FINE STRUCTURE OF SCIARA COPROPHILA SPERM

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ABSTRACT

Though the flagellum of Sciara sperm arises from a blepharoplast and is characterized by doublet tubules with arms, it differs markedly from the familiar type of flagella in the number and arrangement of its tubules. The axial filament complex in sperm from the testis of Sciara consists of approximately 70 doublet tubules, each with an associated singlet tubule. Near the nucleus these tubules are displaced in an oval array. Posteriorly the oval breaks and coils from one free end so that the axial filament complex at posterior levels has the form of a spiral. The singlet tubules do not extend the full length of the sperm but terminate in order from inside the spiral. Farther posteriorly the axial filament complex reverses the direction of coiling, and the doublets terminate from outside the spiral. Four arms are specifically positioned on the singlet and doublet tubules. A single mitochondrial derivative extends most of the length of the sperm; it consists of a large mass of proteinaceous material, a crystalloid located adjacent to the axial filament complex, and peripheral cristae. In the female genital tract, sperm undergo gross morphological changes which include sloughing of practically all the mitochondrial material except the crystalloid, repositioning of the crystalloid, and uncoiling and subsequent recoiling of the axial filament complex into a different configuration. From analysis of serial sections it was determined that the orientation of arms, when the axial filament is viewed from base to tip, is the same as in conventional flagella.

INTRODUCTION

Although the size and shape of the spermatozoa of different animals vary greatly, sperm have the same basic morphology throughout the animal kingdom. Among the most invariable of the sperm components is the flagellum or axial filament complex which has the 9 + 2 pattern characteristic of other cilia and flagella. The sperm of certain flatworms differ slightly; their flagella have a 9 + 1 pattern (16). The present report describes the unusual fine structure of sperm of the fungus gnat Sciara coprophila. These cells have a flagellum which is similar to the flagella of other spermatozoa in that it forms from a blepharoplast (see preceding paper) and contains doublet tubules, but is dissimilar in that it consists of about 70 doublet and the same number of singlet tubules which are not arranged in a 9 + 2 pattern. These tubules are disposed in set geometric patterns at various anterior-posterior levels of the sperm and bear specific spatial relationships to other cytoplasmic components. During maturation in the female genital tract, the sperm flagellum undergoes alterations in form involving not only the tubules of the flagellum but a portion of the mitochondrial derivative (9).

There are several known cases in which the motile apparatus of sperm is anomalous (11, 13, 16); however, these all involve accessory microtubular or membranous systems rather than modifications of the flagellum itself. There are also a few cases of aberrant or mutant flagella and cilia which differ very slightly from the 9 + 2 pat-
tern (1, 10, 14). The axial filament complex of Sciara sperm is, to our knowledge, the only true flagellum (composed of doublet tubules and arising from a blepharoplast) which differs markedly from the 9 + 2 pattern. There have been relatively few fine structural studies of invertebrate sperm, and many other flagellated and ciliated invertebrate tissues have yet to be investigated. As more such studies are done, we may expect to find other variations on the conventional flagellar theme.

MATERIALS AND METHODS

Tissues were dissected in cold (0 to 4°C) OsO₄. Best results were obtained when the testes or spermathecae were cut into several pieces in the fixative. This was especially true of spermathecae, owing to their chitinous lining which, when intact, prevents proper fixation and subsequent infiltration. The fixative was buffered with 0.1 M Sorensen's phosphate buffer. Tissues were fixed for 1 to 2 hr in the cold, dehydrated in cold ethanol, and embedded in Epon 812 according to Luft (8). Sections were cut on a Porter-Blum MT-1 ultramicrotome, stained in 3% aqueous uranyl acetate (6 to 24 hr), poststained with lead citrate employing the method of Reynolds (12) or Venable and Coggeshall (17), and examined with a Siemens Elmiskop I.

RESULTS

The Imaginal Testis

LIGHT MICROSCOPE OBSERVATIONS

Spermatozoa are long tapering cells possessing a small, elongate, anteriorly located, Feulgen-positive nucleus and a single mitochondrial derivative which extends most of the length of the cell (Fig. 1). After sperm were stained with fast green at pH 2 for protein, a thin, strongly-staining stripe was visible along the length of the less heavily staining portion of the cell. Since we know from electron micrographs of the sperm that the cross-sectional area of the axial filament is considerably smaller than that of the mitochondrial derivative, we believe that the thin stripe of densely stained proteinaceous material is the axial filament. The less heavily staining component is the mitochondrial derivative.

