Table 1.--continued.

	dU	BdU	BdU + X-irradiation	dU + X-irradiation	dU + X-irradiation	BdU + X-irradiation
No. lethal chromosomes	1	2	1	4	2	4
Lethals (%)	0.17	0.31	0.26	0.57	0.53	0.46

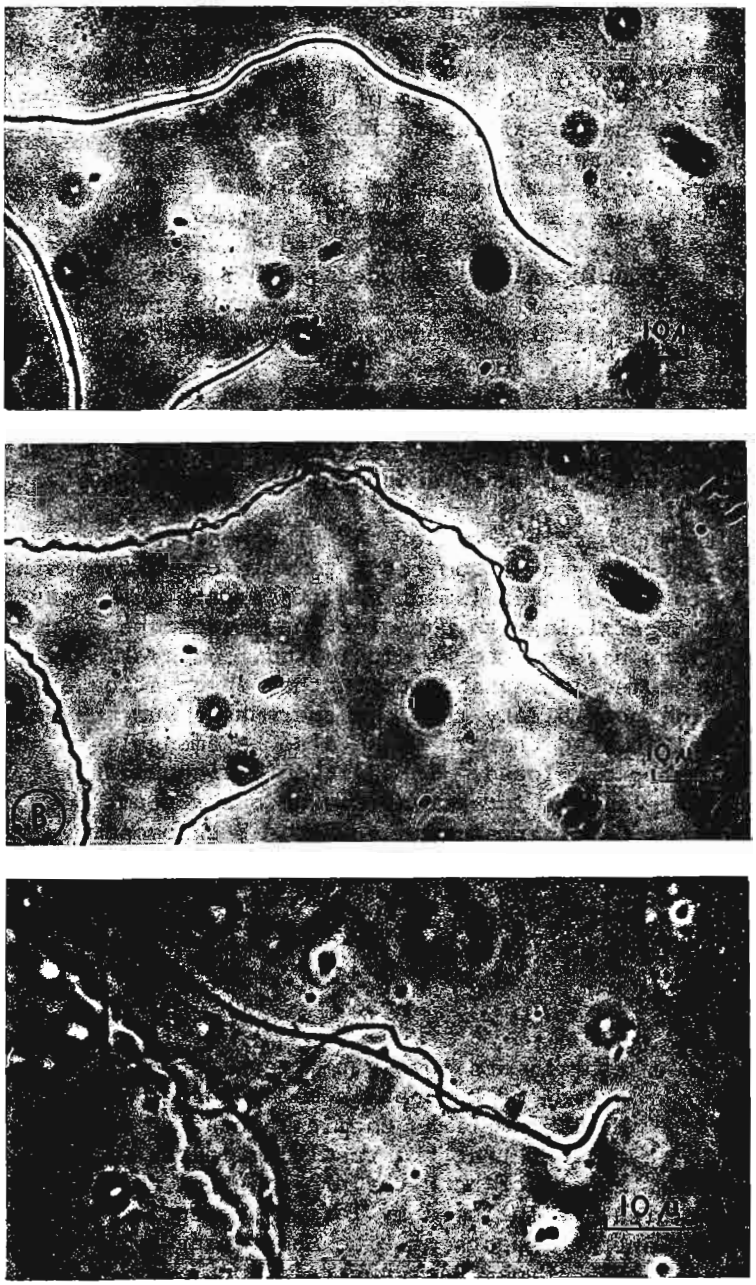
Table 2.--Sex-linked recessive frequencies in Drosophila females induced by X-irradiation (810 r) after larval feeding treatments in the presence of 5-bromodeoxyuridine (BdU) or deoxyuridine (dU).

Treatment	dU	BdU	BdU + X-irradiation	dU + X-irradiation	dU + X-irradiation	BdU + X-irradiation
Concentration of dU and BdU (%)	0.02	0.02	0.02	0.02	0.02	0.02
Concentration of Aminopterin (%)	-	-	0.002	-	0.002	0.002
Hatchability (%)	78.3	73.8	51.1	65.5	56.3	61.6
No. females examined	40	47	55	55	50	45
Average no. chromosomes examined/female	6.8	6.4	4	5.8	7.3	8.6
No. chromosomes examined	273	301	220	320	365	389
No. lethal chromosomes	1	1	0	0	0	1
Lethals (%)	0.36	0.33	0.0	0.0	0.0	0.25

Oster, I. I., J. Duffy and R. Binnard.
The Institute for Cancer Research. Observations on a piece of tail.

During the course of counting the number of spermatozoa utilized in successive matings by males of Drosophila melanogaster in connection with experiments on radiation sensitivity, we found that two structural elements could be recognized in the spermatozoon's tail following fixation. Hitherto, observations by others (Cooper, K. W., 1950, Biology of Drosophila; Yanders, A. F., and J. P. Perras, 1960, DIS; Kaplan, W. D., et al., 1962, DIS; Lefevre, G. Jr. and U. B. Jonsson, 1962, Genetics) had revealed that Drosophila has the type of sperm usually described for insects--that is, a filiform head, no separately discernible mid-piece, and a tail. In fruit flies, the only unique feature which had been noticed until now was the unusual length of the tail (0.2 mm to 6.6 mm, depending on the species, although the diameter is of the order of 0.2μ). Our experiments involved the removal with watch-maker's forceps of the vagina and uterus from a female immediately after copulation to a slide containing a drop of Drosophila Ringer's solution, teasing

open the uterus which allows the spermatozoa to flow out, permitting the sperm sample to dry slowly in air (which facilitates spreading of the sperm mass), fixing in 25% acetic acid, adding a drop of lactic-acetic orcein with fast green, and squashing with a cover slip. Observations with phase optics of material prepared in this manner revealed spermatozoa in which the tails appeared to be composed of two separable fibers (please see Figure 1).



