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Sperm Development in the Teleost *Oryzias latipes*

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Summary. In *Oryzias latipes* the processes of spermatogenesis and spermiogenesis occur within testicular or germinal cysts which are delimited by a single layer of lobule boundary cells. These cells, in addition to comprising the structural component of the cyst wall, ingest residual bodies cast off by developing spermatids. Therefore, they are deemed to be the homologue of mammalian Sertoli cells. The germ cells within a cyst develop synchronously owing to the presence of intercellular bridges connecting adjacent cells. Since bridges also connect spermatogonia, it seems probable that all of the germ cells within a cyst may form a single syncytium and do not exist as individual cells until the completion of spermiogenesis when the residual bodies are cast off. Significant differences between spermiogenesis in *O. latipes* and in the related poeciliid teleosts are discussed.

Key words: Teleost testis – Lobule boundary cell – Sertoli cell – spermatogenesis – spermiogenesis.

Introduction

Within the teleost order Atheriniformes, reproductive modes range from egg scattering to varying degrees of viviparity which have been most thoroughly studied in the family Poeciliidae. The evolution of viviparity in the poeciliid teleosts has involved testicular modification such that sperm bundles, or spermatozeugmata, are transferred to the reproductive tract of the female. Furthermore, the demands of internal fertilization have necessitated modifications in sperm morphology. Poeciliid sperm characteristically possess a "high mitochondrial collar" (Nicander, 1970) and elongated nucleus which is hollowed ventrally by a deep fossa that receives the centriolar complex and proximal portion of the flagellum (see Mattei and Boisson, 1966; Billard, 1970a; Grier, 1973,

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1975b). It has recently been mentioned that sperm nuclear elongation does not occur in teleost families closely related to the poeciliids, namely the cyprinodontids, goodeids and atherinids (Grier, 1975b). However, despite the reproductive diversity observed within the Atheriniformes, scant information is available concerning sperm structure, spermiogenesis and spermatogenesis in fish closely related to the poeciliids and the comparative implications that ultrastructural morphology may reveal.

The present study details sperm formation in *Oryzias latipes,* the Japanese medaka. In this teleost, fertilization is external. The differences observed in sperm formation and structure between *Oryzias* and poeciliid teleosts indicate a parallelism between reproductive modes and sperm diversity in the Atheriniformes.

Materials and Methods

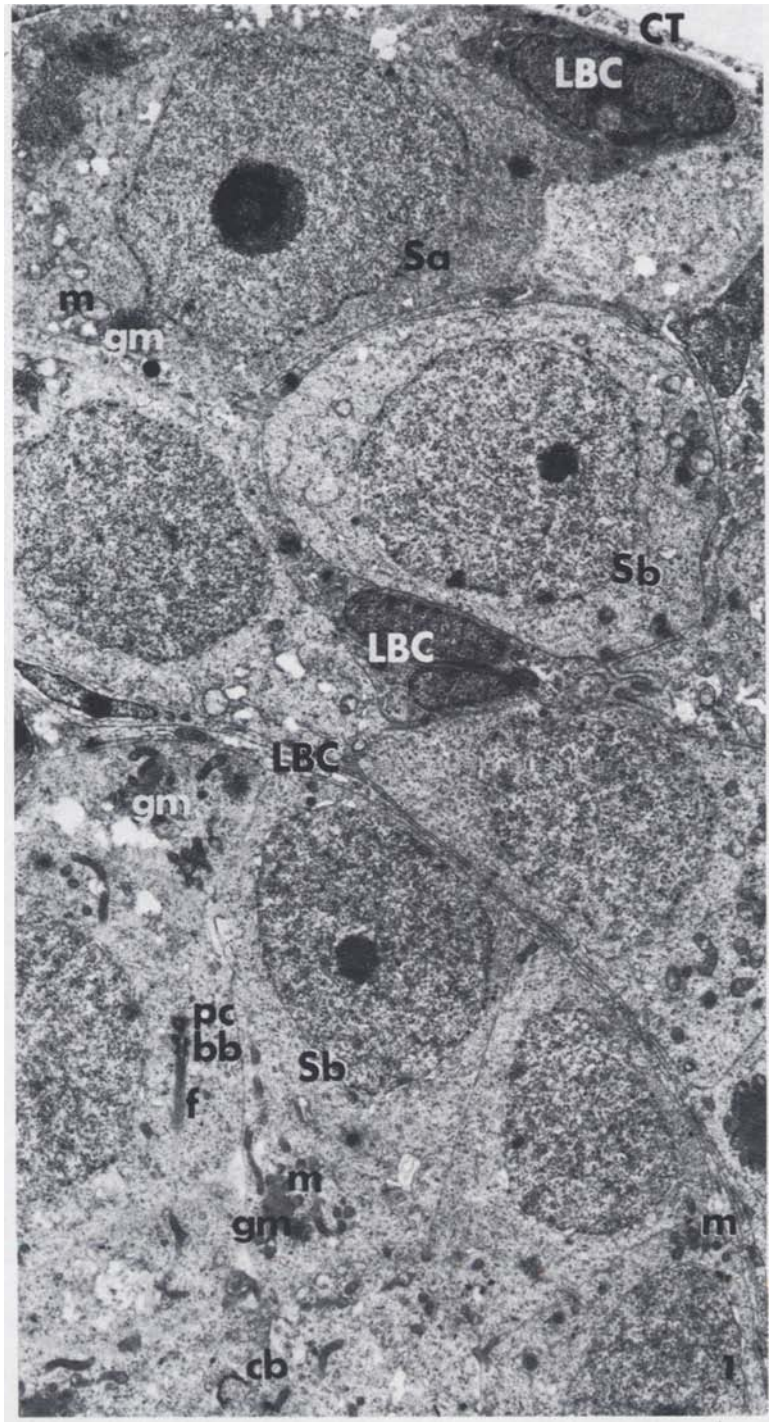
Testes of adult male *Oryzias latipes* were fixed in glutaraldehyde and osmium tetroxide as previously described (Grier, 1975a). They were embedded in Spurr's low viscosity resin. Silver to silver-gold sections were stained with uranyl acetate and lead citrate and examined in a Philips 200 electron microscope.

