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Ultrastructure of ostrich (*Struthio camelus*) spermatozoa: I. Transmission electron microscopy

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ABSTRACT

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The origin and relationships of the tinamous (Order Tinamiformes), ratites (Order Struthioniformes, Rheiformes, Casuariiformes, Apterygiformes) and birds of the order Galliformes and Anseriformes is the subject of much debate and it has been suggested that the ultrastructural analysis of a wide variety of avian sperm may provide information relevant to this problem. This paper describes the fine structure of ostrich sperm and compares the results with published information for other non-passerine birds. Ostrich sperm display a short, conical acrosome which covers the tapered tip of the long, cylindrical nucleus. A nuclear invagination housing an acrosomal rod extends deep within the karyoplasm. A centriolar complex is situated beneath the head and consists of a short proximal centriole and a long (3,0 μm) distal centriole which extends the complete length of the midpiece. The central cavity of the distal centriole contains a pair of microtubules embedded in a rod of electron-dense material. The midpiece is surrounded by a mitochondrial sheath. Concentrations of fine granular material are present between the mitochondria. The principal-piece of the tail is demarcated from the midpiece by a distinct annulus and characterized by a ribbed fibrous sheath enclosing a typical axoneme. Rudimentary coarse fibres are observed between the fibrous sheath and the doublet microtubules of the axoneme in the proximal region of the principal-piece. The end-piece contains a disorganized collection of axonemal microtubules. Ostrich sperm differ in a number of respects from that of other non-passerine birds (the absence of a typical perforatorium; the presence of a ribbed fibrous sheath; a deep nuclear invagination; the structure and length of the distal centriole) but show a close similarity to sperm of the rhea and crested tinamou, both representatives of primitive avian families. These observations add further support to the theory that the ratites and tinamous constitute a monophyletic group. The evidence presented also reinforces the hypothesis that the ratites were the first group to branch off from the main avian stem, to be followed by the Galliformes & Anseriformes. Although it was impossible to determine whether the sperm of the tinamou are more "primitive" than those of the ostrich or rhea, it is clear that ostrich and rhea sperm are closely allied and distinct from tinamou sperm.

INTRODUCTION

During the past 40 years a number of studies have been carried out on the ultrastructure of the sperm of non-passerine birds. Because of their domestic status and economic importance most investigations have concentrated on birds of the order Galliformes. The fine structure of ejaculated chicken sperm (Grigg & Hodge 1949; Bonadonna 1954; Nagano 1962; Lake, Smith & Young 1968; Krustev & Danov 1968; Nicander 1970; Tingari 1973; Bakst & Howarth 1975; Bakst & Sexton 1979; Bakst 1980;

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Lake 1981; Thurston & Hess 1987), turkey sperm (Marquez & Ogasawara 1975; Bakst & Sexton 1979; Thurston & Hess 1987) and guinea fowl sperm (Thurston, Hess, Hughes & Froman 1982; Thurston & Hess 1987) has been thoroughly described, and this information has been supplemented by detailed accounts of the process of spermiogenesis in the chicken and turkey (Nagano 1962; Nicander & Hellström 1967; McIntosh & Porter 1967; Okamura & Nishiyama 1976; Gunawardana & Scott 1977; Maretta 1977; Baccetti, Bigliardi & Burrini 1980; Xia, Clermont, Lalli & Buckland 1986).

The ultrastructure of duck (Order Anseriformes) sperm has also been described (Humphreys 1972; Maretta 1975a; 1975b; 1977). Some attention has been given to aspects of spermiogenesis and sperm morphology in a number of more exotic bird species including the budgerigar (Humphreys 1975; Samour, Smith, Moore & Markham 1986), jacana (Saita, Longo & Tripepi 1983), white-naped crane (Phillips, Asa & Stover 1987), turtle-dove (Mattei, Mattei & Manfred 1972), Coturnix quail (Saita, Tripepi & Longo 1980) and cuckoo (Saita, Tripepi & Longo 1982). Other studies have focused attention on the fine structure of the sperm of the crested tinamou (Asa, Phillips & Stover 1986) and rhea (Phillips & Asa 1989) which according to Van Tyne & Berger (1976) are considered to be representatives of 2 of the most primitive avian families, the Tinamidae and Rheidae, respectively. This study details the ultrastructure of the spermatozoa of the ostrich (*Struthio camelus*), the only representative of an equally primitive avian family, the Struthionidae.

MATERIALS AND METHODS

Semen samples were collected from 20 sexually mature male ostriches from the Oudtshoorn district, Cape Province, Republic of South Africa, by digital massage of the deferent duct papillae (Berens von Rautenfeld 1977; Bertschinger, Burger, Soley & De Lange 1992). A portion of the ejaculate was fixed overnight at 4 °C in 4 % glutaraldehyde in Millonig's phosphate buffer. Employing a method of gentle centrifugation and resuspension, the sperm were subsequently washed once in Millonig's phosphate buffer, post-fixed in similarly buffered 1 % osmium tetroxide for 1 h at room temperature, and given 2 final buffer rinses. After having been pelleted in glass microhaematocrit tubes the sperm were dehydrated through a graded ethanol series (25 %, 50 %, 75 %, 96 %, 100 % x 2–10 min per step), cleared in propylene oxide, and embedded in Polarbed 812 epoxy resin. Thin sections were cut with a Reichert OmU4 ultramicrotome using a diamond knife, stained for 5 min each with a saturated solution of uranyl acetate (Watson 1958) and 0,2 % lead citrate (Reynolds 1963), and examined with a Philips 301 or CM10 transmission electron microscope operated at 80 kV.

