MICROTUBULAR PATTERNS IN SPERMATOZOA OF COCCID INSECTS IN RELATION TO BENDING

W. GERALD ROBISON, JR.

From the Department of Biology, University of Virginia, Charlottesville, Virginia 22903

ABSTRACT

Flagella-like motion occurs in filamentous spermatozoa of coccid insects, which have diameters (0.16-0.65 µ) and lengths (150-300 µ) similar to those of long flagella, but have no doublets or 9 + 2-like arrangements of microtubules. Light and electron microscope investigations of spermatozoa from 10 species reveal many bizarre patterns of microtubules and suggest some basic similarities to flagella. Detailed analyses of spermatozoa which are naturally bent in definable planes during their elongation in the male and their storage in the female provide evidence that a constant topographical relationship is maintained between their unorthodox patterns of microtubules, as viewed in transsections, and the direction of bending. The configuration common to most coccid spermatozoa consists of an acentrically positioned crescent of microtubules surrounded by one to several concentric rings. A line drawn to connect the two ends of the crescent appears to remain perpendicular to the plane of bending, and it defines a plane in which bisection of the spermatozoon produces halves with unequal numbers of microtubules. Bisection of the 9 + 2 motile apparatus in a plane perpendicular to that of bending also appears to produce halves with unequal numbers of microtubules. Therefore, the indispensable elements for flagellar and flagella-like motion may be microtubules arranged in "asymmetric" patterns.

INTRODUCTION

Flagellation has been studied extensively using filamentous cell appendages which contain a pair of singlet microtubules surrounded by nine doublets (5, 9, 20). Flagella have this basic, 9 + 2 complex alone (9, 15), whereas the sperm tails of many insect species have an additional, outer ring of nine singlet microtubules (32), and those of most mammals have an outer ring of nine coarse (dense) fibers (10, 11).

It is accepted generally that flagellation of these organelles includes a basic, two-dimensional component and varying amounts of three-dimensional activity. The two-dimensional bending correlates well with the structural evidence in that it occurs in a plane approximately perpendicular to that formed by a line drawn to connect the two central microtubules of the 9 + 2 configuration (10, 12, 13). On the other hand, the three-dimensional motion is more variable than what might have been predicted from the structural uniformity observed, and has no completely satisfactory explanation.

Questions regarding basic units of flagellation and the significance of the 9 + 2 organization are difficult to approach without comparative material of novel structural organization. Although several cell processes have been reported to vary from the typical 9 + 2 and 9 + 9 + 2 patterns of organization, most of these vary only slightly, and little is known with regard to their motile behavior (1, 3, 6, 18, 19, 31–37, 40).

Recently, the filamentous spermatozoa of coccid
insects ("headless sperm"), which are indistinguishable from long flagella in their diameter, length, and some of their motile activities (21, 22, 40, 46), have been shown to lack a clear-cut ultrastructural resemblance to a flagellum (26, 27, 40-43, 46). Although they have been classified as aflagellate, these spermatozoa possess highly ordered arrays of microtubules, and the entire length of the sperm cell exhibits flagella-like activity.

This report summarizes the ultrastructural information on coccid spermatozoa, includes several previously undescribed patterns of microtubules, and presents evidence which suggests that the two-dimensional component of flagellation maintains a fixed topographical relationship with respect to the unusual patterns of microtubules found in these spermatozoa. The three-dimensional component of flagellation remains unexplained, other than the fact that it must be intrinsic to the filamentous cell, and not a characteristic endowed to an appendage by the shape or control of a "parent cell," as may be the case with some flagella and sperm tails. The contributions of information on flagellar motion and microtubule function which are provided by this new, comparative material will be discussed.

**Materials and Methods**

Stock cultures of *Parlatoria oleae* (Colvèc), *Planococcus citri* (Risso) and *Pseudococcus obscurus* Essig were obtained from Mr. Glenn L. Finney (United States Department of Agriculture Field Experiment Station, Albany, Calif.), Dr. Michael Kosztarab (Virginia Polytechnic Institute, Blacksburg, Va.), and Dr. Uzi Nur (University of Rochester, Rochester, N. Y.), respectively, and were maintained on potato tubers in the laboratory (39, 46).

Most coccid insects cannot be cultured in the laboratory. They are restricted to specific host plants and often to precise localities within the range of a host plant. They complete only one or a few generations per year, and the males usually live only 12-72 hr after emergence from their cocoons. Because of these facts and the expertise required to classify coccid insects correctly, most of the species that were utilized in this study were collected and identified by coccid entomologists. Dr. Michael Kosztarab collected males of *Neostengelia texana* Morrison and *Unaspis euonymi* (Comstock) upon their emergence, on 2 October 1969 in Blacksburg, Va. Dr. John W. Beardsley (University of California, Berkeley) collected males of the following species in California on the emergence dates noted: (a) *Kermes sp.*, 22 January 1968 at Putah Creek; (b) *Matsucoccus bisetosus* Morrison, 11 January 1968 at Mt. St. Helena; (c) *Puto albicans* McKenzie, 3 July 1968 about 6 miles southeast of Placerville; (d) *Puto yuccae* (Coquillet), 26 June 1968 at Mt. Wilson; and (e) *Stomacoccus plantani* Ferris, 10 May 1968 at Berkeley. All material was fixed within a few days of collection.

