A RELATION BETWEEN LARVAL NUTRITION AND THE FREQUENCY OF CROSSING OVER IN THE THIRD CHROMOSOME OF DROSOPHILA MELANOGASTER

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THE frequency of meiotic crossing over in Drosophila females is known to be influenced by such non-genetic factors as the age of the fly, the temperature prevailing during larval, pupal, and adult life, exposure to X-rays, radium radiations, and ultraviolet radiation, treatments designed to render the female temporarily sterile, and the length of time spent in the larval stadium (for reviews of the literature on this subject cf. BRIDGES 1929, STERN 1933, LUDWIG 1938). The investigation described below was undertaken to determine whether or not another environmental factor affecting the frequency of genetic recombination in *D. melanogaster*—and one which could be a source of complication in the last of the above mentioned correlations—might, under certain conditions, be the availability of food during the larval stage.

This question has been briefly touched upon by several earlier investigators. PLOUGH (1917) was unable to detect any influence of prolonged partial starvation of larvae upon crossing over. GOWEN (1919) noted differences in the amount of crossing over shown by flies raised on fermented banana and genetically comparable individuals raised on an artificial food mixture consisting of starch, sugar, peptone, yeast, and water, but these differences were not certainly significant. Finally, the papers of SEREBROVSKY (1927) and BERGNER (1928) contain data which can be interpreted as indicating that food conditions have an effect on the frequency of genetic recombination, although these data, for reasons which will be discussed below, do not permit a satisfactory evaluation of the role of nutrition.

The results of this investigation were reported in a preliminary fashion at the 1940 Christmas meetings of the Genetics Society of America (NEEL 1941).

MATERIALS AND METHODS

The first step in the present work involved a mass mating of approximately 100 wild type females with an equal number of rucuca males. The latter were homozygous for a well known combination of third chromosome genes—namely, roughoid (*ru*, 0.0), hairy (*h*, 26.5), thread (*th*, 43.2), scarlet (*st*, 44.0), curled (*cu*, 50.0), stripe (*sr*, 62.0), sooty (e^s, 70.7), and claret (*ca*, 100.7). When the females were laying freely, eggs were collected

TABLE 1

A summary of the types of third chromosomes recovered from control and experimental flies during successive periods.

PERIOD	REGION(S) INVOLVED IN THE EXCHANGE																										TOTAL
	0		1		2		3		4		1, 2		1, 3		1, 4		2, 3		2, 4		2, 4		1, 2, 3		1, 2, 4		1, 3, 4		
	+	*h*	*h*		*h*		*h*		*h*		*h*		*h*		*h*		*h*		*h*										
	+	*th*		*th*	*th*		*th*		*th*			*th*		*th*		*th*	*th*		*th*				*th*		*th*			*th*	
	+	*cu*		*cu*		*cu*	*cu*		*cu*		*cu*			*cu*		*cu*		*cu*		*cu*								*cu*	
	+	*sr*		*sr*		*sr*		*sr*	*sr*		*sr*		*sr*			*sr*	*sr*				*sr*	*sr*	*sr*				*sr*		
	+	e^s		e^s		e^s		e^s		e^s	e^s		e^s		e^s		e^s		e^s				e^s		e^s			e^s	
A. CONTROL																													
1	310	277	76	91	22	14	40	53	37	36	2	4	13	16	8	14	1	3	3		1			1					1022
2	475	441	93	81	12	9	38	43	37	48	2	2	8	8	9	8	1	1	1										1317
3	381	327	66	75	6	4	28	33	33	52			5	8	10	5				2									1035
4	377	332	79	68	13	6	46	49	40	61		1	7	5	10	5	1						1						1101
5	395	300	55	75	9	7	37	32	25	32	2		5	8	9	3													994
6	286	263	63	45	5	7	16	27	27	25		1	8	6	9	8													796
7	167	128	27	32	2	1	11	13	14	28	1		6	3	2					1	1								437
8	98	74	20	18			11	9	7	7			2	2	1														249
																													6951
B. EXPERIMENTAL																													
1	432	375	101	99	24	21	70	60	52	66	4	4	15	15	14	12	1	2	3		3				1		1	1	1376
2	563	535	118	132	15	16	76	59	54	81	2	2	18	21	13	11	2	1		1	2				1				1723
3	483	447	100	123	13	18	60	52	46	63	1		10	9	8	13	1	1	1										1449
4	362	295	73	66	11	11	40	39	36	42	2	4	7	5	19	11		1	1		1								1026
5	229	223	53	41	2	3	26	23	34	29			3	7	7	4													684
6	155	149	36	42	3	4	18	17	18	20			5	5	5					1									478
7	91	77	21	25	1	1	7	8	13	12			3	2	1	3													265
8	54	34	9	9	1		13	2		10					3	1													136
																													7137

