

# EXCEPTIONS TO THE PREVAILING PATTERN OF TUBULES (9 + 9 + 2) IN THE SPERM FLAGELLA OF CERTAIN INSECT SPECIES

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## ABSTRACT

Various deviations from classical 9 + 2 flagellar structure are found in sperm of insect species. In mature spermatozoa of a psocid, *Psocus*, the outer flagellar tubules are not straight, but are disposed in a long-pitched helix such that they form an angle of about 8° with a single dense rod located in the position usually occupied by the central pair. In young spermatids of *Psocus* the outer tubules are straight; thus, spiraling of the flagellar tubules occurs during the course of spermiogenesis. Spiraling of flagella also occurs in the cat flea *Ctenocephalides felis*. Variations in the number and morphology of the central element or elements occur in other insect species besides *Psocus*. Among the observed deviations from a central pair of tubules are a 9 + 0 tubule pattern in the sperm of three species of mayflies, a 9 + 1 tubule pattern in the sperm of two species of mosquitoes, and 9 + 7 tubules in sperm of two species of caddis flies. Spermatozoa of treehoppers vary in yet another respect from the typical 9 + 9 + 2 insect flagellum. These sperm tails branch into four long tails, three of which each contain two doublet and two singlet tubules while the fourth branch contains three doublet and three singlet tubules. The wide distribution of insects with aberrant flagella suggests that the variant forms have evolved independently.

## INTRODUCTION

With rare exceptions, flagella capable of autonomous motility have the same basic structure, namely a central pair of singlet tubules surrounded by nine parallel doublet tubules. The universality of this pattern in animals and plants suggests that this configuration is uniquely favorable for flagellar motility. Flagella showing deviations from the 9 + 2 pattern are interesting not only as cytological curiosities, but for the clues they provide for identification of those aspects of flagellar structure that are dispensable and those which may not vary without resulting in loss of function. Insect spermatozoa are a useful material in which to search for such deviations, since insects afford a wealth of species variation. Some

700,000 insect species are known which, in the course of 300 million years of evolution, might be expected to have developed some variation in flagellar structure, if, indeed, such variation is consistent with motility.

As will be reported in another paper,<sup>1</sup> sperm flagella of most insect species conform to the 9 + 2 pattern present in typical flagella and cilia of animal cells, but possess, in addition, a peripheral row of nine singlet tubules located outside the nine doublets. These peripheral singlets are in a position analogous to that of the nine outer dense

<sup>1</sup> Phillips, D. M. 1969. Insect spermiogenesis. Submitted for publication.

fibers of mammalian spermatozoa (6, 8) and they may have similar functions. It is noteworthy, however, that in insects they are tubular whereas in mammals they are solid and dense. In a survey of sperm of approximately 185 insect species, several significant variations from the typical 9 + 9 + 2 arrangement of tubules have been encountered and are described in this report.

#### MATERIALS AND METHODS

Most of the insects examined were collected in the vicinity of Boston, Massachusetts.<sup>2</sup> Testes were dissected in cold fixative, usually within a few hours after the specimens were collected. Testes were fixed for 2-4 hr in 5% glutaraldehyde (Eastman Chemicals, Rochester, N. Y.) buffered with 0.2 M collidine (Eastman) at pH 7.4. Testes were then transferred through several changes of cold collidine buffer (10-30 min) and postfixed in 1% collidine-buffered osmium tetroxide. After dehydration in alcohol, tissues were embedded in Epon (13), sectioned on a Servall MT-1 ultramicrotome, stained in 3% aqueous uranyl acetate and lead citrate (26), and examined in a Siemens Elmiskop I.

#### RESULTS

##### *Flagella in Which the Outer Fibers Are Not Parallel to the Long Axis of the Flagellum*

*PSOCUS* SP.: In longitudinal sections of spermatozoa of *Psocus* sp. (Psocoptera), the flagellar

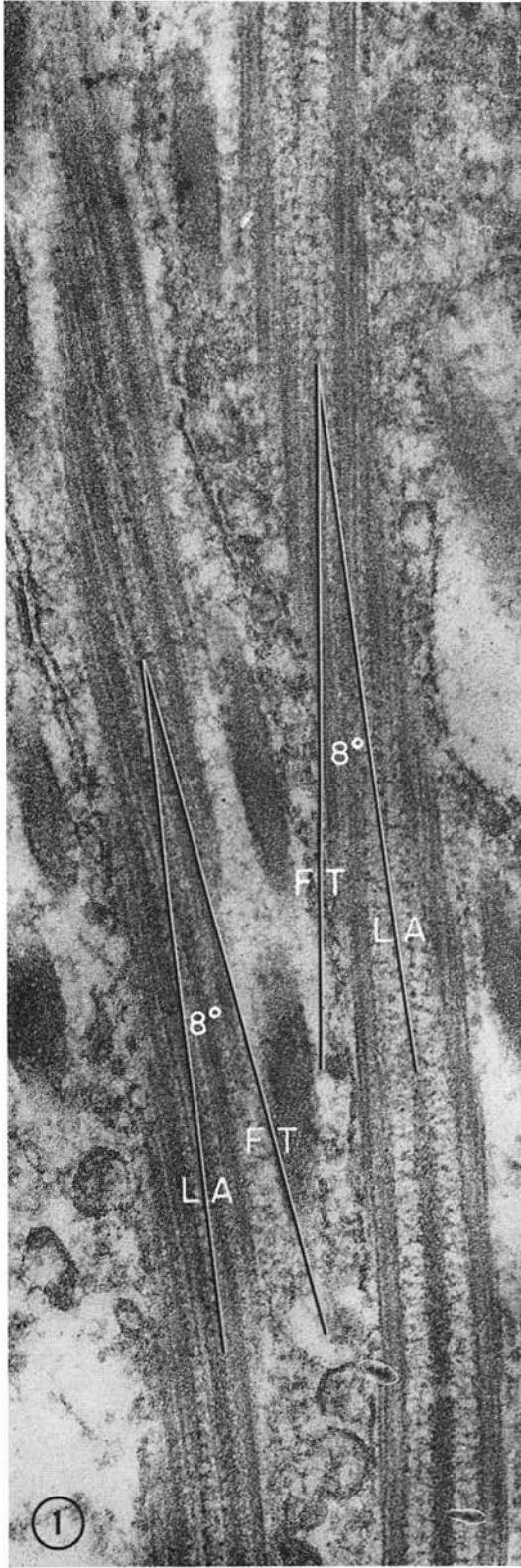
<sup>2</sup> We have examined insects of the following orders: Apterygota, Ephemeroptera, Odonata, Orthoptera, Dermaptera, Psocoptera, Hemiptera, Homoptera, Neuroptera, Coleoptera, Trichoptera, Lepidoptera, Diptera, Siphonaptera, and Hymenoptera. Insects described in this paper were classified according to the following sources: Needham, J. G., J. R. Traver, and Yin-Chi Hosu, *The Biology of Mayflies*, Comstock Publishing Associates, Ithaca, N.Y., 1935. Banks, N., *A Classification of the Psocidae*, *Psyche*, 36, 1929. Britton, W. E. et al., *The Hemiptera or Sucking Insects of Connecticut*, *Conn. State Geol. Natl. Hist. Survey, Bull.* 34, 1923. Horvath, G., and H. M. Parshley, *General Catalogue of the Hemiptera*, Smith College, Northampton, Mass., Fasc. I., 1927. Bettern, C., *The Caddisflies or Trichoptera of New York State*, *N.Y. State Museum Bull.*, 292, 1934. Ross, H. H., *The Caddisflies or Trichoptera of Illinois*, *Illinois Nat. Hist. Survey Bull.* 23, 1944. Matheson, R., *Handbook of the Mosquitoes of North America*, Comstock Publishing Associates, Ithaca, N.Y., 1944. Fox, I., *Fleas of Eastern United States*, Iowa State Press, 1940.

tubules are seen to form an angle of about 8° with the long axis of the flagellum (Fig. 1). In sections which are cut so that the tubules on one side of the central element appear in cross-section as clearly defined circles, the tubules on the opposite side of the central element appear blurred (Fig. 2), and are therefore, obliquely inclined with respect to the plane of the section, so that the hole in the center of the tubule is obscured. Thus, the nine doublet and nine peripheral singlet tubules of *Psocus* spermatozoa are clearly not parallel to the long axis of the flagellum.