Light microscope observations of developing and mature spermatozoa were made from testes and ovaries that were dissected and squashed in Darling-ton and LaCour's Ringer Solution A (8) or in 45% acetic carmine, with or without previous fixation in acetic alcohol. The measurements of spermatozoan and sperm bundle lengths were made from micrographs of phase-contrast or Nomarski contrast images of material lightly squashed in Ringer's. The resolution of spermatozoa with diameters below the theoretical limit of resolution for the light microscope was accomplished by conventional light microscope observations of material dissected and squashed in 45% acetic carmine, which induces lateral swelling as well as some longitudinal shrinking of coccid spermatozoa (21, 26). These preparations permitted studies of the position and alignment of spermatozoa within the sperm bundles. Spermatozoan diameters were not determined from such preparations, but from electron micrographs of transected material prepared especially for electron microscopy. Feulgen staining was carried out as described previously (40). Light micrographs were taken at film magnifications of 40-1500 diameters and then enlarged photographically to 1000-2000 diameters for analysis.

The testes and ovaries studied by electron microscopy were dissected from subadult and adult males or fertilized females immediately following immersion of the insects in one of the primary fixatives to be listed below, which was maintained at 1°C-2°C by a recirculating cold bath. They were fixed for 3-8% hr and then washed in several changes of 0.05 M sodium cacodylate or phosphate buffer (pH 7.2-7.4) with 0.35 M sucrose for 24 hr-4 months at 1°C-4°C. All, except a few that were fixed in dichromate/acrolein (fixative number 2 below), received a secondary fixation (postfixation) in Millonig's 1% OsO4 (25) for 1½-2 hr at 1°C-4°C. They were dehydrated in a cold ethanol series (1°C-2°C) and in room-temperature absolute ethanol, cleared in propylene oxide, and embedded in Epon. Thin sections were obtained with a DuPont diamond knife mounted in a Porter-Blum MT-2 or Reichert OMU2 ultramicrotome. The sections were picked up on uncoated, 200-mesh copper grids, and stained for 2 min with a saturated solution of uranyl acetate in 50% acetone followed by a rinse in distilled water and staining for 1 min with lead citrate prepared according to Venable and Coggshall (32). The stages of development of the spermatozoa were determined by the instar of the male and the
shape of the testis as described by Wieglera (53) who used a related species.

The following solutions were used as primary fixatives for the electron microscope preparations: (a) 0.25 m glutaraldehyde in 0.05 m sodium cacodylate buffer (pH 7.4) with 0.175 m sucrose; (b) 0.268 m acrolein in 0.03 m K_2Cr_2O_7 with 0.175 m sucrose, brought to pH 7.5 with 5 m KOH (7, 44); (c) 0.13 m paraformaldehyde, 0.03 m glutaraldehyde, and 0.036 m acrolein in 0.01 m sodium cacodylate (pH 7.4) with 0.175 m sucrose; and (d) 0.163 m paraformaldehyde in 0.05 m phosphate buffer (pH 7.2) with 0.293 m sucrose.

The electron microscope observations were made with a Philips 200 electron microscope at an accelerating voltage of 60 kv. The micrographs were taken at film magnifications of 4500–23,000 diameters and were printed photographically at three to ten times the original magnifications for analysis. The figure legends include the method of preparation in abbreviated form.

OBSERVATIONS

General Structure of Spermatozoa

The spermatozoa of the 10 species of coccid insects studied are long, filamentous structures which maintain a rather constant diameter throughout most of their length, tapering gradually to minimum diameters near their ends (Figs. 1, 9, and 12). As shown in Table I, they range in size from 0.16 µ in diameter and about 150 µ in length in Stomacoccus plantani (Fig. 3) to 0.65 µ in diameter and 300 µ in length in Neosteingelia texana (Fig. 8). It was not possible to measure the length of individual spermatozoa of Stomacoccus plantani since their diameter is below the theoretical limit of resolution of the light microscope. The measurement presented is an estimate based on the length of the sperm-containing region of the living sperm bundle which, in most species, corresponds quite closely to spermatozoan length, owing to precise alignments of spermatozoa within the bundles. The extent of the sperm-containing region was determined by making squash preparations with acetic acid, which induces longitudinal shrinkage and lateral swelling of coccid spermatozoa. Spermatozoa of Stomacoccus plantani which are made resolvable by lateral swelling appear to be confined to a structurally distinct region of the sperm bundle which could be measured in preparations of living material. Similar techniques were utilized for making an estimate for Puto yuccae, in which the sperm bundle sheaths are too thick to permit clear visibility or isolation of individual spermatozoa for measurement. All other length measurements were obtained from individual spermatozoa, freshly dissected in insect Ringer’s and analyzed with the use of micrographs of phase-contrast or Nomarski contrast images. The diameters were determined from electron micrographs of fixed, transected material.

