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Ultrastructure of the unusual spermatozoon of the Eurasian bullfinch (*Pyrrhula pyrrhula*)

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Abstract

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The Eurasian bullfinch spermatozoon differs from typical passeridan spermatozoa in several major respects. The mature acrosome consists of a concavo-convex vesicle differing from the typical passeridan acrosome, which is a helical structure, is usually longer than the nucleus and has a prominent helical keel. The nucleus differs from that of other oscines in not showing a twisted cylindrical form, in being shorter, and in tending to be an elongate ellipsoid in shape. The chromatin often appears in an uncondensed form reminiscent of a spermatid and consists of discrete fascicles. A small proportion of the mature sperm population however, is characterized by marked chromatin condensation. The midpiece comprises a small group of mitochondria clustered around the nuclear–axonemal junction in contrast to the single, long mitochondrion wound helically around the axoneme that is found in typical Passerida. The presence of a proximal centriole (in addition to the distal one) is a notable difference from all other oscine passerines. We suggest that the unusual morphology of the Eurasian bullfinch spermatozoon, resembling that of a spermatid, is the result of the progressive suppression of the final stages of spermiogenesis and is associated with the likelihood that sperm competition is infrequent in this species.

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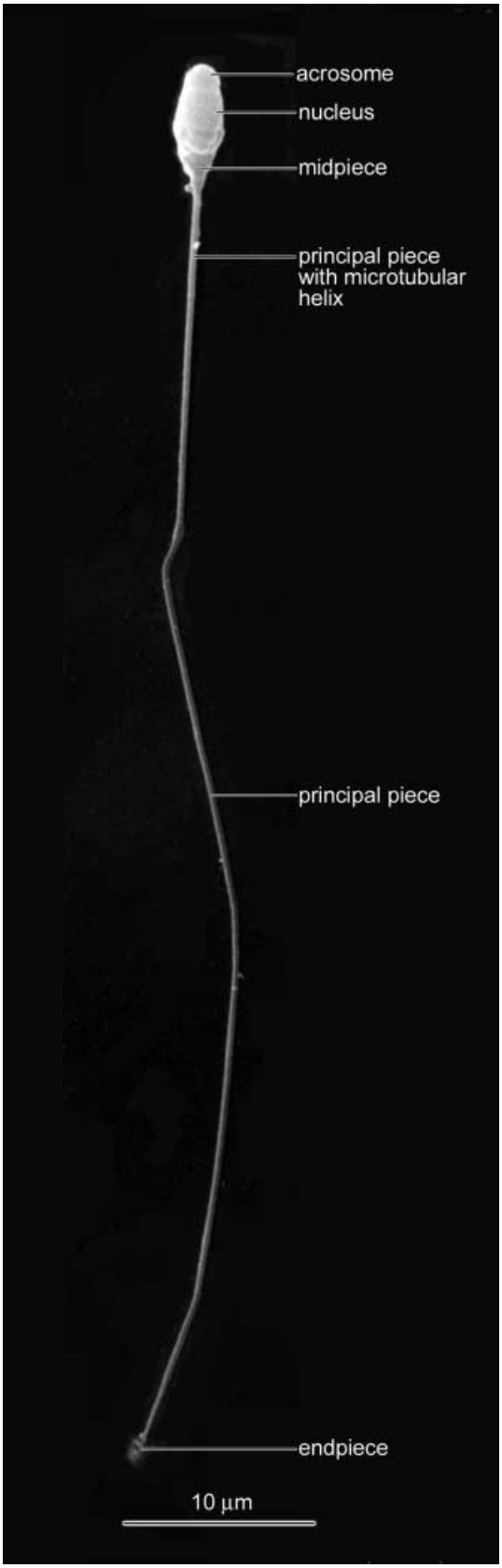
Introduction

Across the animal kingdom the size, shape and ultrastructure of spermatozoa exhibit remarkable variation (Cohen 1977). The gross morphology of spermatozoa (for example, the relative size of the head, midpiece and flagellum) is particularly variable within and between taxa and is thought to have evolved in response to three main factors: (i) fertilization mode (internal vs. external); (ii) post-copulatory sexual selection mediated by sperm competition and cryptic female choice, and (iii) phylogeny (Pitnick *et al.* 1999). With some exceptions, such as the Anura, externally fertilizing species typically have less elaborate spermatozoa than internally fertilizing species, presumably reflecting the more complex environment within the female reproductive tract. The extent to which post-copulatory sexual selection has shaped sperm design varies between taxa and between studies and is currently an active area of research (see Snook 2005). Phylogeny influences both the gross morphology and the ultrastructure of spermatozoa (e.g. Jamieson 1999).

During the course of a comparative study of the gross morphology of passerine spermatozoa Birkhead *et al.* (2006) noticed that the spermatozoa of the Eurasian bullfinch, *Pyrrhula pyrrhula*, differed dramatically from that of other passerines. Instead of having an elongate, pointed form with a single, fused mitochondrion spiralled along the flagellum, as in most passerine birds (see Jamieson 2006), the spermatozoa of the Eurasian bullfinch were relatively short, with a rounded head and no mitochondrial helix around the flagellum. The present paper aims to describe the ultrastructure



of this spermatozoon, to compare it with that of other passerines, and to consider both ultimate and proximate factors that may account for its unusual morphology.

Materials and Methods

We obtained spermatozoa from three captive-bred, sexually mature male Eurasian bullfinches during the breeding season. Spermatozoa were obtained from the distal half of the seminal glomerula (i.e. nearest the cloaca) within 5 minutes of death, dissected in 0.1 M phosphate buffer, pH 7.2, to which 1.8% sucrose was added (PB). After several hours of fixation in 2.5% glutaraldehyde in PB at 4 °C, the material was rinsed several times in PB and then post-fixed in 1% osmium tetroxide for 1 h. The samples were rinsed in distilled water, then stained for 1 h in uranyl acetate at room temperature and, after several washes in distilled water, specimens were dehydrated in alcohol and then embedded in an Epon–Araldite mixture. Ultrathin sections, obtained with the Ultracut Reichert, were routinely stained and observed using a Philips CM 10 transmission electron microscope (TEM) at 80 kV.

