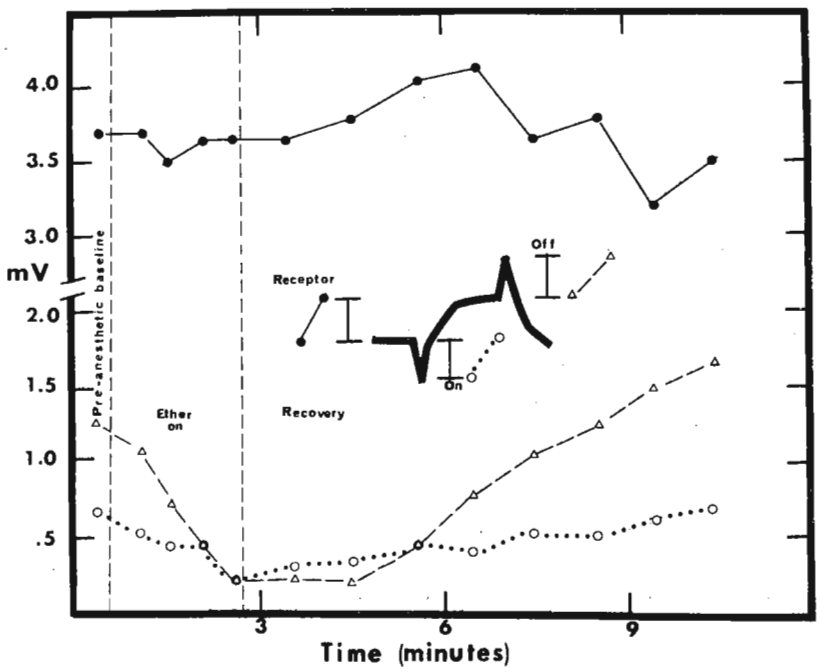
Stark, W.S. University of Wisconsin, Madison, Wisconsin. The effect of ether and carbon dioxide on the components of the ERG of Drosophila.

By fixing adult Drosophila melanogaster on the surface of hardening agar at room temperature, one can prepare specimens for recording the electroretinogram (ERG) without prior anesthetization. This preparation can be used to study the effects of anesthetics on the ERG during recording. The agar fixation is gentle enough to permit subsequent release of flies which can live for several weeks and mate. With the agar block in the bottom of a chamber, ether vapor or pure CO_2, both heavier than air, can be made to surround the preparation and can later be removed by blowing fresh air into the chamber.



The accompanying figure shows the typical effect of anesthetic ether on the ERG of an Oregon-R wild-type male eclosed within the past 24 hr. It was consistently found that the off-transient was diminished most, while the on-transient was also selectively reduced. Short etherization was usually followed by complete recovery. Etherization for longer than 3 min often irreversibly blocked both transients and sometimes also lowered the receptor wave. It cannot be determined how the time scale in these experiments corresponds to the typical 20-30 sec. anesthetization of flies in an etherizer since the agar might protect the fly with a small pocket of air.

Carbon dioxide also diminished both transients selectively and reversibly. The off-transient usually disappeared in less than 5 sec., and the on-transient in less than 10 sec. when pure CO_2 was applied to the preparation. Recovery usually took about the same time as the period of exposure. Long exposure sometimes permanently altered the ERG, although recovery from 5 min exposure has been seen. Probably permanent alterations in the ERG with ether or CO_2 are cuased by near-lethal effects of over-anesthetization and would not take place with normal anesthetization.

These anesthetics cause the ERG to look like the ERGs of the blind mutants such as tan and ebony whose ERGs have been characterized by Hotta and Benzer and Pak, Grossfield, and White. The metabolic and synaptic effects of CO_2 and ether may provide a clue to the mechanism of action of the genes causing blindness without impairing receptor function.

References: Hotta, Y. and S. Benzer 1969 Nature 222:354-356; Pak, W.L., J. Grossfield, and N.V. White 1969 Nature 222:351-354.