RESULTS

The head

The head of ostrich sperm was a slightly curved, cylindrical structure which tapered gradually at its most anterior aspect and measured 13 μm in length and 0,5 μm in width at its widest point. The tip of the head was invested by a 2 μm-long acrosome containing fine homogeneous material of moderate electron density (Fig. 1). A small gap was obvious between the plasmalemma and the outer acrosomal membrane and a wider sub-acrosomal space was present between the inner acrosomal and nuclear membranes. Flocculant material of an electron density similar to that of the acrosome was observed in this space, often in close association with the nuclear membrane (Fig. 2). The plasmalemma was loosely applied over the sperm head, generally appearing smooth in the acrosomal region and ruffled throughout the rest of the head. Ruffling of the plasmalemma has also been reported in chicken sperm (Bakst & Howarth 1975) and is considered to be a fixation artefact. An area of close contact existed between the plasmalemma and nuclear membrane at the caudal extremity of the acrosome, forming a structure similar to the posterior ring of mammalian sperm (Fig. 1).

The nucleus exhibited a cylindrical form along most of its length except for the part covered by the acrosome where it tapered sharply to end in a fine point beneath the tip of the acrosome. From the tip of the nucleus a deep invagination formed a narrow tube-like structure which ran in the centre of the nucleus along the length of the tapered portion and roughly ¼ of the length of the main body of the nucleus. The nuclear invagination was delimited by the nuclear membrane and the centre of the enclosed space contained a rod of material of an electron density similar to that of the acrosome. A thin electron-lucent region separated the nuclear membrane from the rod of dense material (Fig. 1–3). The nuclear chromatin formed a dense compact mass (Fig. 1–3) although small light regions indicative of incomplete condensation appeared throughout the nucleus, particularly in the tapered portion. The base of the nucleus was delimited by a narrow, well defined segment of the nuclear envelope and adopted a characteristic shape which varied according to the plane of section. In most instances it took the form of 2 shallow concave depressions separated by a short medial spine of karyoplasm, although it often appeared as a single large, shallow hollow (Fig. 4) or as a small central depression flanked by 2 shallow cavities. Based on these observations it would appear that the implantation fossa of ostrich sperm consisted of a small central concavity surrounded by a shallow circular moat or series of depressions running around the perimeter of the nuclear base.

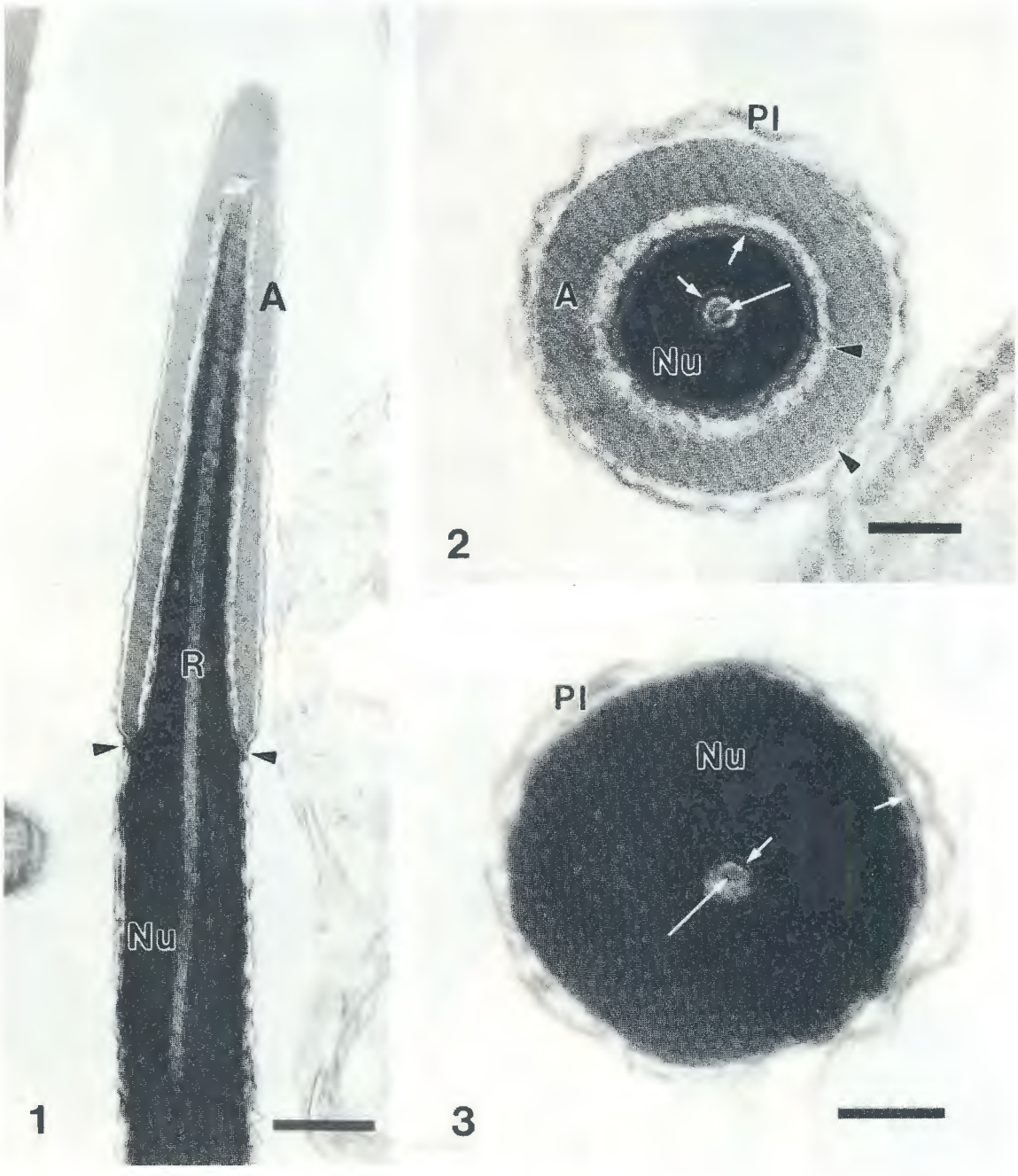
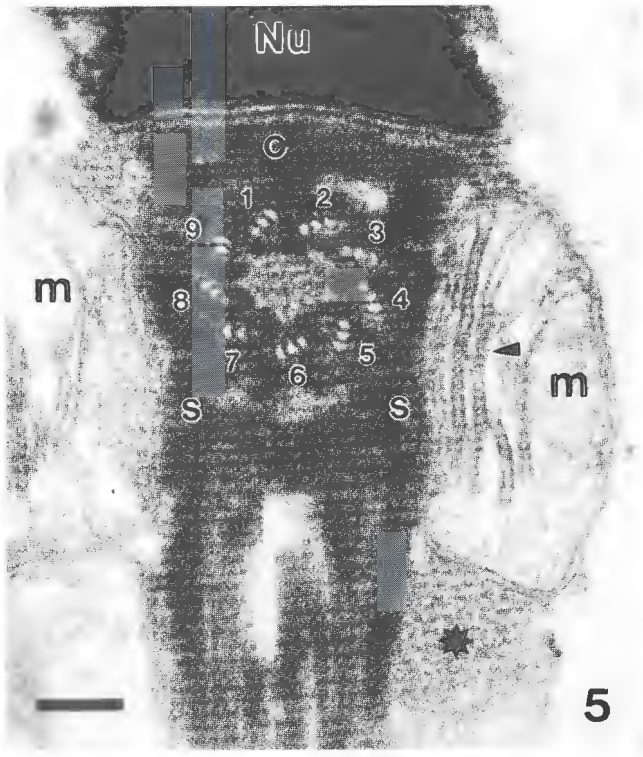


