COMPONENTS AND DEVELOPMENT OF THE CENTRIOLAR COMPLEX DURING AND BEYOND SPERMIOGENESIS IN A PASSERIDAN BIRD, THE MASKED WEAVER (*PLOCEUS VELATUS*)

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Abstract

The fate of the proximal centriole in passeridan birds is an area of controversy and relative lack of knowledge in avian spermatogenesis and spermatology. This study examines, for the first time, spatiotemporal changes in the centriolar complex in various phases of spermiogenesis in a passerine bird, the Masked weaver (*Ploceus velatus*). It also describes the configuration of the centriolar complex and the relationship between it and the granular body in both intra- and extra-testicular spermatozoa.   It is shown that the proximal centriole is retained and attaches, at its free end, to the granular body of spermatids in every step of spermiogenesis, as well as in mature intra-testicular and post-testicular spermatozoa, including those in the lumen of the seminal glomus. As the centriolar complex, along with its attached granular body, approaches the nucleus in the early spermatid, the proximal centriole articulates with the distal centriole at an acute angle of about 45⁰, and thereafter, both centrioles, still maintaining this conformation, implant, by means of their articulating proximal ends, at the implantation fossa of the nucleus. In the mature spermatid and spermatozoon, the granular body winds itself helically around the centriolar complex in the neck/midpiece region of the cell, and, thus, becomes the granular

helix. The significance of this observation must await future studies, including possible phylogenetic re-evaluation and classification of birds.

Key words: centriolar complex – spermiogenetic development – passeridan bird – *Ploceus velatus*

 Introduction

The study of spermiogenesis in birds has lagged considerably behind that of mammals, and the available reports are mainly in non-passerine birds (Aire 2007). The order Passeriformes constitutes more than half (Harshman 2007) of about ten thousand species of birds (Jamieson 2007), and according to Jamieson et al. (2006) even the ultrastructure of the spermatozoon has been reported in only about 40 passerine birds, and, often, superficially. It is therefore clear that our knowledge of spermatogenesis and spermatozoal structure in this order is considerably limited. According to Henley et al. (1978), "it is surprising that so little has been done to reveal the ultrastructure of oscine spermatozoa and to study further the arrangement of sperm within the testes". Yet, in the few reports on passerine spermatogenesis and sperm structure, there are a number of reported differences in organelle disposition and interpretation (Fawcett et al. 1971; Goés and Dolder 2002; Humphreys 1972; Jamieson et al. 2006; Kondo et al. 1988; Nicander 1970; Sotelo and Trujillo-Cenóz 1958; Tripepi and Perotta 1991; Yasuzumi 1956; Yasuzumi and Sugioka 1971). The few reports on the development or morphogenesis of the centriolar complex and flagellum of passerine birds have generated variable data and information, and have yielded to considerable speculation. There is no clarity on the fate of the proximal centriole, which is generally agreed not to be seen in the

differentiating spermatids and spermatozoa in passerine birds of the oscine clade. Sotelo and Trujillo-Cenóz (1958) report the presence of both the proximal and distal centrioles in early spermatids of the house sparrow, but that the proximal centriole disappears thereafter during the process of spermiogenesis. Nicander (1970) considers that passerine birds normally possess only one modified centriole, but Goés and Dolder (2002) are silent on the fate of the proximal centriole in their study of spermiogenesis in the house sparrow. Humphreys (1972) cautiously states that the long midpiece of the tail of the house sparrow 'would appear to be a continuation of a single centriole'. Asa and Phillips (1987) do not report the presence of the proximal centriole in the passerine birds that they studied. Jamieson et al. (2006) observe that the proximal centriole is absent in two passerine birds, *Myrmecocichla formicivora* (the Southern ant-eater chat) and *Philetairus socius* (the Social weaver). A recent report by Birkhead et al. (2007) shows, for the first time to our knowledge, the presence of the proximal centriole in the spermatozoon of a passerine bird, the Eurasian bullfinch (*Pyrrhula pyrrhula*).