at four hour intervals and placed in lots of 60 to 70 on well yeasted corn-meal-molasses agar contained in one-fourth pint milk bottles. Development took place at 26°C. At a mean egg-larval age of 70 hours the larvae were divided into two groups. One group, the controls, completed development under the previously existing conditions. Larvae belonging to the other group were placed in vials containing nothing but Kleenex (an absorbent cleansing tissue) which had been moistened with water. A high proportion of the larvae so treated formed puparia, and puparium formation and the subsequent eclosion took place at approximately the same time or even slightly earlier in the experimental group than in the controls. This observation agrees with the findings of ALPATOV (1930) and BEADLE, TATUM, and CLANCY (1938). The mean weight of 58 newly eclosed females developing from control larvae was 1.11 mg, while that of 171 females developing from partially starved larvae was 0.64 mg.

TABLE 2

The percent of crossing over in the different regions during successive periods.

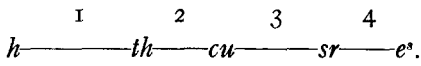
REGION	PERIOD							
	1	2	3	4	5	6	7	8
				A. CONTROL				
1	22.0	16.0	16.3	16.0	15.8	17.6	16.2	17.3
2	4.8	2.1	1.2	2.0	1.8	1.6	1.1	0.0
3	12.4	7.6	7.2	9.9	8.3	7.2	7.8	9.7
4	9.8	7.8	9.8	10.5	6.9	8.6	10.5	6.0
Total	49.0	33.5	34.5	38.4	32.8	35.0	35.6	33.0
				B. EXPERIMENTAL				
1	19.4	18.4	18.2	18.2	16.8	19.4	20.8	16.2
2	4.4	2.3	2.4	2.9	0.7	1.7	0.8	0.7
3	12.2	10.4	9.2	9.1	8.6	9.4	7.5	11.0
4	11.1	9.5	9.0	10.7	10.8	9.2	10.9	10.3
Total	47.1	40.6	38.8	40.9	36.9	39.7	40.0	38.2

Female flies developing from the two types of larvae were collected within 12 hours of eclosion and each placed for two days in a vial containing about a dozen rucuca males. The largest of the control females and the smallest of the experimental group were chosen for this backcross. The two day period in a vial with an excess of the rucuca males was found to be necessary to insure the fertilization of even a minority of the females. At the end of the two days, each female, together with the dozen or so males, was transferred to a one-fourth pint milk bottle containing well yeasted food, allowed to remain there three days, then transferred to a fresh bottle where she was allowed to remain a similar period of time, and

so on until eight successful transfers had been effected. From the offspring of this back cross the frequency of crossing over between *h*, *th*, *st*, *cu*, *sr*, and e^s during successive periods in the two types of females was determined. Only those females were used in the calculation of recombination frequencies which began to lay during the first three day period and continued to lay during at least six successive periods. Six out of 20 attempted control matings and 11 out of 43 matings involving females which had developed from partially starved larvae met this condition.