FIG. 1 A longitudinal section of the tip of the sperm head showing the acrosome (A) covering the tapered tip of the nucleus (Nu), the nuclear invagination containing the acrosomal rod (R), and the posterior ring (arrowheads). TEM X49000. Bar = 0,3 μm

FIG. 2 A cross-sectional enlargement of the acrosomal region of the sperm head revealing an uncharacteristically ruffled plasmalemma (Pl), the outer and inner acrosomal membranes (arrowheads), the outer and inner nuclear membranes (short arrows), and the acrosomal rod (long arrow). Flocculant material occupies the space between the acrosome and nucleus. Ruffling of the plasmalemma and the wide sub-plasmalemmal space may be fixation artifacts. The sub-acrosomal space is considered to be normal. Nucleus (Nu), acrosome (A). TEM X136500. Bar = 0,1 μm

FIG. 3 Cross-section of the main body of the nucleus (Nu) sectioned at the level of the nuclear invagination containing the acrosomal rod (long arrow). Note the ruffled appearance of the plasmalemma (Pl) and the inner and outer nuclear membranes (short arrows). TEM X157500. Bar = 0,1 μm



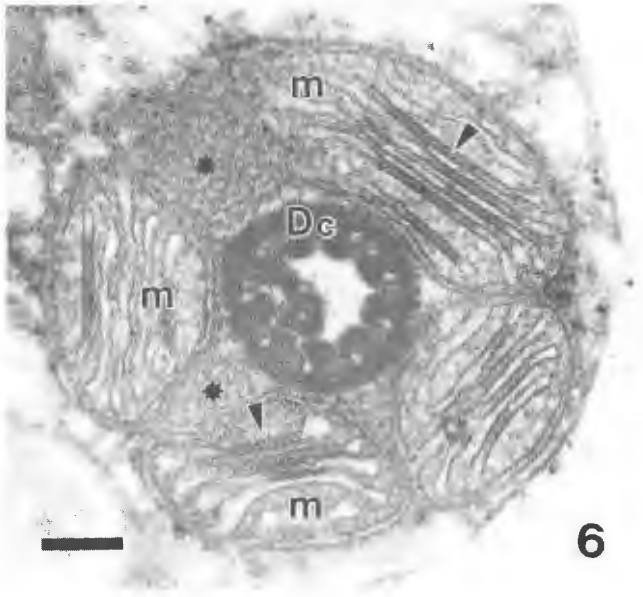


FIG. 4 A longitudinal section through the midpiece showing the mitochondrial sheath (M), the proximal centriole (Pc), the dense walls of the distal centriole (arrowheads), the inter-mitochondrial cement (asterisks) and the annulus (An). Note the termination of the inner dense rod of the distal centriole in the vicinity of the annulus (arrow). A cytoplasmic droplet (D) is present. TEM X43500. Bar = 0,5 μm

FIG. 5 A longitudinal section through the neck region of a sperm cell. The proximal centriole has been cut in cross-section to reveal the 9 sets of triplet microtubules (1–9). The central cavity of the centriole is filled with fine granular material similar to that situated between the mitochondria (asterisk). Note the atypical cristae (arrowhead) within one of the mitochondria (m). Non-segmented columns (s), capitellum (c), nucleus (Nu). The plasmalemma of the cell is missing. TEM X112000. Bar = 0,1 μm

FIG. 6 A transverse section through a midpiece surrounded by a cytoplasmic droplet. The dense wall of the distal centriole (Dc) contains nine sets of triplet microtubules. A dense rod of material containing 2 singlet microtubules is eccentrically positioned within the central cavity of the centriole. Mitochondria (m), atypical cristae (arrowheads), inter-mitochondrial cement (asterisks). TEM X101500. Bar = 0,1 μm

The neck and midpiece

Beneath the base of the nucleus lay a short (0,3 μm) proximal centriole which displayed the characteristic nine sets of triplet microtubules embedded in a ring of dense amorphous material. The central cavity of the centriole generally appeared empty, although it sometimes contained flocculant or granular material similar to that observed between the mitochondria of the midpiece (Fig. 5). Dense material associated with the juxta-nuclear surface of the proximal centriole filled the centre of the shallow nuclear fossa, merging with similar peripheral material provided by dense non-segmented columns emanating from the walls of the proximal and distal centrioles (Fig. 4 & 5). The distal centriole was positioned beneath, and at right angles to, the proximal centriole, and occupied the entire length of the midpiece. In transverse section it was seen to consist of a narrow ring of electron-dense material from which nine evenly spaced dense projections jutted into the centriolar cavity. Nine sets of characteristically arranged triplet microtubules were situated between the projections. Within the centriolar cavity was a rod of dense material containing a pair of microtubules. The rod was generally located in the centre of the centriolar cavity although it often adopted an eccentric position against the centriolar wall (Fig. 6). This explains why some longitudinal sections of the centriole failed to reveal its existence. The rod was occasionally seen in the form of 2 closely apposed but separate units, each containing a single microtubule. The dense material surrounding the central microtubules disappeared in the vicinity of the annulus (Fig. 4) and the organelles extended throughout the rest of the tail as the typical central pair of microtubules of the axial filament complex.