Results

Gross spermatozoal morphology

By light microscopy (not illustrated here, but see Birkhead *et al*. 2006) the spermatozoa of the Eurasian Bullfinch appear short relative to those of most other passerines. The total length of the spermatozoa is 48.93 ± 4.02 µm (± SD), of which 5.41 µm is the 'head' (here consisting of acrosome, nucleus and midpiece) and 43.53 µm is the free flagellum (based on 50 spermatozoa from each of 10 captive-bred males). The spermatozoon, as seen by scanning electron microscope (SEM; Figs 1 and 2A), has a rounded, cap-like acrosome surmounting a short cylindrical or fusiform nucleus, at the base of which is a small group of mitochondria, comprising the midpiece, followed by the free flagellum.

Spermatozoal ultrastructure

In the following account, the ultrastructure of what are considered normal, presumably functional, Eurasian bullfinch spermatozoa is described as observed by SEM and TEM. In addition, micrographs of abnormal spermatozoa are included in view of the unusually high frequency of their occurrence, which is relevant to the hypothesized reduced sperm competition in this species (see below and Birkhead & Immler, in press).

Acrosome. The ultrastructure of the spermatozoon depicted by SEM (Figs 1 and 2A) is consistent with the morphology

Fig. 1—Eurasian bullfinch, *Pyrrhula pyrrhula*. Scanning electron micrograph of the entire spermatozoon.

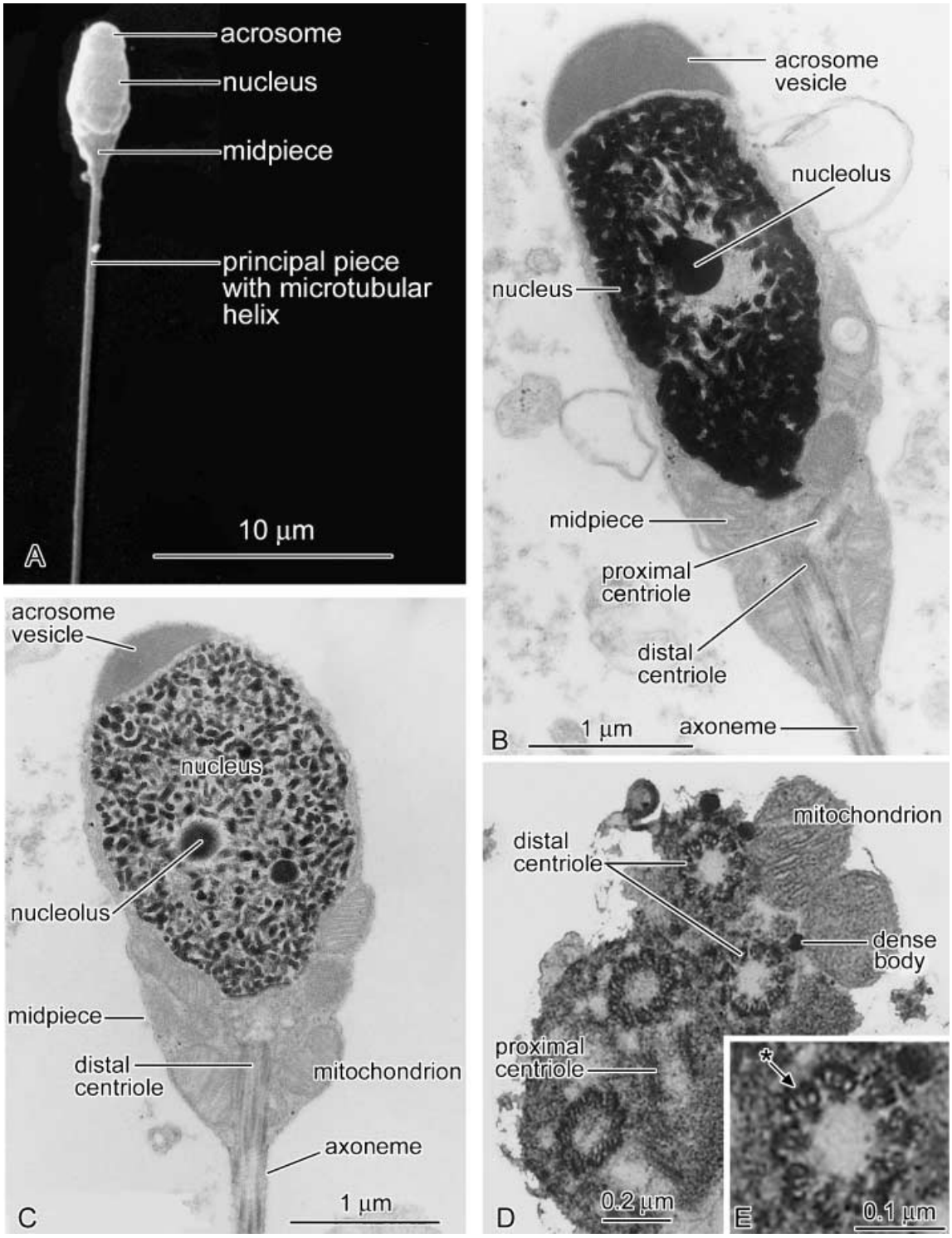


Fig. 2—Eurasian bullfinch, *Pyrrhula pyrrhula*. Ultrastructure of the spermatozoon. —**A**. Scanning electron micrograph of the sperm head and anterior region of the flagellum, showing its spermatid-like appearance. Note the cap-like acrosome differing from the elongate helical acrosome of other passerines. —**B**. Corresponding longitudinal section (LS) by transmission electron microscopy (TEM) of the spermatozoon showing retention of the proximal centriole which is lost in other passerine sperm. —**C**. Similar LS of a late spermatid. —**D**. TEM section of an abnormal spermatozoon with multiple centrioles. —**E**. Higher magnification of a centriole from D, showing nine triplets, each with a small peripheral density (asterisked arrow) identified as a dense fibre.

of that shown by TEM (Fig. 2B). The mature acrosome consists of a thickly concavo-convex vesicle with homogeneous, moderately to strongly electron-dense contents (Figs 2B,C and 3A,B). Its concave posterior face is closely applied to the nucleus. There is no perforatorium.