The present report reveals the retention of both the proximal and distal centrioles in spermatids as well as spermatozoa in the Masked weaver, and also describes spatiotemporal changes in the centriolar complex during spermiogenesis in this bird.

Materials and methods

Three male, sexually mature and active Masked weaver birds were sacrificed by an overdose of Sagatal (May and Baker Ltd) that was administered intraperitoneally. The entire bird was immediately, thereafter, perfused intravascularly with 3% glutaraldehyde buffered in sodium cacodylate, at pH 7.4, using the left ventricle as point of entry into the cardiovascular system.

Tissues were taken from the testis and epididymis and processed for electron microscopy, employing standard, conventional methods. Ultrathin sections of tissues from the testis and various parts of the excurrent ducts of the testis, including the seminal glomus, were cut, stained and viewed in the electron microscope.

Results

In the early round spermatid, both the proximal and distal centrioles occur in the peripheral part of the cytoplasm, typically, lying perpendicular to each other (Fig. 1a). During this phase of spermiogenesis, a dense, retiform and granular globular structure, the granular body (GB), appears close to the nucleus and Golgi complex (Fig. 1b). As the pro-acrosome attaches to the nucleus, the centriolar complex moves inward, with the free end of the proximal centriole attaching to the GB. Subsequently, the centriolar complex, along with the GB attaches to the nucleus, close to the pro-acrosomal attachment, at the definitive cranial pole of the nucleus (Fig. 1b). The attached centriolar complex of the round spermatid thereafter migrates to the definitive caudal pole of the nucleus, along with the GB which, itself, remains attached to the free end of the proximal centriole. At this stage, the latter articulates with the distal centriole at an acute angle (of about 45⁰) and its GB-attached end projects caudo-laterally (Fig. 1c). This configuration and relationship between the centrioles and GB are maintained during the elongating phase of the spermatid (Figs. 1d, 2b, c). As the spermatid continues to elongate, and the tail segment differentiates, the GB becomes more finely granular, and also begins to wind round the neck/midpiece of the tail in a helical manner (Fig. 2a). The attachment of the

elongated and helical GB, now known as the granular helix (GH), is evident in mature spermatids in the testis and mature spermatozoa in the seminal glomus (Figs. 2 c, d).

Discussion

A granulofilamentous structure, known as the granular body in the spermatid, and granular helix in the spermatozoon, is described for most species of the Passeroidea. This structure is referred to as the 'granular body' (Humphreys 1972; Tripepi and Perotta 1991; Jamieson et al. 2006) and the 'centriolar adjunct' (Goés and Dolder 2002) in various passeridan birds. In mammals, Fawcett et al. (1971) describes a centriolar adjunct, which structure is attached to the free end of the proximal centriole in rodents. The centriolar adjunct of rodents is obviously different structurally from the one described in this study in the Masked weaver, and, besides, in rodents, it is transient, whereas that of the passerine is an integral and permanent component of the spermatid as well as the mature spermatozoon. The term, granular body, already adopted by a number of authors (Humphreys 1972; Tripepi and Perotta 1991; Jamieson et al. 2006; Jamieson 2007) is also adopted in this report in order to avoid confusion in the literature, especially as the homologous structure in the rodent appears to be different from that of birds. The granular body of round spermatids becomes the granular helix in elongating spermatids (present report) as well as in mature spermatozoa. The granular helix invests the distal centriole and the proximal region of the axoneme (Jamieson 2007), extending between the base of the nucleus and the mitochondrial helix, in those passeridan birds that possess it. The granular body is not present in the spermatozoa of all passerine birds, being absent in the

southern ant-eater chat (*Myrmecocichla formicivora*) and in suboscine birds. The function of this organelle is not known.

