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Spermatozoal Ultrastructure in Three Species of Parrots (Aves, Psittaciformes) and Its Phylogenetic Implications

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ABSTRACT ***Background:*** **DNA-DNA hybridization studies suggest that Psittaciformes are highly, but not the most, derived nonpasserines. Multilocus protein electrophoresis indicates that cockatoos (Cacatuinae) form a monophyletic lineage distant from the other Australo-Papuan psittacids (Psittacinae).**

Methods: **Transmission electron microscope procedures are applied to the spermatozoa of three parrots, in the Cacatuninae and Psittacinae, to investigate these relationships.**

Results: **Psittaciform sperm have the following characteristics: (1) conical acrosome vesicle; rodlike perforatorium; cylindrical, highly condensed nucleus; proximal and distal centriole embedded in dense material; elongate periaxonemal mitochondrial midpiece, (2) nine dense peripheral axonemal fibers (coarse fibers), (3) no fibrous sheath around the axoneme, (4) mitochondria with linear cristae, lacking intra- (or inter-) mitochondrial dense bodies, (5) restriction of the endonuclear perforatorial canal to the anterior region of the nucleus, (6) a short distal centriole, and (7) nucleus abutting on but not penetrating the acrosome.**

Conclusions: **(1) These features are tetrapod symplesiomorphies, (2) is an amniote synapomorphy; the fibers differ from those of reptiles in being uniform in size, (3) loss of the fibrous sheath is an apomorphy known elsewhere only in columbiforms, (4) are apomorphies relative to basal amniotes (Chelonia, *Sphenodon*, and Crocodilia), (5) is an apomorphic condition shared with other nonpasserines (galliforms and the white-naped crane) and crocodilians, (6) the latter taxa differ from parrots in a plesiomorphic elongation of the distal centriole, and (7) is a unique apomorphy of parrot sperm relative to other nonpasserines and reptiles. The short midpiece of *N. hollandicus* distinguishes this cacatuine from the two psittacines.**

Key words: Cacatuinae, Psittacinae, Endonuclear canal, Perforatorium, Transmission electron microscopy

The Psittaciformes (parrots), which form the third largest nonpasserine avian order, are an old lineage that probably arose in the Australasian section of Gondwanaland (Homberger, 1990). From DNA-DNA hybridization studies, the Psittaciformes are recognized as the sister group of the most derived avian assemblage. This assemblage consists of two monophyletic sister groups, one of which consists of several families ranging from the Apodiformes to the Caprimulgidae, the other consisting of the Passeriformes and the sister group of these. The latter group contains many families ranging from the Columbidae to the Procellariidae, of which the Columbidae appears to be the most plesiomorphic (Sibley et al., 1988; Sibley and Ahlquist, 1990). From DNA hybridization parrots thus appear to be a moderately apomorphic group within the nonpassserines. They are highly derived relative to the Phasianidae (Galliformes), which lie in a clade that is the sister group of the ratites, but are less derived than the Columbidae (Sibley and Ahlquist, 1990). The Galliformes, exemplified by the domestic fowl (Grigg and Hodge, 1949; Nagano, 1960, 1962; McIntosh and Porter, 1967; Lake et al., 1968; Harris et al., 1973; Tingari, 1973; Bakst and Howarth, 1975; Gunawardana and Scott, 1977; Okamura and Nishiyama, 1976, 1978; Bakst and Sexton, 1979; Bae and Kim, 1987; Thurston and Hess, 1987; Sprando and Russell, 1988), the turkey (Marquez and Ogasawara, 1975; Thurston and Hess, 1987), and the guinea fowl (Thurston et al., 1982; Thurston and Hess, 1987) and the Columbidae, exemplified

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by various pigeons and doves (Mattei et al., 1972; Yasuzumi and Yamaguchi, 1977; Jamieson, unpub.), have been examined for sperm ultrastructure and form a useful basis for comparison in the present study. Further comparison is made with the sperm of palaeognaths (ratites) (Phillips and Asa, 1986, 1989; Baccetti et al., 1991) and passerines, of which several descriptions exist (see references in Asa and Phillips, 1987).

With regard to internal relationships of the Psittaciformes, Christidis et al. (1991) have demonstrated, largely on the basis of multilocus protein electrophoresis, that cockatoos (Cacatuinae) form a monophyletic lineage distant from the other Australo-Papuan psittacids, the Psittacinae, consisting of parrots (Platycercini and Psittaculini) and lorikeets (Loriini). They show also that lorikeets are monophyletic, although clustered among the other parrots.