ELECTRON MICROSCOPE OBSERVATIONS

THE AXIAL FILAMENT COMPLEX: The axial filament complex extends caudad from an oval groove in the nuclear membrane for the en-
FIGURE 3  Enlargement of the axial filament complex of cell A in Fig. 2. Four arms can be discerned on each set of these tubules: one prominent arm on subfiber B which is directed towards the cell membrane and perpendicularly to the long axis of the doublet; one arm of the peripheral singlet points towards the arm on the adjacent subfiber B; and two small arms on subfiber A are directed towards subfiber B of the adjacent doublet. This section was cut caudally to the termination point of the last three singlets and the arms on the adjacent three B subfibers in the spiraling gyre ($n_1$). × 125,000.

tire length of the cell. In cross-section near the nucleus the axial filament complex takes the form of an indented oval (Fig. 2, cell B). It consists of about 70 doublet tubules each with a peripherally associated singlet tubule (Fig. 2). In the posterior portions of the cell, the singlet is lacking. One member of the doublet pair is larger, more clearly defined, and less dense centrally than the other tubule of the doublet. This larger tubule, subfiber B, bears an arm directed away from the center of the oval and perpendicular to the cell membrane. Subfiber A, the smaller tubule of the doublet pair, has two smaller arms which are directed towards subfiber B of the adjacent doublet. The singlet also has an arm which is directed towards the arm on subfiber B (Fig. 3). The singlet is slightly larger in cross-sectional diameter than subfiber B of the doublet.

In cells sectioned farther caudal, the oval appears broken at one point, and the axial filament forms two spirals, one from each free end (Fig. 3). One of the pair of spirals, the gyre farthest away from the mitochondrial crystalloid, continues to coil at farther posterior levels while the other portion of the double spiral, the gyre nearer to the mitochondrial crystalloid, simultaneously uncoils (Fig. 4). As a result of this coiling and uncoiling, the axial filament at a more caudal level is disposed in a single spiral (Figs. 5 and 6).

At the level where the oval of the axial filament breaks and begins the spiralization-despiralization process, a few axial filament singlets are seen to terminate (Fig. 3). The singlets terminate more or less in order, commencing from the end of the axial filament where spiralization occurs. As each singlet terminates, the arm on subfiber B of the adjacent doublet disappears. Thus, in a cross-section of a cell in which some doublets are lacking both the singlets and arms on subfiber B these doublets always occur in a row at the spiraling end of the double spiral (cells A and C of Fig. 4). A clear region appears around each doublet immediately caudal to the place where the associated singlet terminates (Figs. 3 and 4).
In the most posterior regions of the spiral, the innermost subfiber of each doublet is subfiber A (Figs. 8 to 10) whereas in the anterior regions the innermost subfiber is subfiber B (Figs. 5 and 6). This reversal of direction is accomplished through an intermediate stage where the spiral is double, apparently spiraling from one end and despiraling from the other. Such double spirals may be seen in sections cut in posterior regions of the cell (Fig. 7).

In the most posterior region, part or all of subfiber B of the outermost doublet of the spiral is often missing (Figs. 8 to 10). Sometimes all of subfiber B and part of subfiber A are missing from the outermost doublet. Therefore, doublets apparently terminate in order from the outside of the spiral. Subfiber B of each doublet terminates before subfiber A.

Dense bodies occur in the center of part of the anterior portion of the axial filament complex (Fig. 2). These bodies extend from the nucleus caudad to the level where the oval becomes discontinuous and the axial filament complex describes a double spiral (Fig. 2). A smooth double membrane encompasses the row of dense bodies (Figs. 2 and 11). Discontinuities occur in this double membrane and the interstices are sometimes dilated (Fig. 3, cell C).

The mitochondrial derivative: The single mitochondrial derivative which occupies most of the volume of the testicular spermatozoon is composed of three distinct components: homogeneous material; cristae; and crystalloid. These components are discussed separately.

The mass of the mitochondrial derivative consists of a homogeneous, moderately electron-opaque material (Fig. 2). This material stains moderately heavily with fast green at pH 2, indicating that it is proteinaceous. The material is negative for the PAS reaction and Sudan stain, carried out after various types of fixation (acetic-alcohol, alcohol, OsO4).

A thin rim of cristae composes the periphery of the mitochondrial derivative. Although the rim is narrow, the total volume of cristae is probably considerable because the organelle is so large.