The 3,0 μm-long midpiece was slightly wider (0,65 μm) than the nucleus and contained about 20 mitochondria arranged in a helical pattern around the proximal and distal centrioles (Fig. 4 & 12). In both longitudinal and transverse sections of the midpiece the mitochondria presented a flattened, rectangular profile (Fig. 4), although round or oval forms were sometimes observed (Fig. 5 & 6). In tangential sections they appeared as rectangular or polygonal structures (Fig. 12). The mitochondria displayed longitudinal cristae embedded in a dense matrix. Atypical cristae containing paracrystalline inclusions were observed in some mitochondria (Fig. 5 & 6). The inclusions displayed 2 forms depending on the plane of section. Those sectioned longitudinally presented the appearance of tight junctions (Fig. 6) while oblique sections revealed a pattern of parallel fibres with a regular spacing and direction (Fig. 5 & 6). Sandwiched between the mitochondria were conspicuous accumulations of granular material (Fig. 4–6 & 12).

The principal-piece

A well developed annulus (Fig. 4, 7, 8 & 12) demarcated the boundary between the midpiece and the principal-piece, although a retro-annular recess was not apparent. The annulus was situated beneath the last row of midpiece mitochondria in close association with the plasmalemma and was composed of homogeneous, electron-dense material. The principal-piece formed the longest segment of the tail (50 μm) and consisted of an axial filament complex (axoneme) surrounded by a ribbed fibrous sheath (Fig. 7–15). The axoneme displayed the typical arrangement observed in mammalian sperm (Fawcett 1975) and consisted of 9 regularly spaced outer doublet microtubules surrounding a central pair of singlet microtubules. The A microtubule of each outer doublet was a circular structure filled with dense material whereas the B microtubule formed an incomplete lucent cylinder. Dynein arms projected from each A microtubule towards the neighbouring doublet and radial links formed a connection with the central microtubules (Fig. 10, 11 & 15).

The principal-piece was a tapered structure and the gradual decrease in diameter along its length, coupled with changes in the composition of the fibrous sheath, allowed 3 regions to be distinguished. The first region lay immediately beneath the annulus where the tail abruptly narrowed to a diameter of 0,5 μm. The axoneme was surrounded by a loosely arranged fibrous sheath consisting of 2 dense longitudinal columns connected by circumferential bands of dense material. The longitudinal columns and the interconnecting ribs appeared to be composed of alternating layers of electron-dense and loosely packed material, giving both structures a laminated appearance. The columns lay in line with the 2 central microtubules in the position occupied by coarse fibres 3 & 8 in mammalian sperm. Peculiar to this region was the presence of small, dense, coarse fibres lying between the fibrous sheath and the axonemal doublets, in close association with the A microtubules. A prominent cytoplasmic layer containing fine flocculent material was interposed between the fibrous sheath and the cell membrane (Fig. 7, 8, 10 & 12).

In the second region the diameter of the tail narrowed to 0,4 μm, the rudimentary coarse fibres disappeared, and the ribs of dense material appeared as solid structures. The longitudinal columns, however, retained their laminated appearance while the cytoplasmic layer became narrower. Septum-like inward extensions of the longitudinal columns were seen to make contact with the adjacent microtubular doublets (Fig. 15).

The third region of the principal-piece was characterized by a progressive decrease in diameter, from 0,3 μm to approximately 0,2 μm. The longitudinal