Nucleus. The chromatin of the nucleus presents an appearance that is highly unusual for functional animal spermatozoa. Instead of being homogeneously electron-dense or consisting of compacted granules, the chromatin usually consists of a loose assemblage of small bundles or fascicles around a chromatin-less central region (Fig. 2B). This contains a large electron-dense spherical body, the putative nucleolus. There is, however, considerable compaction of the chromatin at the periphery and particularly posteriorly. A similar fasciculate appearance of the chromatin is seen in two spermatozoa in Figs 3(A) and 4(I). In a clearly abnormal spermatozoon characterized by at least three centrioles (Fig. 3C), the nucleus, seen approximately in transverse section, is again fasciculate with a large central area with only sparse chromatin. In a further abnormal spermatozoon (Fig. 3D) a large block of chromatin appears to have become detached and displaced. In a presumably younger spermatozoon or late spermatid (Fig. 2C) the nucleus has finer, closely compacted granules. Nevertheless, strong chromatin condensation, which gives an almost homogeneously electron-dense nucleus of normal appearance, has been observed (Fig. 3B) in a spermatozoon which shows normal morphology, albeit of the unusual type seen in this species.

Midpiece. The midpiece consists of a granular body, mitochondria and centrioles. The granular body lies posterior to the nucleus (Fig. 3E) and is considered further in the Discussion. The mitochondria comprise a small group of discrete organelles clustered around the tapered base of the nucleus (SEM, Figs 1 and 2A) and containing the proximal and distal centriole with the proximal region of the axoneme (TEM, Figs 2B,C and 3B). The number of mitochondria observed in longitudinal sections of apparently normal spermatozoa is not constant but is in the order of three or four in anterior–posterior sequence on each side (Fig. 2B,C). The mitochondria are variable in shape but have well-developed, oblique cristae. In transverse section (Fig. 3C) there appear to be as many as nine mitochondria in a circlet around the base of the nucleus. Some fusion of mitochondria appears to have occurred in some spermatozoa (Fig. 4A). Unlike the spermatozoa of other oscines, in which a proximal centriole is absent at maturity (Asa & Phillips 1987; Jamieson 2006), the spermatozoon of the Eurasian bullfinch has a proximal and a distal centriole (Figs 2B and 3B) as found in spermatids generally and in the spermatozoa of suboscine birds. Both centrioles are short, though the distal is the longer, and the central singlets of the axoneme do not penetrate the distal centriole. In transverse section the distal centriole in normal spermatozoa (Fig. 4D–F) and in abnormal spermatozoa (Figs 2D,E and 3E,F) is seen to have the usual nine triplets of microtubules. Dense material adheres to the outer face of each triplet (Fig. 3E–G – asterisked arrows, 4D–H). These densities appear to be rudimentary dense fibres but peripheral enlargement of each of these as a subcircular transverse section, typical of oscine spermatozoa, is absent not only from the centriole but also from the succeeding principal piece of the flagellum. Occasional dense bodies, circular in section (Figs 2D and 3F) in the vicinity of the centriole are not to be confused with dense fibres. The section of the abnormal spermatozoa in Fig. 2(D) displays four centrioles with the configuration of distal centrioles and one apparent proximal centriole approximately at right angles to the latter. In a further abnormal spermatozoon (Fig. 3C), three centrioles, of which two appear to be distal and one proximal, are present and in an additional abnormal spermatozoa (Fig. 3D,E) two distal centrioles are seen.

Flagellum. The flagellum or tail is defined as that region of the axoneme that is free and not contained in the midpiece. Division into a principal piece and an end-piece depends on the existence of some type of external sheath or other specialization for the principal piece, which contrasts with the naked condition of the axoneme, apart from its plasma membrane, diagnosing the end-piece. Such differentiation occurs in the spermatozoon of the Eurasian bullfinch. The axoneme has the typical structure of nine doublets of microtubules and two central singlets. It is accompanied by the microtubular helix (Fig. 4C,H; see below).

Microtubular helix. A microtubular helix diagnostic of immature passerine spermatozoa is here demonstrated for the Eurasian bullfinch spermatozoon (Fig. 4A–C,G–I). In the figures in which its helical form is not apparent it is given the more neutral term of microtubular bundle (Fig. 4B,G – sperm head) but the spiral configuration of the microtubules is evident in Fig. 4(A,I). It is also deduced to be spiral where it accompanies the axoneme unilaterally (Fig. 4C,G,H).

Principal piece. The principal piece is demarcated in this species by the persistence of the dense fibres of the distal centriole as, albeit minute, fibres peripheral and adherent to the nine doublets (Fig. 3G, left axoneme; Fig. 4C,H).

End-piece. An end-piece, which by definition is that part of the flagellum in which the axoneme is bounded only by the plasma membrane, is observable in this species. As is usual in spermatozoa, its posterior end is marked by disruption of the 9 + 2 arrangement of microtubules. Here it is marked first by disappearance of the central singlets (Fig. 3G, right axoneme) and then by loss from the nine doublets of the B subtubule (Fig. 3B, bottom left).