RESULTS

Although crossing over was followed between six genes, recombination between two of them, *th* and *st*, was so rare that for all practical purposes they marked a single locus, and so *st* will be omitted from further consideration. The section of the chromosome studied may thus be divided into four regions, as shown below:



The offspring of the backcross were classified as to which, if any, of the above regions of their maternally derived third chromosome had undergone crossing over. The results are summarized in tables 1 and 2, where the data from individual females have been averaged together by periods. The total number of flies involved in the two series is 14,088. As is apparent from table 1, complementary crossover and non-crossover classes of flies were for the most part represented by approximately equal numbers. The complementary classes arising from no crossing over (normal and *h th cu sr* e^s) and from single crossing over in region 4 (*h th cu sr* and e^s) showed the largest deviations from equality. Since such significant viability differences between classes as did obtain were the same in the two series, they cannot be a factor in any inequality in the amount of crossing over observed in the two groups.

Figure 1 summarizes graphically the data of tables 1 and 2. The abscissa of this figure is the time axis of the experiment; the ordinate represents the observed mean frequency of recombination per chromosome recovered or, if the decimal is moved two places to the right, total observed map distance between *h* and e^s. The controls showed an initial high in crossing over, followed by a sharp drop and irregularly sustained low. The curve given by the experimental flies appears to be a "damped" replica of the control curve, with a lower initial frequency of recombination but higher later values. The general shape of the two curves is remarkably similar, even to the minor inflections. From the second to the eighth period there is on the average 13 percent more crossing over in the experimental flies than in the controls.

Two questions at once arise concerning these data. First, of what significance are the differences between successive points on the two curves—that is, in this experiment what is the most probable true relation between age and crossing over, from which the data obtained represent random deviations? Second, are these two curves significantly different from one another?

In order to provide an answer to the first question, two row contingency tables were constructed, comparing the frequency and distribution of

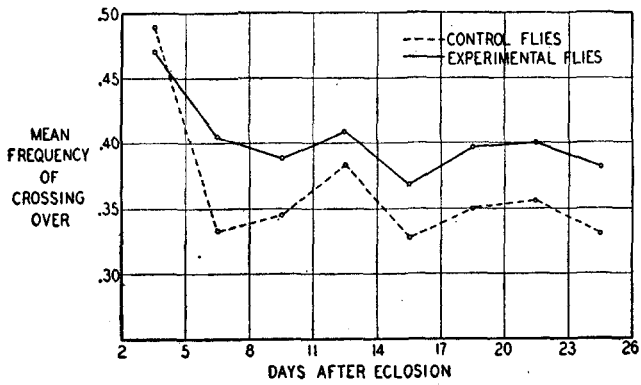


FIGURE 1.—A comparison of control flies and flies developing from partially starved larvae, with respect to the relation between age and the observed mean frequency of recombination in the third chromosome.

crossing over in chromosomes recovered from control flies during successive periods. Thus, a comparison was made of the amount of crossing over observed during the first and second periods, the second and third, the third and fourth, etc. The experimental series was treated in the same way. A χ^2 test of the significance of the differences observed between successive periods was then applied to each table. The analysis revealed that for both series there is a significant difference in the frequency and distribution of crossing over during the first two periods. The only other instance where such a significant difference was noted is between the fourth and fifth periods of control flies. Not only are the successive values observed in the latter seven periods not significantly different, with one exception, but also no clearly defined trend in the data is apparent. Otherwise put, between the fifth and twenty-sixth days of the experiment, the points of the two curves shown in figure 1 do not deviate significantly from a straight line of zero slope. In the case of the control flies, the straight line which best fits the latter seven points would intercept the ordinate at about 0.350, while a similar line of fit to the experimental results would cross the ordinate in the neighborhood of 0.395. In these experiments, then, an initial

high in crossing over is quickly followed by a sustained low. Any assumption of a more complicated relationship seems gratuitous. The relation of these findings to the observations of other workers will be discussed in a later section of the paper.