A crystalloid extends the length of the mitochondrial derivative and projects anteriorly into the nucleus (Fig. 12) where it is separated from the

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**Figure 4** If an observer follows the row of tubules of the axial filament complex (i.e. in cell A or C) from the spiraling gyre (s1) of the double coil to the despiraling gyre (s2), it is apparent that singlets and arms on subfiber B always terminate in order from the s1 gyre. In cell B the axial filament complex has coiled out instead of in. The axial filament has broken sharply in two places. The occurrence of aberrant breaking of this type leads us to believe that the tubules of the axial filament complex are connected by a rigid material. × 30,000.

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nucleoplasm by the mitochondrial and nuclear membranes. Farther caudad the crystalloid is always located in the portion of the mitochondrial derivative adjacent to the part of the indented oval which contains the dense solids (Fig. 2), and still farther caudad it is oriented adjacent to the despiraling gyre of the double spiral (Figs. 2 and 4). Posterior to this the crystalloid is adjacent to the outermost few doublets in the single spiral of the axial filament (Fig. 6). In longitudinal section the crystalline material of the mitochondrial derivative has a herringbone pattern (Fig. 11). The center-to-center spacing of the dense lines is 90 Å and the distance from peak to peak is 450 Å (Fig. 11). Cut in cross-section, the crystalloid appears as hexagonally packed circles or hexagons with a center-to-center spacing of 90 Å (Fig. 12). Obliquely sectioned, the crystalloid appears as parallel lines with a center-to-center spacing of 90 Å (Fig. 13).

THE NUCLEUS: The spermatozoan nucleus is about 1 μ in diameter and 20 μ in length (Fig. 14). The nucleus tapers anteriorly where an acrosome fits into a nuclear groove (Figs. 14 and 15). An oval posterior nuclear groove accommodates the tubules of the axial filament complex. The nucleus also possesses two prominent lateral grooves which extend the entire length of the nucleus. Membranous whorls which appear during nuclear condensation (see preceding paper) are present in the grooves (Fig. 5). In longitudinal section the whorls appear as faint lines (Fig. 17). They bear no direct relation to the axial filament complex. The nucleus often contains spherical areas of very dense chromatin which may be surrounded by a region of low electron opacity.

THE ACROSOME: A minute acrosome about 2 μ in length and 0.5 μ in diameter is located at the anterior end of the Sciara spermatozoon where it fits into a groove in the nuclear membrane (Figs. 14 to 16). The acrosome is homogeneous and moderately electron opaque. No membranes such as have been described in spermatozoa of other species (5) were resolved around the acrosome.

Maturation of Spermatozoa after Insemination

LIGHT MICROSCOPE OBSERVATIONS

Once two flies begin copulation, they will usually continue in copulo even if picked up and placed under a cover slip. Spermatozoa showed no observable autonomous movement during insemination when viewed with high-dry phase optics. Active sperm movement was observed only during oviposition. Adult gnats live but a few days, and inseminated females generally retain spermatozoa for almost their entire lifetime. Fertilization and oviposition take place simultaneously shortly before death (4), but can be induced in old inseminated females by injury (i.e., by amputating wings or legs). Biocuting impregnated females at the level of the thorax serves as a strong inducment to oviposition (E. Rasch, personal communication). At the time of oviposition, sperm are activated by the female, and within a few seconds of the onset of egg laying the spermatozoa inside the female seminal receptacles move so rapidly that it is difficult to resolve individual sperm cells.

When the spermathecae were teased apart or squashed in saline, some spermatozoa moved for several minutes, but most cells were immobilized immediately or after a few seconds. Numerous attempts to obtain a medium in which sperm remain active have failed. We have, therefore, been able to observe only the more obvious characteristics of sperm movement. Motile spermatozoa are long, needle-shaped cells which do not appear to taper along most of the length of the cell (Fig. 18). It is evident that these sperm are very rigid and are incapable of complex contortions.

Figure 5 Cross-section of a nucleus showing whorled configurations in the lateral grooves (arrows). The whorls bear little resemblance to the axial filament complex, which can be seen in the adjacent cell, and are not directly related to the axial filament complex. X 54,000.

Figure 6 Cross-section of the caudal portion of several spermatozoa. The axial filament complex is disposed in a single spiral. Note that the mitochondrial crystalloid in each cell is oriented near the last few tubules of the axial filament spiral. The section is cut at a level posteriorly to the termination point of the singlets and the arm on subfiber B. The two arms on subfiber A remain. X 50,000.
FIGURE 7 In the posterior region of the testicular spermatozoan the axial filament complex undergoes a reversal of direction. As a result of this, the innermost doublet becomes the outermost doublet and the direction of coiling is reversed. × 53,000.