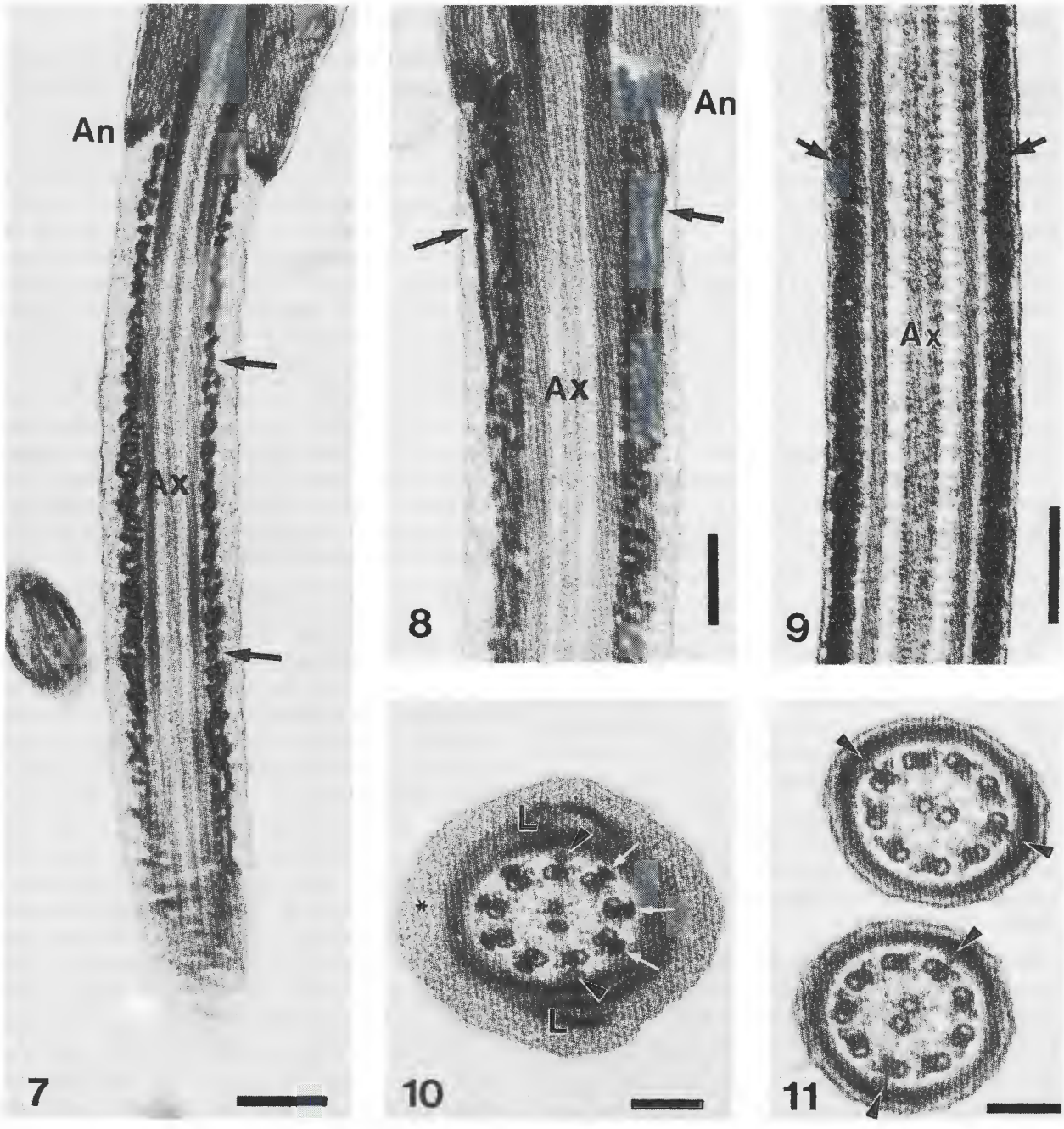


FIG. 7 A longitudinal section through the proximal region of the principal-piece of the sperm tail. The ribs of the fibrous sheath (arrows) are shown in cross-section. Note the layer of fine amorphous material between the fibrous sheath and the plasmalemma. Annulus (An), axoneme (Ax). TEM X45000. Bar = 0,3 μm

FIG. 8 A section similar to that shown in Fig. 7. In this instance the longitudinal columns of the fibrous sheath have been sectioned to reveal their loose arrangement (arrows). Annulus (An), axoneme (Ax). TEM X70000. Bar = 0,2 μm

FIG. 9 A longitudinal section through a more distal region of the principal-piece showing the axoneme (Ax) and the longitudinal columns of the fibrous sheath (arrows). Note that the plasmalemma is closely applied to the fibrous sheath. TEM X90000. Bar = 0,2 μm

FIG. 10 A cross-section of the proximal principal-piece showing the lamellar nature of the longitudinal columns (L) of the fibrous sheath. The small dense fibres of this region are situated against the outer face of the axonemal doublets (arrows). Dense fibres 3 and 8 have been incorporated into the longitudinal columns (arrowheads). Note the cytoplasmic layer (asterisk) containing fine flocculent material. TEM X111000. Bar = 0,1 μm

FIG. 11 A cross-section through the distal region of 2 adjacent sperm tails demonstrating the typical structure of the axoneme. The longitudinal columns of the fibrous sheath are inconspicuous but retain contact with the underlying doublets (arrowheads). TEM X109000. Bar = 0,1 μm

dense columns became solid, less conspicuous, structures which eventually disappeared leaving a thin dense band of material surrounding the axoneme. The plasmalemma was closely applied to the layer of dense material (Fig. 9, 11 & 13).

The end-piece

The end-piece formed the short, narrow (0,15 μm), termination of the tail and consisted of the axoneme covered by the plasmalemma. Because the transition from principal-piece to end-piece was gradual, remnants of the fibrous sheath were sometimes seen around the axoneme (Fig. 14). The organized structure of the axoneme was disrupted towards the end of the tail. The specific orientation of the axonemal microtubules was lost, the dynein arms and radial spokes linking the microtubules had disappeared, the doublets separated into individual components, and the dense contents of the A microtubules disappeared (Fig. 16). The disorganized collection of 20 lucent microtubules which resulted from these changes displayed a random decrease in number at the tip of the end-piece.

DISCUSSION

In a study of the development of spermatozoa in the rhea Phillips & Asa (1989) note the continuing debate concerning the origin and relationship of the tinamous, ratites, Galliformes and Anseriformes (Sibley & Frelin 1972; Cracraft 1974; 1981; De Boer 1980; Sibley & Ahlquist 1981; Stapel, Leunissen, Versteeg, Wattel & De Jong 1984; Jacob & Hoerschelmann 1985; Houde 1986). Evidence has been presented (Sibley & Frelin 1972; De Boer 1980; Stapel *et al.* 1984) to show that the ratites form a monophyletic group while other studies link the ratites and tinamous within a monophyletic taxon (Cracraft 1974; 1981; Sibley & Ahlquist 1981). The general opinion has been expressed that the tinamous and ratites are the most primitive birds, with the tinamou possibly representing the most ancient lineage. Asa *et al.* (1986) have suggested that the ultrastructural analysis of a wide variety of avian sperm may provide information relevant to taxonomic problems.

When viewed with the transmission electron microscope the sperm of the ostrich are structurally similar to those of the crested tinamou and the rhea but display striking differences when compared with the sperm of other non-passerine birds.