Results

The germinal cells of *Oryzias latipes* are packed into cysts which are organized at the periphery of the testis (Fig. 1). The most peripheral germ cells are the spermatogonia which are separated from each other by intervening strands of lobule boundary cell cytoplasm except at those points where cells are conjoined by persistent cytoplasmic bridges (Fig. 2). At least two types of spermatogonia are observed, indicating that these cells divide a number of times by mitosis before they are organized into cysts (Fig. 1). In each individual cyst, the processes of meiosis and spermiogenesis occur synchronously. After each meiotic division, cytokinesis fails to go to completion resulting in a branching network of interconnected germ cells which persist as a syncytium through spermiogenesis (Fig. 6). The germ cells probably do not exist as separate entities until after the residual bodies are cast off.

The centriolar complex is a common cytological feature in the spermatogonial cytoplasm. It consists of the paired centrioles and their associated satellites (Fig. 2). The centrioles differ functionally. One often acts as a basal body by generating what appears to be a nonfunctional flagellum that does not always extend beyond the periphery of the cell. A striated satellite, or intercentriolar

Fig. 1. Spermatogonia (*Sa, Sb*) beneath connective tissue capsule (*CT*) of testis separated from each other by lobule boundary cell (*LBC*) cytoplasm or organized into cysts bounded by lobule boundary cells. Note granular material (*gm*) associated with mitochondria (*m*). Cytoplasmic bridges (*cb*) connect spermatogonia which often contain a single flagellum (*f*). (*bb*), basal body; (*pc*), proximal centriole. ×6,000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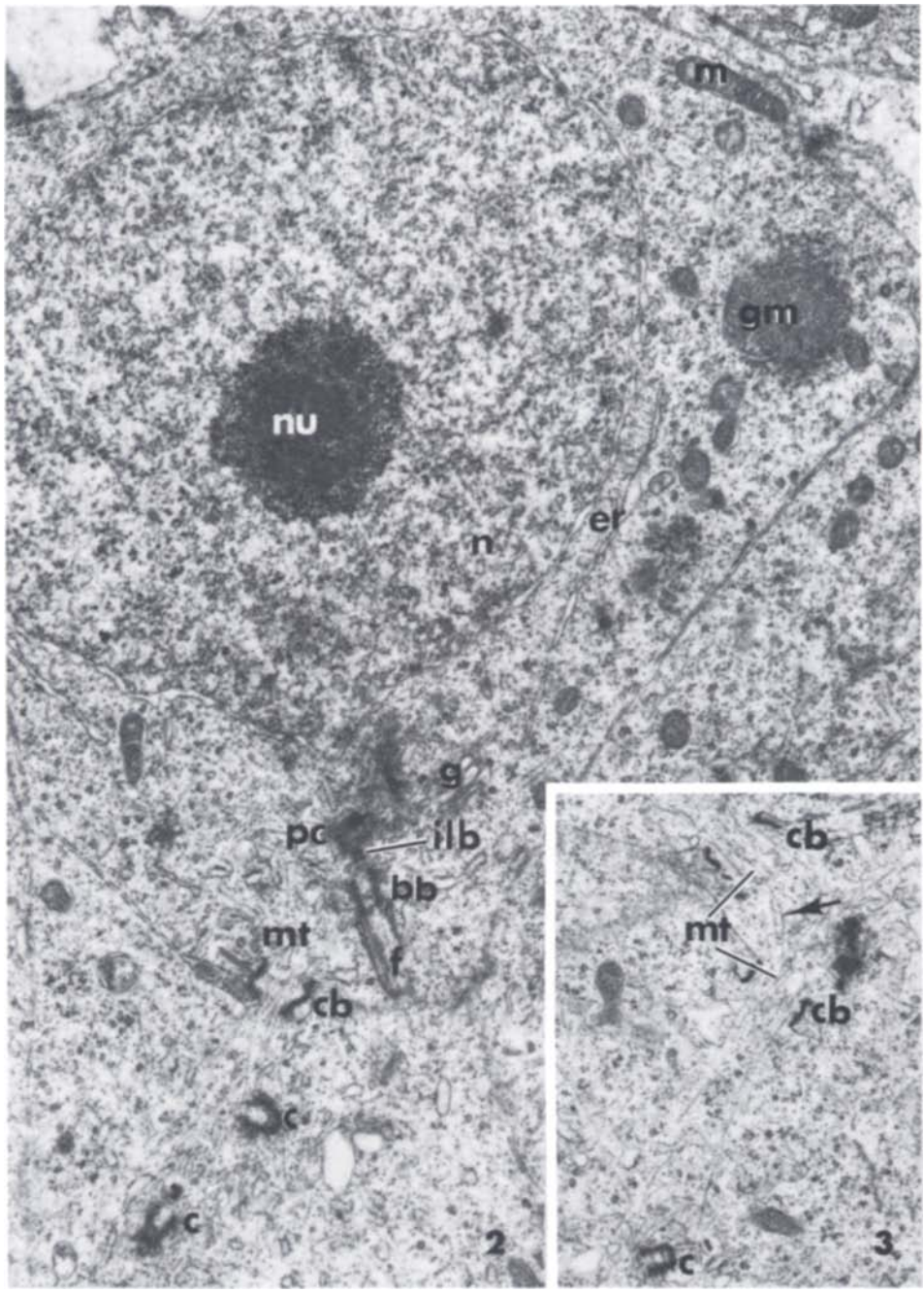

Fig. 2. Spermatogonial nucleus (*n*) with nucleolus (*nu*). Mitochondrion (*m*) associated with granular material (*gm*). A flagellum (*f*) is produced by basal body (*bb*). Interposed between proximal centriole (*pc*) and basal body (*bb*) an intercentriolar lamellated body (*ilb*). (*g*), Golgi complex. Microtubules (*mt*) pass between cells. × 14,200

Fig. 3. Microtubules (*mt*) pass through cytoplasmic bridges (*cb*) of spermatogonia. One (arrow) passes between three cells, others pass to centrioles (*c*). × 15,000