FIGURES 8, 9, and 10 In cross-sections very near the posterior end of the cell it is always the outermost doublet (arrows) which appears as a partial doublet or partial singlet thus demonstrating that the doublets terminate in order from outside the spiral of the axial filament complex. Here the innermost subfiber of each doublet is subfiber A whereas in anterior regions the innermost subfiber is subfiber B. Subfiber B terminates before subfiber A. × 77,000.

ELECTRON MICROSCOPE OBSERVATIONS
THE MITOCHONDRIAL DERIVATIVE: The cristae and the homogeneous, moderately electron-opaque component of the mitochondrial derivative are sloughed during maturation of the spermatozoon in the spermatheca. This sloughed material occupies most of the volume of the spermatheca. The crystalloid, which extends most of the length of the mature spermatozoon, is not sloughed during maturation but is left with a surrounding double membrane resembling the mitochondrial double unit membrane. Anteriorly the crystalloid projects into the nucleus. Directly caudal to the nucleus the crystalloid lies adjacent to the axial filament complex (Fig. 19, cell A, and Fig. 20). At more caudal levels the axial filament is partially wrapped around the crystalloid (Fig. 19, cells B and C, and Fig. 21), and at still more posterior levels it completely encircles the crystalloid so that the double-membrane-bounded crys-

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FIGURE 11 Longitudinal section of the axial filament complex and adjacent mitochondrial crystalloid. The row of dense bodies (b) in the center of the axial filament complex is bounded by a double membrane (arrows). In favorable longitudinal sections the crystalloid has a herringbone pattern with a center-to-center spacing of 90 Å and a peak-to-peak spacing of 450 Å. Fig. 11, × 74,000; inset, × 173,000.
FIGURE 12 Cross-section through a portion of a sperm nucleus. The mitochondrial crystallloid extending into the nucleus appears as hexagonally packed circles or hexagons. Center-to-center spacing is 90 Å. X 151,000.

FIGURE 13 Cut obliquely, the mitochondrial crystallloid is seen as parallel lines with a center-to-center spacing of 90 Å. X 151,000.

taloid lies in the center of the axial filament complex (Fig. 19, cell D, and Fig. 22).

THE AXIAL FILAMENT COMPLEX: In the anterior part of the cell the axial filament complex is composed of two rows of tubules (doublets and associated singlets) arranged in spiral configuration (Fig. 17, cell A). The displacement of singlets and doublets with respect to each other can be seen in Fig. 20. The dense double-membrane-bounded row of spheroids is located in the center of the spiral.

Farther caudad the axial filament is partially wrapped around the crystallloid (Fig. 19, cell B), and slightly more posteriorly the dense spheroids and their associated double membrane terminate and, at the same level, the crystallloid becomes surrounded by the tubules of the axial filament (Fig. 19, cell C). Still farther posteriorly, in contrast to the testis spermatozoa in which the singlets terminate in order starting from the inside of the spiral, the singlets terminate more or less in order from the outside of the spiral simultaneously with the termination of the arm of subfiber B of the adjacent doublet (Fig. 21). Farther caudad where all the singlets have terminated, the membrane-bounded crystallloid is located in the center of the spiral. As in testicular spermatozoa, the two arms on subfiber A do not terminate before the subfiber bearing them does (Fig. 22).

THE NUCLEUS: During maturation in the female genital tract, the cell membrane and the "whorls" are no longer seen with methods that revealed them in the testicular spermatozoon (Fig. 23). The acrosome is apparently unchanged.

DISCUSSION

The morphology of Sciara sperm before and after maturation in the spermatheca is diagrammed in Figs. 24 and 25, respectively.

In low-power micrographs including cross-sections of the axial filament complex of several spermatids (i.e. Fig. 15 of the preceding paper), it is evident that neighboring axial filament complexes spiral in the same direction (i.e., are all either right-handed or left-handed spirals). This is also usually true of spermatozoa in the testis of mature flies (Figs. 2 and 6). Therefore, we assume that spermatids and spermatozoa are oriented in
FIGURE 14 Anteriorly (right) a small acrosome is contained in an indentation in the nuclear membrane. Posteriorly (left) an oval groove accommodates the tubules of the axial filament complex. \( X \ 7000 \).

FIGURE 15 The acrosome (a) in longitudinal section. \( X \ 28,000 \).

FIGURE 16 The acrosome (a) in cross-section partially surrounded by the nucleus. \( X \ 47,000 \).