A tube-like structure containing a rod of dense material situated centrally within the nucleus is characteristic of ostrich sperm. A similar structure has been observed in the sperm head of the rhea (Phillips & Asa 1989) and tinamou (Asa *et al.* 1986). In the tinamou the tube runs the entire length of the nucleus, whereas in the rhea it extends from the anterior part of the acrosome to deep within the nucleus, in similar fashion to that observed in the ostrich (present study). It would appear from this study that the tube-like structure represents a deep invagination of the nuclear envelope, and that the dense contents of the tube form the acrosomal rod described by Phillips & Asa (1989) in rhea sperm. A structure analogous to the acrosomal rod, the acrosomal spine or perforatorium, has been observed in most non-passerine birds (Nagano 1962; Lake *et al.* 1968; Humphreys 1972; 1975; Bakst & Howarth 1975; Saita *et al.* 1980; Thurston *et al.* 1982; Samour *et al.* 1986; Thurston & Hess 1987; Phillips *et al.* 1987; Asa & Phillips 1987). The perforatorium is a solid, well-defined structure which fits into a shallow nuclear depression posteriorly and is covered by the acrosome anteriorly. The perforatorium is thus partly enclosed by the nucleus and partly by the acrosome, in contrast to the situation in the ostrich, rhea and tinamou, where the acrosomal rod is completely surrounded by the nucleus. No definite function has yet been ascribed to the avian perforatorium and it would appear merely to represent a residual structure. The loose arrangement of the plasmalemma around the ostrich sperm head and the resulting wide sub-plasmalemmal space has also been described in chicken sperm (Lake *et al.* 1968; Tingari 1973; Bakst & Howarth 1975) and is considered to be a fixation artefact. In duck sperm (Maretta 1975a) the plasmalemma is tightly applied over the head.

An obvious sub-acrosomal space appears to be a consistent feature of non-passerine bird sperm and is also present in ostrich sperm. This space is established during the early stages of acrosome development in ostrich spermatids (Personal observation) and its presence in mature sperm is unlikely to be the result of fixation procedures.

Another feature common to ostrich, rhea and tinamou sperm is the presence of a long distal centriole which extends the entire length of the midpiece. This organelle is situated immediately beneath, and at right angles to, a short proximal centriole. Although other non-passerine birds such as the fowl (Nagano 1962; Lake *et al.* 1968; Tingari 1973; Bakst & Howarth 1975; Okamura & Nishiyama 1976; Maretta 1977), turkey (Thurston & Hess 1987) and duck (Humphreys 1972; Maretta 1975b; Maretta 1977) also display a relatively long distal centriole it occupies only the anterior part of the midpiece. The guinea fowl is unique in that it apparently possesses only a distal centriole situated anteriorly within the midpiece (Thurston *et al.* 1982; Thurston & Hess 1987). In these species, and in the tinamou, the centriolar cavity appears to be empty and the central pair of singlet microtubules of the axoneme originate independently at the posterior extremity of the organelle. In both the ostrich and

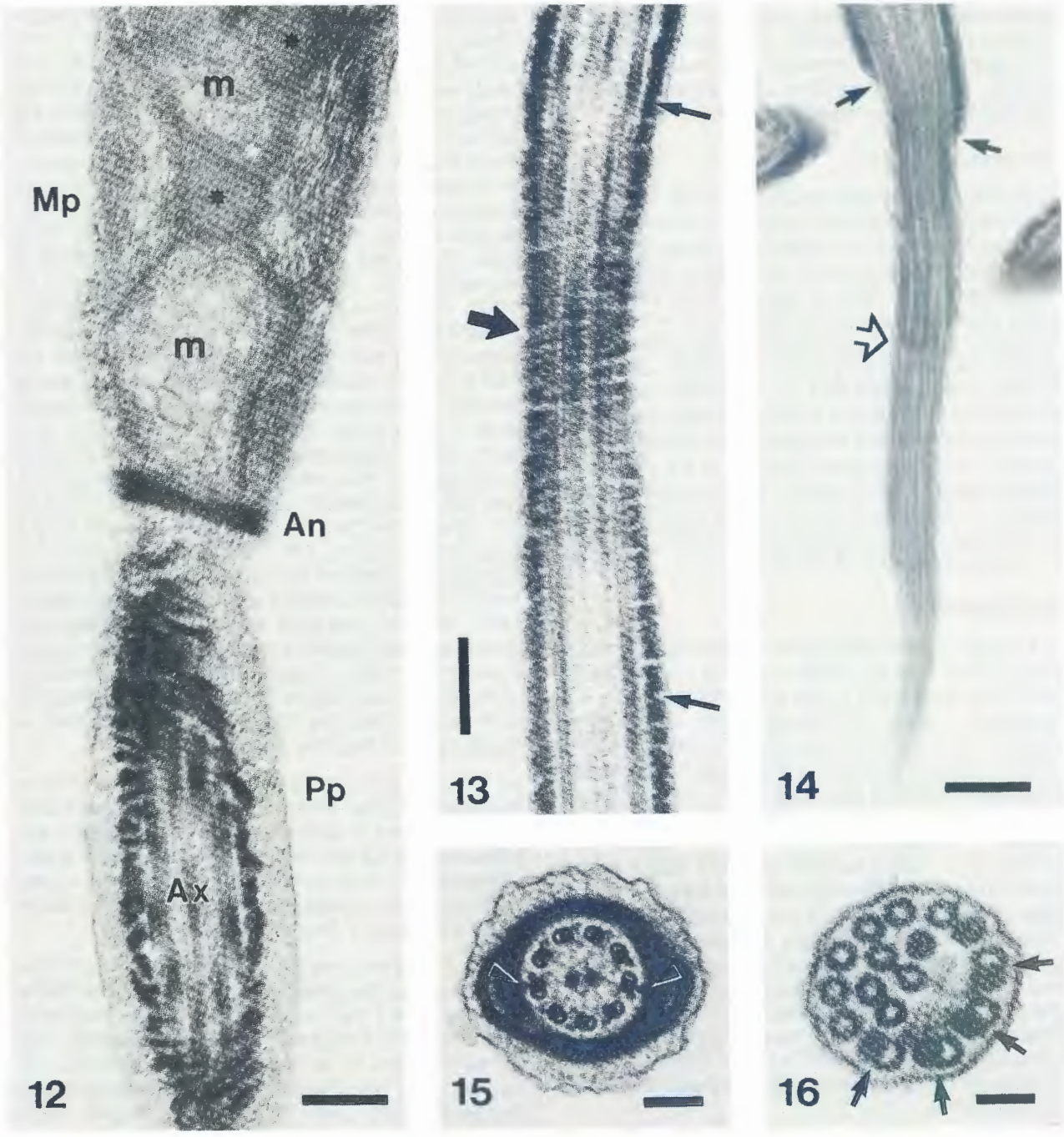


FIG. 12 A longitudinal section through the junction between the midpiece (Mp) and the principal-piece (Pp). The midpiece has been cut superficially revealing some of the mitochondria (m) and the annulus (An) in surface view. Inter-mitochondrial substance (asterisks), axoneme (Ax). TEM X65500. Bar = 0,2 μm