The phylogenetic position of birds has been provisionally assessed, using spermatozoal ultrastructure and parsimony analysis, within the context of all amniote classes by Jamieson and Healy (1992). The sperm of the budgerigar has been briefly described by Samour et al. (1986) and its spermiogenesis by Humphreys (1975). In the present study we investigate the ultrastructure of the spermatozoa of three contrasted psittaciforms: *Nymphicus hollandicus,* the cockatiel (Cacatuinae, Calopsittacini), *Melopsittacus undulatus,* the budgerigar (Psittacinae, Platycercini), and *Agapornis roseicollis,* the peach-faced lovebird (Psittacinae, Psittaculini), for its interest per se, to assess the phylogenetic position of psittaciforms relative to other nonpasserines which have been investigated for spermatozoal ultrastructure and to provide data for future cladistic analysis of the internal relationships of psittaciforms. These aims recognize the need "to depend increasingly on collaborative, multidisciplinary and non-dogmatic approaches rather than on the use of single character complexes, techniques or methods to unravel the evolutionary history of birds" (Homberger, 1990).

MATERIALS AND METHODS

Specimens of *Melopsittacus undulatus, Agapornis roseicollis,* and *Nymphicus hollandicus* obtained from aviaries were euthanased and portions of the ductus deferens removed. This tissue was fixed in cold (4°C) 3% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.2) for 2 hours and then rinsed in three changes of cold buffer (5 minutes each). It was postfixed in similarly buffered 1% osmium tetroxide for 80 minutes, rinsed three times (5 minutes each) with cold buffer, and dehydrated in an ascending ethanol series (20%, 40%, 60%, 70%, 80%, 90%, and two changes of 100%), 30 minutes in each. The ethanol solutions up to 70% were kept at 4°C and the rest at room temperature. The tissue was then immersed in a 50% ethanol/50% Spurr's resin solution and left on a rotator for 1 hour. Spurr's resin was then added to this mixture to increase the resin concentration to 75%, and this was again rotated for 1 hour. The 75% mixture was then replaced by 100% Spurr's resin and rotated for 2 hours. The tissue was left overnight in a final change of 100% resin and then transferred to silicon moulds filled with fresh resin and polymerized in a 60°C oven for at least 8 hours.

The blocks were trimmed and survey sections were cut using a glass knife on an LKB III Ultratome. Ultrathin sections were cut with a Dehmer diamond knife on an LKB UM IV Ultratome and mounted on copper grids coated with 1% colloidion in amyl acetate. These were stained using the following technique: 30 seconds in lead citrate, 2 minutes in uranyl acetate, and 1 minute in lead citrate, with two rinses in distilled water between each stain. The grids were dried and micrographs taken on an Hitachi H-300 electron microscope at 75 kV.

RESULTS

Melopsittacus undulatus, Budgerigar

Throughout this report, the region of the acrosomal tip is referred to as the "anterior" or "proximal" end of the spermatozoon, whereas the "posterior" or "distal" extremity signifies the tip of the tail.

General

The spermatozoon of *Melopsittacus undulatus* is a filiform cell consisting of a head region containing the nucleus and acrosomal structures, a midpiece, and a tail region. Its ultrastructure is summarized, semidiagrammatically, in Figure 3, which is referred to throughout the description. The anteriorly tapering, cylindrical head was estimated from both light microscopy and transmission electron microscopy to be ~13 μm long.

Acrosome Complex

The anteriormost region of the head consists of the acrosomal complex, which is composed of a smooth, elongated, conical acrosomal vesicle and a rod-like perforatorium lying free in the subacrosomal space (Figs. 1A, C, D, G, H; see also Fig. 3). The acrosomal vesicle is 1.9 μm long (n = 2) and terminates anteriorly in a blunt tip. Its width is greatest at its distal end. Here, its diameter is 0.5 μm and its walls are ~0.15 μm thick (Fig. 1C, D). The perforatorium extends proximally, very nearly to the tip of the spermatozoon, and distally extends within a cylindrical endonuclear canal another 0.6 μm into the anterior region of the nucleus. At its widest point, the perforatorium measures 0.2 μm (Fig. 1C). The contents of the acrosomal vesicle are homogeneous and of moderate electron density. The perforatorium is slightly more electron-dense, but of a less uniform composition, with irregularly spaced, electron-lucent channels penetrating it. Granular material surrounds the perforatorium within the subacrosomal space (Fig. 1C, D). The space between the outer membrane of the acrosomal vesicle and the plasma membrane is also occupied by granular material. This gap is ~20 nm wide at the distal end of the acrosomal vesicle. The acrosomal vesicle terminates adjacent to the proximal end of the nucleus. These two structures do not overlap or join directly. It appears that only the plasma membrane and the shared perforatorium hold them in position.

Nucleus

The nucleus, which is a gently curved cylinder (Fig. 1A), has an approximate length of 10 μm, a width of 0.5 μm at its proximal end, and 0.6 μm at the nuclear-midpiece junction (Fig. 1C–F). The endonuclear canal (and perforatorium) occupies roughly only the proxi-