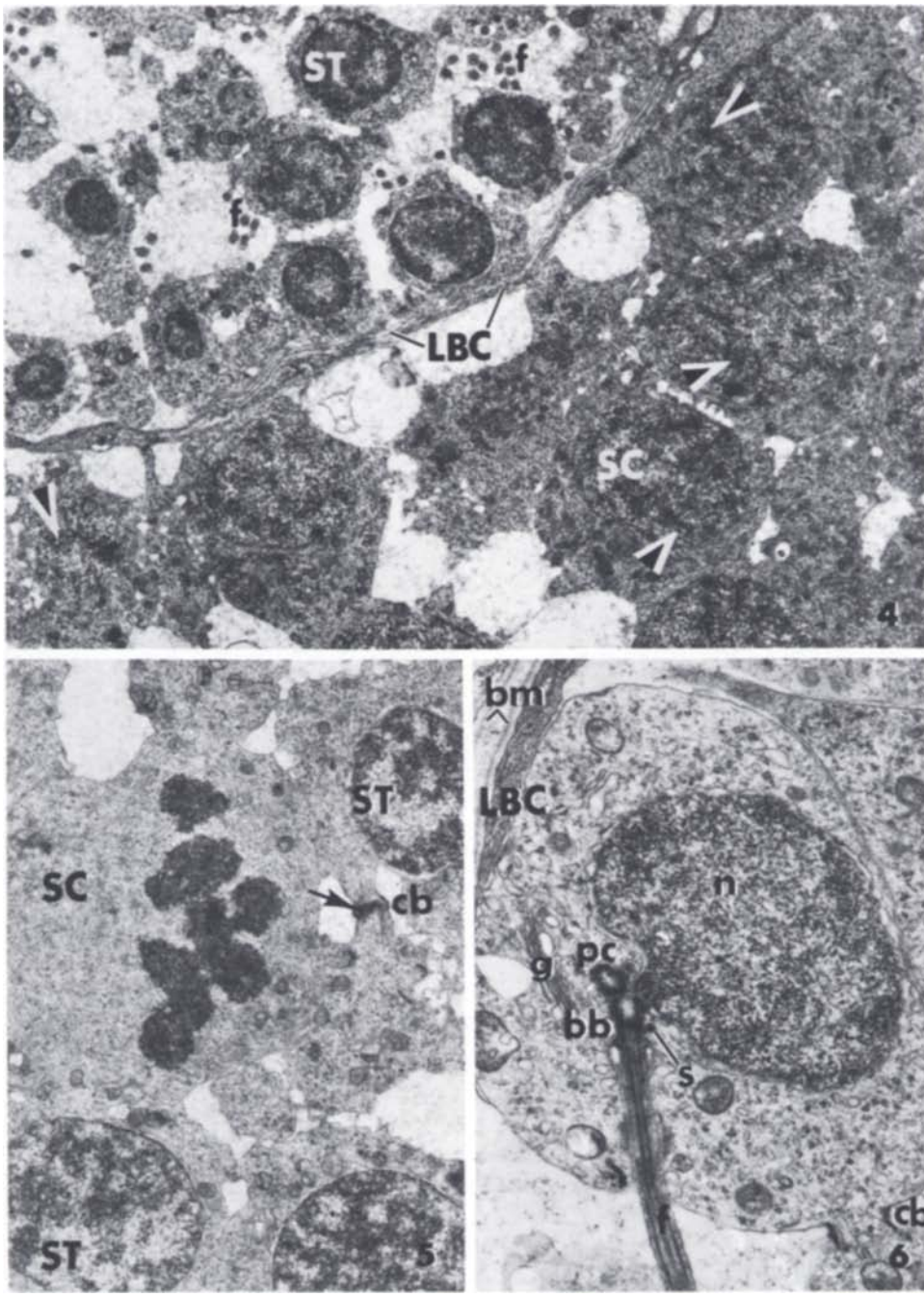

Fig. 4. Lobule boundary cells (*LBC*) form walls of adjacent cysts containing early pachytene spermatocytes (*SC*) or spermatids (*ST*). Arrows indicate synaptinemal complexes. (*f*), flagellum. × 3,700

Fig. 5. Dividing secondary spermatocyte adjacent to early spermatids (*ST*). Arrow points to cytoplasmic bridge (*cb*) containing darkened midbody. × 7,000

Fig. 6. Spermatid nucleus indents upon association with centriolar complex. Golgi apparatus (*g*) becomes displaced from juxtacentriolar position. Cytoplasmic bridge (*cb*) connects spermatids. (*bb*), basal body; (*bm*), basement membrane; (*LBC*), lobule boundary cell; (*n*), spermatid nucleus; (*pc*), proximal centriole; (*s*), satellite of basal body. × 16,000

lamellated body, extends from the proximal centriole toward the basal body to which it becomes attached by striae which are barely visible in the micrograph (Fig. 2). The Golgi complex and numerous microtubules are associated with the centriolar complex. Microtubules from the centrioles radiate into the spermatogonial cytoplasm; many pass through the cytoplasmic bridges which connect adjacent spermatogonia (Figs. 2, 3).

Synchrony of germ cell differentiation is illustrated in Figure 4. Each of the depicted cysts is bounded by strands of lobule boundary cell cytoplasm approximately 0.3 μm thick. Within each cyst, all of the germ cells are in the same stage of differentiation. The lower cyst contains spermatocytes in early pachytene while the upper one contains young spermatids with developing flagella and rounded, undifferentiated nuclei. While there is a synchrony of germ cell differentiation, meiotic divisions appear to be initiated in a group of spermatocytes and then spread throughout the cyst. Thus, actual cell divisions are not perfectly synchronous (Fig. 5). A midbody persists within the cytoplasmic bridge for a period of time after cell division. This is transitory and disappears from the bridge as cell development proceeds (Fig. 6).