In young spermatids of *Psocus*, cross-sectional profiles of the flagellar tubules are similar in appearance to those seen in other species, i.e. all tubules of a flagellum appear to be parallel to each other and to the long axis of the flagellum (Fig. 3). Only in late spermatid stages (Fig. 4) are the tubules realigned into the arrangement characteristic of mature spermatozoa.

The sperm of *Psocus* also deviate from the typical 9 + 9 + 2 insect tubule pattern in the morphology of the central element. Instead of a pair of central tubules, *Psocus* sperm flagella possess a single central dense rod (Fig. 2). Young spermatids, however, possess no central element at all (Figs. 3), and late spermatids in which the rearrangement of tubules has occurred may still lack a central element (Fig. 4). Thus, the dense central rod appears quite late in spermiogenesis after the peripheral tubules have already assumed the configuration characteristic of mature sperm.

*CTENOCEPHALIDES FELIS*: Cross-sections of sperm flagella of the cat flea *Ctenocephalides felis* (Siphonaptera) are reminiscent of those of *Psocus* in that some flagellar tubules always appear blurred when others in the same cell appear in true transverse section (Fig. 5). However, it is apparent from longitudinal sections that the sperm of *C. felis* are morphologically more complicated than the spermatozoa of *Psocus*. When the mitochondrial derivative appears in longitudinal section, the flagellum appears as obliquely sectioned tubular profiles alternating in position between one side of the mitochondrial derivative and the other. In instances in which the flagellar tubules are seen in longitudinal section, they appear to be oblique to the long axis of the flagellum itself (Fig. 6). An additional unusual feature of mature flagella of *C. felis* sperm is the absence of the nine peripheral singlet tubules which generally characterize insect sperm flagella.<sup>1</sup>



### Variations in the Central Elements

**CADDIS FLIES; A 9 + 7 TUBULE PATTERN:** Testes of nine species of caddis flies (Tricoptera) were examined. Testes of all individuals examined apparently contain only mature spermatozoa; spermiogenesis probably occurs during pupation. Sperm of seven caddis fly species possess typical 9 + 9 + 2 flagellar tubule patterns. However, two species, *Polycentropus* sp. and *Hydrosyche* sp., possess unusual flagellar tubule patterns. In cross-sections of spermatozoa of these two species, seven tubules are found in the position usually occupied by the central pair of tubules (Fig. 7). One of these seven tubules is truly central and the other six are symmetrically disposed around it.

Caddis fly sperm possessing the 9 + 7 tubule pattern are morphologically peculiar in several other respects. Spermatozoa of both species lack the nine peripheral singlet tubules which characterize almost all mature insect sperm flagella which we have observed or which have been described (3-5, 10-12, 16, 27, 28), except for the peculiar sperm of the scale insects (17, 22) and the flea *C. felis*. Roughly concentric lamellar structures loosely encircle the 9 + 7 axial filament of *Polycentropus* (Fig. 7), and a zone of fibrous material occurs in an analogous position in *Hydrosyche*. In addition, sperm of these species lack a crystalloid-containing mitochondrial derivative such as is found in most insect sperm.

**MOSQUITOES AND PSOCUS; 9 + 1 FLAGELLAR TUBULE PATTERNS:** The two species of mosquitoes we have examined, *Culex* sp. and *Aedes canadensis canadensis*, possess a single central element. The central element appears more solid than tubular, and the diameter is slightly larger than that of the peripheral singlet tubules or subfibers A or B of the doublet (Fig. 8). The central element is more regularly shaped than the single central element of the flagellum of *Psocus* (Fig. 2) described earlier in this paper. Similar central "fibers" have been described in other species of mosquitoes by Breland, Gassner, Riess, and Biesele (5).

**MAYFLIES; 9 + 0 FLAGELLA:** We have examined mature spermatozoa in the vas deferens

FIGURE 1 Longitudinal section of two sperm flagella of the psocid *Psocus*. Lines (LA) drawn parallel to what appears to be the long axis of the flagellum form an angle of about 8° with lines (FT) drawn parallel to the flagellar tubules. × 63,000.