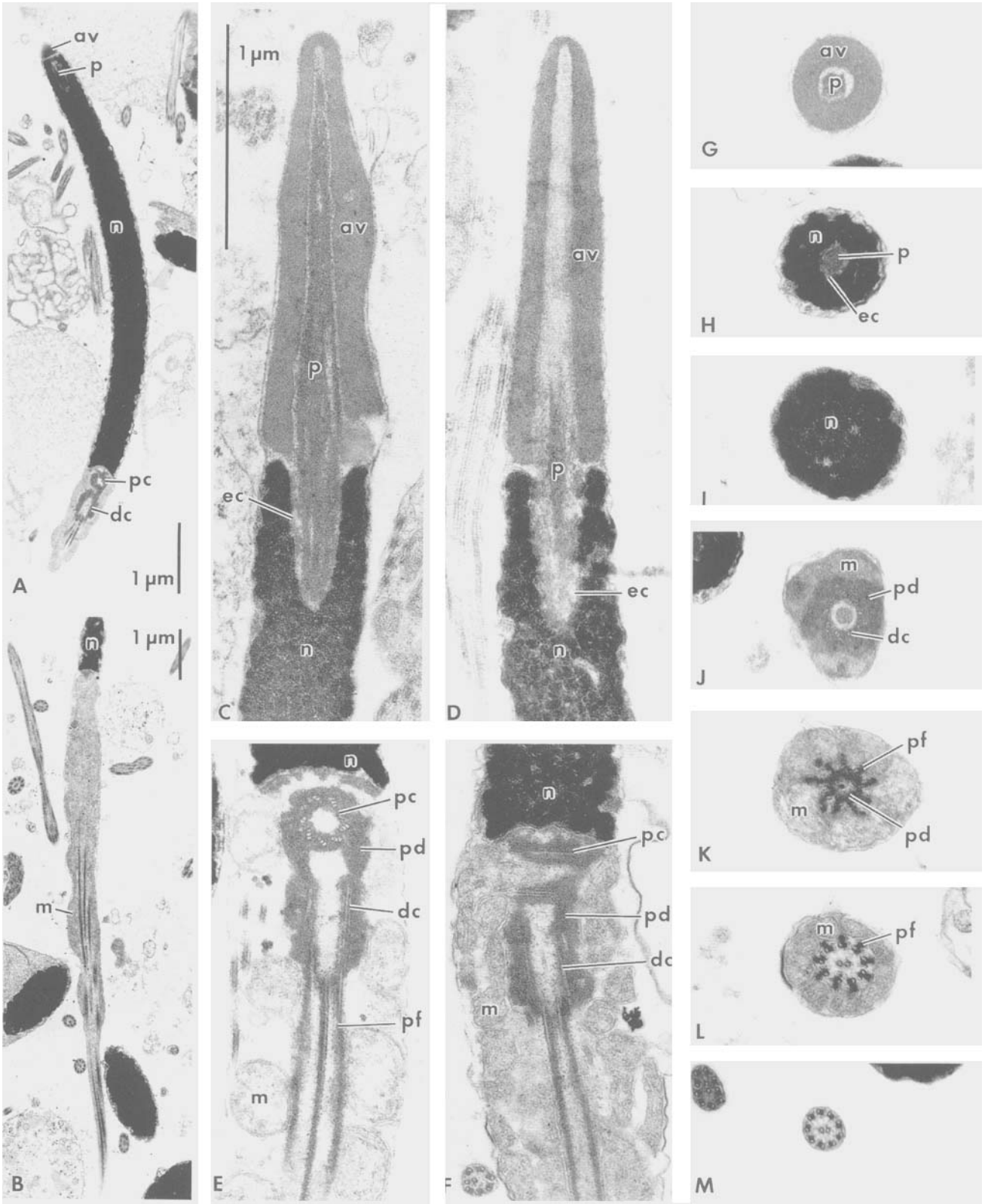

Fig. 1. *Melopsittacus undulatus*. **A.** Longitudinal section (LS) of the full length of the nucleus. **B.** LS of the full length of the midpiece. **C** and **D.** LS of the acrosomal complex and the anterior region of the nucleus. **E** and **F.** LS of the posterior end of the nucleus and part of the midpiece. Note the nine triplets of the transversely sectioned proximal centriole (E), which lies at right angles to the distal centriole, the latter forming the basal body of the axoneme. **G.** Transverse section (TS) of the acrosomal region through the perforatorium. **H.** TS nucleus through the endonuclear canal. **I.** TS nucleus posterior to the endonuclear canal. **J.** TS through the distal centriole showing granular central element and surrounding mitochondria. **K.** TS midpiece at the point of transition between distal centriole and axoneme. **L.** TS midpiece posterior to the distal centriole showing the 9 + 2 axoneme with peripheral dense fibers. **M.** TS of the axoneme behind the midpiece. Scale bars = 1 μm (C–M same scale). av, acrosome vesicle: dc, distal centriole; ec, endonuclear canal; m, mitochondrion; n, nucleus; p, perforatorium; pc, proximal centriole; pd, pericentriolar density; pf, dense peripheral fiber (coarse fiber).

mal 5% of the nuclear length and is 0.2 μm wide (Figs 1C, D). The chromatin is compact and electron-dense, although sporadic, small, electron-lucent areas are interspersed throughout the nucleoplasm. The surface is rough and the nuclear membrane is in close association with the plasma membrane (Fig. 1C, D, H, I). The distal edge of the nucleus forms a broad, shallow, concave fossa (Fig. 1E,F).