FIGURE 17 In longitudinal section the whorl of the longitudinal nuclear groove (arrows) appears as faint lines less electron-opaque than the tubules of the axial filament complex (ax). \( X \ 47,000 \).

the testis with anterior ends of adjacent cells facing the same direction and that the axial filament complex has the same enantiomorphic form in all cells.

The direction of coiling in the spermathecal spermatozoon is invariably the mirror image of the direction observed in the anterior portion of the testicular sperm. Fig. 26 depicts schematically the rearrangement that the axial filament undergoes in the spermatheca. This is the only rearrangement which will result in singlets lying outside instead of inside each doublet, and in subfibrils A and B in the relative positions which are observed. As a result of these contortions the innermost doublet of the testicular spermatozoon becomes the outermost doublet of the spermathecal spermatozoon and the enantiomorphic form is reversed. The direction of the arms on subfiber A, however, is the same. The form of the axial filament complex in the spermathecal spermatozoon is identical with
FIGURE 18 Phase-contrast photomicrograph of spermatozoa immediately after being teased into saline from the spermatheca of a female fly 2 days after insemination. The cells are needle-shaped and very rigid. When not tangled with other cells, they are almost perfectly straight (regardless of the tonicity or pH of the saline). When bent by contact with other cells, they form smooth arcs. X 425.

The form observed in the most posterior region of the testicular spermatozoon.

In the mature spermatozoon from the female genital tract, the axial filament complex spirals virtually the entire length of the cell. The direction of coiling cannot be determined from any single electron micrograph since we do not know in a given case whether the anterior ends of the cells are above or below the plane of section. When a spermatozoon is cut in cross-section at slightly different levels, the axial filament may appear wrapped more fully around the crystalloid in some sections than in others. We know that the farther caudad we look in the cell, the farther the axial filament will have wrapped around the mitochondrial crystalloid (see diagram, Fig. 25). It is also true that the more singlets which have terminated, the farther caudal the level of the section. Figs. 27 and 28 are taken from a series of serial cross-sections all placed with the same side up on grids.
FIGURE 20  Anterior portion of the axial filament complex from a sperm 2 days after insemination.

FIGURE 21  Singlets terminate in order from outside the spiral. The singlets and arms on subfiber B are in evidence up to a point (arrow), after which all singlets and adjacent arms on subfiber B are missing.  × 48,000.

FIGURE 22  In the posterior part of the cell the double-membrane–bounded crystalloid lies in the center of the axial filament complex.  × 79,000.

FIGURE 23  In the spermatheca whorled configurations are apparently obliterated by the close apposition of the plasma membrane to the nuclear membrane in the lateral grooves.  × 16,000.

numbered in the order that they were cut from the block. The section in Fig. 27 was cut before that in Fig. 28. The sections shown are sufficiently distant from each other to show significant differences in the degree of coiling of the axial filament complex around the crystalloid and in the number of doublets lacking associated singlets. The plane of section of Fig. 27 transects cells A and C more posteriorly than the plane of section of Fig. 28, since in Fig. 27 the axial filament complex is...
FIGURE 24 Diagrammatic representation of a sperm from the testis of an adult fly. Discontinuities in the diagram indicate that the cell is much longer, relative to its width, than depicted. An axial filament of singlets and doublets is represented as two rows of dots and of doublets alone as one row of dots. Acrosome, A; nucleus, N; mitochondrial crystalloid, CR; mitochondrial homogeneous material, H.

FIGURE 25 Diagrammatic representation of a sperm in the spermatheca 2 days after insemination. Symbols are the same as in Fig. 24.
Figure 26 Diagrammatic representation of the changes undergone by the axial filament complex during maturation in the female genital tract. The diagram has been drawn from the point of view of an observer looking down the spermatozoon shaft from tip to base. Given that the innermost subfiber of each doublet is subfiber B along most of the length of the testicular spermatozoon and subfiber A in the spermathecal spermatozoon and that the singlets lie outside rather than inside the doublets in both testicular and spermathecal sperm, the axial filament complex must rearrange as depicted in the figure. This is identical to the uncoiling and recoiling which is observed in the most caudal region of testicular spermatozoa. As a result of the rearrangements, the innermost doublet of the testicular spermatozoon becomes the outermost doublet of the spermathecal spermatozoon, and the resulting spiral is the mirror image. The direction of the arms on subfiber A remains the same (counterclockwise when viewed from tip to base).