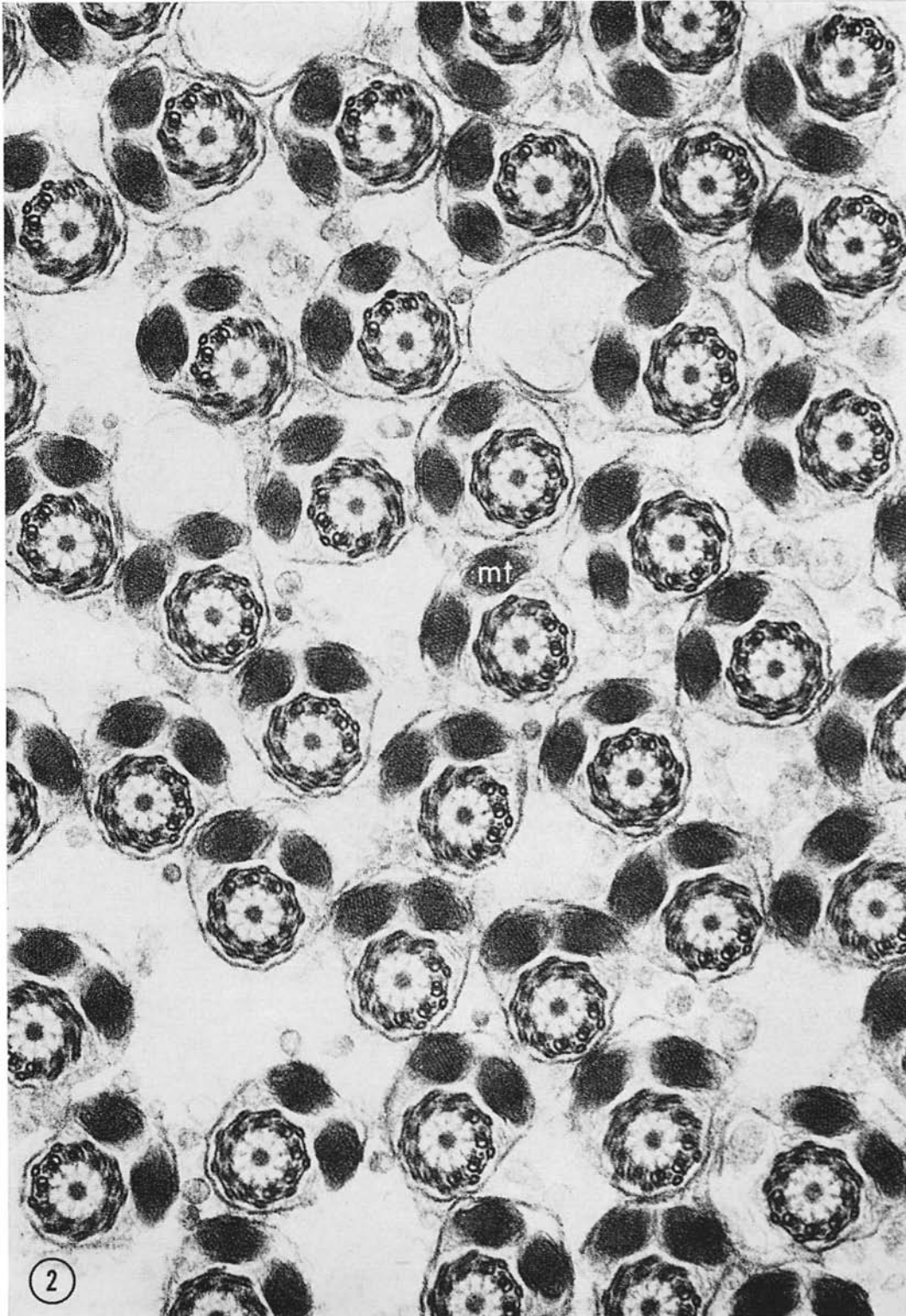


FIGURE 2 In sections which perpendicularly transect the tubules on one side of the sperm flagellum of *Psocus* so that the tubules appear as circular profiles, tubules on the opposite side of the flagellum appear to be oblique to the plane of section. This indicates that the tubules describe a helical course. In this flagellum a dense rod occupies the position usually occupied by the central pair of tubules. Mitochondrial derivative (*m*).  $\times 54,000$ .

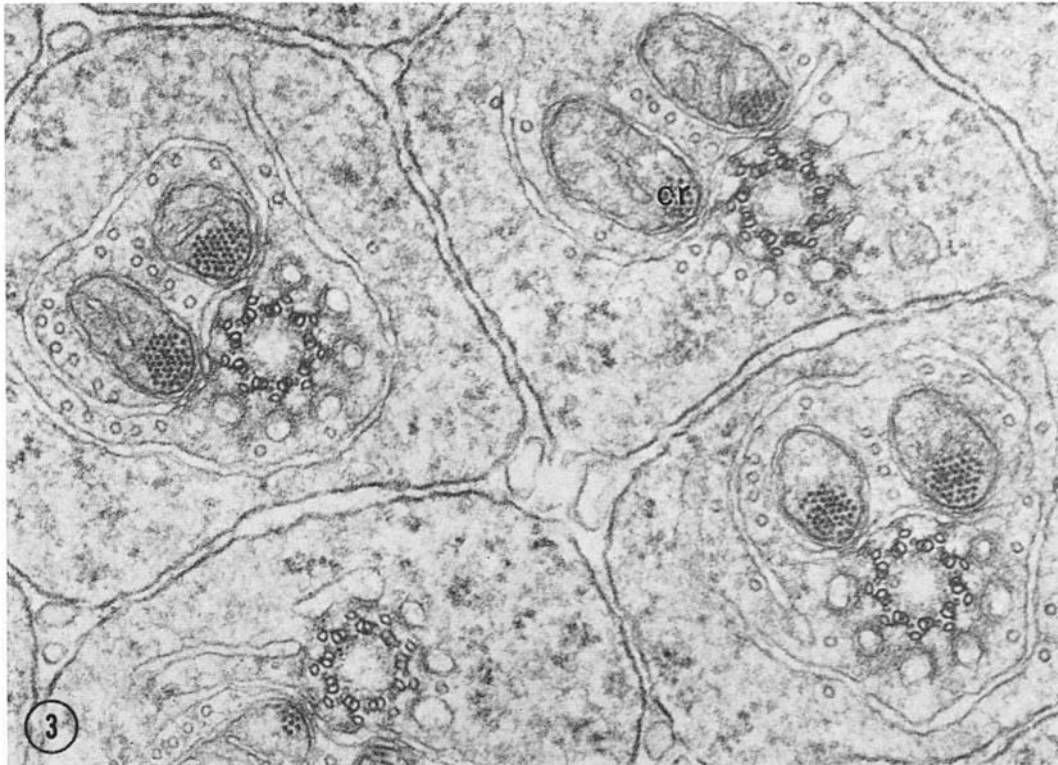


FIGURE 3 Viewed in transverse section, the flagellar tubules of young spermatids of *Psocus* generally all appear as circular profiles. Spiraling is, therefore, thought to occur later in spermiogenesis after the flagellar tubules have formed. The central element forms late in spermiogenesis and is not represented in these cells. Mitochondrial crystalloid (*cr*).  $\times 49,000$ .

of three mayfly (Ephemeroptera) species: *Pentagenia vittigera* (Ephemeridae), *Hexagenia* sp. (Ephemeridae), and *Tricarythodes* sp. (Baetidae) represent two of the three families in this order. The flagella of all three species lack the central pair of tubules. In place of the central pair is a single large, faintly staining cylinder from which faint lines radiate to subfiber A of each doublet (Figs. 9 and 10). The single large central element is much less well defined than the other flagellar tubules and should probably not be considered a flagellar tubule. These flagella of the three mayfly species studied may be considered 9 + 0 flagella. In *Tricarythodes*, subfiber A of the flagellum appears slightly smaller and more irregularly shaped than subfiber A of other insect sperm flagella. This is the only case that we have observed of a deviation from the normal shape of the doublet tubules.

FLAGELLAR BRANCHING: Testes of four

species of treehoppers (Membracidae) were examined: *Ceresa* sp., *Ceresa dicerus*, *Stictocephala* sp., and *Telamona* sp. Living treehopper spermatozoa viewed in Goulden's saline solution (21) with phase optics have the general form of minute cat-o-nine-tails, where a handle-like anterior portion of the sperm branches into four long whip-like tails. The anterior part of the cells appears to beat rapidly with vigorous undulations, and the four thin tails appear to be straight and stiff and move passively.

In some electron micrographs of treehopper testes in which a number of aligned spermatids are seen in cross-section and appear to be slightly out of register longitudinally, several different types of cross-sectional profiles are seen (Fig. 11). Some cells display the typical 9 + 9 + 2 tubule pattern characteristic of insect sperm; presumably, these are cells which have been cut anterior to the point of flagellar branching. Other cross-