Correlations between Feulgen-positive regions of whole sperm bundles and the presence of electron-opaque spermatozoan centers, as determined by consecutive transections from different regions of sperm bundles, indicate that the spermatozoa of the coccids studied possess chromatin in the form of an electron-opaque, threadlike core. A single, threadlike core extends throughout most of the cell’s length in the spermatozoa of Matsucoccus bistosus, Planococcus citri, Pseudococcus obscurus: and Stomacoccus plantani, but extends only about half the cell’s length in Neosteingelia texana, Parlatoria oleae, and Unaspis euonymi (Figs. 2–8 and 10). A multistranded core is present in Kermes sp., Puto albicans, and Puto yuccae (43).

None of the thousands of ultrathin sections of spermatozoa examined, which include representatives from 10 species of coccids, from various types of fixation, and from various regions within the spermatozoa, has shown the presence of centrioles, mitochondria, or a typical flagellum (Figs. 2–8). A structure of unknown composition and origin is found occasionally in transections of Stomacoccus plantani spermatozoa (Fig. 3), but its function is unknown and a relation to any of these structures may not be as probable as a relation to an acrosome, since preliminary analyses of consecutive transections suggest that it is limited to a relatively short, anterior region.

Patterns of Microtubules

Eight different arrays of microtubules were found in the spermatozoa of the 10 species of coccid insects examined, none of which contain doublet microtubules or closely resemble the 9 + 2 pattern that is typical of flagella. The observations are presented with respect to three regions along the length of a spermatozoon, which can be distinguished in transections: (a) the nuclear region, which is distinguishable by the presence of an electron-opaque core and usually by the greatest diameter; (b) the nonnuclear region, which has no dense core, but usually has a diameter similar
FIGURE 1 Sperm bundle of *Matsucoccus bisetosus* with 60 or more filamentous spermatozoa. In living preparations a continuous train of undulations passes from anterior (right) to posterior (left), a fact which suggests synchronous, two-dimensional motion of the spermatozoa. The bundle sheath has been ruptured in preparation of the squash, and the spermatozoa have become deranged posteriorly. Ringer's phase contrast. × 840.

to or only slightly less than that of the nuclear region; and (c) the extremities, whether anterior or posterior, which are distinguishable by their obviously decreased diameter. In instances in which an extremity is classified as anterior or posterior, the determination was made by studies of consecutive sections from several regions of a sperm bundle of known orientation. All the microtubular numbers and patterns reported were determined on the basis of 25-100 unambiguous observations. More recordings were made from species which displayed a great deal of variability than from those that did not. Attempts were made to avoid counting the microtubules of a single spermatozoon more than once for a given region. The structure of the extremities was not analyzed in all the species reported owing to the difficulty involved in obtaining enough sections for meaningful counts.

Transections from the spermatozoa of *Parlatoria oleae* (Figs. 2, 11, 14, and 15), whether taken from

<table>
<thead>
<tr>
<th>Species</th>
<th>Length</th>
<th>Diameter</th>
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<tr>
<td><em>Kermes sp.</em></td>
<td>200</td>
<td>0.3</td>
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<tr>
<td><em>Matsucoccus bisetosus</em></td>
<td>170</td>
<td>0.5</td>
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<tr>
<td><em>Neosteingelia texana</em></td>
<td>300</td>
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<tr>
<td><em>Parlatoria oleae</em></td>
<td>300</td>
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<tr>
<td><em>Planococcus citri</em></td>
<td>300</td>
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<tr>
<td><em>Pseudococcus obscurus</em></td>
<td>300</td>
<td>0.25</td>
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<tr>
<td><em>Puto albicans</em></td>
<td>—</td>
<td>0.35</td>
</tr>
<tr>
<td><em>Puto yuccae</em></td>
<td>~300</td>
<td>0.35</td>
</tr>
<tr>
<td><em>Stomacoccus plantani</em></td>
<td>~150</td>
<td>0.16</td>
</tr>
<tr>
<td><em>Unaspir euonymi</em></td>
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<td>0.25</td>
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the nuclear region which is known to be the anterior region in this species (40), or from the nonnuclear, posterior region, show 43-52 (47 average) microtubules arranged in a simple spiral of approximately 1½ revolutions. The incomplete
revolution is composed of 8–18 (13 average) microtubules in the nuclear region and 13–22 (16 average) microtubules in the nonnuclear region. Transections taken from near the posterior extremity have 28 or fewer (usually 23) microtubules which are arranged in two concentric rings of about 17 and 7 microtubules each, rather than in a spiral. There appears to be relatively little variation in microtubular number in this species, but about 10% of the spermatozoa have their 43–52 microtubules distributed between a spiral with about 36 microtubules, and a crescent with about 11 microtubules which is interposed between the overlapping portions of the spiral (Figs. 11 and 14). Less overlap occurs in such specimens. Multiple sections from several individual, aberrant spermatozoa indicate that the crescent modification may extend the majority of a spermatozoon’s length (Fig. 11).

