*CHROMOSOME STRUCTURE AND THE MECHANISM OF CROSSING OVER**

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Chromosome structure and the mechanism by which genes recombine through crossing over are intimately related. A knowledge of one should shed light on the other. Further, an understanding of the mechanism of crossing over should contribute to our conception of the delineation of genetic units within the chromosomes. The principal hypotheses proposed to explain the mechanism of crossing over[1] utilize certain mechanical features of chromosome structure to explain the way in which crossing-over occurs. One drawback of these hypotheses lies in their not being amenable to strict genetic and cytological proof.

It has been demonstrated[2, 3] that the spontaneous chromosome breakage in *Tradescantia* can be increased approximately seventeen times under calcium-deficient conditions. Other cation deficiencies do not have this effect. Furthermore, it has been shown[4] that the salivary gland chromosomes of *Drosophila melanogaster* can be broken in vitro by treatment with a chelating agent. Because calcium and magnesium are major cationic constituents of nuclei,[5] Mazia[4] has suggested that they play a fundamental role in the structure of chromosomes. He has proposed that chromosomes are divided into particulate units linked through divalent cations, either calcium or magnesium. This model for chromosome structure has been extended by Steffensen,[3] who suggests that deoxyribonucleic acid (DNA) molecules, after the structure proposed by Watson and Crick,[6] can be linked in a linear fashion through their terminal phosphate groups, these groups being capable of chelating calcium ions. Data to be presented here suggest that cations, particularly calcium, may play a major role in chromosome stability, perhaps at the sites where calcium ions are presumed to be bound into the structure of the chromosome. The validity of a hypothesis considering such a function for divalent cations can be tested by attempts to alter, in a specific manner, conditions to which the structure of chromosomes is believed to be sensitive, and to measure the effects genetically, by crossover analysis, and cytologically, by examination of the chromosomes themselves. The purpose of this paper is to present in summary the results of genetic experiments which support the hypothesis mentioned above. Though most of the evidence to be presented is indirect or perhaps circumstantial, it points in such a direction as to support the hypothesis under consideration.

Three sorts of experiments on crossing over in *D. melanogaster* will be mentioned here. All of them have been carried out in this laboratory, and certain of them have been done independently by Dr. Dale M. Steffensen, of the Brookhaven National Laboratory. Since space does not permit the full development of experimental methods or the statistical analysis of the results, a detailed presentation will be published elsewhere.

The first genetic experiment is summarized in Table 1. It concerns the effect on crossing over of the feeding of calcium chloride to adult females carrying the

X-chromosome markers *y ct ras f* (*yellow* body color, *cut* wings, *raspberry* eye color, and *forked* bristles). The usual corn meal, molasses, agar, and yeast medium was prepared in a 0.1 *M* calcium chloride solution. The females were permitted to remain on this medium for four days, after which they were transferred to normal media. Thereafter they were transferred to fresh media every two days for a period of sixteen days. Appropriate statistical tests have demonstrated that the differences between the various treatments and their controls in this and in the other genetic experiments considered in this paper need not be attributed to viability differences. In all cases the significance of the results was tested by variance analysis, following a procedure similar to that of Levine and Levine.[7] Feeding of excess calcium results, as shown by a heterogeneity chi square (Table 1), in a significant decrease in crossing over between the control and treated series from the sixth to the fourteenth day after feeding (see last column, italicized figures).

TABLE 1

CROSSING OVER IN FEMALES FED AS ADULTS ON THE USUAL MEDIUM SUPPLEMENTED WITH 0.1 *M* CALCIUM CHLORIDE

TWO-DAY EGG-LAYING PERIODS		NO. FLIES	PER CENT CROSSING OVER				χ^2	*P*
			y-ct	*ct-ras*	*ras-f*	Total		
1	T*	1,278	14.63	10.48	25.19	50.30	1.72	0 18
	C	1,433	17.94	10.00	24.91	52.85		
2	T	1,847	16.40	10.98	25.77	53.15	0.96	0.95
	C	1,858	18.46	11.52	24.81	54.79		
3	T	2,162	16.42	9.30	24.74	50.46	6.80	*0.008*
	C	2,189	17.95	11.43	23.16	52.54		
4	T	1,771	16.02	10.66	22.18	48.86	12.01	*0.0005*
	C	2,157	18.08	11.58	24.69	54.35		
5	T	1,846	13.87	9.26	22.64	45.77	7.73	*0.004*
	C	1,988	16.30	10.77	23.25	50.32		
6	T	954	13.52	10.48	22.64	46.64	8.77	*0.003*
	C	1,177	16.65	10.96	25.41	53.02		
7	T	1,179	12.38	8.73	22.99	44.10	98.59	*< 0.0001*
	C	1,273	14.45	10.53	23.10	48.08		
8	T	864	14.94	7.76	25.47	48.17	0.04	0.85
	C	1,011	14.83	9.49	23.44	47.76		
9	T	624	15.54	11.38	25.63	52.55	0.00	...
	C	870	17.46	9.99	25.17	52.62		
10	T	357	18.20	10.92	24.37	53.49	0.84	0.36
	C	658	15.20	11.40	24.02	50.62		

* "T" represents the treated series; "C" represents the control series.

It has been noted in small-scale experiments that crossing over is not altered with the feeding of magnesium chloride but can be increased with feeding potassium chloride.