Midpiece, Centrioles, and Axoneme

The cylindrical midpiece region is 5.1 μm long (n = 1) and ~0.6 μm wide along much of its length. At its anterior end, a basal lamina lines the concave surface of the nuclear base. Associated with this lamina is a band of granular, electron-dense material (Fig. 1E). Immediately posterior to the lamina is a 0.3-μm-long proximal centriole, positioned centrally, parallel to the base of the nucleus. Lying perpendicular to the proximal centriole is a 0.4-μm-long distal centriole, occupying only a very small fraction of the midpiece length. Both centrioles display the typical pattern of nine triplets of microtubules arranged in a cylinder, and both are embedded in electron-dense pericentriolar material (Fig. 1E, F, J, K). Whereas the centre of the proximal centriole appears completely electron-lucent, mottled granular material occupies the lumen of the distal centriole (Fig. 1J).

The axoneme begins at a point ~1 μm along the length of the midpiece (Fig. 1E, F), continuous with the distal centriole, and extending the remainder of the length of the spermatozoon. It is organized according to the usual "9 + 2" pattern (Fig. 1K–M). The A microtubule of each doublet is completely circular and electron-dense and bears two dynein arms, whereas the lumen of the incomplete subunit B is electron-lucent. In the axoneme of the midpiece, associated peripherally with each doublet and in the same radius is a dense peripheral fiber (coarse fiber). In cross section, these fibers, which are approximately equal in size, are circular with a diameter of approximately 30 nm (Fig. 1K, L).

A single layer of mitochondria surrounds the centrioles and the axoneme along the length of the midpiece. In longitudinal section, the mitochondria appear elongate elliptical and rather loosely aggregated, particularly in the centriolar region, with flocculent material present between them. They are apparently arranged longitudinally in a long period spiral (Fig. 1B, E, F). In transverse section, they appear more circular, and approximately nine are contained, tightly juxtaposed, around the axoneme (Fig. 1J–L). Mitochondrial dimensions are roughly 0.3 × 0.15 × 0.15 μm. The mitochondrial matrix is moderately electron-dense and the cristae, although difficult to discern, appear to be linear or slightly curved.

No annulus is discernible at the distal end of the midpiece, but the beginning of the tailpiece can be identified by a reduction in the diameter of the spermatozoon, to ~0.2 μm (Fig. 1B). The length of the tail has been estimated from light micrographs to be 53–55 μm (n = 5), for an entire sperm length of ~70–71 μm (n = 7). Transverse sections of the flagellum reveal the typical "9 + 2" microtubular axoneme, but the peripheral accessory fibers seen in the midpiece region do not exist (Fig. 1M). A narrow, 10-nm-wide ring of cytoplasm with granular inclusions separates the doublets from the surrounding plasma membrane.

Nymphicus hollandicus, *Cockatiel, and* Agapornis roseicollis, *Peach-faced Lovebird*

The spermatozoa of *Agapornis roseicollis* and *Nymphicus hollandicus* essentially agree with the above account for *Melopsittacus undulatus,* with some differences in acrosome vesicle size and midpiece length (Fig. 2A–D). Thus a hollow conical acrosome vesicle encloses a stoutly rodlike perforatorium around which is a small or negligible amount of subacrosomal granular material. The perforatorium, which is penetrated by pale canals, extends almost to the tip of the acrosome vesicle and posteriorly for a short distance, within an endonuclear canal, into the anterior end of the nucleus (Fig. 2A, C). The cylindrical nucleus has a shallow concave basal fossa housing only the anterior region of the proximal centriole. This centriole lies parallel to the base of the nucleus and approximately at right angles to the distal centriole. Both centrioles are embedded in dense material. The axoneme, arising from the distal centriole, again lacks a fibrous sheath or an amorphous sheath. In the midpiece, nine coarse fibers are attached externally to the doublets in the same radii. The axoneme, with its coarse fibers is surrounded by a single layer of mitochondria, few and ovoid in cross section but seen in longitudinal section to be slightly elongated and apparently arranged in a long period spiral. No annulus has been demonstrated. The coarse fibers do not extend behind the midpiece. The midpiece is very short in *N. hollandicus* (~2 μm (n = 4)) (Fig. 2B), but in *A. roseicollis* (Fig. 2D) the midpiece has similar dimensions to that of the budgerigar, ~7 μm (n = 1). Nuclear and acrosomal dimensions for both species are in the same order as those of *M. undulatus.* They are: for *N. hollandicus,* length of acrosome 1.4 μm (n = 3); length of nucleus 6.5 μm (n = 2); width of nucleus proximally 0.5 μm, distally 0.6 μm; for *A. roseicollis,* length acrosome 1.5 μm (n = 2); length nucleus 9.3 μm (n = 1); width of nucleus proximally 0.5 μm, distally 0.6 μm.

DISCUSSION

The spermatozoon of the Psittaciformes, as exemplified by *Nymphicus hollandicus, Melopsittacus undulatus* and *Agapornis roseicollis,* retains many features of "reptilian" sperm (see Jamieson and Healy, 1992), whereas others are unique to birds or are limited, in the present state of our knowledge, to Psittaciformes.