Transections from all but the extremities of the spermatozoa of *Stomacoccus plantani* (Fig. 3) invariably contain 17–20 microtubules (always 20 in the nuclear region) arranged in 13–15 (usually 14) concentric rings in the nuclear region and in 12–17 (usually 13) rings in the nonnuclear region. The outer ring is complete and usually has 15–16 microtubules in the nuclear region and 13–14 in the nonnuclear region. The inner ring is incomplete and forms a crescent which usually has three to five and five to six microtubules in the nuclear and nonnuclear regions, respectively.

The spermatozoa of *Planococcus citri* (Fig. 4), like those of *Pseudococcus obscurus*, which have already been described in detail by Ross and Robison (46), contain an average of 56 microtubules in approximately 2½ concentric rings with little variation, except in the gradually tapered regions at either end, where three concentric rings with 56 or fewer tubules are found. Transections from the midregions of the spermatozoa of these two species generally have about 56 microtubules distributed with 28 in the outer ring, 21 in the inner, complete ring, and seven in the incomplete ring.

Transections from the nuclear region of the spermatozoa of *Kermes sp.* possess 47–58 (54 average) microtubules which are disposed in 17/8, 2 or 21, 4 (usually 2) concentric rings, whereas transections from the nonnuclear region have 41–
FIGURE 8  Spermatozoon of a subadult male of Neoteingelia texana transected in its nonnuclear region. This specimen has 401 microtubules arranged in seven complete rings and two partial rings. See text for the variability which occurs in this species. Note the subunit structure of the microtubules and the apparent interconnecting arms. Glutaraldehyde, osmium tetroxide. X 210,000.

53 (46 average) microtubules which are usually arranged in 2\(\frac{1}{4}\)–2\(\frac{3}{4}\) (usually 2\(\frac{1}{2}\)) concentric rings (see micrograph by Robison in reference number 43). Considerable variation was observed, especially in the nonnuclear region where a few transections have a pattern consisting of one complete and two incomplete rows of microtubules, and others have patterns with two complete and two incomplete rings in which one or two “extra” microtubules occur just inside the inner row of the 2\(\frac{1}{2}\) ring pattern, suggesting the commencement of a fourth ring. In these few examples where two incomplete rows of microtubules were observed, both the crescents that they formed were found on the same side of the spermatozoon.

Transections from the nuclear region of the
FIGURE 9 Developing spermatozoa from a disc-shaped, testis cyst of *Parlatoria oleae* (fourth instar), which are bent in a definable plane (see text). The cells that normally form enveloping sheaths have been ruptured, and the cyst contents have been spread to demonstrate approximately 32 filamentous spermatozoa in two bundles that are coiled to make two to three revolutions each (three to four before spreading). One sperm bundle is oriented clockwise and the other counterclockwise. Ringer's, phase contrast. X 1480.

FIGURE 10 Transections of three disc-shaped cysts from *Parlatoria oleae* with naturally bent spermatozoa like those in Fig. 9. Each of the two developing sperm bundles in the middle cyst has been transected six to eight times, twice per revolution. The upper cyst is unusually small and probably contains only one sperm bundle which has been sectioned 10 times. The dense cores in the spermatozoa represent their nuclear material which runs only half their length and, therefore, is not seen in all transections. The plane of sectioning is perpendicular to the direction of spermatozan binding. Dichromate/acrolein, osmium tetroxide. X 4500.

spermatozoa of *Puto albicans* (see micrograph by Robison in reference number 43) have 100–116 (107 average) microtubules which are arranged in 2⅔–3⅓ (usually 3) concentric rings, and transections from the nonnuclear region (Fig. 5) have 101–111 (106 average) microtubules arranged in 3–4⅔ (usually 3½–3⅔) concentric rings. Transections from the extremities show
FIGURE 11 Transection through the rim of a disc-shaped testis cyst from Parlatoria oleae, showing similarly aligned microtubular patterns in all the spermatozoa of two developing sperm bundles, one of which has 15 spermatozoa and has been transected three times and another of which has 17 spermatozoa and has been transected four times. The spermatozoa of one bundle are probably viewed in an anterior-to-posterior direction, while those of the other bundle are probably viewed from posterior to anterior, since the orientations of their spiral microtubular patterns are clockwise and counterclockwise, and an opposite orientation of the bundles is seen in squash preparations (see Fig. 9). The center of the cyst lies outside the micrograph, almost directly below it, but slightly to the left of center. All the spermatozoa are aligned so that their spiral pattern of microtubules has two rows toward the periphery of the cyst and one toward the center. Therefore, each spermatozoon is bent at right angles to a reference plane defined by a straight line drawn to connect the two ends of its spiral microtubular pattern. Even the aberrant spermatozoa (one per sperm bundle in this preparation), which have a crescent of microtubules interposed between the overlapped regions of their spiral pattern (arrow), show a similar relationship if their reference plane is defined by a line connecting the inner extremity of the crescent with the outer end of the spiral. Dichromate/acrolein, osmium tetroxide. × 30,000.
Figure 12  Spermatozoa coiled around the nucleus of a specialized cell (vestibule cell) at the base of an ovariole in the female reproductive tract of Parlatoria oleae, where they apparently await maturation of the oocyte. Acetocarmine squash, phase contrast. × 2900.