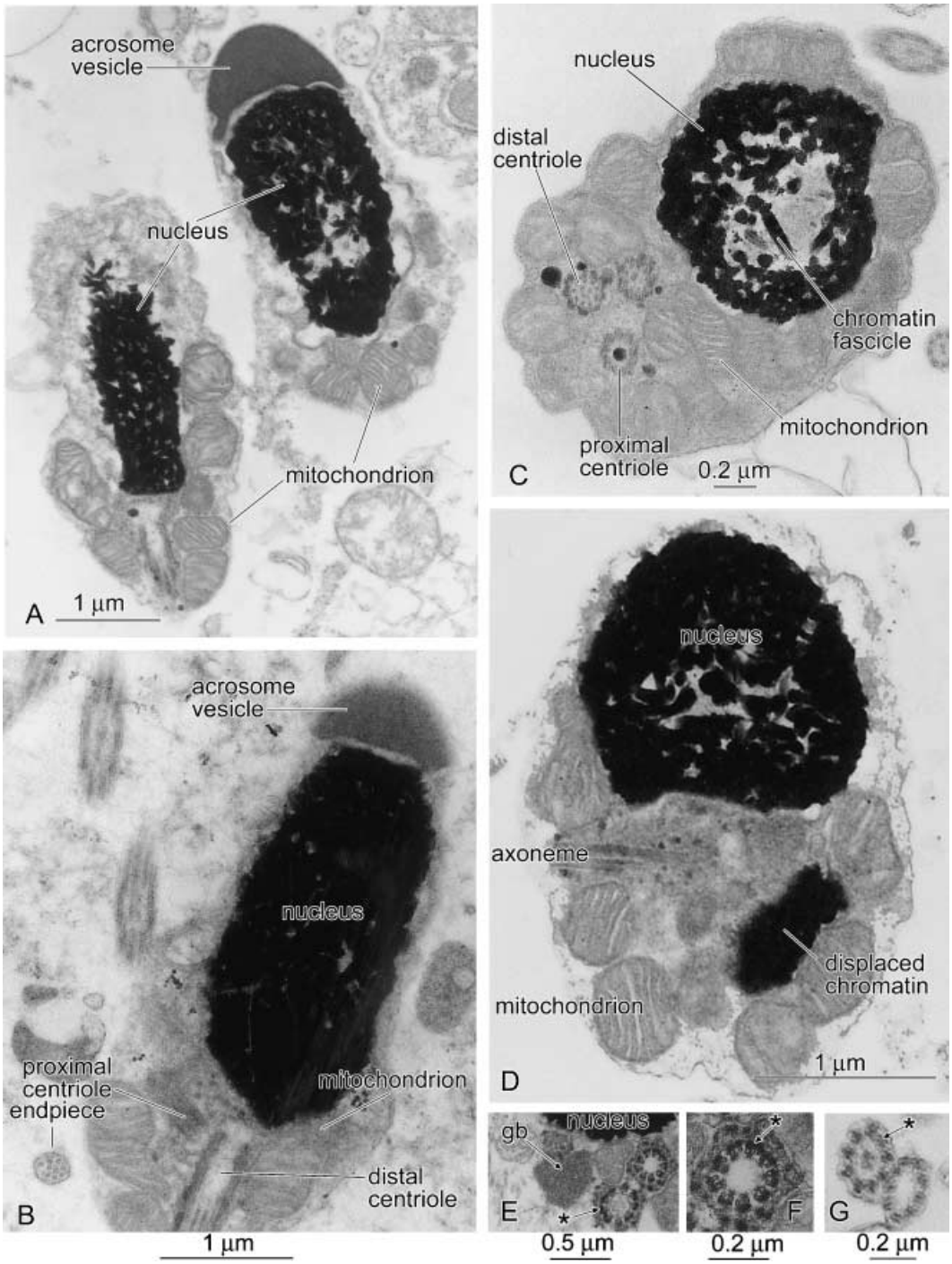


Fig. 3—Eurasian bullfinch, *Pyrrhula pyrrhula*. Ultrastructure of the spermatozoon, continued. —**A**. Longitudinal sections (LS) of two spermatozoa, the right of which shows the cap-like acrosome. In both the chromatin consists of electron-dense fascicles, which in places are loosely arranged. Mitochondria of the midpiece are loosely grouped around the base of the nucleus and proximal axoneme. —**B**. LS of a well-formed spermatozoon in which the cap-like acrosome has become electron-dense, chromatin is condensed, two centrioles are visible and the midpiece is formed by a compact group of mitochondria. An end-piece in transverse section is also seen. —**C**. An approximately transverse section of an abnormal spermatozoon that has multiple centrioles. —**D**. Oblique section of an abnormal spermatozoon that has a fragmented nucleus and eccentric axoneme. —**E**. Post-nuclear region of an abnormal spermatozoa with two distal centrioles visible. Minute dense fibres (asterisked arrow) are adherent to the triplets. A granular body (gb) typical of some passeroids and certhioids is seen. —**F**. Detail of same. —**G**. Transverse sections of, left, the principal piece, in which the axoneme has small dense fibres, and right, an end-piece consisting only of doublets.