A conical acrosome vesicle penetrated almost to its tip by a subacrosomal space, which contains a rodlike perforatorium, as in parrot sperm, is a feature not only of reptiles (Healy and Jamieson, 1992; Jamieson and Healy, 1992; Jamieson and Scheltinga, 1993) and nonpasserine birds, but also of the primitive frog *Ascaphus truei* (Jamieson et al., 1993) and of the lungfish *Neoceratodus forsteri* (see Jespersen, 1971). It is therefore a basic tetrapod plesiomorphy. In birds, it has been demonstrated ultrastructurally in the nonpasserines turkey, *Meleagris gallopavo,* chicken, *Gallus domesticus,* and guinea fowl, *Numida meleagris* (e.g., Thurston and Hess, 1987), the mallard duck, *Anas platyrhynchos* (Humphreys, 1972), the turtle dove *Streptopelia roseogrisea* (Mattei et al., 1972, Fig. 18) and the quail

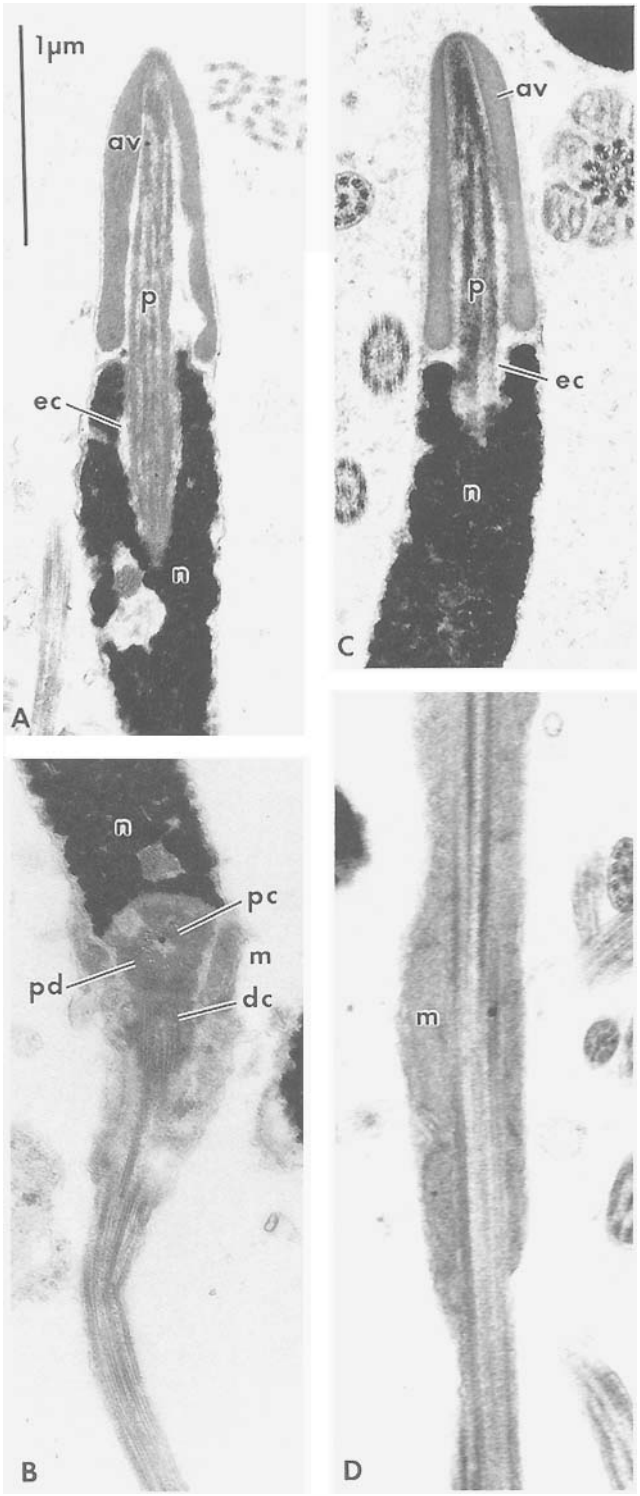

Fig. 2. *Nymphicus hollandicus:* **A.** Longitudinal section (LS) of acrosome vesicle and anterior end of nucleus penetrated by the perforatorium. **B.** LS of the posterior end of the nucleus and short midpiece region with two centrioles. *Agapornis roseicollis:* **C.** LS of acrosome vesicle and anterior end of nucleus penetrated by the perforatorium. **D.** LS (slightly oblique) of the distal end of the elongated midpiece. Scale bar = 1 μm (A–D same scale).