Figure 13  Transection of a vestibule cell in the female reproductive tract of Parlatoria oleae, with approximately two spermatozoa coiled around the nucleus. Note that the alignment of spermatozoa into a coil is not as precise as that observed in the cysts of the testis. Glutaraldehyde, osmium tetroxide. × 8700.
great variability and may have as few as 17 microtubules in two concentric rings.

The spermatozoa of *Puto yuccae* appear to be essentially the same as those of *Puto albicans*. Transections from the nuclear region show 95-109 (102 average) microtubules which are disposed in 2½-3½ (usually 3) concentric rings; and transections from nonnuclear regions have 94-110 (103 average) microtubules arranged in 3-4 (usually 3½-3¾) concentric rings.

The number of microtubules does not correlate closely with the number of concentric rows in either *Puto albicans* or *Puto yuccae*, and often a negative as well as a positive correlation is found. For example, in *Puto albicans* two transections from nonnuclear regions have 108 and 111 microtubules in 4½ and 3½ concentric rows, respectively, and a transection from the nuclear region has 116 microtubules in only three rings. In *Puto yuccae* two transections from nonnuclear regions have 101 and 110 microtubules in 4 and 3½ concentric rows, respectively.

Transections from the nuclear region of the spermatozoa of *Unaspis euonymi* contain 47-59 (50 average) microtubules arranged in 1½-2 (usually 1¾-1½) concentric rings. Transections from the nonnuclear region (Fig. 6) have 44-55 (50 average) microtubules arranged in one of two basic configurations: (a) a pattern with one outer, complete ring of microtubules plus one inner ring which may be partial or complete; (b) a pattern with one outer, complete ring plus two inner, partial rings. Microtubules which form the first type of pattern are disposed in 1¾-2¼ (usually 1¾-2) concentric rings; and microtubules of the second pattern type are distributed in 1½ plus ½ rows to 1½ plus ¾ rows with a multitude of variations in between. The partial rows form two crescents on the same side of the spermatozoon in all the transections of this type which were observed. Transections interpreted to be from the extremities of these spermatozoa have 27-30 microtubules in two complete rings.

Transections from both nuclear and nonnuclear regions of the spermatozoa of *Matsucoccus bisetosus* (Fig. 7), except for those interpreted to be from the tapered regions near the extremities, are essentially similar. They have 142-224 (180 average) microtubules which form an array composed of 22-27 (24 average) whorled radii that are surrounded by a ring of 39-56 (50 average) microtubules and are composed of four to eight (usually five to six) microtubules each. The microtubules within a radius appear to be interconnected, as do the 50 which form the outer ring. The microtubules on one side of the spermatozoon are cut obliquely, while those on the other side are cut transversely. The extremities and regions

**Figure 14.** Transection through a rim of a disc-shaped vestibule cell of *Parlatoria oleas*, showing some of the multiple transections of two or more spermatozoa with similarly aligned microtubular patterns. Apparently some are viewed from anterior to posterior and the others from posterior to anterior since some have a clockwise and others have a counterclockwise orientation of their spiral microtubular pattern. Both simple spirals of the usual type for this species and spirals with crescent aberrations are seen in this preparation. A straight line drawn across the simple spiral pattern to connect its two ends, or across the aberrant pattern to connect the inner end of its crescent with the outer end of its spiral, serves to define a reference plane for each pattern. Since the center of the vestibule cell is essentially below the bottom of the page and all reference planes are approximately horizontal to the page, the bending of a spermatozoon appears to occur in a plane approximately perpendicular to its reference plane, and towards its single row of microtubules. Compare with Figs. 11 and 15 and see the text. Glutaraldehyde, osmium tetroxide. X 90,000.

**Figure 15.** Micrograph of material similar to that in Fig. 14. Here the center of the vestibule cell is towards the lower left, and more variability in the alignment of microtubular patterns is observed. One transection of a spermatozoon (double-stemmed arrow) appears to be 180° off from the usual alignment. However, a distinctive indentation in its outer row of microtubules suggests that it may be from the same spermatozoon as the adjacent transection to its right. If so, the fact that these "companion" transections show clockwise and counterclockwise spirals as well as oppositely oriented double rows of microtubules suggests that they represent a spermatozoon which doubled back on itself without enclosing the nucleus of the vestibule cell. Note that the spermatozoa are encircled by two membranes (double-stemmed arrow), one of which may be its plasma membrane and the other of which may represent a vacuolar membrane of the vestibule cell. Glutaraldehyde, osmium tetroxide. X 90,000.
which were interpreted to taper into them, on the basis of their diameter, show much variability in microtubular number and pattern. They may have as few as 12 whorled radii with 80 microtubules, but where only 15-50 microtubules are present patterns of two to three concentric rings with no whorled radii are found.