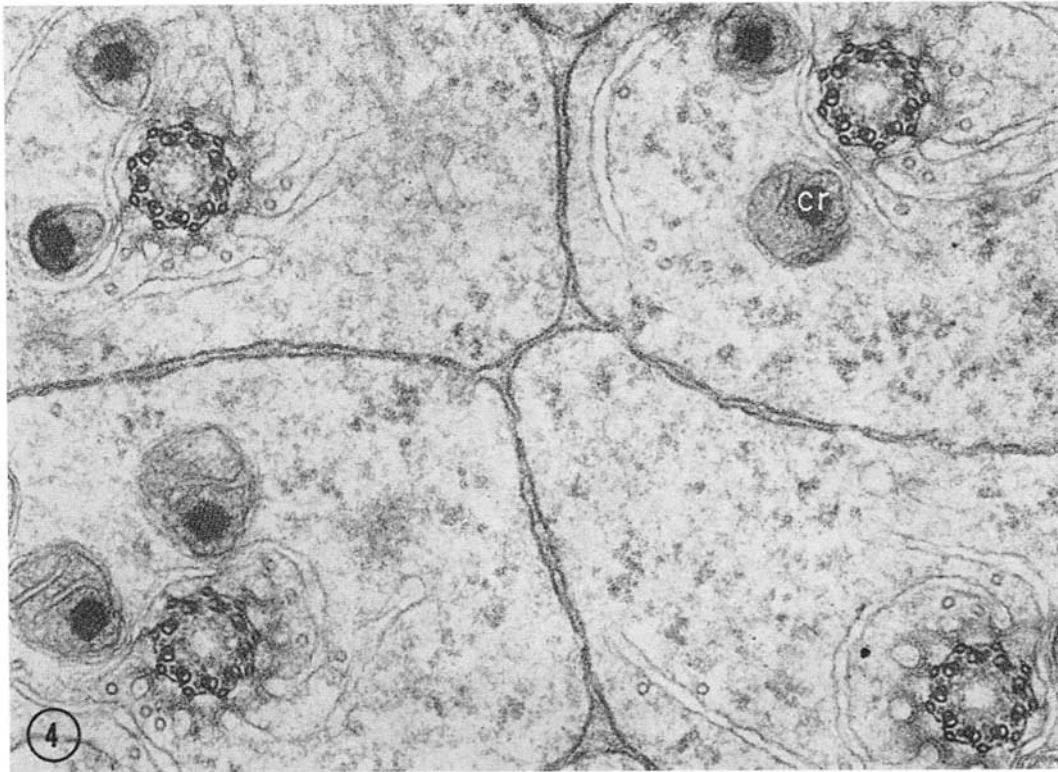


FIGURE 4 Spermatids of *Psocus*. A later stage than that shown in Fig. 2. (The stage of spermiogenesis is determined by the degree of chromatin condensation in nuclei of spermatids in the same cyst). Spiraling has occurred but the central rod has not yet formed at this stage. Mitochondrial crystalloid (*cr*).  $\times 49,000$ .

sectional profiles contain two doublet and two singlet tubules, or three doublet and three singlet tubules—these profiles are believed to represent cross-sections of the four flagellar branches observed in living spermatozoa. In Fig. 11 some transected flagellar branches occur in groups of four. In such groups, three of the four profiles contain two doublet and two singlet tubules and the fourth contains three doublet and three singlet tubules. Fig. 12 illustrates cells which are presumably transected at the level of flagellar branching. Various levels of the process are seen. In micrographs in which numerous cross-sections of flagellar tails are observed, the profiles are of two types: those which contain two doublet and two singlet tubules, and those which have three doublet and three singlet tubules (Fig. 13). (There are less than 1% exceptional flagella—these are assumed to be aberrant). In five separate micrographs of testes of two treehopper species, we

counted the number of profiles with two doublet and two singlet tubules and the number with three doublet and three singlet tubules and found ratios of 68:19 (+ two exceptions), 46:14, 360:124 (+ one exception), 280:91, and 76:21. A total of 1099 tails was counted, of which 830 had two doublet and two singlet tubules and 269 contained three doublet and three singlet tubules, a ratio of about 3:1.

#### DISCUSSION

##### *Helical Arrangement of Flagellar Tubules*

The structure of cilia and flagella has been analyzed in detail (1, 9, 23, 24). To our knowledge, in all cases previously described, all the flagellar (or ciliary) tubules were orientated parallel to the long axis of the flagellum. *Psocus* and *Ctenocephalides* sperm flagella seem to be exceptions to this rule. In longitudinal sections of the sperm

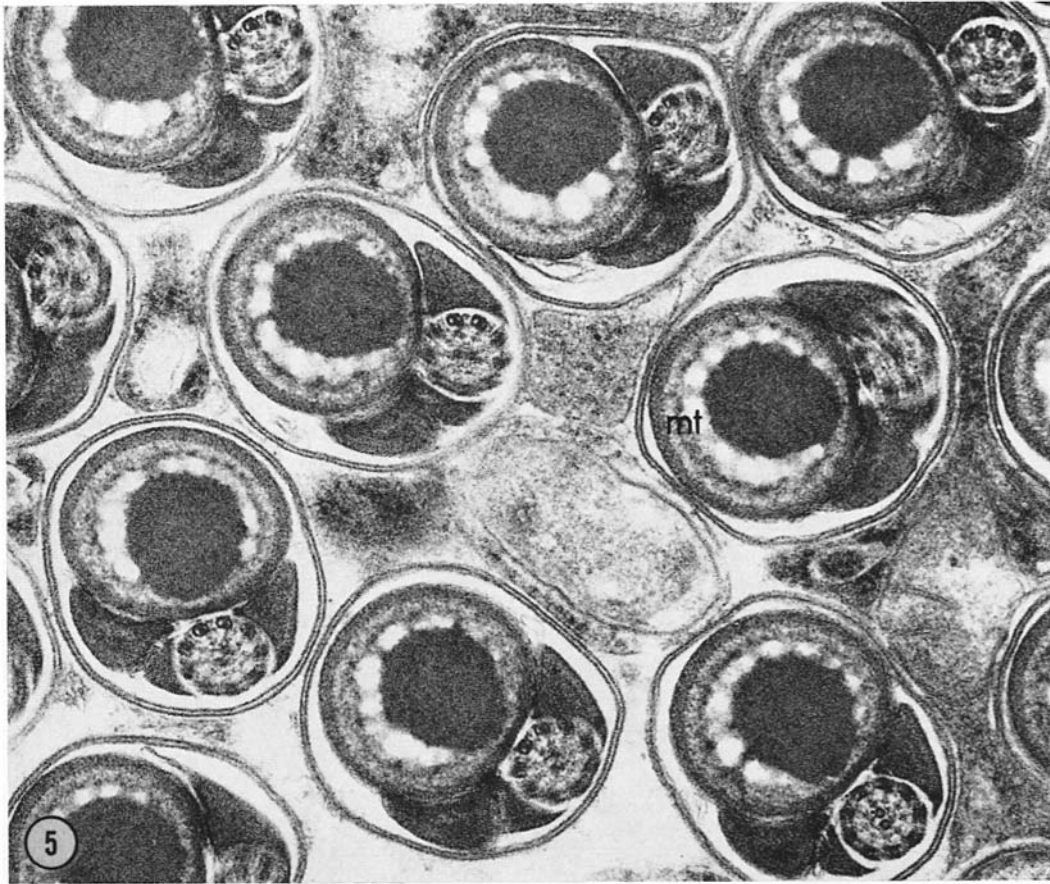


FIGURE 5 Spermatozoa of the flea *Ctenocephalides felis*. Flagellar tubules on one side of the flagellum appear in cross-section while the flagellar tubules on the opposite side of the flagellum appear to be oblique to the plane of section. This indicates that the flagellar tubules spiral around the central pair. Mitochondrial derivative (*mt*).  $\times 47,000$ .

flagellum of *Psocus*, the flagellar tubules appear to form an angle of about  $8^\circ$  with the long axis of the cell. In addition, images in which all the flagellar tubules of a cell are seen in perfect cross-section (i.e. as circles), which would be expected if all the tubules were parallel to each other and to the long axis of the cell, are never obtained. Indeed, images with tubules on one side of a flagellum transversely sectioned and those on the opposite side obliquely sectioned could not be obtained by any plane of section through a cell in which all tubules were parallel to the long axis. This suggests that the flagellar tubules of *Psocus* spermatozoa describe a long-pitched helix around a central rod (see Fig. 14 for further explanation). The triplet tubules of

animal centrioles have also been reported to be disposed in a helix (2, 7). The relationship between the possible helically disposed centriolar tubules is unclear, but, if the basal body is involved in the helical arrangement of flagellar tubules in *Psocus*, this involvement does not occur during flagellar formation because the tubules are initially parallel to the long axis of the cell.