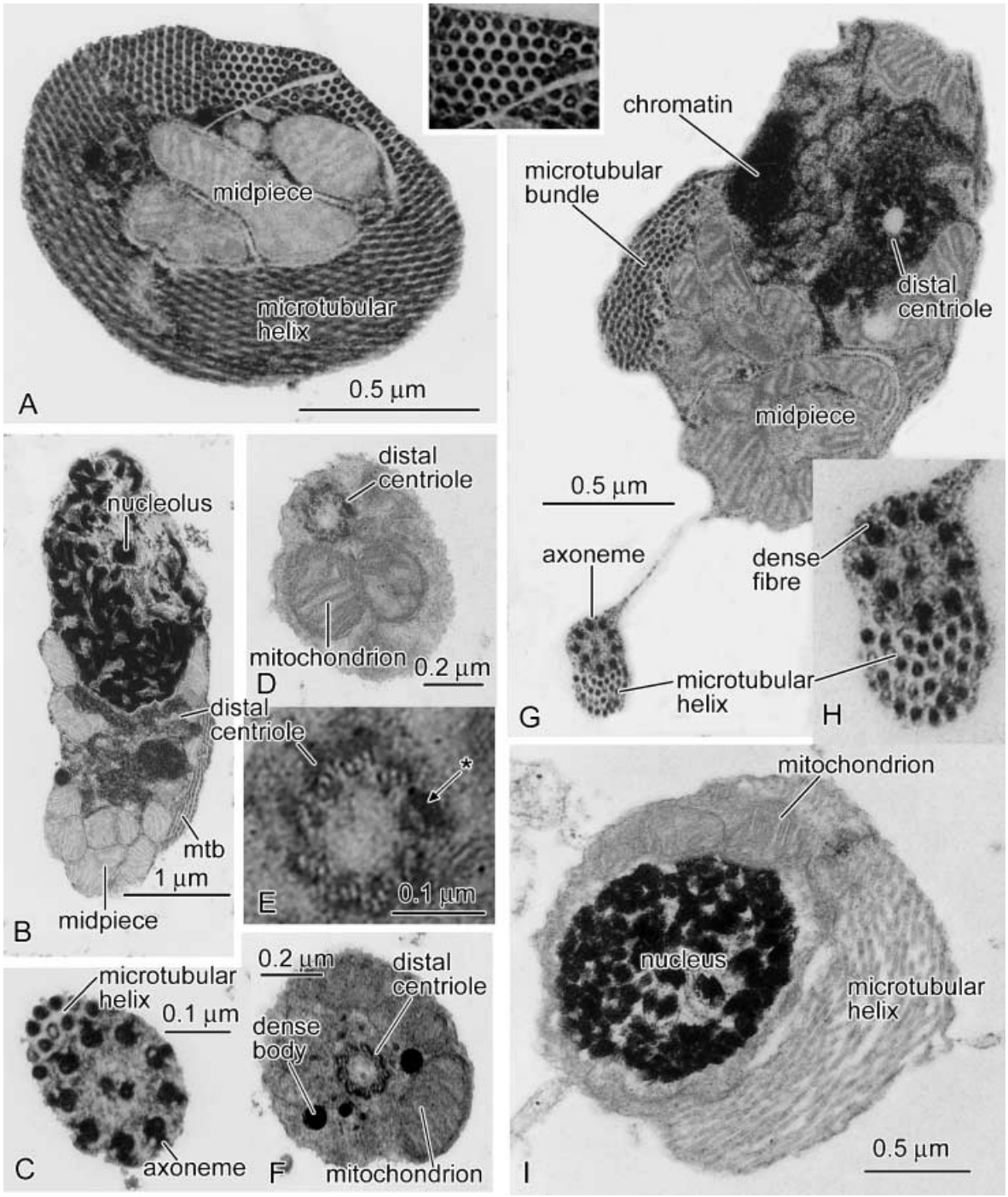


Fig. 4—Eurasian bullfinch, *Pyrrhula pyrrhula*. Ultrastructure of the spermatozoon. —**A**. Transverse section (TS) of the midpiece showing a surrounding well-developed microtubular helix. Inset shows detail of microtubules. —**B**. Approximately longitudinal section of the sperm head, with midpiece, showing many discrete mitochondria and a microtubular bundle (mtb). —**C**. TS of axoneme accompanied by the microtubular helix. The doublets of the axoneme each have a very small dense fibre. —**D**. TS through the distal centriole and mitochondria of the midpiece. —**E**. Detail of same distal centriole showing densities peripheral to the triplets. —**F**. TS of a distal centriole and surrounding discrete mitochondria. Some dense bodies of unknown nature are present in the midpiece. —**G**. Oblique section of a spermatozoon to show the microtubular bundle, distal centriole, a presumed block of detached chromatin, and many mitochondria. Continuous with it (bottom left) is a section of the axoneme and microtubular helix. —**H**. Detail of same axoneme, showing very small dense fibres peripheral to the doublets, and the lateral microtubular helix. —**I**. TS of the base of the nucleus, showing mitochondria of the midpiece and prominent microtubular helix.

Discussion

In the Passeriformes, the spermatozoa of oscines (Passeri) differ from those of suboscines (Tyranni), and from non-passerines including palaeognaths, in lacking a proximal centriole and in having a single mitochondrial strand along the anterior region of the axoneme (Corvida) or wound for a great distance along the axoneme (Passerida) (see Barker *et al*. 2004 for details of avian phylogeny). Passerines differ further from non-passerines in possessing in the spermatid or immature spermatozoon a 'helical membrane'. This consists of multiple microtubules that form a thick strand coiled around at least the flagellum. The spermatozoa of the Passerida differ from those of the Corvida, with some exceptions, in that the ratio of acrosome length to nucleus length exceeds 1 (Asa and Phillips 1987; Jamieson 2006).

The spermatozoon of the Eurasian bullfinch differs from the typical passeridan spermatozoon in major respects discussed below.