The spermatozoon of Neostegingleia texana (Fig. 8) is the largest coccid spermatozoon described, and it is the most variable with respect to microtubular number and pattern, especially in its nonnuclear regions. Transections from the nuclear regions have 233-443 microtubules arranged in 5-7½ concentric rings. 52% of the transections counted had 233-352 (310 average) microtubules arranged in 5-5½ concentric rows; 35% had 291-422 (360 average) microtubules in 6-6½ concentric rows; and 13% had 356-443 (390 average) microtubules in 7-7½ concentric rows. One transection had 608 microtubules in five complete rings and five incomplete rings. Transections from the nonnuclear regions have 139-510 microtubules arranged in 4-9½ concentric rings. 4% of the transections counted had 139-194 (166 average) microtubules arranged in 4-4½ concentric rows. 31% had 151-227 (190 average) microtubules in 5-5½ concentric rows; 17% had 225-352 (290 average) microtubules in 6-6½ concentric rows; 37% had 304-310 (397 average) microtubules in 7-7½ concentric rows; and 8% had 413-480 (440 average) microtubules in 8-8½ concentric rows; and 3% had 406-450 (428 average) microtubules in 9-9½ concentric rows. Although there appears to be a positive correlation between the average numbers of microtubules and the numbers of concentric rings, a great deal of variation in microtubular number occurs among transections which exhibit a given number of concentric rings, and a negative correlation can be shown if comparisons are made using individual counts which are at the extremes of the ranges.

For example, transection has 406 microtubules in nine rings, another has 480 microtubules in 8½ rings, and a third has 510 in 7½ rings. Whether from the nuclear or nonnuclear regions, some transections have two rather than one partial ring of microtubules in addition to their complete rings, and both partial rings are located on the same side of the spermatozoon. Perhaps part of the variation observed in this species is related to the fact that some of the spermatozoa examined were probably obtained from subadult males.

Microtubular Arrangements in Bent Spermatozoa

During two stages of their ontogeny, the spermatozoa of Parlatoria oleae form flattened coils in which they are bent in a plane that can be defined at both light and electron microscope levels of observation. These stages were analyzed in order to determine if the microtubular pattern of the spermatozoon of this species maintains a constant topographical relationship with respect to the direction of bending. The microtubular pattern in this species is particularly favorable for such a study because it shows very little variation throughout most of the spermatozoon's length, and a relatively unambiguous reference plane can be defined by a line drawn to connect the two free ends of the spiral of microtubules (Fig. 2).

Each cyst of the testis in a subadult male of Parlatoria oleae contains 32 developing spermatozoa whose extensive elongation requires that they make three to four revolutions in order to be accommodated within its confines. The cyst is sphere shaped in testes removed from late third instar and some early fourth instar males. However, soon after the insect's third molt the spermatozoa appear to be grouped into two bundles of approximately 16 and the cyst becomes disc shaped, as if deformed by the spermatozoa which now occupy its thickened rim (Figs. 9 and 10). The two sperm bundles of a cyst usually show opposite orientations, clockwise and counterclockwise. Similar observations were made by Nur (28) in Pseudococcus obscursus and by Weglarska (53) in Quadraspidiotus ostenformis, which are also members of the superfamily Coccoidea (the coccids). The spermatozoa remain in this natural state of two-dimensional bending for several hours, thus permitting chemical fixation and analytical studies.

A section which passes perpendicular to the disc-shaped cyst and through its center transects the rim of the cyst twice, once on each side of the cyst center. Each transection of the rim is populated with transections of bundled spermatozoa which are cut once for each of their revolutions of the cyst (Fig. 10). The plane of bending is known since it must coincide with a line drawn to connect the two transections of the rim, and the
direction of bending is towards the center of the
cyst, or the midpoint of this line. Thus, the orienta-
tion of the microtubular pattern in every sper-
matozoon can be compared with the plane and
direction of bending.

Sections of 100 different disc-shaped cysts in
which the microtubular patterns were un-
ambiguous, were analyzed with respect to possible
alignments of the component spermatozoa relative
to the plane of the disc. Very little variation was
found. As seen in Fig. 11, which is representative
of the many cysts examined, the spermatozoa are
not oriented randomly. The spiral of microtubules
has essentially the same orientation in every transected spermatozoon. A reference line drawn
to connect the free ends of the spiral is aligned
perpendicular to the plane of the disc-shaped
cyst, and therefore to the plane of bending. The
side of the spermatozoon with two rows of micro-
tubules occurs at the periphery of the disc, or the
outside of the bend.

The other stage in which the spermatozoa of
Parlatoria olea form coils takes place within the
female’s reproductive tract. Examination of
fertilized females reveals that most of the sper-
matozoa occur in the vagina and spermaducta as
motile bundles. However, other spermatozoa
occur individually and as small, unbundled groups
in the oviducts and also “within the cytoplasm”
of a specialized cell which is located at the base of
each ovariole (Figs. 12 and 13). Apparently, this
specialized, sperm-containing cell was first de-
scribed in the scale insects (superfamily Coccoidea,
family Diaspididae) by Krassilstschik in 1893
(23). It was given various names by early in-
vestigators and is referred to as the “cellule de
Krassilstschik” by Pesson in 1951 (30). Since this
cell probably plays a role in the storage of sper-
matozoa in the basal region of the ovariole until
oogenesis is completed, it will be referred to as the
vestibule cell in this report.