Flagellar outgrowth occurs in the early spermatid when the centriolar complex is situated at the cell periphery (Fig. 7). At this time, a distinct intercentriolar lamellated body is situated between the centrioles, but this structure is transitory and is not observed after a centriolar complex nuclear relationship is formed (Fig. 6). The association of the centriolar complex with the initially rounded spermatid nucleus results in the formation of a fossa (Figs. 6, 8, 9) which completely surrounds the proximal centriole. The outline of the fossa is scalloped; its concavities are associated with electron dense material (Fig. 9). During spermiogenesis, the density and amount of electron dense material increase. This apparently acts as a means of anchoring the proximal centriole to the nucleus (Figs. 14, 15). In addition to the dense material, a microtubule extends from the proximal centriole to the nuclear envelope lining the fossa in the mature sperm (Fig. 15). Electron dense material also anchors the basal body

Fig. 7. Forming flagellum (*f*). Note intercentriolar lamellated body (*ilb*) located between proximal centriole (*pc*) and basal body (*bb*). (*n*), spermatid nucleus. × 17,000

Fig. 8. Basal body (*bb*) flanked by satellites (*s*); attached to nucleus (*n*) by electron dense material (arrow heads). Flagellum (*f*) flanked by dense material (*dm*). × 13,500

Fig. 9. Proximal centriole (*pc*) within nuclear fossa with scalloped outline containing electron dense material (*dots*). Electron dense material connects basal body (*bb*) to nucleus (*n*). × 54,900

Fig. 10. Cross section of spermatid flagellum (*f*) at level denoted by line in Figure 8. Dense material comprised of concentric lamellae. (*m*), mitochondrion. × 32,000

Fig. 11. Spermatids (*S*) shed residual bodies (*rb*) into cyst lumen whereupon they are phagocytized by lobule boundary cells (*LBC*) to become digestive vacuoles (*dv*). Degenerating spermatid (*x*) in digestive vacuole. × 4,400

Fig. 12. Sperm (*s*) surrounded by cytoplasmic processes of lobule boundry cell (*LBC*) at time of residual body formation. × 13,600