Acrosome

The mature acrosome consists of a thickly concavo-convex vesicle – differing greatly from the typical passeridan acrosome, which is a helical structure with a prominent helical keel. The passeridan acrosome is longer than the nucleus except, so far as is known, in some Hirundinidae (McFarlane 1963) and possibly in the Blackbird, *Turdus merula* (Furieri 1961), reaching four times the nuclear length in the Summer tanager, *Piranga rubra* (McFarlane 1971; *fide* Koehler 1995; see Jamieson 2006). In contrast, in the Eurasian bullfinch the acrosome is a thickly concavo-convex vesicle. As in all other passerines, there is no perforatorium or corresponding endonuclear canal. The simple, cap-like form of the acrosome in the bullfinch is reminiscent of the acrosome of a spermatid, as exemplified by the Cirl bunting, *Emberiza cirlus* (Tripepi & Perrota 1991; Jamieson 2006). With its cap-like acrosome and compact midpiece the bullfinch spermatozoon also resembles the mature spermatozoa of certain non-passerines, specifically typical Charadriiformes and Falconiformes (see Jamieson 2006). However, molecular phylogenetic analysis conducted so far appears to confirm that the Eurasian bullfinch lies among the cardueline finches (e.g. Fehrer 1996; see also Arnaiz-Villena *et al*. 2001).

Nucleus

The nucleus differs from that of other passerines in not showing a helical, or at least slightly twisted, cylindrical form and in being shorter and tending to an elongate ellipsoid shape. The chromatin, instead of being homogeneously electron-dense or consisting of compacted granules, often differs in consisting of a loose assemblage of small bundles or fascicles around a chromatin-less central region. This uncondensed form is again reminiscent of a spermatid. Nevertheless, marked chromatin condensation, to give an almost homogeneously electron-dense nucleus of normal appearance, occurred in at least a small portion of the sperm population.

Midpiece

In Passerida, the midpiece consists of a single mitochondrion wound helically around the greater part of the length of the axoneme (e.g. Humphreys 1972; Asa and Phillips 1987; Jamieson 2006). In contrast, in the Eurasian bullfinch the midpiece appears to consist of a small group of mitochondria clustered around the tapered base of the nucleus, the proximal and distal centriole and only the proximal region of the axoneme. Any fusion or elongation of mitochondria in the Eurasian bullfinch appears to be limited. Similarly, in the rounded spermatids of the Cirl bunting, there are several mitochondria clustered around the base of the axoneme but as maturation and elongation progress the mitochondrial helix develops (Tripepi and Perrotta 1991).

Granular body

A granular mass, posterior to the nucleus, seen in one longitudinal section of a spermatozoon of this species, is here presumed to be the homologue of the granular body previously described for spermatids of several species of the Passeroidea, and a certhioid, which at maturity becomes a spiral structure anterior to the mitochondrial helix (Humphreys 1972; Tripepi and Perrotta 1991; Koehler 1995). The granular body was reported to be absent from Tyranni, Corvida and Sylvioidea (Tripepi and Perrotta 1991; Koehler 1995; see review in Jamieson 2006). Although reported for the Cirl bunting by Tripepi and Perrotta (1991), it was not seen in another emberizid, the Cardinal (*Cardinalis cardinalis*), and while present in the icterid Common grackle (*Quiscalus quiscala*), it was not seen in the cofamilial Red-winged blackbird (*Agelaius phoeniceus*, Koehler 1995). Its presence as a compact mass in the Eurasian bullfinch is again characteristic of a spermatid, while confirming its passeridan status.

Microtubular helix

In an earlier study, Birkhead *et al*. (2006) indicated that if external sperm morphology alone had been used to infer phylogeny, the Eurasian bullfinch might not have been classified as a passerine. However, the existence of a microtubular helix, a form of manchette known only in the Passeriformes, unequivocally confirms the bullfinch's status as a passerine. The helical configuration of microtubules is clearly visible in Fig. 4(A,F) and in the latter figure it closely resembles the arrangement illustrated for the spermatid of the Cirl bunting by Tripepi and Perrotta (1991).

Centrioles

The presence of proximal and distal centrioles has been unequivocally demonstrated here for the spermatozoon of the Eurasian bullfinch. This is a notable difference from all other passerines for which centriolar features have been documented, in which only a distal centriole (the basal body of the axoneme) is present in the mature spermatozoon. In a review of avian spermiogenesis, Aire (2006) states that centriolar complex development in passerine birds is reported to be generally similar to that in mammals and non-passerines (Sotelo and Trujillo-Cenóz 1956). He notes that Góes and Dolder (2002) consider that in the House sparrow (*Passer domesticus*) the proximal centriole lodges in an implantation fossa of the nucleus while the distal centriole extends, caudally, to the cell membrane and forms an annulus at the contact junction, but that Sotelo and Trujillo-Cenóz (1956) state for the same species that one of the centriolar pair disappears. Nicander (1970) also states that passerine birds possess only one, modified centriole and this, and the absence of an annulus, is confirmed by Jamieson (2006) for the muscicapoid *Myrmecocichla formicivora* (Muscicapoidea) and the ploceid *Philetairus socius* (Passeroidea).

Retention of two centrioles in the mature spermatozoon of the Eurasian bullfinch is therefore a further feature characteristic of the spermatid, although it is normal in mature spermatozoa of non-passerines (e.g. Aire 2006; Jamieson 2006).

The shortness of the distal centriole in the Eurasian bullfinch is a major difference from the very long (midpiece length) centriole of palaeognaths and the elongate, though shorter, centriole of the Galloanserae but it is also short in some other non-passerine orders, e.g. Psittaciformes (Jamieson 2006).

Flagellum

In oscines, within the midpiece, and therefore in Passerida extending for most of the length of the axoneme, there are nine very large dense fibres, one peripheral and adherent to each doublet. The dense fibres are approximately circular in cross-section. In contrast, Eurasian bullfinch spermatozoa have only minute dense fibres along the axoneme, which lacks the mitochondrial helix.

Microtubular helix

The bundle of microtubules that winds around the sperm head and the axoneme in the immature spermatozoa of passerines (see Jamieson 2006 for a review) has here been demonstrated in the spermatid-like spermatozoon of the Eurasian bullfinch.