In the Masked weaver, the granular body appears in the cytoplasm of the round spermatid quite early, in close proximity to the Golgi complex, and later on, it attaches to the free end of the proximal centriole of the centriolar complex as it approaches the nucleus for attachment or implantation. A major agreement among investigators, until recently, is the absence of a proximal centriole in the developing spermatid and mature spermatozoon in members of the Passeroidea studied (see Jamieson 2007). It has not been difficult demonstrating the proximal centriole in non-passerine birds (see Jamieson 2007), not even in the guinea-fowl and quail in which it is in-line aligned with the distal centriole (Aire and Soley 2003; Jamieson 2007). Birkhead et al. (2007) demonstrate the presence of the proximal centriole in the spermatozoon of the Eurasian bullfinch (*Pyrrhula pyrrhula*). The present study, for the first time, describes the centriolar complex, including the proximal centriole, and its spatiotemporal changes in the various phases of spermiogenesis in the Masked weaver. The structural configuration of the centriolar complex, including the acute angle of articulation between the proximal and distal centrioles, and their relationship with the granular body are also observed and described in mature spermatozoa of this bird. In the bullfinch, the proximal centriole articulates with the distal centriole nearly perpendicularly, or, as one micrograph indicates, at an obtuse angle, in normal spermatozoa (Birkhead et al., 2007). It is not known why the free end of the proximal centriole is attached to the granular body in round/oval or early spermatid, or granular helix in the elongated spermatid, during and beyond spermiogenesis, but, as previously stated, the proximal centriole is not present in the spermatozoon of some other passeridan birds that

possess and display the granular helix e.g. in *Taeniopygia guttata* (Tripepi and Perotta 1991) and *Philetairus socius* (Jamieson et al. 2006). The significance of this observation must await future studies, including possible phylogenetic evaluation and classification of birds. It is, however, advocated that spermiogenesis and sperm structure in more species of birds in general and, specifically in passerine birds, be studied in order to enhance both knowledge and understanding of spermatogenesis and sperm structure in this large group of birds, constituting about 60% of all birds (Sibley and Monroe 1990).

Acknowledgement

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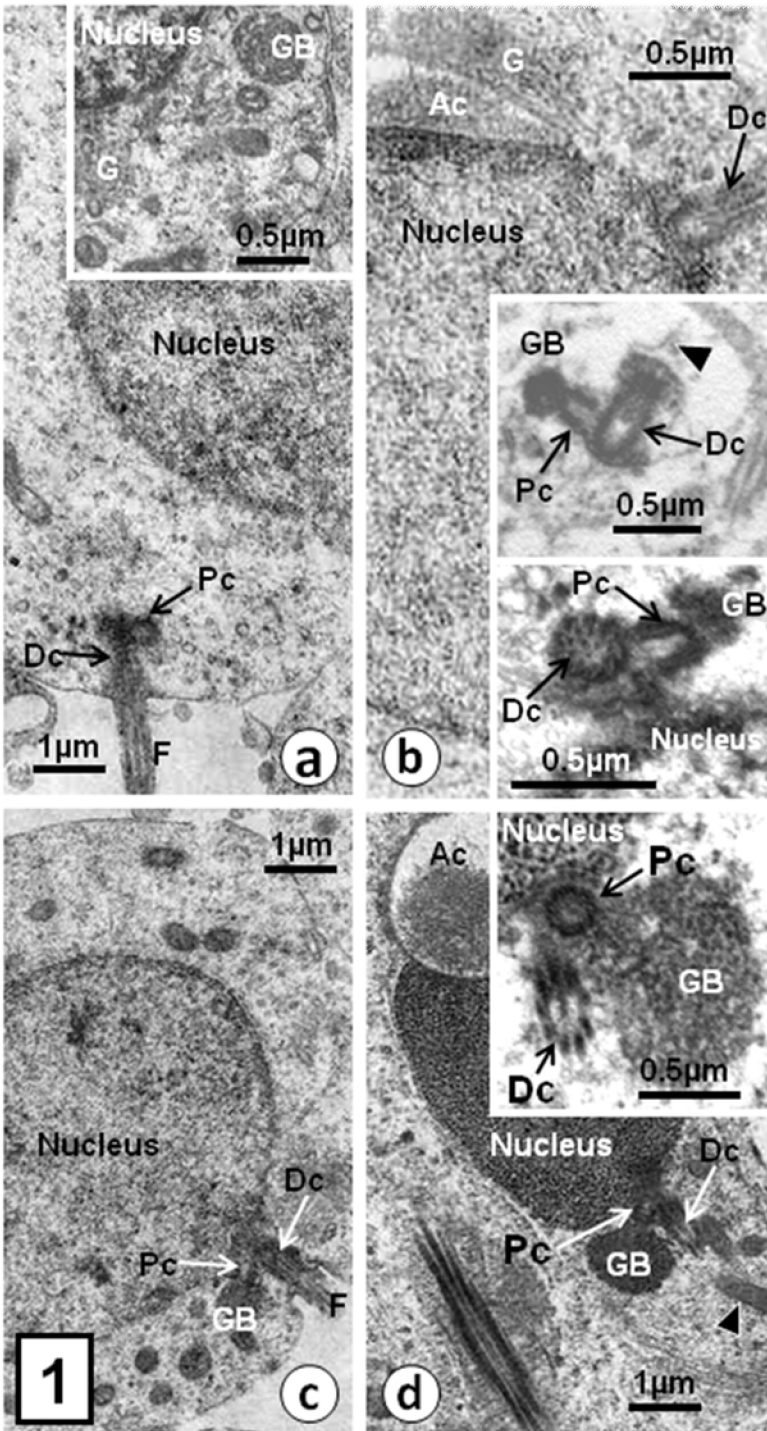
Plate 1: Fig. **a**: A young, round spermatid, displaying within its peripheral cytoplasm, the centriolar complex, proximal centriole (Pc) and distal centriole (Dc), arranged in the usual configuration. **Inset** is part of a spermatid showing the close relationship between the granular