The spermatozoa are coiled 4–10 times around
the nucleus of the vestibule cell. If dissected in
insect Ringer’s, they undulate and appear to be
“swimming” around the nucleus, but the ap-
parent forward motion could not be demonstrated
to be more than an illusion. Whether they are
active during storage in the undissected female or
are stimulated by dissection in Ringer solution
could not be determined. Electron microscopy
suggests that the spermatozoon is separated from
the ground cytoplasm of the vestibule cell by a
vacuolar membrane and by its own plasma mem-
brane (Fig. 15). The important facts for the
present investigation are that the spermatozoa in
the vestibule cell remain in a relatively stable
state of two-dimensional bending and that, unlike
the developing spermatozoa in the male, they are
capable of autonomous bending, and they form a
coil with more revolutions.

The coiled spermatozoa in the female, like those
in the male, provide an opportunity to compare
the direction of bending with the spermatozoon’s
microtubular pattern. Sections of 50 different
vestibule cells were analyzed by comparing the
orientations of the microtubular patterns of their
component spermatozoa relative to the plane of
the coil. The majority of transections of such
spermatozoa show an alignment of their micro-
tubular pattern similar to that observed in the disc-
shaped cysts of the testis. Lines drawn to connect
the two terminal microtubules of the spirals are
approximately perpendicular to the plane of the
cell, and the double row of microtubules occurs on
the periphery of the coil. More variation in
alignment is seen in the vestibule cell than in the
testis cyst, and some spermatozoa are aligned 180°
different from the majority. Fig. 14 is representa-
tive of many vestibule cells in which a relatively
precise alignment was found, whereas Fig. 15
shows the variation which occurs in other vestibule
cells. Often the spermatozoa are somewhat less
precisely coiled in the female than in the male,
as seen by light microscopy, which fact may ex-
plain the decreased ultrastructural uniformity in
pattern alignment in the vestibule cell as com-
pared to the disc-shaped cyst of the testis. The
variants which are 180° off (Fig. 15) may represent
spermatozoa which bend back on themselves
without surrounding the nucleus of the vestibule
cell, or they may indicate an alternate functional
alignment.

The spermatozoa in the female have slightly
indented, or scalloped, outlines which may be
characteristic of the motile stages, or may be
artifacts of fixation. However, even when cyst cells
and vestibule cells are fixed in the same manner,
this structural difference between nonmotile and
motile stages is maintained.

DISCUSSION

Filamentous Spermatozoa and Motility

Unlike the spermatozoa of most organisms,
those of all the species of coccid insects that have
been studied so far have no distinguishable head, middle-piece, or tail, and they lack centrioles, mitochondria, and a typical flagellum (21, 22, 26, 40, 43, 45, 46). Although coccid spermatozoa may be classified as aflagellate, on the basis of their lack of distinct "head" and "tail" regions, their filamentous morphology is very similar to what one might expect from a long, "headless" flagellum or sperm tail.

We have reported that the spermatozoa of Parlatoria oleae (40) and those of Pseudococcus obscurus (46), which are probably representative of coccid spermatozoa, exhibit a motile behavior throughout most of their length which appears to mimic that of a flagellum. A three-dimensional component of the motion is demonstrated by the fact that forward movement in a viscous medium involves rotation around the axis of propagation as well as anterior-to-posterior undulations (46). Two-dimensional bending is inferred from the spermatozoon's ability to form a natural coil during two stages of its ontogeny, and an artificially induced coil when isolated in buffered saline at maturity (40, 41). Targioni-Tozzetti in 1867 (51) and Berlese in 1893 (4) drew coccid spermatozoa with loops near their ends, which could be interpreted to demonstrate two-dimensional bending. Such loops are not static, but are rapidly formed and reformed (40, 43).

**Microtubular Patterns and Direction of Bending**

Regardless of whether or not the microtubules of flagella and most sperm tails are directly involved in the motile mechanism, their patterns of organization appear to influence motile activities. Except for a few possible deviations (38), the two-dimensional component of flagella and sperm-tail movements occurs in a plane approximately perpendicular to a reference plane that connects the two central microtubules of the 9 + 2 motile apparatus (1, 9, 10, 12, 13). As suggested by several investigators (49, 20), if one assumes equal spacing1 of the doublets in the 9 + 2 pattern, the reference plane should divide the complex into one "half" with five and another with four doublet microtubules:

1 The assumption of equal spacing seems especially justified since very good superimposition of doublet images is obtained by 40° rotations with the Markham technique on micrographs of flatworm flagella (48).

doublet numbers one, two, three, eight, and nine would constitute the major "half" (compartment) and numbers four, five, six, and seven would constitute the minor "half." Therefore, the 9 + 2 motile apparatus appears to bend in a plane perpendicular to a line which bisects it into halves with unequal numbers of microtubules.