TABLE 3

Test of the significance of the total difference in frequency and distribution of crossing over in control and experimental flies. Details in text.

PERIOD	1	2	3	4	5	6	7	8	TOTALS
χ^2	4.706	14.958	10.479	11.922	8.216	5.416	2.082	2.928	60.707
Degrees of freedom	7	7	6	6	5	4	4	3	42
P	<.7	<.05	<.2	<.1	<.2	<.3	<.8	<.5	<.03 & >.02

We may now consider the second of the two questions raised above—namely, what is the probability that the differences between the two series are of significance? To answer this, contingency tables similar to those described above were prepared, comparing for each period the frequency and distribution of crossing over in chromosomes recovered from control and experimental flies, and a χ^2 obtained for the difference between the two. Table 3 summarizes the results of the analysis. Differences in the number of degrees of freedom in the various periods are due to the fact that when certain crossover classes were poorly represented (fewer than seven individuals), they were combined with adjacent classes in order to get significant numbers. It is apparent that for only one of the eight periods, the second, are the differences between the two series beyond the accepted level of significance. But since both χ^2 and the degrees of freedom are additive quantities, they may be totaled and an estimate obtained of the significance of the differences observed over the entire course of the experiment. When this is done, the probability that crossing over is the same in the two series is less than 0.03 but greater than 0.02.

This method of analysis fails to take into account the consistency of the difference between the two series—that is, the uniformly higher rate of crossing over in the experimental flies, except for the first period. Our previous analysis has shown that between the second and eighth periods the two curves approximate straight lines of zero slope. It should therefore be permissible, for both the control and experimental series, to combine the data of the latter seven periods and set up a single table comparing the chromosomes recovered from the two groups from the fifth to the twenty-sixth days of the experiment. This treatment is shown in table 4. The probability that the two series are not different during this interval is, according to this method of analysis, less than 0.01.

TABLE 4

A comparison of crossing over in control and experimental flies, during the fifth to twenty-sixth days of adult life.

SERIES	REGION								TOTAL
	0	1	2	3	4	1 3	1 4	OTHERS	
Control	4044	797	81	393	436	81	79	18	5929
Exp'l	3697	848	99	440	458	95	99	25	5761

$\chi^2 = 23.671$ Degrees of freedom = 7 $P < 0.01$

In view of the small number of parental flies used in the derivation of the curves (six control and 11 experimental females), the possibility existed that the differences between controls and experimentals were due to the existence in either or both series of one or several aberrant females showing recombination frequencies quite different from those found in the others. However, analysis of the data for consistency by the usual methods employed in the analysis of variance revealed no evidence for a lack of homogeneity in either the control or experimental females.

In the studies dealing with the relation of recombination to age, extremes of temperature, and X-rays, it was found that the region of the chromosome most susceptible to the effects of these agencies was in the vicinity of the centromere (PLOUGH 1917; MULLER 1926; BRIDGES 1929; MATHER 1939). Clear cut evidence for a similar localization of effect was not found in the present work. During the period of the sustained low, the ratio of experimental to control crossing over in the various regions was as follows: *h-th*, 1.121 ± 0.045; *th-cu*, 1.271 ± 0.171; *cu-sr*, 1.166 ± 0.063; and *sr-e*, 1.119 ± 0.065. This grouping of all the data of the latter seven periods bearing on crossing over in a given region seems justified, since, as is apparent from table 2, during these periods the differences between control and experimental results for any given region were rather consistently in the same direction. All of the ratios are above 1.00, two of the four significantly so. The highest ratio is found in the short *th-cu* region, which contains the centromere, but at the same time this ratio differs less significantly from unity than any of the others. This may in part be due to the statistical methods employed, since there is a relatively larger error in the estimation of the length of a small segment than of a large segment. Accordingly, too literal emphasis should not be placed upon the comparative estimates of significance obtained. None of the ratios differs significantly from any of the others. We may therefore conclude that the increase in crossing over is well distributed along the length of the chromosome

studied, with the possibility of a maximum effect in the region of the centromere not excluded.