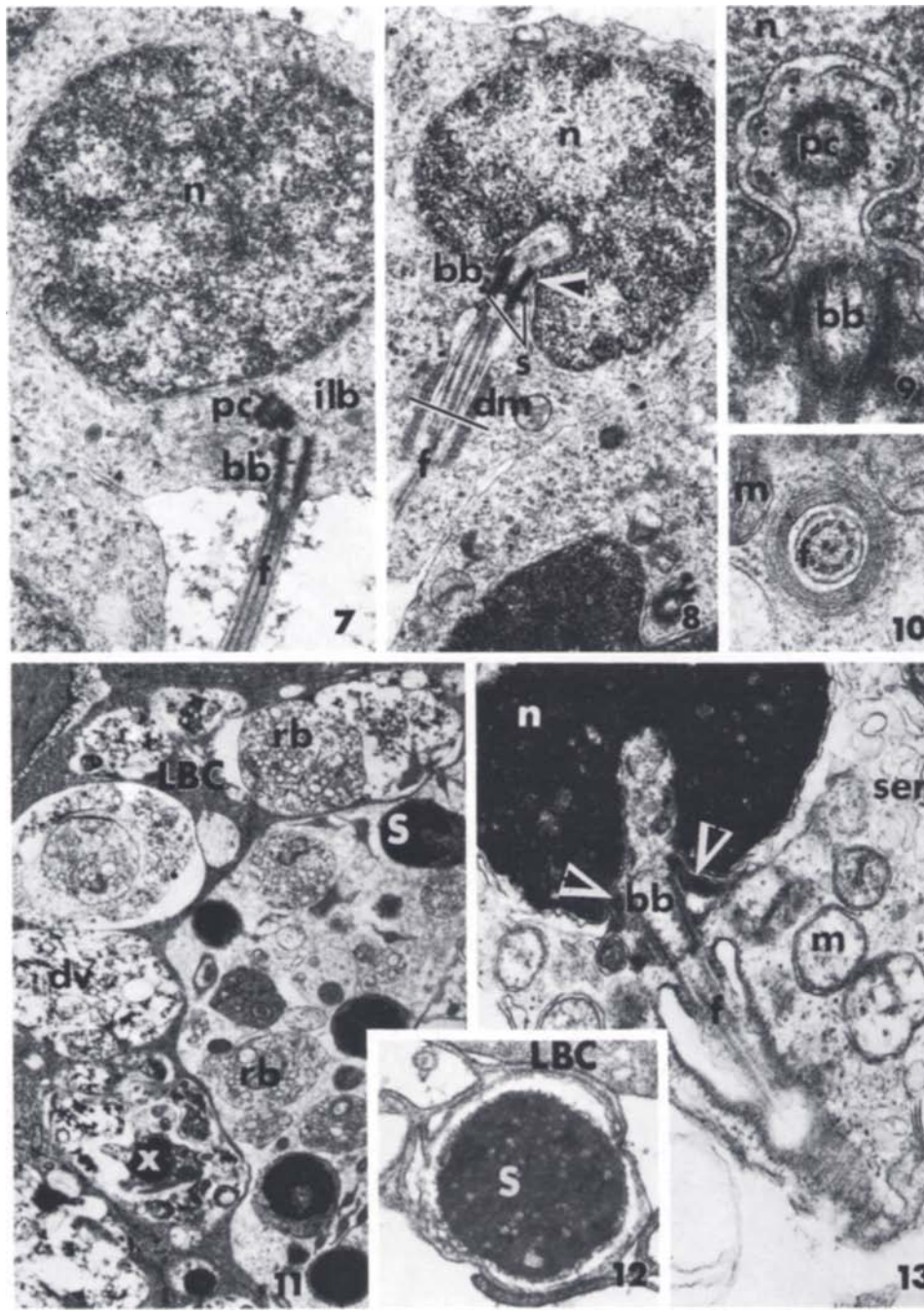

Fig. 13. Basal body (*bb*) attached to spermatid nucleus (*n*) by electron dense material (arrow heads). Mitochondria (*m*) remain rounded and loosely associated with flagellum (*f*); (*ser*), smooth endoplasmic reticulum. × 32,200