In mature spermatozoa of the cat flea, *Ctenocephalides*, the flagellar tubules appear to run obliquely with respect to the long axis of the axial filament complex, and sections which transect perpendicularly all flagellar tubules of a cell are never obtained. Thus, it appears that in *Ctenocephalides* spermatozoa, as well as *Psocus* sperm, the outer flagellar tubules spiral around the

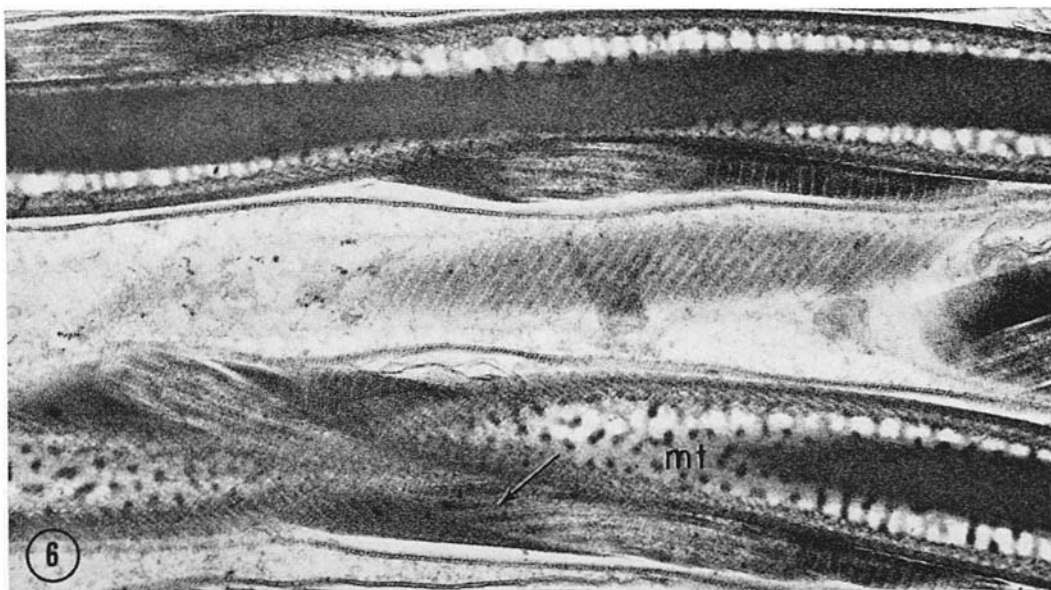


FIGURE 6 Longitudinal sections of two spermatozoa of *C. felis*. In the upper cell, the obliquely cut flagellum occurs alternately on one side of the mitochondrial derivative (*mt*) and the other. Thus, it appears that the flagellum spirals around this structure. In the lower cell, the flagellar tubules are seen at one point (arrow) to form an angle with respect to the long axis of the flagellum. This is frequently observed in oblique sections of flagella. This is another indication that the flagellar tubules spiral around the central pair.  $\times 31,000$ .

central elements. However, in *Ctenocephalides* it appears that the main axis of the flagellum also describes a helix and does not run parallel to the long axis of the cell as in *Psocus* and spermatozoa of other species.<sup>1</sup> Instead the mitochondrial derivative is centrally located and the flagellum spirals around it.

#### *Flagellar Branching*

Flagella with long, slender whiplash tips and various surface-amplifying specializations of the flagellar membrane have been reported (14, 15, 20, 25), but to our knowledge no instances have been described of branching of the axial filament complex such as occurs in treehopper sperm. The branching of the flagellum appears to occur in a regular manner. The frequent appearance of flagella with branches in groups of four, of which three contain two doublet and two singlet tubules and the fourth contains three doublet and three singlet tubules, and the consistent finding of a 3:1 ratio between the two types of branches suggest that the manner in which the 18 peripheral tubules are distributed among the

four flagellar branches is, in fact, identical in each spermatozoan. It is assumed that the central pair of tubules terminates at the level of flagellar branching and is not represented in the distal part of the tails. Occasional profiles (Fig. 14, cell *D*) in which cells transected at the level of branching appear to possess all the peripheral tubules but lack the central pair support this belief.

#### *Phylogenetic Considerations*

Since sperm of relatively few of the estimated 700,000 insect species have been studied with the electron microscope, only very preliminary statements can be made regarding the phylogenetic relationships of exceptional flagellar forms. We have observed variant flagella in species from six of the fourteen insect orders we have examined: Ephemeroptera, Psocoptera, Homoptera, Trichoptera, Siphonaptera, and Diptera (18, 19), and exceptional flagella have been described in other species of Diptera (5) and Homoptera (17, 22). In cases in which exceptional flagella of any given type are found in more than one species,



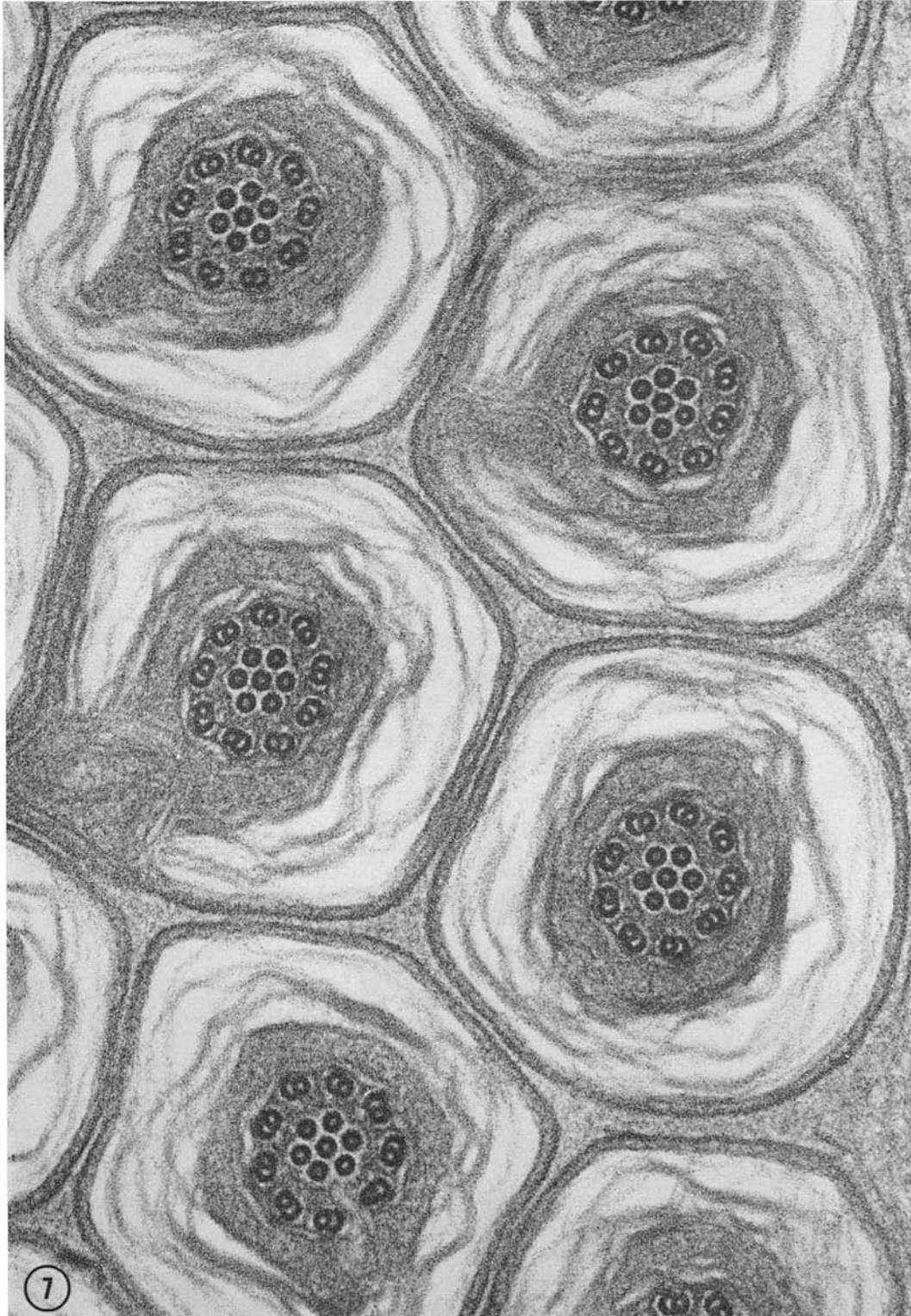


FIGURE 7 Spermatozoa of the trumpet-net caddisfly *Polycentropus* have seven central tubules in the position usually occupied by the central pair and lack the peripheral tubules generally present in insect sperm. The concentric configuration of lamellar structures around the flagellum is also peculiar to this species.  $\times 101,000$ .