Of the possible ways of accounting for this generally distributed increase, two are outstanding: (1) a decrease in the interference between adjacent points of crossing over, or (2) a general increase in the coefficient of crossing over, resulting in proportionately greater numbers of both single and multiple exchange chromosomes. These two explanations are not mutually exclusive; the observed results may be due to a suitable combination of both these factors. If the first possibility obtains to any significant extent, then both coincidence values and the proportion of chromosomes which are the result of multiple crossing over within any two regions should be higher in the experimental than in the control series. Unfortunately for the purposes of this experiment, the regions for which it is feasible to calculate coincidence and the proportion of multiples—namely, regions 1 and 3, and 1 and 4—are on opposite sides of the centromere, and it is well known (and confirmed by these data) that as a rule there is no interference between crossing over on opposite sides of this region. A decision between the two possibilities is thus not practical on the basis of the material at hand. It seems unlikely that decreased interference alone can account for the whole of the effect.

DISCUSSION

To the list of agencies known to influence the frequency of crossing over in Drosophila females should be added still another, the type of larval nutrition. However, the detailed effects of nutrition appear to differ from those of the other agencies, which in turn differ among themselves. Thus, the effects of age, temperature, and irradiation are most strongly apparent —or even sharply localized—in the region of the centromere; nutritional effects appear to differ from all these in that the increase was observed to be more uniformly distributed over the entire section of the chromosome under surveillance. KIKKAWA (1934) has stressed the possibility that any experimentally produced increase in crossing over in the region near the centromere is attended by a compensatory decrease in the more remotely situated regions. This possibility also emerged from the earlier irradiation experiments of MAVOR (1923a, 1923b) and MULLER (1926). The present data offer no support to this hypothesis, but it should be pointed out that crossing over was not followed in the most distal portions of the chromosome. The nutritional effects also differ from those of temperature and possibly irradiation in that the former persist longer than the latter after the cessation of treatment.

SEREBROVSKY (1927) and BERGNER (1928) have published data indicating that the frequency of crossing over is correlated with the length of

larval life; the first investigator obtained a negative correlation and the latter a positive. A reexamination of their data in the light of the present findings suggests that the apparent correlation between the length of the larval stage and the frequency of crossing over may be largely or entirely due to a mutual dependence of these two phenomena on a third variable,

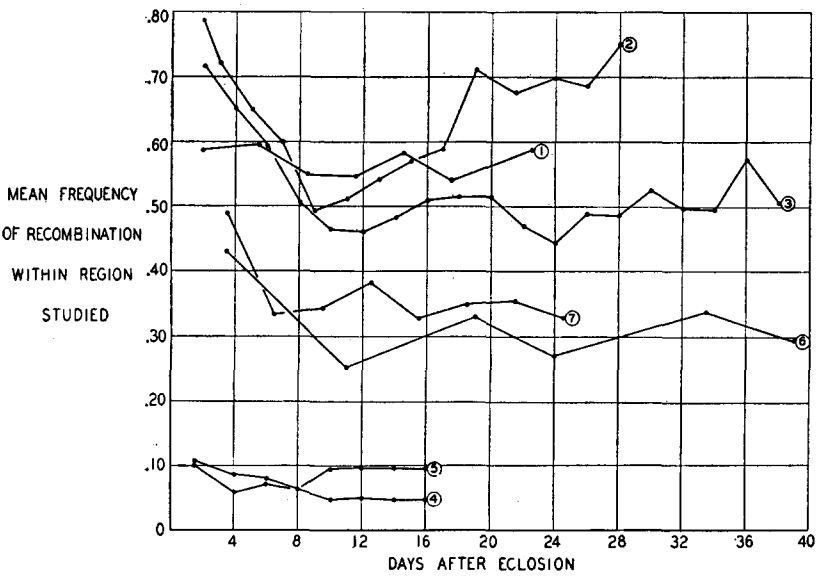


FIGURE 2.—A graphic comparison of the results of five investigators who have studied the relation between age and crossing over in the third chromosome of *Drosophila melanogaster*. The numbers affixed to the various curves refer to the following studies:

Investigator	Region covered
1. PLOUGH, 1921	*se-ss-e^s-ro*
2. BRIDGES, 1929, experiment 1	*ru-D-st-p^p-ss-e^s*
3. BRIDGES, 1929, experiment 2	*ru-h-D-st-p^p-ss-e^s*
4. BERGNER, 1928, control 1	*D-cu*
5. BERGNER, 1928, control 2	*D-cu*
6. POLITZER, 1940	*se-cp-gl^3-e''*
7. NEEL, this paper, controls	*h-th-cu-sr-e^s*

a nutritional effect, since the lengthened larval periods in each case were due to poor nutritional conditions. It is not apparent why SEREBROVSKY's results should differ in direction from those of BERGNER and the present investigator.

The work of STADLER (1926) and OEHLKERS and his students and collaborators (cf. OEHLKERS 1937) renders it probable that the amount of crossing over in plants is likewise influenced by nutritional circumstances.

The tendency toward increased crossing over under adverse living conditions is not without adaptive significance to the organism. It would lead to a larger number of new factor combinations under just those conditions

where it is imperative that the organism explore its genetic potentialities to the fullest extent possible.

Finally, we may turn to a consideration of the relation between age and crossing over. This subject has been considered by a number of investigators, and particularly by BRIDGES (1929), KIKKAWA (1934), and POLITZER (1940). These investigators state that the initial high and subsequent decline in the frequency of crossing over are followed by a second, less pronounced high, and a second decline—that is, that with increasing age there are significant periodic fluctuations in the amount of recombination. In figure 2 are summarized graphically the results of five different investigators who have studied under standard conditions the relation between age and crossing over in some portion of the third chromosome of *D. melanogaster*. In examining this collection of curves, one is impressed by two points: (1) the fact that in all cases but one there is an initial high in the frequency of crossing over, followed by a decline, and (2) the considerable degree of divergence in the course of the latter, and numerically less reliable, portions of the curves. An attempt has been made to analyze, insofar as it is possible from the published data, the latter portions of all these curves with respect to possible significant trends, particularly when they are considered in conjunction with one another. This is a rather difficult procedure, but it would appear quite probable that if these curves are all expressions of the same fundamental relationship, then the nature of that relationship in this particular chromosome is an initial high in the frequency of crossing over, followed by a sustained low. Certainly the "periodic fluctuations" can at this stage of the analysis be as well attributed to the operations of chance and uncontrolled environmental variables as to the effect of some inherent physiological rhythm of the female.

Much of this work was carried out during the summer of 1940 at the Department of Genetics, CARNEGIE INSTITUTION OF WASHINGTON, Cold Spring Harbor, New York; I am greatly indebted to the members of this institution for facilities and suggestions.

SUMMARY

The frequency of crossing over in the *h-e*[8] region of the third chromosome of *Drosophila melanogaster* females was significantly increased in flies which during the larval stage had been removed from all food from the seventieth hour of egg-larval life until the time of puparium formation (development at 26°C). Except for the first few days, this increase in the amount of crossing over was consistently apparent throughout the entire 26 day period that the females were under observation.

The increase was distributed along the entire portion of the third chro-

mosome studied; the possibility of a maximum effect in the region of the centromere remains open.

In both the control and the experimental series, the relation of crossing over to age was characterized by an initial high in the frequency of recombination, followed by a sharp decrease and sustained low.

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