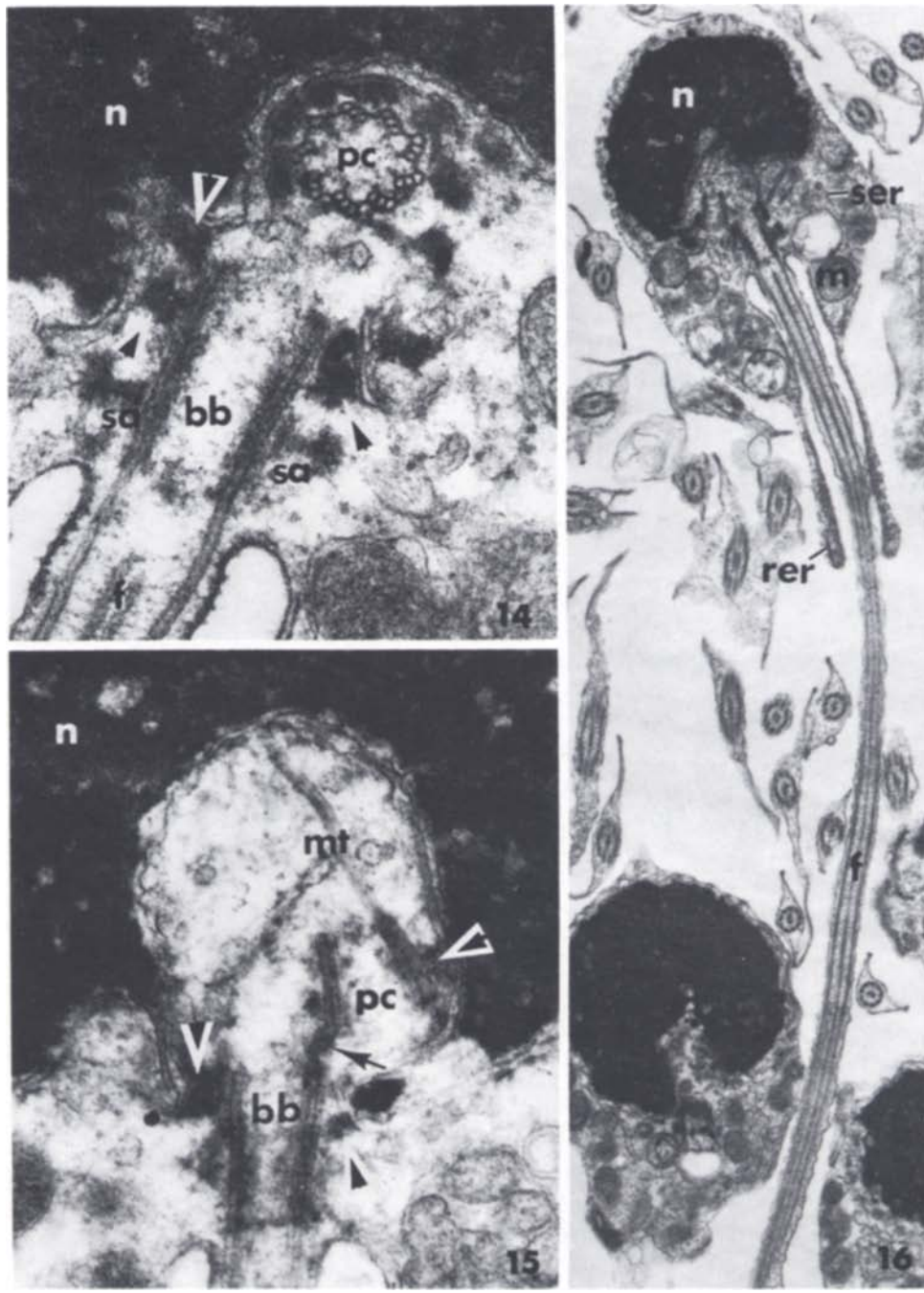

Fig. 14. Mature sperm illustrating attachment of proximal centriole (*pc*) to nucleus (*n*) by electron dense material. Basal body (*bb*) attached in a similar manner (arrow heads). Satellites (*sa*) flank basal body. Central flagellar tubules (*f*) end behind basal body. × 79,300

to the nuclear envelope lining the nuclear fossa. The scant amount of electron dense material that initially attaches the basal body to the nucleus becomes quite extensive as spermiogenesis proceeds (Fig. 13). It persists in mature sperm (Figs. 14, 15). In addition, electron dense material also connects the two centrioles (Fig. 15), thus stabilizing the positional relationship between them.

Toward the completion of spermiogenesis, the residual bodies are cast off into the cyst lumen by the developing spermatids. Shortly thereafter, the residual bodies are phagocytized by lobule boundary cells and transform into digestive vacuoles within the lobule boundary cell cytoplasm (Fig. 11). The digestive vacuoles disappear completely from the lobule boundary cell within a comparatively short period. They are rarely seen in cysts containing mature sperm. Occasionally, sperm are ingested along with residual bodies (Fig. 11) and undergo degenerative changes. Other individual sperm may be surrounded by lobule boundary cell processes (Fig. 12), or may actually be embedded in lobule boundary cell cytoplasm. These sperm have not been observed to degenerate as in the former case.

The nucleus of mature sperm of *O. latipes* is rounded and contains dense chromatin interspersed with irregular spaces (Figs. 14–16). No acrosome is formed which is typical of teleost sperm (Nicander, 1970; Mattei, 1970). Aside from the expansion of electron dense material which anchors the centriolar complex