PHENOTYPIC EFFECTS OF Y CHROMOSOME MUTATIONS IN *DROSOPHILA MELANOGASTER*

I. SPERMIOGENESIS AND STERILITY IN *KL-1*⁻ MALES¹

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A previous paper (KIEFER 1966) has described ultrastructural abnormalities during spermiogenesis in *X/0 Drosophila melanogaster*. In the absence of the *Y* chromosome the development and organization of the mitochondrial elements (*Nebenkern* derivatives) is defective, and some flagella either lack axial fibers or the fibers are disoriented. Additionally, there is a reduced number of germ cells per cyst. The present study is an attempt to assign functions to different *Y*-loci by means of light and electron microscopic studies of spermiogenesis in males deficient in specific regions of the *Y* chromosome. This paper describes the effects of a deletion in the proximal region of the *Y*^L fertility complex (*kl-1*, BROUSSEAU 1960) on sperm maturation and fertility. This mutant stands out from the other six derived by BROUSSEAU (1960) in that motile sperm are produced. A report on the other *Y*-loci will follow.

MATERIALS AND METHODS

Stocks: The sterile *Y* tester stock used in this study is the *kl-1*⁻ of BROUSSEAU which has been described in detail elsewhere (BROUSSEAU 1960). Briefly, a deletion (or mutation) in the proximal region of *Y*^L is maintained in a stock in which the females have attached -*X* chromosomes and a free sterile *Y* (attached -*X/Y*^{kl-1}), and the males have the long arm of the *Y* attached to the *X* and a free sterile *Y* (attached -*XY*^{Lc/Y^{kl-1}). A similar stock derived from the above was obtained from Dr. E. NOVITSKI's laboratory and used in parallel experiments. Sterile (*KL-1*⁻) males are obtained by crossing stock males with virgin wild-type females.}

The flies were grown on either standard cornmeal-molasses-agar medium or Instant *Drosophila* Medium (Carolina Biological Supply Co.) at 25°C. Upon emergence the males were collected at 12-hour intervals and aged for four days.

Light Microscopy: Testes from 4-5 day old males and female reproductive tracts were dissected in BEADLE-EPHROSSI saline, transferred to lacto-aceto-orcein (25 ml lactic acid, 25 ml glacial acetic acid, 0.5g orcein) and examined both before and after squashing. Other preparations were examined directly in the saline solution for the presence of motile sperm. All observations and photographs were made with phase contrast or dark field optics.

Electron Microscopy: Testes from 4-5 day old *kl-1*⁻ males were dissected as above and fixed for 30 min in cold 6% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) and post-fixed for 1 hr in cold 1% OsO₄ in veronal-acetate buffer (pH 7.5). After dehydration in a series of cold ethanols, the tissues were treated with propylene oxide and embedded in Epon 812 or Maraglas 655. Thin sections were obtained using a Porter-Blum MT-2 ultramicrotome with a glass knife, stained with uranyl-acetate, and studied in a Zeiss EM-9A electron microscope.

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OBSERVATIONS

Light Microscopy: The testes, seminal vesicle, and vas deferens of mature *kl-1*⁻ males are fully formed, pigmented and, at the gross level, morphologically indistinguishable from the wild type. Many sperm reach morphological maturity, enter the seminal vesicle and vas deferens and become fully motile (Figure 1). At the level of the light microscope, the development of the germ cells very closely approximates the wild-type situation.

Because the production of motile sperm by *kl-1*⁻ mutants had not been reported by Brosseau (1960), two different stocks (see MATERIALS AND METHODS) were tested separately, and each of these reordered and retested a second time. Motile sperm were found in all *kl-1*⁻ males examined. To test the fertility of these males, 91 pair matings were observed. In all cases copulation occurred and hundreds of eggs were laid. However, no larvae were produced.

Females were examined immediately after mating with *kl-1*⁻ males and at selected time intervals thereafter. Immediately after copulation the uterus is filled with sperm (Figure 2). These sperm move up to the junction of the seminal receptacle with the uterus, but do not enter the seminal receptacle. The sperm remain in the uterus until the first egg is laid at which time they are presumably forced out by the passage of the egg.

Thirty female reproductive tracts were examined 24 hrs after copulation. Four were found to contain motile sperm in the seminal receptacle. Of these four seminal receptacles only four sperm were detected in one, two in another, and one in each of the remaining two (Figure 3). All the other regions of the reproductive tract in these females were devoid of sperm. No sperm were seen in any of the 26 remaining females.