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Chinnici, J.P. Virginia Commonwealth University, Richmond, Virginia. The effect of age on crossing over in the X-chromosome of Drosophila melanogaster.

Recently, I have published the results of a bi-directional section experiment (involving both family and chromosomal selection) which resulted in an increase and a decrease in the amount of crossing over between the sex-linked genes sc and cv in Drosophila melanogaster (Chinnici 1971). Recombination of the regions cv-sn^3 and sn^3-m were also followed throughout the course of the experiment. In odd-numbered generations, the progeny of 14 to 37 single-pair (family)

Table 1. Regression coefficients of standardized crossover values on age of the female parent. The L and H notations indicate low and high lines, respectively.

Generation	Region of X-Chromosome and Rank [(1) > (2) > (3)]		
	sc-sc	cv-sn3	sn3-m
1	+0.0206 (1)	+0.0125 (2)	-0.0021 (3)
3L	+0.0192 (1)	+0.0045 (3)	+0.0097 (2)
3H	+0.0088 (2)	+0.0120 (1)	+0.0003 (3)
5L	+0.0139 (2)	+0.0259 (2)	-0.0056 (3)
5H	+0.0204 (2)	+0.0406 (1)	+0.0039 (3)
7L	+0.0104 (1)	+0.0057 (2)	+0.0030 (3)
7H	+0.0150 (1)	+0.0080 (3)	+0.0113 (2)
9L	+0.0228 (1)	+0.0154 (2)	-0.0016 (3)
9H	+0.0261 (1)	+0.0202 (2)	+0.0014 (3)
11L	+0.0191 (1)	+0.0146 (2)	+0.0104 (3)
11H	+0.0214 (1)	+0.0117 (2)	-0.0026 (3)
13L	+0.0165 (1)	+0.0092 (3)	+0.0099 (2)
13H	+0.0303 (1)	+0.0031 (3)	+0.0063 (2)
15L	+0.0443 (1)	+0.0196 (3)	+0.0246 (2)
15H	+0.0328 (1)	-0.0124 (3)	+0.0229 (2)
17L	-0.0112 (3)	+0.0533 (1)	+0.0029 (2)
17H	+0.0158 (2)	+0.0485 (1)	+0.0062 (3)
19L	+0.0327 (1)	+0.0074 (2)	-0.0140 (3)
19H	+0.0078 (1)	-0.0066 (3)	-0.0009 (2)
21L	+0.0163 (2)	+0.0272 (1)	+0.0029 (3)
21H	+0.0118 (1)	-0.0649 (3)	+0.0054 (2)
23L	+0.0225 (2)	+0.0509 (1)	+0.0047 (3)
23H	+0.0225 (2)	+0.0504 (1)	-0.0052 (3)
25L	+0.0270 (2)	+0.0356 (1)	+0.0130 (3)
25H	+0.0093 (1)	+0.0056 (2)	+0.0001 (3)
27L	+0.0411 (2)	+0.0536 (1)	-0.0092 (3)
27H	+0.0191 (1)	+0.0039 (3)	+0.0077 (2)
29L	-0.0098 (2)	-0.0150 (3)	-0.0077 (1)
29H	+0.0106 (2)	+0.0323 (1)	-0.0152 (3)
31L	+0.0282 (2)	+0.0365 (1)	+0.0229 (3)
31H	+0.0033 (2)	-0.0305 (3)	+0.0097 (1)
33L	+0.0074 (2)	+0.0406 (1)	+0.0045 (3)
33H	+0.0215 (2)	+0.0370 (1)	-0.0031 (3)
45L	+0.0006 (3)	+0.0249 (1)	+0.0194 (2)
45H	+0.0185	+0.0097 (1)	+0.0054 (3)

Table 2. Analysis of rankings of regression coefficients found in Table 1 by the ranking correlation W.