Subfiber A, A; subfiber B, B; singlet, S.

farther wrapped around the crystallloid. However, in Fig. 27, cell B has been cut further anteriorly than it has in Fig. 28, since all doublets possess a singlet in Fig. 27, but some outer singlets are missing in Fig. 28. This is to be expected if cells A, B, and C all spiral in the same direction, since the spiral in cell B is the mirror image of the spiral in cells A and C. The sections were picked up from above on the underside of the grid so that viewing the grid from above was equivalent to looking into the block from the front. The grids were placed section-side-down in the microscope. The plates were printed with the emulsion side up, so the prints appear as the image did on the microscope screen. (Electron microscopes and photographic enlargers do not produce mirror images.) Therefore, if Fig. 27 were held in front of Fig. 28, the two figures would be oriented as the sections were in the block. Cell C of the two figures appears as an observer would see it when viewing the sperm from head to tail. Figs. 20, 21, and 22 are also presented as if a viewer were observing the sperm from head to tail. Note that the arms on subfiber A point in a clockwise direction. We have found this to be true in all cells in which comparisons of serial sections can be made. Therefore, we feel that we are correct in our assumption that the axial filament spirals in the same direction in all spermatozoa in the spermatheca. Gibbons and Grimstone (7) observed that in conventional cilia the arms on subfiber A also point in a clockwise direction when the cilium is viewed from base to tip.

Satir (15), working with cilia of the gill filaments in the fresh water mussel Elliptio, has shown that the termination point of the nine peripheral fibers with respect to each other is dependent on the position of the cilium (effective or recovery stroke). He concluded that the fibers slide with respect to one another during the ciliary beat. One might argue that in Sciara spermatozoon the singlet fibers may be approximately the same length and only appear to terminate at different points because the cell coils and thereby causes the singlet fibers to slide with respect to one another. Since the arm on subfiber B of the doublet adjacent to each singlet terminates simultaneously with the singlet, one would conclude that the doublets may also all be approximately the same length and that their orderly termination in the posterior regions of the cell is produced at least partially by sliding. If this were true, one would expect that in the
Figures 27 and 28 Serial sections of the same three spermatozoa. By means of such serial sections we have determined the direction of coiling of the axial filament complex (see text). × 54,000.
testicular spermatozoon, where the direction of coiling reverses in the posterior regions of the cell, the doublets would terminate in order from outside the spiral. This is indeed the case; thus it is not possible to determine whether the termination of fibers at different levels is due to a sliding or to an actual difference in length of the tubules.

Although the number and size of mitochondria vary considerably in different species, mitochondria or mitochondrial derivatives are almost universally present in motile spermatozoa (1) and probably function in energy utilization for motility. Sciara sperm have only one mitochondrial derivative, and the major part of it, consisting of the moderately electron-opaque material and the cristae, is sloughed in the spermatheca before the sperm become motile (see Figs. 24 and 25). Only the double-membrane-bounded crystalline component and a few cristae remain as part of the spermatozoon. It is possible that the mitochondrial crystalloid represents a mitochondrial enzyme or enzymes in high concentration, as there is no other mitochondrion in motile Sciara sperm.

Boveri (2) theorized that the essential aspect of fertilization was the introduction of the sperm centrosome (centriole) into the egg. Although the centriole is no longer considered the principal element in egg activation, the role of the sperm centriole is still unclear. Many classical light microscope studies indicate that sperm centrioles are brought into the egg at fertilization, and Wilson states that "something is introduced into the egg by the middle-piece of the sperm that either is a central body or has the power to incite the formation of one" (18). Fine structural observations of vertebrate sperm lend some support to this view (5, 6). Spermatozoa have two centrioles. One, the distal centriole, serves as a basal body for the flagellum and becomes greatly modified. The proximal centriole, however, retains the characteristic centriole morphology and does not appear to be involved in the structure of the axial filament complex. This centriole is perhaps transported into the egg. The facts, however, that centrioles occur in cytasters of unfertilized sea urchin eggs (3) and are almost certainly present in parthenogenic animals, provide evidence that centrioles not of sperm origin, which are either present or formed within the activated egg, are capable of supporting cleavage. The spermatozoa of Sciara coprophila have only one centriole. This centriole, which serves as a blepharoplast for the axial filament complex (see preceding paper), is not a conventional centriole but a very odd one composed of many tubules. Because more or less typical 9-membered centrioles do occur in somatic tissues of Sciara embryos (see preceding paper), they must derive either from the giant centriole of the sperm, from centrioles already present in the unfertilized egg, or from de novo centriole formation at the time of fertilization.

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