Electron Microscopy: Ultrastructural studies support the general observation reported above in that spermiogenesis is much more normal than that observed in *X/0* testes, and fully mature sperm are found (Figure 4).

The only structural abnormality similar to those reported for *X/0* spermiogenesis is a low incidence of axial fiber complexes which are either missing peripheral fibers or are disorganized (Figures 5). It is worth noting that, when found, such abnormal complexes usually occur in groups. No flagella lacking only the central fibers were found. Occasionally, aberrant mitochondrial development is observed (Figure 6), but this occurrence is no more frequent than in the wild type.

The average cross-section of a *kl-1*⁻ testis is indistinguishable from the wild type. However, sections of the testis taken near the testicular duct and through

FIGURE 1.—Motile sperm from break in seminal vesicle of *kl-1*⁻ males. 300 ×

FIGURE 2.—Motile sperm from break in the uterus (u) of a wild-type female which had mated with a *kl-1*⁻ male; s = spermathecae, sr = seminal receptacle. 300 ×

FIGURE 3.—Single motile sperm (arrow) in seminal receptacle of wild-type female 24 hrs after mating with *kl-1*⁻ male. 4,800 ×

FIGURE 4.—“Typical” cross section of *kl-1*⁻ testis showing sperm cysts in various stages of maturation; a fully mature cyst is indicated by the arrow. 5,200 ×

FIGURE 5.—Disorganized axial fiber complexes. Circles represent arbitrary groupings of the fibers to account for the $9 + 2$ of the three flagella; c = central pair. $54,000 \times$

FIGURE 6.—An example of aberrant mitochondrial development (arrows). $18,000 \times$

the seminal vesicle reveal masses of mature sperm in various stages of degeneration (Figures 7–13). The first sign of degeneration is the breaking away of the mitochondrial element from the axial fiber complex (Figure 8). This is followed by an increased density, disorganization and subsequent loss of fibers (Figure 9). The mitochondrial element does not begin to lose its characteristic shape until the final stages of degeneration. During this time the previously undetectable paracrystalline detail becomes visible (Figure 10). The structure at this point resembles that seen in negatively stained preparations (MEYER 1964). The degeneration of the axial fiber complex can and usually does take place while the plasma membrane surrounding each cell remains intact. When the membrane is destroyed, projections appear along the outer surface of the mitochondrial ele-

FIGURE 7.—Low magnification micrograph showing the extent of degeneration. Though not completely obvious at this magnification, almost all the sperm pictured are in some stage of degeneration. $5,200 \times$

FIGURE 8.—First sign of degeneration: the breaking away of the mitochondrial element (arrows) from the axial fiber complex; most mitochondrial elements remain surrounded by plasma membrane. $36,000 \times$

FIGURE 9.—Beginning of fiber loss and disorganization mid-way in the degenerative process. $36,000 \times$

ment (Figure 11). The regular arrangement and consistent size of these projections suggest that they may be structural elements made visible by the removal of the membrane rather than being a response to the degenerative processes.

Counts were made of the number of mature sperm per cyst to compare with the previously reported X/Y and $X/0$ data. 50 cysts were counted giving a mean of 62 sperm per cyst. This is equivalent to the normal (X/Y) figures.

DISCUSSION

Motile yet sterile sperm: The most unexpected findings are that motile sperm are produced by *kl-1* males, that insemination takes place, and that many motile sperm reach the upper area of the uterus but rarely get further and are not used in fertilization. The sequence of events involved in the transfer, storage, and utilization of sperm in *Drosophila melanogaster* has been the subject of many investigations: most recently, the work of LEFEVRE and JONSSON (1962) and PEACOCK and ERICKSON (1965). It is generally agreed that the seminal receptacle is the major sperm storage organ with the spermathecae serving a secondary role in the females of this species. The total number of sperm stored in a well inseminated female is from 500 to 700 (LEFEVRE and JONSSON 1962; KAPLAN, TINDERHOLT and GUGLER 1962), although the amount actually deposited by the male in the uterus of the female can be as high as 3,000 to 4,000 (KAUFMANN and DEMEREC 1942). No attempts were made to count the number of sperm per insemination in the matings involving *kl-1* males, but the general impression is that there are less than that seen in the wild-type matings. This would be expected in light of the observed degeneration of mature sperm in the *kl-1* testes.

