossing over is discussed.

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