FIG. 13 A longitudinal section through the distal region of the principal-piece. The ribs of the fibrous sheath are shown in cross-sectioned (arrows) and longitudinal (squat arrow) profile. TEM X75500. Bar = 0,2 μm

FIG. 14 A longitudinal section through the end-piece. Note the staggered termination of the fibrous sheath (arrows) and the loss of contents of the axonemal microtubules towards the tip (open arrow). TEM X66000. Bar = 0,2 μm

FIG. 15 A cross-section through the intermediate region of the principal-piece. The coarse fibres have disappeared. Doublets 3 and 8 are connected to the longitudinal columns by splines of electron-dense material (arrowheads). The longitudinal columns contain a core of less dense material. The cytoplasmic layer surrounding the axoneme is thinner than that observed in the proximal region of the principal-piece (See Fig. 10). TEM X88500. Bar = 0,1 μm

FIG. 16 A cross-section of the end-piece showing disruption of the axoneme resulting in a collection of microtubular doublets (arrows) and dense and translucent singlet microtubules. TEM X183000. Bar = 50 nm

rhea 2 centrally positioned tubules, which are continuous with the central pair of axonemal microtubules in the rest of the tail, are observed within the central cavity of the distal centriole. Ostrich sperm differ from those of the rhea, however, in that the central tubules are embedded in a core of dense material which only disappears in the vicinity of the annulus. The eccentric position often adopted by this structure in ostrich sperm may be a fixation artefact.

The mitochondria of the midpiece of non-passerine birds display a variety of forms. In the ostrich these organelles are rectangular or polygonal plate-like structures similar to those seen in the chicken (Lake *et al*. 1968; Bakst & Howarth 1975), turkey (Thurston & Hess 1987) and guinea fowl (Thurston *et al*. 1982; Thurston & Hess 1987). It is interesting to note that in both the ostrich and rhea modifications of the mitochondrial membranes have been observed, with those of the rhea (Phillips & Asa 1989) resembling myelin figures and those of the ostrich adopting the form of atypical cristae containing paracrystalline material. The significance of these structural modifications is unknown. The number of mitochondria (20) seen in the midpiece of ostrich sperm tallies with that observed in the tinamou (Asa *et al*. 1986). However, both species display fewer mitochondria than the 25–30 organelles reported in the chicken, turkey, guinea fowl, duck, budgerigar and rhea (Lake *et al*. 1968; Bakst & Howarth 1975; Marquez & Ogasawara 1975; Maretta 1975b; Thurston *et al*. 1982; Samour *et al*. 1986; Thurston & Hess 1987; Phillips & Asa 1989). The substantial aggregations of fine granular material situated between the mitochondria of the ostrich midpiece have also been noted in the tinamou (Asa *et al*. 1986) and illustrated in the rhea (Phillips & Asa 1989). Similar material has not been identified in the other non-passerine bird species studied.

The principal-piece and end-piece of the ostrich sperm tail, as is the case in the rhea and crested tinamou, closely resemble the corresponding segments of the mammalian sperm tail. As in mammalian sperm the boundary between the midpiece and the principal-piece of ostrich sperm is demarcated by a distinct annulus. This structure is also present in sperm of the rhea and tinamou and would appear to be a feature of many non-passerine bird sperm, although it is only in the rooster (Nagano 1962; Lake *et al*. 1968; Tingari 1973; Bakst & Howarth 1975; Thurston & Hess 1987), guinea fowl (Thurston *et al*. 1982; Thurston & Hess 1987), turkey (Thurston & Hess 1987), duck (Maretta 1975b) and turtle dove (Mattei *et al*. 1972) that it has been specifically described. In ostrich sperm the principal-piece is invested by a well developed fibrous sheath which resembles that of mammalian sperm (Einarsson & Nicander 1961; Telkka, Fawcett & Christensen 1961; Fawcett 1965). This structure consists of paired longitudinal columns running the length of most of the principal-piece and interconnecting ribs of dense material. Although organized and arranged in a fashion similar to that of mammalian sperm, the ribbed sheath of ostrich sperm is more flimsy in structure, resembling that described in the rhea (Phillips & Asa 1989) and tinamou (Asa *et al*. 1986).

The occurrence of a ribbed fibrous sheath in ostrich, rhea and tinamou sperm is unique as this structure is not present in the sperm of other non-passerine birds. In the chicken (Nagano 1962; Lake *et al*. 1968; Tingari 1973; Okamura & Nishiyama 1976; Thurston & Hess 1987), turkey (Thurston & Hess 1987), guinea fowl (Thurston *et al*. 1982; Thurston & Hess 1987) and duck (Maretta 1975b) an amorphous sheath of granular material, which shows no definite signs of specialization, surrounds the axoneme. The proximal segment of the principal-piece of ostrich sperm displays a prominent cytoplasmic layer situated between the axoneme and the plasmalemma. This layer is filled with fine amorphous material and resembles a similar region seen in rhea sperm (Phillips & Asa 1989). Tinamou sperm are structurally similar in this respect although particulate material which morphologically resembles glycogen is found throughout this region (Asa *et al*. 1986). A similar region is absent in other non-passerine birds.