to the sperm nucleus, relatively few changes take place in the centriolar complex by termination of sperm maturation. The proximal centriole retains a definitive structure of nine triplets (Fig. 14); the basal body and its associated satellites are essentially the same as observed in the early spermatid. During spermiogenesis, the concentric lamellae that surround a portion of the spermatid flagellum (Figs. 8, 10) disappear and are not to be found within the residual cytoplasm which constitutes the sperm midpiece (Fig. 16). Rather, the residual cytoplasm contains scattered, unmodified mitochondria, ribosomes and both smooth and rough endoplasmic reticulum. The latter is often prevalent within the slender cytoplasmic extensions that trail alongside of the flagellum (Fig. 16). Just behind the sperm midpiece, the membrane of the flagellum is thrown into ridges that are in the same plane as the central flagellar doublets (Figs. 16).

Discussion

Several ultrastructural studies in poeciliid teleosts have revealed a similarity in the processes of spermiogenesis, sperm morphology (Mattei and Boisson,

Fig. 15. Sperm nucleus (*n*) at right angle to previous illustration. Electron dense material (large arrows) attaches proximal centriole (*pc*) and basal body (*bb*) to nucleus (*n*) and to each other (small arrow). Single microtubule (*mt*) attaches proximal centriole to apex of nuclear fossa. ×64,700

Fig. 16. Mature sperm illustrating the nucleus (*n*) and midpiece residual cytoplasm which contains smooth (*ser*) and rough (*rer*) endoplasmic reticulum, mitochondria (*m*) loosely associated with flagellum (*f*) and centriolar complex. Cross sections through flagella reveal typical 9+2 axonemal doublet configuration and fin-like, membranous extensions in plane of central axonemal doublets. ×18,000