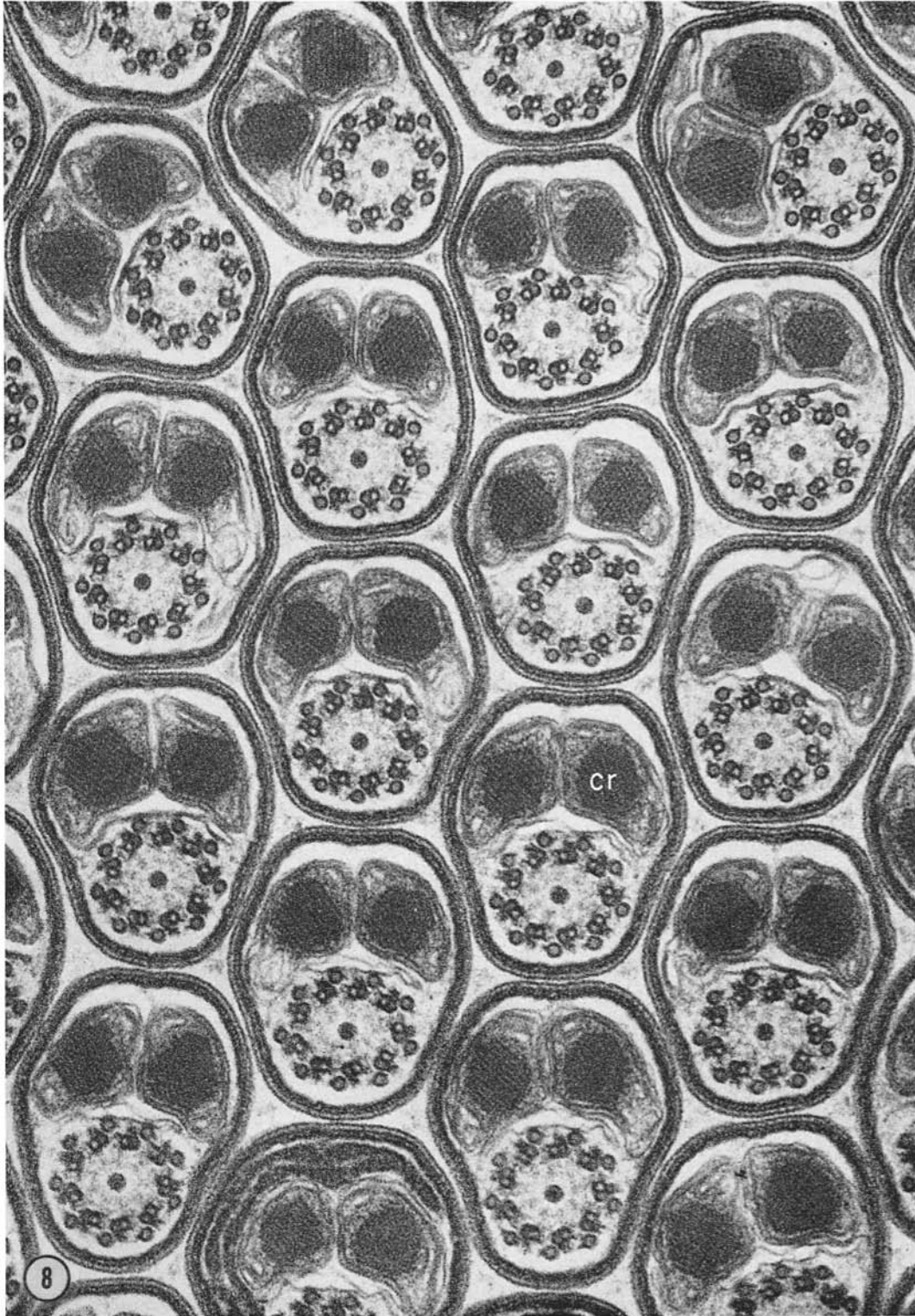
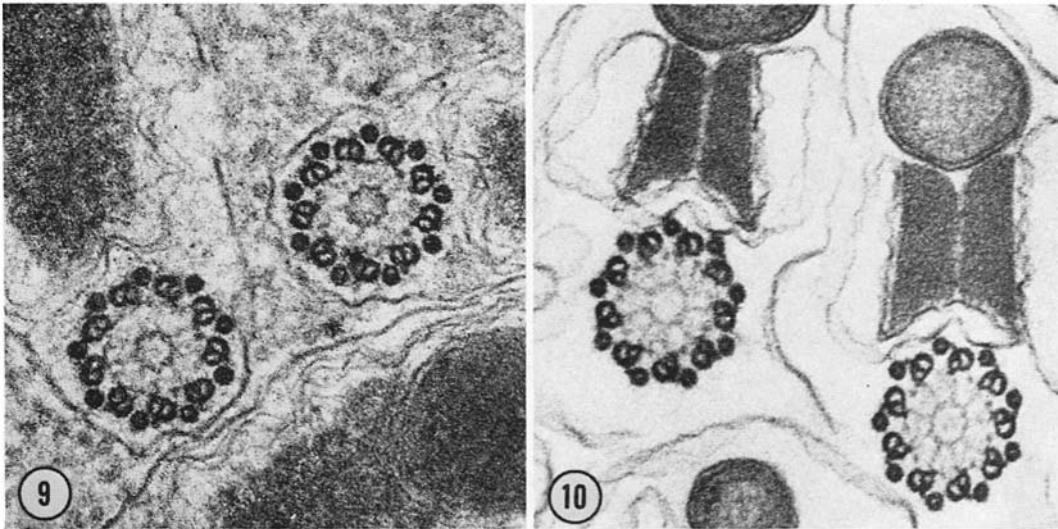


FIGURE 8 9 + 9 + 1 spermatozoa of the mosquito *Culex* sp. Mitochondrial crystalloid (*cr*).  $\times 73,000$ .



FIGURES 9 and 10 9 + 9 + 0 sperm of two species of mayflies. The large central element is so indistinct that it probably should not be considered a flagellar tubule. Fig. 9. *Pentagenia vittigera*.  $\times 82,000$ . Fig. 10. *Tricorythodes* sp.  $\times 82,000$ .