The end-piece of ostrich sperm reveals a pattern of termination of the axonemal microtubules that is similar to that reported in mammals (Nicander & Bane 1962; Fawcett 1965; Phillips 1970; Woolley & Nickels 1985). Disruption of the axonemal microtubules of the end-piece is also described in the duck (Maretta 1975b) and chicken (Lake *et al*. 1968). However, according to Woolley & Brammall (1987) the mode of termination of the axonemal microtubules in the chicken differs significantly from that observed in the ostrich. In the chicken the A-tubule of the axonemal doublets loses its dynein arms and its dense core after which the B-tubule ends as a loose strand. The A-tubules continue to the tip of the flagellum as singlets. The central pair of microtubules terminates before dissociation of the doublets and merges into "a central electron-dense fusiform mass." In contrast, disruption of the axoneme in ostrich sperm results in the formation of 20 lucent singlet microtubules which show a random decrease in number towards the tip of the flagellum.

Although it is apparent from the morphological information presented that ostrich, rhea and tinamou sperm are structurally similar, and exhibit features which are distinctive when compared to the sperm of other non-passerine birds, the evidence favouring the more primitive status of tinamous remains contradictory. It has been proposed, for example, that

the "evolutionary pattern favours diminuation of the perforatorium" (Thurston & Hess 1987). If it is accepted that the acrosomal rod, which is a characteristic feature of ostrich, rhea and tinamou sperm, is analogous to the perforatorium of other birds, then it would appear to be true that the reduction in length of this structure is a reflection of evolutionary development. In the tinamou the acrosomal rod extends the entire length of the nucleus (Asa *et al.* 1986) whereas in the ostrich (present study) and rhea (Phillips & Asa 1989) this structure is shorter, although it penetrates deep within the nucleus. In other non-passerine birds the well-defined perforatorium is extremely short and is confined to the anterior tip of the nucleus, although it also extends beneath the acrosome beyond the confines of the nucleus (Nagano 1962; Lake *et al.* 1968; Humphreys 1972; 1975; Tingari 1973; Maretta 1975a; Bakst & Howarth 1975; Saita *et al.* 1980; Thurston *et al.* 1982; Samour *et al.* 1986; Thurston & Hess 1987; Phillips *et al.* 1987; Asa & Phillips 1987). In some non-passerine birds, for example, the jacana (Saita *et al.* 1983), cuckoo (Saita *et al.* 1982) and turtle-dove (Mattei *et al.* 1972) the perforatorium is reported to be totally absent, a situation that is characteristic of passerine birds (Nicander 1970; Henley, Feduccia & Costello 1978).

The more primitive nature of tinamou sperm is also supported by the report that no dense fibres are present in the sperm tail (Asa *et al.* 1986) in contrast to the rudimentary dense fibres observed in the proximal segment of the principal-piece of ostrich and rhea (Phillips & Asa 1989) sperm. Small dense fibres have also been reported in the sperm of the chicken (Lake *et al.* 1968; Bakst & Howarth 1975), duck (Humphreys 1972; Maretta 1975b), turkey (Thurston & Hess 1987) and guinea fowl (Thurston *et al.* 1982), but these are found in the distal portion of the midpiece. The particular location of the dense fibres in these birds does not appear to be significant, as in each case the fibres begin at the point of termination of the distal centriole. As the distal centriole occupies the entire midpiece of ostrich and rhea sperm the dense fibres are confined to the proximal part of the principal-piece. Chicken and duck sperm possess a much shorter distal centriole (1,5 μm and 2,0 μm, respectively) (Maretta, 1975b; Okamura & Nishiyama 1976) and the dense fibres therefore occupy the remaining (distal) part of the midpiece. Dense fibres have also been reported in sperm of the jacana (Saita *et al.* 1983) and are a prominent and characteristic feature of passerine bird sperm (Yasuzumi & Sugioka 1966; Nicander 1970; Fawcett & Phillips 1970; Humphreys 1972; Henley *et al.* 1978; Asa & Phillips 1987). The progressive development of the outer dense fibres of the axoneme would therefore appear to be a characteristic feature in the evolution of avian sperm. However, the reports that dense fibres are absent in mature sperm of the turtle-dove (Mattei *et al.* 1972) and white-naped crane (Phillips *et al.* 1987) places a question mark on the importance of this feature as a determinant of primitive status.

In contrast, ostrich and rhea sperm appear to be more primitive than those of the tinamou in respect of the structure of the distal centriole. In these birds a central pair of microtubules occupy the centriolar cavity, a feature which is typical of chelonian (Furieri 1970; Hess, Thurston & Gist 1991) and most mammalian sperm (Fawcett & Phillips 1969; Pedersen 1970; Gordon 1972; Fawcett 1975). Tinamou sperm lack this microtubular arrangement and display an empty distal centriole (Asa *et al.* 1986) in similar fashion to that described in other non-passerine birds. Although many more species will have to be studied before a trend in the evolution of bird sperm can be determined, the following general tendencies in the development of non-passerine bird sperm are evident:

- A reduction in the length of the perforatorium (acrosomal rod) and the eventual disappearance of this structure.
- A concomitant decrease in the depth of the nuclear recess housing the perforatorium and a shortening of the tapered portion of the nucleus leading to penetration of the perforatorium beneath the acrosome.
- Simplification and shortening of the distal centriole.
- Disappearance of the fibrous sheath covering the principal piece of the sperm tail.

Based on ultrastructural characteristics, ostrich sperm are strikingly similar to rhea and tinamou sperm adding further support to the theory that the ratites and tinamous constitute a monophyletic group. The evidence presented also reinforces the hypothesis (Stapel *et al.* 1984) that the ratites were the first group to branch off from the main avian stem, to be followed by the Galliformes and Anseriformes. Although it was impossible to determine whether the sperm of the tinamou was more "primitive" than that of the ostrich or rhea, it was clear that ostrich and rhea sperm are closely allied and distinct from tinamou sperm.

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