Coccid spermatozoa display no 9 + 2 or closely related organizational pattern of microtubules which might relate them directly to flagella and explain their flagella-like motility. Nevertheless, their bizarre microtubular patterns do have some characteristics in common with the 9 + 2 configuration. Comparisons among the spermatozoa from several species of coccid insects reveal a significant recurrence of incomplete rings, or crescents of microtubules. The usual microtubular patterns appear to consist of one to several concentric rings of singlet microtubules surrounding acentrically located crescents. A straight line drawn to connect the two terminal microtubules of the crescent defines a plane in which bisection of the spermatozoon divides it into halves with unequal numbers of microtubules. The "asymmetry"2 which can be demonstrated in this manner is similar to that described for the 9 + 2 motile apparatus.

Unlike most coccid spermatozoa, the spermatozoon of Parlatoria oleae has no crescent. However, it is "asymmetric" and apparently it bends, as does a flagellum, in the plane of "asymmetry." Chemical fixation of this spermatozoon during the two stages of its ontogeny when it forms coils permitted ultrastructural analyses during natural states of sustained bending in clearly definable planes. A straight line drawn to connect the two end microtubules of its spiral pattern divides the spermatozoon of Parlatoria oleae into one "half" (compartment) with two rows of microtubules, and another with one row of microtubules (Fig. 2). This line defines a relatively unambiguous reference plane for the spiral pattern. In almost all transections of naturally bent spermatozoon the reference plane was found to be approximately perpendicular to the planes of bending in the coils formed by the immature spermatozoon during its development in the male and by the mature spermatozoon

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2 The word asymmetry and all of its grammatical forms are placed in quotation marks throughout this report since lack of radial symmetry and any easily definable symmetry does not in itself justify the designation asymmetric.
during its storage in the female. These coiled spermatozoa are bent towards the single row of microtubules of their spiral pattern, leaving the double row of microtubules at the periphery of the coil (Figs. 11, 14, and 15). If one can assume that a flagellating spermatozoon bends in the same plane with respect to its microtubular pattern as does a coiled spermatozoon, then the results suggest that the spermatozoo of Parlatoria oleana may be related to flagella in that their microtubular configuration may influence, or even control, the two-dimensional component of their flagella-like motion. That this assumption is not unreasonable is suggested by the work of Lindahl and Drevius (24) who reported that permanent bending of the bull sperm tail induced by a hypotonic medium appears to be directly related to normal motion in that topographical relationships are maintained which are similar to those found in cilia that are fixed during and shortly after motility (14, 15, 47).

The pattern of whorled radii found in Matsucoccus bisetosus provides another exception to usual coccid spermatozoon structure. It may be considered to be an array of radially arranged crescents surrounded by a complete ring of microtubules. The crescents within the same spermatozoon vary with respect to their numbers of microtubules, a fact which could provide unequal numbers of microtubules on different sides of the spermatozoon, depending on the distribution, but no decisive evidence is available. It is interesting to note that an array which consists of seven whorled radii of microtubules occurs in the tentacles of certain suctorians (17) and that the spiral tilt, or pinwheel pattern which has been described for centrioles and basal bodies (2, 29, 50), shows some similarities to the pattern of whorled radii found in the spermatozoon of Matsucoccus bisetosus.

Ross and Robison (46) observed that the regions in the spermatozoon of Pseudococcus obscurus that have radially symmetrical patterns of microtubules show little motility compared to the regions that have "asymmetric" patterns. An extreme "asymmetry" is exhibited in some transections of the Unaspis euonymi spermatozoon in which two partial rings of microtubules are located on the same side of the spermatozoon, giving it grossly different numbers of microtubules in its opposite halves (Fig. 6). The discovery of 2½-3 concentric rows of microtubules in the spermatozoa of the coccid Statoecoccus tuberculatus (26) is consistent with the findings reported here.

Therefore, most of the microtubular patterns found in the spermatozoa of coccid insects appear to be related to each other and to the 9 + 2 pattern in that they have an inner, partial row of microtubules which disrupts their radial symmetry in somewhat the same manner as the two central microtubules disrupt that of a typical flagellum. Results from the species tested suggest that bending occurs, as in the 9 + 2 motile apparatus, in a plane approximately perpendicular to a reference plane which divides the patterns into "halves" with unequal numbers of microtubules. Three-dimensional motion appears to arise intrinsically.

It appears that all motile filaments, except the spermatozoo of certain Crustacea (36), have basic structural and functional similarities. Probably they all exhibit two-dimensional and three-dimensional components of motility, all are composed of microtubules, and a line drawn to bisect any of their microtubular patterns in a plane perpendicular to the plane of bending divides the pattern into halves with unequal numbers of microtubules. This may hold even for the 9 + 0 and 9 + 1 configurations. Whereas, it appears that motile ability may be inherent in the singlet microtubule, the ability to effect the two-dimensional component of flagellar and flagella-like motility may depend on the organization of microtubules into "asymmetric" patterns. In any case, general theories on mechanisms of flagellar motion should include a consideration of coccid spermatozoa.

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REFERENCES