The second experiment is one in which larvae were raised on the usual medium, which was made 0.01 *M* for the chelating agent ethylenediamine tetra-acetic acid (EDTA). Crossing over was studied in the X chromosome marked with *y ct ras f*. The results are summarized in Table 2. EDTA has the effect of causing significantly increased crossing over during the early egg-laying periods. These results are similar to those of Steffensen.[8] The feeding of EDTA to adults has not brought about significant changes in crossing over.[8]

The final experiments in this series have been an extension of work previously reported[9] in which decreased crossing over was observed between the second-chromosome loci *black* and *cinnabar* in *D. melanogaster* as a result of exposing pupae to extreme water loss at low relative humidity. Data have been obtained

for other loci on the second chromosome as well as for the X and third chromosomes. In each case crossing over was significantly decreased in the first egg-laying period when the female pupae had been maintained over concentrated sulfuric acid. The results of one experiment are given in Table 3 for crossing over between the second-chromosome loci *a px or* (*arc* wings, *plexus* wings, and *orange* eye color). In addition to significantly reduced crossing over, it has been observed that the nuclei of the primary oöcytes and nurse cells of desiccated animals have been reduced about eight times in volume when compared with control animals.[10]

TABLE 2

CROSSING OVER IN FEMALES FED AS LARVAE ON THE USUAL MEDIUM SUPPLEMENTED WITH 0.01 *M* EDTA

Two-Day Egg-Laying Periods		No. Flies	Per Cent Crossing Over				χ^2	*P*
			y-ct	*ct-ras*	*ras-f*	Total		
1	T	549	17.67	12.02	24.96	54.65	0.40	0.52
	C	5,158	19.15	11.38	25.47	56.00		
2	T	2,166	18.33	13.29	26.31	57.93	7.29	*0.006*
	C	8,897	19.06	11.39	24.27	54.72		
3	T	3,649	20.80	12.49	24.83	58.12	2.10	0.15
	C	9,874	19.99	11.43	25.31	56.73		
4	T	2,580	21.42	12.24	25.46	59.12	17.38	*< 0.0001*
	C	6,499	18.67	11.05	24.55	54.27		
5	T	2,803	18.99	9.92	25.58	54.49	9.59	*0.002*
	C	6,207	16.22	10.74	24.01	50.97		
6	T	2,114	19.30	10.83	24.02	54.15	0.87	0.35
	C	5,140	17.66	10.01	25.27	52.94		
7	T	2,034	18.29	10.27	23.94	52.50	0.05	0.85
	C	4,954	16.81	10.96	25.03	52.80		
8	T	1,621	19.24	9.31	24.42	52.97	0.34	0.60
	C	4,358	16.03	10.27	25.81	52.11		
9	T	1,295	17.07	11.05	26.34	54.46	0.15	0.70
	C	3,468	17.76	10.98	25.05	53.79		
10	T	1,293	18.71	10.83	25.06	54.60	1.13	0.30
	C	3,001	17.56	10.64	24.59	52.79		

TABLE 3

CROSSING OVER IN FEMALES TREATED AS PUPAE TO ZERO PER CENT RELATIVE HUMIDITY AT 25° C.

Two-Day Egg-Laying Periods		No. Flies	Per Cent Crossing Over			χ^2	*P*
			a-px	*px-or*	Total		
1	T	3,849	1.92	4.31	5.23	40.84	*< 0.0001*
	C	4,253	3.34	5.55	8.89		
2	T	3,767	0.93	4.91	5.84	3.02	0.08
	C	4,317	1.27	5.49	6.76		
3	T	3,281	0.85	5.06	5.91	0.24	0.60
	C	3,970	1.18	5.04	6.22		
4	T	3,430	0.55	5.80	6.35	3.43	0.06
	C	4,393	0.39	4.96	5.35		

If one interprets crossing over as resulting from genetic exchanges at the sites where, as Steffensen suggests, calcium is bound, then one would predict that the amount of crossing over or stability of the chromosome should be directly related to the amount of calcium present. The data reported here support such a prediction. Thus, when the amount of calcium is increased by feeding calcium chloride to adult flies, crossing over is reduced. The removal of water from the primary oöcytes by desiccation, with a resultant visible effect on their nuclear structure, could result in increased calcium concentration and reduced crossing-over. On the other hand, a calcium deficiency, as might arise with the feeding of EDTA, results in increased crossing over.

Though Steffensen's model suggests that the crossovers will occur at the sites where calcium might be bound into the chromosome, it must be emphasized at this point that generalized changes in ionic strength or composition could affect crossing over in a manner other than that predicted by the model. Regardless of this, the summarized available experimental data as presented here suggest that crossing over can be modified by ionic changes according to predictions as to their effects on chromosome structure.

Summary.—The following facts are interpreted as related to a mechanism of crossing-over in which it is assumed that genetic exchanges will occur as the result of altering cationic conditions (particularly with respect to calcium) to which chromosomes may be sensitive:

1. Decreased crossing over in *D. melanogaster* when excess calcium is fed to young adult females.
2. Increased crossing over in *D. melanogaster* with the feeding of EDTA during the larval stage.
3. Decreased crossing over in *D. melanogaster* following loss of water from the nuclei of the ovaries and a possible concomitant increase in ionic concentration.
4. Increased chromosome breakage in *Tradescantia* with calcium deficiency (Steffensen[2, 3]).
5. In vitro chromosome breakage in *D. melanogaster* with EDTA (Mazia[4]).

These facts are discussed in terms of a model for chromosome structure, as proposed by Steffensen, in which it is assumed that genetic exchanges will occur at sites where calcium ions are presumed to be bound into the structure of the chromosome.

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