Abnormalities

Frequent abnormalities of the spermatozoa of this species reported above are incomplete compaction of the chromatin of the nucleus, fragmentation of the nucleus, and the presence of multiple centrioles. The frequency of intra-male gross abnormalities in Eurasian bullfinch spermatozoa has been quantified and compared with some other passerine species by Birkhead & Immler 2006).

The neotenous spermatozoon

Our observations strongly suggest that the unusual morphology and ultrastructure of the Eurasian bullfinch spermatozoa, resembling that of a spermatid, is the result of the suppression of the final stages of spermiogenesis. Normally in a passeridan these would involve spiralization of the sperm head, elongation of the acrosome and development on this of a helical keel; condensation of the nucleus and its acquisition of a more or less helical form; loss of the proximal centriole; and great extension of a single mitochondrion as a mitochondrial helix along much of the axoneme. None of these changes occurs in the spermatozoa of *P. pyrrhula*: the acrosome remains a simple cap-like vesicle; the nucleus is an ellipsoidal cylinder; the chromatin consists of dense fascicles, which become compacted; the proximal and distal centrioles persist; and multiple discrete mitochondria form a group around the nuclear–flagellar junction. These features, with the possible exception of nuclear compaction, are typical of a spermatid before elongation and lead us to conclude that the spermatozoon may be regarded as neotenous, retaining at maturity the features of an immature stage.

Origin of neoteny

The spermatozoon of Beavan's bullfinch (*Pyrrhula erythaca*), like those of the Eurasian bullfinch, is short (length 49.11 μm) but instead of a rounded head it has a more pointed, spiral-shaped head. Like the Eurasian bullfinch, it has an extremely small midpiece (Birkhead *et al*. 2006). This contrasts with the elongated mitochondrial helix that forms the midpiece in the Passerida, which is almost all other passerines with the exception of Corvida and Tyranni (which have a short midpiece). The fact that the spermatozoon of Beavan's bullfinch also shows features of a spermatid-like morphology suggests that some modification, including reduction of the midpiece, had already occurred in a putative common ancestor of the two species. If this is true, it seems likely that the suppression of the final stages of spermiogenesis and the retention of an immature morphology in the Eurasian bullfinch evolved gradually rather than through a saltatory change from a typical passeridan spermatozoon, involving a single cytogenetic 'accident' or mutation. The adaptive significance of such suppression may be that it represents an energetic saving in response to reduced sperm competition (see below).

Functionality of the neotenous spermatozoon

The frequency of gross abnormalities and the peculiar form of the normal, neotenous spermatozoa of the Eurasian

bullfinch might call into question their ability to successfully fertilize ova. However, the spermatozoa are known to be motile and there is no evidence for an unusually high proportion of unhatched eggs, either in the wild or in captivity.

Birkhead *et al.* (2006) could find no gross features of the female reproductive tract, which might account for the peculiar morphology of the spermatozoon of the Eurasian bullfinch. These authors consider the possibility that a low level of sperm competition in the Eurasian bullfinch may have played a role in its unusual sperm morphology. Relative testis mass provides an index of the intensity of sperm competition in birds (Møller and Briskie 1995) and the same is true for the dimensions of the cloacal protuberance and the male's extragonadal sperm stores, the seminal glomera, which reside in the cloacal protuberance of passerine birds (Wolfson 1954; Birkhead *et al.* 1993). Birkhead *et al.* (2006) demonstrated that the testes of the Eurasian bullfinch are extremely small relative to its body size, as are the cloacal protuberance, the paired seminal glomera (Birkhead *et al.* 1993) and the number of stored sperm. These features strongly suggest that sperm competition might be absent or rare in the Eurasian bullfinch. If that were the case it is possible that relaxed selection may have resulted in the evolution of the unusual sperm morphology.

Although the neotenous spermatozoon appears, so far, to be unique in birds, it is interesting that a very similar situation occurs in some rodents with relatively small testes examined by Breed (1993, 1997, 2002). As in the Eurasian bullfinch, the spermatozoa of these rodents also had atypical head morphology, poorly condensed chromatin and exhibited a high degree of intra-male variability in gross morphology. The possibility exists that in both taxa the relatively small testes reflect a low incidence of sperm competition, and hence relaxed selection on sperm design and 'quality control'. Two alternative hypotheses are: (i) that these unusual features are the result of some constraint on sperm design and quality control dictated by relatively small testes, and (ii) that they are the result of a genetic bottleneck and/or inbreeding (see Wildt *et al.* 1983; Barone *et al.* 1994; Gomendio *et al.* 2000). We are currently examining these hypotheses (see also Birkhead & Immler 2006).

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References

Aire, T. A. 2006. Spermatogenesis and testicular cycles. In Jamieson, B. G. M. (Ed.): *Reproductive Biology and Phylogeny of Birds*, pp. 279–347. Science Publishers, Enfield, New Hampshire, USA.

Arnaiz-Villena, A., Guillen, J., Ruiz-del-Valle, V., Lowy, E., Zamora, J., Varela, P., Stefani, D. and Allende, L. M. 2001. Phylogeography of crossbills, bullfinches, grosbeaks, and rosefinches. – *Cellular and Molecular Life Sciences* **58**: 1159–1166.

Asa, C. S. and Phillips, D. M. 1987. Ultrastructure of avian spermatozoa: a short review. In Mohri, H. (Ed.): *New Horizons in Sperm Cell Research*, pp. 365–373. Japan Scientific Societies Press, Tokyo; Gordon and Breach Scientific Publishers, New York.

Barker, F. K., Cibois, A., Schikler, P., Feinstein, J. and Cracraft, J. 2004. Phylogeny and diversification of the largest avian radiation. – *Proceedings of the National Academy of Sciences, USA* **101**: 11040–11045.