Several different stocks of Drosophila melanogaster, including Oregon-R (wild type) as well as individuals carrying different recessive and dominant mutations, consistently showed this pattern. One fiber is spiralized, and the other appears to be fairly straight. The two fibers generally seem to be of the same thickness at the head end but the spiralized one gradually becomes thicker as it approaches and finally joins the straight fiber at the tail end of the sperm. Also, the gyres of the spiral appear to be smaller at the anterior end. The degree to which such differences in thickness and spiralization may be due to the fixation is not yet certain. In well-spread preparations the doubleness of the tail can be observed and traced along the entire length of the spermatozoon.

Since it was possible that this effect was the outcome of the method by which the material had been prepared, variations of the original technique were tried. Following the observation that air-drying per se did not have any effect, we were able to develop a fairly simple procedure which yields consistent results. In practice this consists of removing either the testis and/or the seminal vesicles from a male, transferring the organs to a drop of Drosophila Ringer's solution on a slide, gently teasing apart the organs to facilitate separation of the spermatozoa, and covering the sample with a cover-slip. The excess Ringer's solution can then be drawn off by holding a piece of filter paper at one side of the cover-slip while introducing a 25% aqueous solution of acetic acid along the opposite side. As the acid flows over the sperm, their heads become darker and distinctly visible (all observations should be made with phase

Figure 1: Drosophila melanogaster spermatozoa.
A. Live spermatozoa in Drosophila Ringer's solution.
B. The same spermatozoa following treatment with 25% acetic acid. (Photographed at 430X using phase optics).
C. Anterior portion of an acid-treated spermatozoon as seen under oil immersion. (Photographed at 970X using phase optics).
(Note that in A and B a head is located at the lower left and a tail-end can be seen at the upper right of the photographs).

optics) and one fiber appears to pull away from the other, the former becoming convoluted. Weaker solutions of acetic acid produce only a slight separation of the fibers. Twenty-five percent solutions of formic or lactic acid as well as a 1:1 solution of acetic acid and ethyl alcohol act similarly to that of aqueous acetic acid. On the other hand, a 1:3 solution of acetic acid in ethyl alcohol, hydrochloric acid, butyric acid, ammonium hydroxide, and formaldehyde have no effect on the tail. We have also found that the enzyme pronase produces a separation of the fibers of the sperm tail but with this treatment there is only a partial separation (i.e., sections of the tail seem to be affected) and both fibers appear to be non-spiralized. However, application of a 25% solution of acetic acid to such separated fibers causes one element of each pair to become convoluted. This uniform differential reaction to acetic acid indicates that the spiralization of one of the fibers of the tail following immediate acid fixation is not somehow related to the actual separation which takes place, but reveals a structural difference between the two elements.

Among the few previously reported accounts of a similar nature on insect spermatozoa were those made by Ballowitz (1890, Zeit. für Wissen. Zool. Leipzig) and Retzius (1909, Biologische Untersuchungen, Neue Folge XIV, Stockholm). Ballowitz had found that subjecting macerated samples of beetle spermatozoa to hypertonic salt solutions or osmic acid for several days revealed a number of fibers in the tail. We have also tried such drastic treatments with Drosophila, but at best only obtained a partial splitting accompanied by much cross-wise fragmentation of the tails of the spermatozoa.

After numerous observations of the preparations, it was noticed that the tails of Drosophila spermatozoa which had only been in Ringer's solution often exhibited separation along small areas of their length. In these cases neither fiber appeared spiralized. The slight degree with which this occurs makes it evident why it had not attracted the attention of other investigators who had studied non-fixed material.

At the present time it seems probable to suppose that one of the fibers represents an elongated mitochondrion. On the other hand, it may yet be too early to rule out the possibilities that either the two fibers correspond to the axial filament and its sheath or to any (or all) of the groups of bundles which are discernible with the electron microscope. The functional aspects of these structural relations have yet to be elucidated. However, the fact that a portion of the spermatozoon which had seemed impregnable to further observation by visible light has been dissociated enhances the possibility that the sperm head and the orientation and state of the chromosomal material located therein might also be amenable to such observation provided the proper techniques can be developed. In addition, it should be of interest to determine the nature of these tail elements in spermatozoa of other genotypes (particularly in those bearing two Y chromosomes or disarrangements of the Y chromosome), in spermatozoa which had been treated with various mutagens, and in spermatozoa of other species of Drosophila.

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Oster, I. I. The Institute for Cancer Research. Improved strains for detecting somatically-induced damage.

Experiments involving treatment of immature stages of Drosophila melanogaster with physical as well as chemical mutagens led us to suggest that the resulting increase in mortality of both the pre-imaginal and post-imaginal stages was largely due to chromosome breakage followed by loss of essential parts of the genome (Muller, H. J., 1958, Conf. on the Genetic Aspects of Life Shortening by Radiation, Dec. 13 and 14, 1958, Ames, Iowa; Oster, I. I., 1958, Proc. Sec. Austral. Conf. on Rad. Biol.; Oster, I. I., 1959, Science; Oster, I. I., 1960, Science; Oster, I. I., 1960, in Proc. of Conf. on Research on the Radiotherapy of Cancer; Oster, I. I., 1961, The Sec. Int. Conf. of Human Genetics; Oster, I. I. and A. Cicak, 1958, DIS). Several genetical schemes, involving comparisons between males and females, rod-X- and ring-X-bearing males, and normal rod-X-carrying males and attached-X females, were utilized to obtain evidence for this view. Soon thereafter, additional confirmatory data was obtained by Ostertag and Muller (1959, Science), who also extended the work to include comparisons of normal individuals with those heterozygous for small deficiencies (Ostertag, 1963, Zeit. für Vererbungsl.).