High Line (H)

Ranking	Frequency of Ranking Region of X		
	sc-cv	cv-sn3	sn3-m
1	11	6	1
2	7	5	6
3	0	7	11
Sum	25	37	46

W = 0.3425
z = 1.4469
P > 0.01

Low Line (L)

Ranking	Frequency of Ranking Region of X		
	sc-cv	cv-sn3	sn3-m
1	8	9	1
2	8	5	5
3	2	4	12
Sum	30	31	47

W = 0.2803
z = 0.9450
P > 0.01

matings were scored to determine the amount of crossing over in the heterozygous female parent. Since each family mating was set up so that the parents were allowed four consecutive 3-day laying periods, the data could be analyzed to determine the effect of female ageing on crossing over between these sex-linked genes. All female parents were two days old at the beginning of the first laying period.

The data thus obtained were analyzed in the following manner: 1) The crossover frequency in each region of the X for each age group was determined for each family.

2) Each crossover percentage was transformed into angular values and the mean crossover value for each region was determined using the transformed data.

3) The three X-regions were standardized for differences in mean crossover values so that they could be directly compared for change in recombination over time per map distance. For each region, this was accomplished by dividing the mean crossover value for each laying period by the value obtained for the first laying period. This gave each region a mean crossover value of 1.0 for the first laying period, with the values for the other laying periods being expressed as proportions of this standardized map length.

4) Regression analysis of standardized crossover values for each laying period on mean age of the female parent for that period (3 days old for the first, 6 days for the second, 9 for the third, and 12 for the fourth) was performed for each region. These regression coefficients (b) are listed in Table 1. In each generation, these b values were ranked, with a value of 1 given to the highest b, 2 to the second highest, and 3 to the lowest b.

5) The Coefficient of Concordance (W; see Kendall, 1962) for the total rankings of the 18 low line family mating generations and the 18 high line family mating generations for each region were calculated. A non-parametric ranking statistic (W) was used because regression coefficients calculated by using standardized values may not be parametrically compared. W was used to test the significance of ranking orders by employing Fisher's z-test (Kendall, 1962). These statistics are presented on Table 2.

These data may be summarized as follows. For all of the three X-chromosome regions analyzed, ageing of the female parent has a very small positive effect on recombination frequency. By rank correlation analysis (W), it has been shown that sc-cv $>$ cv-sn^3 $>$ sn^3-m in the magnitude of this ageing effect.

Bridges (1915) and Plough (1921) both tested the effect of increasing age of the female parent on recombination in the X-chromosome of D. melanogaster and both concluded that ageing did not affect the rate of recombination in the X. However, Plough's data show a very small change in recombination in the entire chromosome. This small change is directional since the sc-ec (most distal) region shows a small increase with age, ec-ct shows a smaller increase, ct-v shows a slight decrease, and v-g (most proximal region) shows a slightly greater decrease. He also observed a large increase in crossing-over in the g-f region, which is quite close to the centromere. The very small magnitude of these changes led Plough to disregard them. Rendel (1957), however, found that increasing age of female parents caused a significant decrease in crossing-over between sex-linked genes, with or without the presence of the second chromosome inversion Curly.

The data gathered in the present experiment agree well with those of Plough (1921). In each of the three X-chromosome regions studied, recombination increased very slightly, though significantly, with sc-cv $>$ cv-sn^3 $>$ sn^3-m in the magnitude of increase. It appears, therefore, that female ageing in Drosophila, besides affecting the frequency of recombination significantly in chromosomes 2 and 3 (Plough, 1921; Bridges, 1915, 1927), also affects recombination in the X-chromosome, but in a polarized fashion and to a smaller extent. Rendel's observations are inconsistent with this statement, for reasons which are not understood.

References: Bridges, C.B., 1915 J. Exp. Zool. 19:1-21; _____, 1927 J. Gen. Physiol. 8:689-700; Chinnici, J.P., 1971 Genetics 69(1) in press; Kendall, M.G., 1962 Rank Correlation Methods, 3rd Edition, Hafner, N.Y.; Plough, H.H., 1921 J.Exp. Zool. 32:187-202; Rendel, J.M., 1957 Genetics 43:207-214.