Obviously, there is more to the fertilization process than the deposition of motile sperm in the female tract. LEFEVRE and JONSSON (1962) have demonstrated sperm displacement in twice mated females, and have interpreted this displacement in terms of a continual circulation of the sperm within the female organs. Also, sperm from different males have differing displacement abilities. PEACOCK and ERICKSON (1965) have provided evidence which is consistent with the prediction of NOVITSKI and SANDLER (1957) that one half of the sperm produced by a *D. melanogaster* male are regularly nonfunctional in the ability to fertilize an egg. There appears to be a competition for storage between the two classes of sperm as well as a selective utilization of stored sperm in fertilization. All these findings point to the fact that the entry of the sperm into the female storage organs and the subsequent utilization of these sperm in fertilization involves some processes which are dependent upon the nature of the individual

FIGURE 10.—Final stages of degeneration; note paracrystalline appearance of mitochondrial element. 76,500 $\times$

FIGURE 11.—Projections from mitochondrial element which appear when plasma membrane (arrow) ruptures. 140,000 $\times$

FIGURE 12.—Large myelin figure (M) adjacent to three groups of degenerating sperm. 12,000 $\times$

FIGURE 13.—Three sperm which appear to be contained within a cytoplasmic inclusion (arrow). 54,000 $\times$

sperm. Additionally, the spermatid fluid probably plays an important role in sperm transfer and storage. Because so little is known about the mechanisms which guide the sperm into the seminal receptacle and spermathecae, speculations regarding an apparent breakdown of these mechanisms are of limited value.

The *kl-1*⁻ sperm are apparently incapable of entering the seminal receptacle, or at least do so only very rarely. This could be a reflection of a comparatively weak or inefficient motile apparatus. Since the most obvious effect on spermiogenesis of the loss of the entire *Y* chromosome is defective sperm mitochondria (KIEFER 1966), it is possible that although the mitochondria of the *kl-1*⁻ sperm appear structurally normal they might be physiologically defective (e.g. a lowered ATP production).

LEFEVRE and JONSSON (1962) have argued convincingly that the accessory gland secretion plays an important role in sperm transfer, and BAIRATI (1966) and ACTON (1966) have shown the presence of filamentous structures in the lumen of the gland as well as along the male genital duct and in the female receptacles. It is suggested that the accessory glands of the male secrete these filamentous structures as a component of the spermatid fluid which aids in the transfer of sperm along the female reproductive tract. Preliminary studies have shown that these structures are present in the accessory gland of the *kl-1*⁻ males; a thorough study of the accessory glands and spermatid fluid of the *kl-1*⁻ males is in progress. It is, at any rate, an intriguing possibility that the *Y* chromosome may be affecting the functional development of an organ other than the testes, but one just as essential for fertility.

Degeneration of Sperm: The finding of masses of sperm in the process of degeneration was also not anticipated because this situation is not observed in the *X/0* testis. However, it is only fully mature sperm which degenerate and this level of sperm development is never attained in the *X/0* male (KIEFER 1966). A degenerating bundle is occasionally seen in a wild-type testis (personal observation and BAIRATI 1967), and it is felt that the larger scale degeneration observed in the *kl-1*⁻ testis is simply an intensification of a process which can and sometimes does occur normally, as opposed to being a direct manifestation of this mutation. The morphologically mature sperm of *kl-1*⁻ males appear to be somewhat restricted in their ability to move out of the testes and, thus, begin to accumulate. Such accumulation could initiate cellular "clean-up" mechanisms. Many cellular inclusions resembling various autophagic vacuoles (lysosomes, cytolosomes, etc.) are found in the vicinity of the degenerating sperm (Figure 12) and frequently sperm are seen within these structures (Figure 13).

The degeneration of the axial fibers closely resembles that reported by NAGANO (1963) for cryptorchid rat testes. In this case the fragmentation or disappearance of the plasma membrane was apparently prerequisite to the degeneration of the fibers. This does not seem to be true in the present study, although ultimately the membranes are destroyed. However, the removal of the plasma membrane does appear to be important in the degeneration of the mitochondrial element.