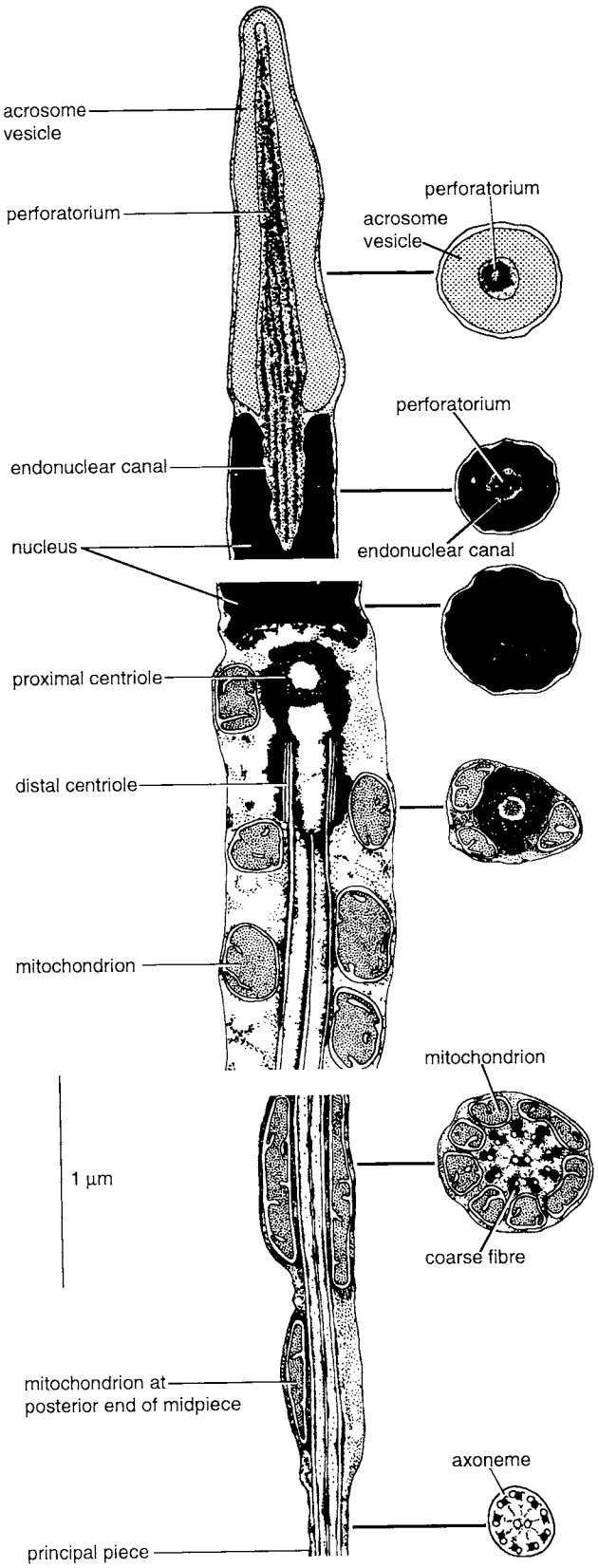

Fig. 3. *Melopsittacus undulatus*. Semidiagrammatic representation of the ultrastructure of a spermatozoon from the ductus deferens.

Coturnix coturnix (Saita et al., 1980), and in the ratites (palaeognaths) tinamou, *Eudromia elegans* (Asa et al., 1986), ostrich, *Struthio camelus,* and emu, *Dromaius novaehollandiae* (Baccetti et al., 1991). However, unlike these other species, the parrot acrosomal vesicle does not overlap the nucleus, thus giving a psittaciform synapomorphy and autapomorphy. No more than a tenuous connection, by the plasma membrane and the shared perforatorium, exists between these two structures. This fragile connection is considered to form a point of mechanical weakness and "decapitated" spermatids and isolated acrosomes are often found in testicular sections (Humphreys, 1975). Parrot sperm, like those of ratites and other birds, differ from reptiles in reduction of the subacrosomal material (subacrosomal cone) to a negligible amount. In the columbiforms *Ocyphaps lophotes* and *Geopelia striata,* even a perforatorium is absent (Jamieson, unpub.). The psittaciform restriction of the endonuclear canal, housing the perforatorium, to the anterior region of the nucleus is here recognized as an apomorphic condition shared with other nonpasserines (galliforms and the white-naped crane, Phillips et al., 1987) and crocodilians (Saita et al., 1987).

The nonpasserine and suboscine spermatozoon acrosome is short relative to the nucleus, as in reptiles (Jamieson and Healy, 1992; Jamieson and Scheltinga, 1993) in contrast to the oscine spermatozoon, which has an extremely large acrosomal complex. The nuclear and acrosomal lengths reported for the budgerigar in this study and from the work of Samour et al. (1986) are close to those ascertained for the three galliform species studied (Thurston and Hess, 1987).

Not all nonpasserines possess a conical acrosome. A small, approximately spherical acrosome has been described for the white-naped crane, *Grus vipio* (Phillips et al., 1987), for *Jacana jacana* (Saita et al., 1983), for the woodpecker *Melanerpes carolinus* (Henley et al., 1978), and for most Charadriiformes (Fawcett et al., 1971). These latter avian taxa are considered to be advanced nonpasserines on the basis of the DNA hybridization studies (Sibley et al., 1988; Sibley and Ahlquist, 1990).