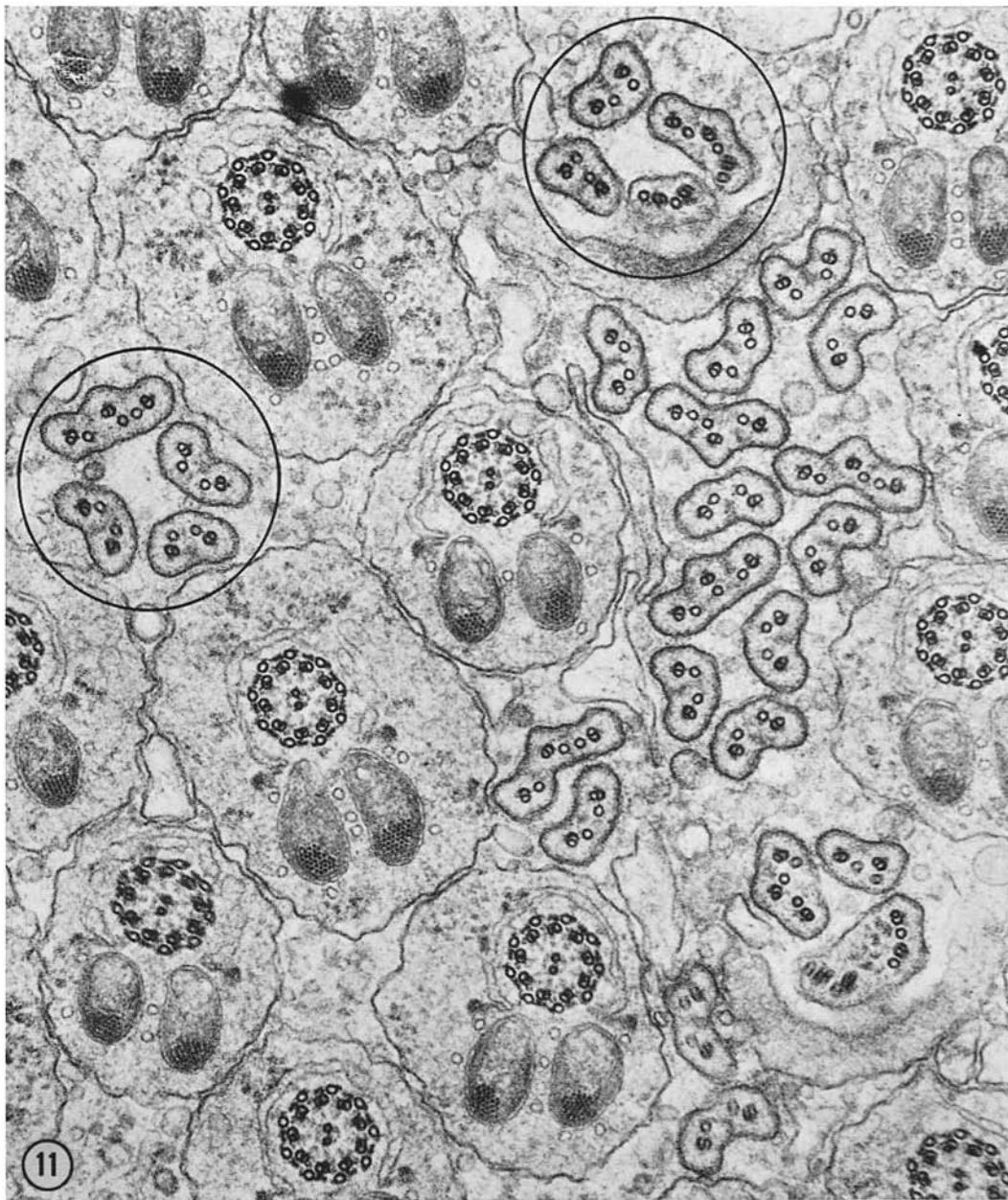


FIGURE 11 The flagellar tubules of treehopper sperm in the four species examined dissociate into four membrane-bounded branches. Three of the branches contain two doublet and two singlet tubules while the fourth has three doublet and three singlet tubules. The central pair terminates at this level and is not represented in the four smaller flagellar branches. In this section, the flagellar tubules of the treehopper, *Ceresa*, are cut close to the level of branching. Cross-sections of cells transected anteriorly possess a nine-membered flagellum, whereas cells sectioned more posteriorly show groups of four flagellar branches. Circles have been drawn around two such groups.  $\times 54,000$ .

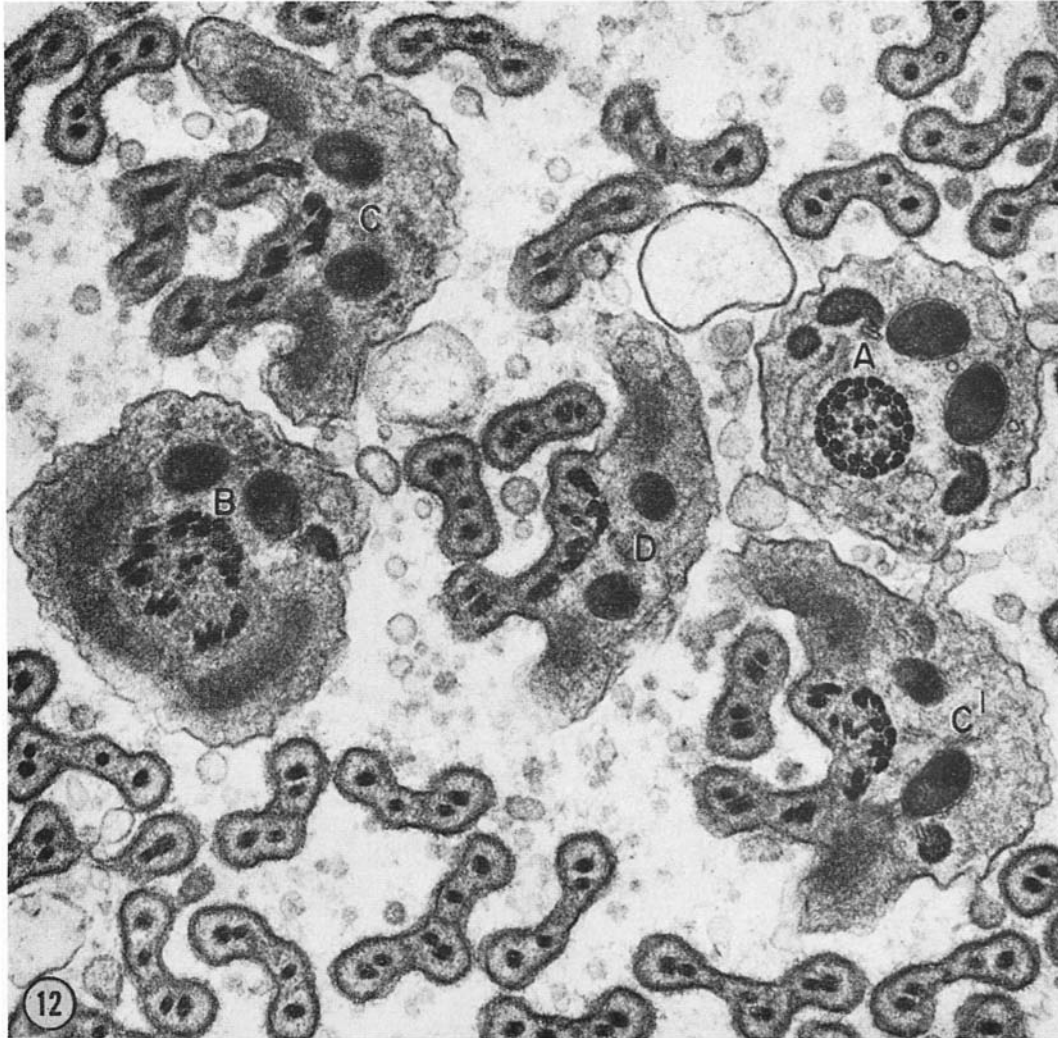


FIGURE 12 Transverse sections of what appear to be various planes of sections through late treehopper spermatids at the point of flagellar branching. Cell *A* has been transected just anterior to the point of branching, and Cell *B* is believed to be cut just at the point at which branching begins. In Cells *C* and *C'*, cut further posteriorly, one branch has separated, and in Cell *D* two of the four branches are separated. Cell *C* appears to be transected caudal to the point of termination of the central pair. *Ceresa*.  $\times 40,000$ .