body (GB) (already present in the cytoplasm at this early stage), the Golgi complex (G) and the nucleus.

Fig. **b**: The main figure shows the centriolar complex attaching to the nucleus close to the pro-acrosome (Ac) of an older round spermatid; Dc = distal centriole; G = Golgi complex. <u>Upper</u> **inset** shows the free end of the proximal centriole (Pc) of the centriolar complex attaching to the granular body (GB) in the young round spermatid. Dc = distal centriole; arrowhead shows the flagellar canal.

<u>Lower</u> **inset** shows the centriolar complex (Pc, proximal centriole; Dc, distal centriole), along with attached granular body (GB) lying very close to the nucleus.

Fig. **c**: The centriolar complex, along with the articulated granular body (GB), has attached to the caudal pole of the nucleus, with the proximal (Pc) and distal (Dc) centrioles retaining the 45° angulation between them. F = flagellum.

Fig. **d**: The nucleus of a spermatid at an early stage of elongation displays fine granulation of the chromatin. The centriolar complex (Pc, proximal centriole; Dc, distal centriole), along with the granular body (GB) are in place at the caudal pole of the nucleus. Ac = acrosome; Arrowhead = mitochondrion. **Inset:** shows transverse sections of the proximal centriole (Pc) inserting at the implantation fossa, and distal centriole (Dc). GB = granular body.
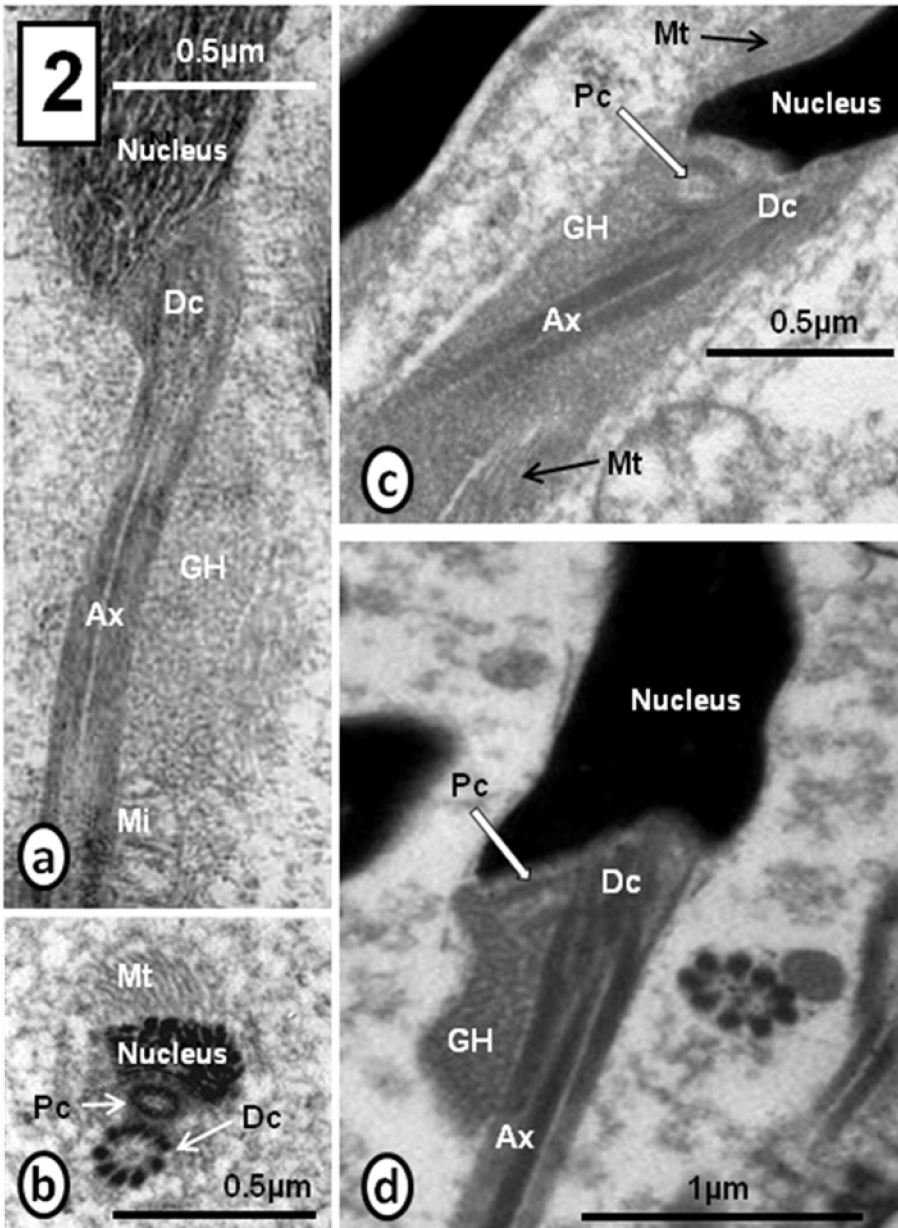
Plate 2: Fig. **a**: shows an elongated spermatid with long, dense, chromatin strands in the nucleus, and granular body (GB) winding round the neck/midpiece region. Note the attachment of the distal centriole (Dc) at the implantation fossa of the nucleus. Mi = mitochondrial helix; Ax = axoneme. Fig. **b** displays transverse sections of both the proximal (Pc) and distal (Dc)

centrioles at the stage of development of the spermatid in Fig. **a**, above. Mt = microtubular helix.

Fig. **c**: A longitudinal section of a mature testicular spermatozoon showing both the proximal (Pc) and distal (Dc) centrioles inserting at the implantation fossa. The helical granular helix (GH) is still very closely related to the centrioles. Mt = microtubular helix; Ax = axoneme.

Fig. **d**: a longitudinal section of a spermatozoon in the seminal glomus (at the distal end of the ductus deferens) of this bird. The centriolar complex is present in its peculiar angular configuration, and association with the granular helix (GH). Note that the microtubular helix has disappeared.