The extent of the perforatorium of parrot sperm, penetrating almost the entire length of the acrosomal vesicle and extending distally into the anteriormost end of the nucleus, is very similar to that shown by the guinea fowl (Thurston and Hess, 1987). In the other Galliformes studied (turkey and chicken), the perforatorium extends forward only approximately half the length of the acrosomal vesicle. However, the length of the endonuclear canal, limited to the anterior region of the nucleus, is approximately equivalent in all four species. The endonuclear canal in ratite spermatozoa extends the entire length of the nucleus (Asa et al., 1986; Baccetti et al., 1991), as in Chelonia and *Sphenodon* (Healy and Jamieson, 1992; Jamieson and Healy, 1992), a plesiomorphic state relative to the lesser extent in nonpasserines.

Apical narrowing of a cylindrical nucleus within the acrosome is a feature also seen in the anuran *Ascaphus truei* (Jamieson et al., 1993) and in *Neoceratodus forsteri* (Jespersen, 1971) and must be considered plesiomorphic for tetrapods. Although parrot sperm retain the cylindrical form of the nucleus they are, as noted above, exceptional among nonpasserines, and resemble passerines, in lacking the anterior extension of the nucleus into the acrosome. The concave nuclear fossa (at the distal surface of the nucleus) evident in parrot sperm is again consistent with observations made of the three Galliformes and the mallard duck. However, this can be contrasted to the situation in the ostrich, tinamou, and rhea where the base of the nucleus is characterized by two (possibly three in the rhea) parallel implantation fossae (Asa et al., 1986; Phillips and Asa, 1989; Baccetti et al., 1991). Interestingly, the emu appears to lack an implantation fossa (Baccetti et al., 1991).

Presence of a proximal and distal centriole at right angles, often embedded in dense material as in parrots, is a reptilian feature that is also a basic feature of spermatozoa throughout the animal kingdom (see Afzelius, 1979; Jamieson, 1987, 1991). The perpendicularly orientated proximal and distal centrioles seen in budgerigar sperm are also found in the ostrich, emu (Baccetti et al., 1991), and tinamou (Asa et al., 1986), and similarly in the neognaths: mallard duck (Humphreys, 1972), crane (Phillips et al., 1987), chicken and turkey (Thurston and Hess, 1987). The guinea fowl sperm differs by seemingly lacking a proximal centriole (Thurston and Hess, 1987). Of all the above-mentioned avian species, the parrots possess the shortest distal centriole, although that of the guinea fowl is not very much longer. By contrast, the distal centriole of the mallard duck and the chicken occupies two-thirds and three-quarters, respectively, of the length of the midpiece (Humphreys, 1972; Thurston and Hess, 1987). A distal centriole extending along the entire midpiece length is considered an autapomorphic characteristic of the Palaeognaths by Baccetti et al. (1991) but has been shown (Healy and Jamieson, 1992; Jamieson and Healy, 1992) to be a plesiomorphic feature for the Amniota, being seen also in Chelonia, Crocodilia, and *Sphenodon.* In passerines, the proximal centriole is absent in oscines, but both centrioles are present in suboscines as exemplified in the great-crested-flycatcher, *Miarchus griseisticta* (see Asa and Phillips, 1987).

An elongate midpiece in which mitochondria surround the axoneme is a feature of many internally fertilizing sperm, including those of amniotes (Jamieson and Healy, 1992), and appears to be a tetrapod symplesiomorphy (Jamieson, 1991). The estimate of 28–30 mitochondria in budgerigar sperm made by Samour et al. (1986) may be a severe underestimate as the midpiece may reveal as many as nine mitochondria in transverse section and eight or more in longitudinal section. Hence, it appears that the budgerigar spermatozoon possesses significantly more mitochondria than the 26–30 that have been ascertained for the Galliformes, for which only five appear in cross sections of the midpiece (Thurston and Hess, 1987). The mitochondria of galliforms are significantly more elongate than those observed in the budgerigar. Using the number of mitochondria as a spermatological character for reconstructing phylogenies may be misleading; large differences exist within the ratites (Baccetti et al., 1991; Asa et al., 1986). Like the advanced reptilian groups, the ratites and other nonpasserines, the cristae of parrot sperm are linear or slightly curved, whereas in Chelo-

nia, Crocodilia, and *Sphenodon*, they are concentric (Healy and Jamieson 1992; Jamieson and Healy, 1992). Parrot sperm also differ from those of squamates in lacking intermitochondrial dense bodies (mitochondrial transformations). By seemingly lacking an annulus, which in reptiles terminates the midpiece (Jamieson and Scheltinga, 1993), parrot sperm differ from those of palaeognaths (e.g., Baccetti et al., 1991) and primitive nonpasserines including the mallard duck (Humphreys, 1972), and the turkey, chicken, and guinea fowl (Thurston and Hess, 1987). An annulus is basal to amniotes and is seen in most investigated nonpasserines. Absence is therefore an apomorphic feature of budgerigar sperm but appears homoplasic relative to absence in passerines. A more advanced nonpasserine, on the basis of DNA studies (Sibley et al., 1988; Sibley and Ahlquist, 1990), the turtle dove, *Streptopelia roseogrisea,* does appear to possess an annulus (Asa and Phillips, 1987).