Barone, M. A., Roelke, M. E., Howard, J., Browne, J. L., Anderson, A. E. and Wildt, D. 1994. Reproductive characteristics of male Florida panthers: comparative studies from Florida, Texas, Colorado, Latin America and North American zoos. – *Journal of Mammalogy* **75**: 150–162.

Birkhead, T. R., Briskie, J. V. and Møller. A. P. 1993. Male sperm reserves and copulation frequency in birds. – *Behavioral Ecology and Sociobiology* **32**: 85–93.

Birkhead, T. R. and Immler, S. 2006. Making sperm: design, quality control and sperm competition. In Roldan, E. and Gomendio, M. (Eds): *Spermatology, SRF (Supplement 65)*. Nottingham University Press, Nottingham.

Birkhead, T. R., Immler, S., Pellatt, E. J. and Freckleton, R. 2006. Unusual sperm morphology in the Northern bullfinch (*Pyrrhula pyrrhula*). – *Auk* **123**: 383–392.

Breed, W. G. 1993. Novel organization of the spermatozoon in two species of murid rodents from Southern Asia. – *Journal of Reproduction and Fertility* **99**: 149–158.

Breed, W. G. 1997. Unusual chromatin structural organization in the sperm head of a murid rodent from Southern Africa: the red veld rat, *Aethomys chrysophilus* type B. – *Journal of Reproduction and Fertility* **111**: 221–228.

Breed, W. G. 2002. Variation in sperm form in rats and mice (Subfamily Murinae). In van der Horst, G., Franken, D., Bornman, R. T. Bornman, S. and Dyer, S. (Eds): *9th International Symposium on Spermatology*, pp. 269–274. Monduizzi Editore, Bologna.

Cohen, J. 1977. *Reproduction*. Butterworths, London and Boston.

Fehrer, J. 1996. Conflicting character distribution within different data sets on cardueline finches: artifact or history? *Molecular Biology and Evolution* **13** (1): 7–20.

Furieri, P. 1961. Caratteri ultrastrutturali di spermi flagellati di anfibi e uccelli. Studio al microscopio elettronico. – *Archivio Zoologico Italiano (Napoli)* **46**: 123–147.

Góes, R. M. and Dolder, H. 2002. Cytological steps during spermiogenesis in the house sparrow (*Passer domesticus*, Linnaeus). – *Tissue and Cell* **34**: 273–282.

Gomendio, M., Cassinello, J. and Roldan, E. R. S. 2000. A comparative study of ejaculate traits in three endangered ungulates with different levels of inbreeding: fluctuating asymmetry as an indicator of reproductive and genetic stress. – *Proceedings of the Royal Society of London Series B* **267**: 875–882.

Humphreys, P. N. 1972. Brief observations on the semen and spermatozoa of certain passerine and non-passerine birds. – *Journal of Reproduction and Fertility* **29**: 327–336.

Jamieson, B. G. M. 1999. Spermatozoal phylogeny of the vertebrata. In Gagnon, C. (Ed.): *The Male Gamete: From Basic Science to Clinical Applications*, pp. 304–331. Cache River Press, Vienna, IL.

Jamieson, B. G. M. 2006. Avian spermatozoa: structure and phylogeny. In Jamieson, B. G. M. (Ed.): *Reproductive Biology and Phylogeny of Aves*, pp. 249–511. Science Publishers, Inc., Enfield, New Hampshire, USA. Plymouth, UK.

Koehler, L. D. 1995. Diversity of avian spermatozoa ultrastructure with emphasis on the members of the order Passeriformes. *Mémoires du Muséum National d'Histoire Naturelle, Paris* **166**: 437–444.

McFarlane, R. W. 1963. The taxonomic significance of avian sperm. In Sibley, G. C. (Ed.): *Proceedings of the XIII International Ornithological Congress*, pp. 91–102. American Ornithologists' Union, Ithaca, NY.

McFarlane, R. W. 1971. *The ultrastructure and phylogenetic significance of avian spermatozoa*. Unpublished PhD Thesis. University of Florida, U.S.A. (*Fide* Koehler 1991).

Møller, A. P. and Briskie, J. V. 1995. Extra-pair paternity, sperm competition and the evolution of testes size in birds. – *Behavioral Ecology and Sociobiology* **36**: 357–365.

Nicander, L. 1970. Comparative studies on the fine structure of vertebrate spermatozoa. In Baccetti, B. (Ed.): *Comparative Spermatology*, pp. 47–55. Academic Press, New York.

Pitnick, S., Markow, T. and Spicer, G. S. 1999. Evolution of multiple kinds of female sperm-storage organs in *Drosophila*. – *Evolution* **53**: 1804–1822.

Snook, R. R. 2005. Sperm in competition: not playing by the numbers. – *Trends in Ecology and Evolution* **20**: 46–53.

Sotelo, J. R. and Trujillo-Cenóz, O. 1956. Electron microscope study of the kinetic apparatus in animal sperm cells. – *Zeitschrift für Zellforschung* **48**: 565–601.

Tripepi, S. and Perrotta, E. 1991. Spermiogenesis and sperm of passerine birds. In Bacetti, B. (Ed.): *Comparative Spermatology Twenty Years After*, pp. 1021–1023. Raven Press, New York.

Wildt, D., Bush, M., Howard, J. G., O'Brien, S. J., Meltzer, D., Van Dyck, A., Ebedes, H. and Brand, J. 1983. Unique seminal quality in the South African cheetah and a comparative evaluation in the domestic cat. – *Biological Reproduction* **29**: 1019–1025.

Wolfson, A. 1954. Sperm storage at lower-than-body temperature outside the body cavity of some passerine birds. – *Science* **120**: 68–71.