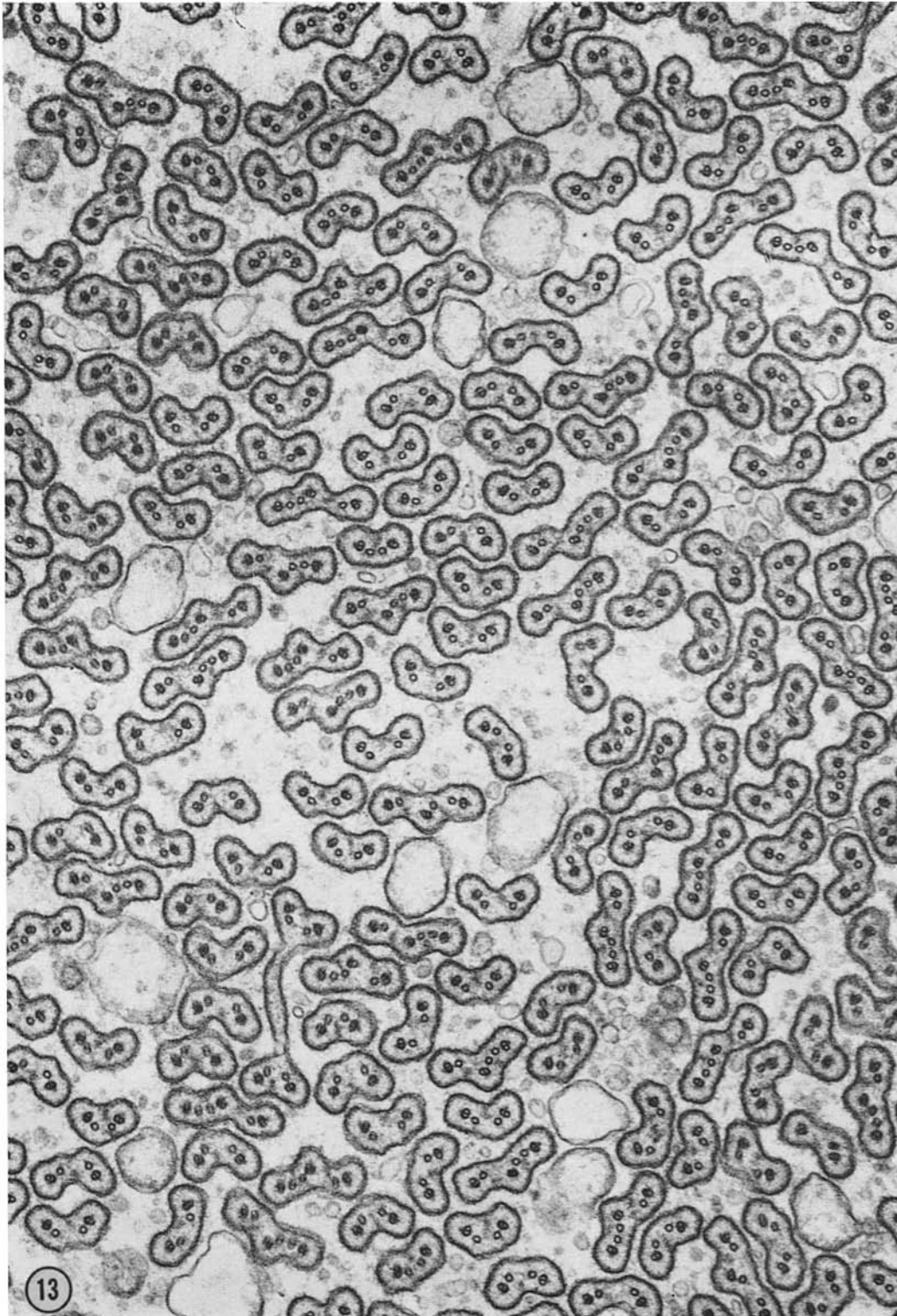


FIGURE 13 In areas containing many cross-sections of posteriorly transected treehopper spermatids, we find 3:1 ratio between the number of flagellar branches which contain two doublet and two singlet tubules and those that have three doublet and three singlet tubules. This suggests that the dissociation of flagellar tubules occurs consistently and in a nonrandom manner in these sperm. *Ceresa*.  $\times 40,000$ .

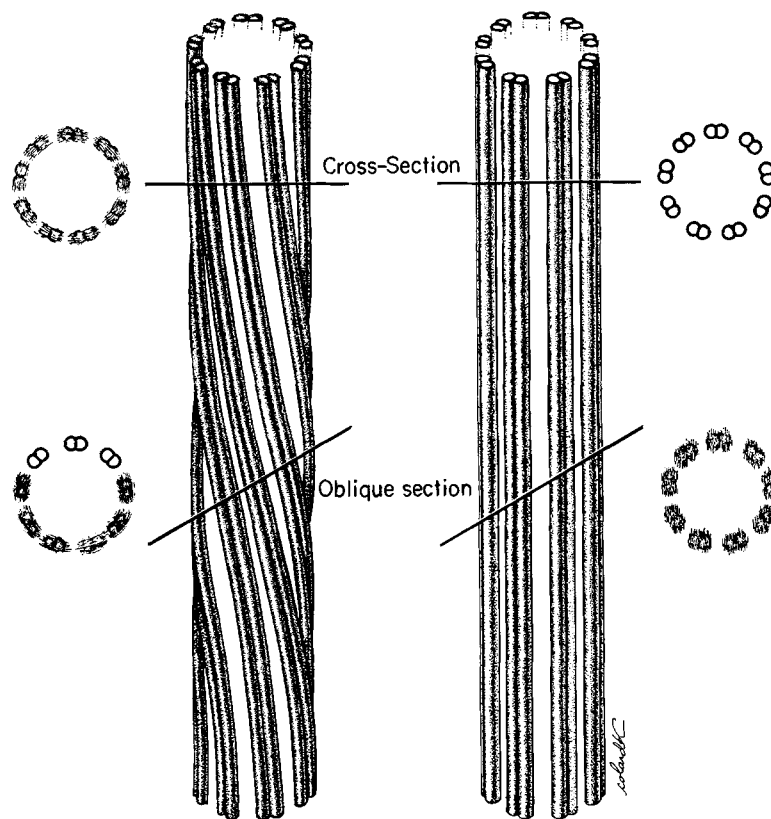


FIGURE 14 Diagram illustrating the images which one would expect to obtain by transverse and oblique sections through insect sperm flagella with straight, parallel tubules (right) and through flagella with helically disposed tubules (left).

all species with that type of flagellum are in the same order. However, there appear to be some cases in which exceptional flagella are widely distributed within the order. All mayflies (Ephemeroptera) examined possess similar  $9 + 0$  flagella, and branching flagella appear to be a characteristic of an entire family (Membracidae). Breland et al. (5) report a  $9 + 1$  flagellum in several genera of mosquitoes, and we have observed a similar tubule pattern in two mosquito genera. In the family Sciaridae, we have found similar giant flagella in several species of the genus *Sciara*, and a similar giant flagellum has been observed in the genus *Rhynchosciara* (J. Michael Price, personal communication). There is no apparent similarity in the type of aberration found in the giant flagellum of the Sciaridae (18, 19) and the  $9 + 9 + 1$  flagellum which is

found in several genera of mosquitoes (Culicidae), though these families are both lower dipteran families. Similarly, the peculiar flagella observed in treehopper sperm depart from the usual in a manner very different from that of the strange motile apparatus which has been described in two other Homopterans, the coccids *Steatococcus tuberculatus* (17) and *Parlatoria oleae* (22). The wide distribution of insect species with aberrant flagella and the observation that, in many of the orders which contain exceptional forms, most species examined possess typical flagella<sup>1</sup> suggest that the several types of exceptional flagella observed in insects evolved independently.

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