The presence of nine dense peripheral fibers (coarse fibers) external to each axonemal doublet is an amniote synapomorphy (Jamieson and Healy, 1992). It is seen convergently in some fish (Jamieson, 1991), in heterobranch gastropods (Healy, 1988), and in cephalopod sperm (Healy, 1993). These dense fibers have been demonstrated in the Galliformes (e.g., Thurston and Hess, 1987), the mallard duck (Humphreys, 1972), and in the anteriormost region of the principal piece of ratite spermatozoa (Asa et al., 1986; Asa and Phillips, 1987; Baccetti et al., 1991). The dense fibers in nonpasserine sperm are small relative to those present in the spermatozoa of mammals and passerine birds (Asa and Phillips, 1987). In birds, all nine are approximately equal in size, whereas in mammals they are of various sizes in the same axoneme and in reptiles those at doublets three and eight are enlarged (Healy and Jamieson, 1992; Jamieson and Healy, 1992).

Despite lacking an annulus, the midpiece-tail junction in parrot sperm is clearly demarcated, because at this point there is a rather abrupt narrowing of the spermatozoon. Only a narrow rim of cytoplasm separates the plasma membrane from the axonemal complex throughout the length of the axoneme behind the midpiece. The fibrous sheath is also absent in the columbiforms *Ocyphaps lophotes* and *Geopelia striata* (Jamieson, pers. obs.) and is not mentioned or figured for the turtle dove, *Streptopelia roseogrisea,* by Mattei et al. (1972). The functional significance of loss of the fibrous sheath is unclear. In contrast, an amorphous sheath surrounds the axoneme in the tail of the galliform sperm (Thurston and Hess, 1987) and that of the mallard duck (Humphreys, 1972), whereas the ratites have retained the reptilian-like, dense, annulated fibrous sheath (Asa et al., 1986; Asa and Phillips, 1987; Baccetti et al., 1991). Birds (ratites) differ from squamates but agree plesiomorphically with nonsquamate reptiles in failure of the fibrous sheath to penetrate the midpiece.

The features and parameters of the ductal spermatozoa of *Melopsittacus undulatus* correspond very closely with those previously reported for semen samples by Samour et al. (1986) who conclusively confirmed the presence of seminal glomera in the cloaca of this species. Conventionally considered a passerine feature, the seminal glomera are situated on either side of the cloaca of male birds and are formed by convolutions of the terminal end of the ductus deferens. These sacs have been likened to the eutherian epididymis in that they serve as sites for sperm storage and appear to play a role in sperm maturation (Middleton, 1972). The results of the present study and of that of Samour et al. (1986) indicate that in *M. undulatus* no significant changes to sperm morphology occur within the seminal glomera. Nevertheless, the claim of McFarlane (1963) that avian sperm maturation is characterized by an increase in tail length is neither confirmed nor denied and the possibility of more subtle changes in organization of the spermatozoon in the glomera should not be discounted on the basis of the present study. Despite the fact that the budgerigar possesses seminal glomera typical of the more advanced passerine species, the results of this present study reveal that its spermatozoon conforms to the phylogenetically older, nonpasserine spermatozoal design. However, the transverse acrosome-nucleus junction is similar, although possibly homoplasically, to the passerine condition.

Passerine spermatozoon differ significantly from the more primitive, simple, sperm of the ratites and nonpasserines, by possessing a helical nuclear region and an "undulating membrane," although within the passerine order, the nature of the "undulating membrane" differs between the oscines and the suboscines (see Asa and Phillips, 1987). Correspondingly, passerine spermatozoa move in corkscrew fashion, whereas the sauropsid-type avian sperm propel themselves by vigorously lashing the flagellum from side to side (Humphreys, 1972).

There is a paucity of information on avian sperm ultrastructure. Most of the research has hitherto focused on domestic birds of commercial importance, with the aim of improving artificial insemination techniques. The majority of these birds belongs to the most primitive nonpasserine avian taxa, e.g., the Galliformes and the Anseriformes (Sibley et al., 1988; Sibley and Ahlquist, 1990). Nevertheless, the spermatological approach has already proved useful in avian systematics. Ratites can be differentiated from nonpasserines on the basis of the retention of plesiomorphic spermatozoal character states such as the reptilian-like annulated fibrous sheath,, or conversely the apomorphic modification of these in nonpasserines, whereas an obvious dichotomy exists between the passerines and nonpasserines with respect to spermatozoal features.

Ultrastructural examination of the spermatozoa of many other avian species is now necessary to reveal whether characteristics individually and in combination which are at present known only for psittaciform sperm may be more widespread among avian species. The morphology of the spermatozoa of the three species here investigated indicates the existence of a distinctive psittaciform sperm. With regard to the existence of features that might be phylogenetically valuable in distinguishing between the two subfamilies, the brevity of the midpiece of the cacatuine *N. hollandicus* constitutes a spermatozoal distinction from the two psittacines.

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