Genetic Function: The main question which led to this study remains essen-

tially unanswered in that the role of the *kl-1*⁻ region does not specifically pertain to any of the obvious defects of spermiogenesis observed in the absence of the entire *Y* chromosome. Nevertheless, this locus is necessary for the production of fully functional sperm. The low incidence of missing or disoriented axial fibers is also found in some of the other *Y*-mutants (manuscript in preparation) and, therefore, is not peculiar to *kl-1*⁻. The occurrence of this same phenotypic effect in several different *Y*-mutants and in *X/0* males may indicate redundancy, especially since most of the axial fiber complexes are normal. MEYER (1968) has recently published an investigation on spermiogenesis in *Y*-deficient *Drosophila hydei*. In *X/0* males of this species spermatogenesis is already blocked at the spermatocyte stage. The morphogenetic effects observed in partially *Y*-deficient *D. hydei* are similar to those seen in the various *Y*-mutants of *D. melanogaster* and these will be compared in detail in the next paper of this series. One of the mutants studied by MEYER (1968) produces comparatively large numbers of mature sperm of which some exhibit weak motility. However, the possibility of sperm transfer to females was not investigated. This particular mutant is a deficiency for the second most distal of the four *Y*^L regions studied. The *melanogaster* mutant described in the present paper is a deletion of the most proximal of five regions on the long arm. The partial similarities in the morphogenetic effects of these two mutants could reflect differences in the linear arrangement of similar fertility genes in the two species, or similarities of deletion size in a chromosome which is at least partially redundant.

The production of motile sperm and the degeneration of some mature sperm indicate that the role of the *kl-1*⁻ region is subtle and one which is perhaps more amenable to a biochemical than a morphological investigation. At present, we can only define the function of this region in terms of phenotypic effects *not* attributable to it. As has been true with every past study regarding the *Y* chromosome, we find there is more to the function of this chromosome than had been previously thought.

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SUMMARY

Spermiogenesis and fertility were studied in *Drosophila melanogaster* males deficient for a specific region of the *Y* chromosome (*kl-1*⁻ BROSEAU). Light and electron micrographs have revealed that spermiogenesis very closely approximates that seen in wild type, and many morphologically mature and motile sperm are produced. The *kl-1*⁻ males are capable of inseminating wild-type females, but the sperm are not stored, and, hence, are not used in fertilization. Sections through the seminal vesicle show many mature sperm in various phases of degeneration. The sterility and degeneration of the sperm are discussed in terms of an inefficient motile apparatus, and comparisons are made between *kl-1*⁻ sperm maturation and that of other *Y*-mutants and *X/0*.

LITERATURE CITED

- ACTON, A. B., 1966 An unusual ciliumlike process. *J. Cell Biol.* **29**: 366-369.
- BAIRATI, A., 1967 Struttura ed ultrastruttura dell'apparato genitale maschile di *Drosophila melanogaster* Meig. *Z. Zellforsch.* **76**: 56-99. — 1966 Filamentous structures in spermatid fluid of *Drosophila melanogaster* Meig. *J. Microscopie* **5**: 265-268.
- BROSSEAU, G. E. JR., 1960 Genetic analysis of the male fertility factors on the Y chromosome of *Drosophila melanogaster*. *Genetics* **45**: 257-274.
- KAPLAN, W. D., V. E. TINDERHOLT, and D. H. GUGLER, 1962 The number of sperm present in the reproductive tracts of *Drosophila melanogaster* females. *Drosophila Inform. Serv.* **36**: 82.
- KAUFMANN, B. P., and M. DEMEREC, 1942 Utilization of sperm by the female *Drosophila melanogaster*. *Am. Naturalist* **76**: 445-469.
- KIEFER, B. I., 1966 Ultrastructural abnormalities in developing sperm of X/O *Drosophila melanogaster*. *Genetics* **54**: 1441-1452.
- LEFEVRE, G., JR., and U. B. JONSSON, 1962 Sperm transfer, storage, displacement, and utilization in *Drosophila melanogaster*. *Genetics* **47**: 1719-1736.
- MEYER, G. F., 1964 Die parakristallinen Körper in den Spermienschwänzen von *Drosophila*. *Z. Zellforsch.* **62**: 762-784. — 1968 Spermiogenese in normalen und Y-defizienten Männchen von *Drosophila melanogaster* und *D. hydei*. *Z. Zellforsch.* **84**: 141-175.
- NAGANO, T., 1963 Fine structural changes in the flagellum of the spermatid in experimental cryptorchidism of the rat. *J. Cell Biol.* **18**: 337-344.
- NOVITSKI, E., and I. SANDLER, 1957 Are all products of spermatogenesis regularly functional? *Proc. Natl. Acad. Sci. U.S.A.* **43**: 318-324.
- PEACOCK, W. J., and J. ERICKSON, 1965 Segregation-Distortion and regularly nonfunctional products of spermatogenesis in *Drosophila melanogaster*. *Genetics* **51**: 313-328.