1966; Grönberg and Telkkä, 1968; Billard, 1969; Asai, 1971; Grier, 1973; Russo and Pisano, 1973) and testis structure, particularly with regard to the testicular lobules in which sperm cysts develop (Billard, 1969; Hurk, 1973, 1974; Grier, 1975a). It has been suggested that the formation of an intercentriolar lamellated body (ILB) between centrioles of developing spermatids may be of phylogenetic significance since it occurs in spermatids of the related poeciliids, atherinids and cyprinodonts (Grier, 1973) and now in a fourth member of the order Atheriniformes, *O. latipes* (phylogenetic classification of Greenwood et al., 1966). The ILB has not been described in more distantly related teleosts. However, the occurrence of striated structures, the intercentriolar formation, in spermatids and sperm of two unrelated teleosts, *Dactylopterus volitans* (Boisson et al., 1968) and the carp, *Cyprinus carpio* (Billard, 1970b), indicates that intercentriolar structures in developing sperm may be more generally distributed among teleosts than heretofore supposed. Further ultrastructural investigations are therefore required to determine if the ILB has significant phylogenetic or taxonomic importance.

The ILB in spermatids of *O. latipes* differs significantly from that described in poeciliids (Mattei and Boisson, 1966; Grönberg and Telkkä, 1968; Billard, 1970a; Asai, 1971; Grier, 1973, 1975b), being both smaller and disappearing earlier during spermiogenesis. This may be a reflection of the angle assumed by the proximal centriole with respect to the basal body. While the poeciliid centrioles are perpendicular to each other, and the ILB is well developed, the centrioles of *O. latipes* spermatids are at an angle of approximately 45°. The ILB is subsequently poorly developed and does not appear to play an important role during spermiogenesis. It disappears from an intercentriolar position before the centriolar complex becomes associated with the spermatid nucleus. In the poeciliids, the ILB persists until the completion of spermiogenesis (Grier, 1973), apparently contributing to the formation of an electron dense cap at the proximal end of the basal body from which the central flagellar doublets originate (Grier, 1973, 1975a, b). This may be an important sperm adaptation in poeciliids resulting in greater efficiency of flagellar movement and sperm motility within the female reproductive tract. In *O. latipes,* a species in which fertilization is external, an electron dense cap does not form at the proximal end to the basal body nor do the flagellar central doublets extend through the length of the basal body. The function of an ILB in spermatogonia of *O. latipes* and in spermatocytes of *Gambusia affinis* (Grier, 1975b) and *P. reticulata* (Grönberg and Telkkä, 1968) cannot be resolved with the information presently available.

Besides the ILB, significant differences exist with regard to the centriolar complex between poeciliid teleosts and *O. latipes.* An obvious difference centers on the structure of the proximal centriole which retains a configuration of nine triplets in mature sperm of *O. latipes,* but which is reduced to a remnant in poeciliid sperm (Billard, 1970a; Nicander, 1970; Grier, 1973, 1975b). Whereas electron dense material is responsible for the stabilization of the spatial relationship between the nucleus and centriolar complex in *O. latipes* spermatids and sperm, this function is almost entirely taken over by microtubules in poeciliid spermatids (see Grier, 1975b) except for the period prior to the formation of microtubules. Then, electron dense material in poeciliid spermatids (Grier,

1973, 1975b) is of importance in establishing the association between the nucleus and the centriolar complex and flagellum.

In stark contrast to the poeciliids, where sperm possess an elongated nucleus and a well developed midpiece, the sperm of *O. latipes* show neither of these characteristics. Nuclear elongation does not occur. The midpiece is composed of residual cytoplasm containing ribosomes, smooth and rough endoplasmic reticulum and an array of rounded mitochondria which do not undergo significant enlargement or shape alterations as is seen in the poeciliids. Thus, despite the occurrence of an ILB in both spermatogonia and young spermatids of *O. latipes,* and the presence of this structure in poeciliid spermatocytes and spermatids (Grönberg and Telkkä, 1968; Billard, 1969; Asai, 1971; Grier, 1973, 1975b), both sperm morphology and the process of spermiogenesis differ significantly between these related teleosts. Aside from the ILB, there is practically no resemblance in sperm morphology in these teleost species that would suggest a close phylogenetic relationship. Sperm morphology reflects reproductive modes, i.e., external as opposed to internal fertilization.

Cells lining testis cysts ingest residual bodies cast off by developing spermatids in poeciliid (Grier, 1975a) and cyprinodontid (Grier and Linton, 1976) teleosts. This has been previously reported in *O. latipes* (Gresik et al., 1973) and substantiated in the present study. However, Gresik et al. (1973) erroneously state that the teleost lobule boundary cell "is a fibroblastic derivative which lies in the intercellular space and not within the epithelial basement membrane". Accordingly, they introduced the descriptive term "cyst epithelial cell" in reference to the cells lining the testicular cysts in *O. latipes.* There is no evidence to assume that testis structure in *O. latipes* and *Fundulus heteroclitus,* a related cyprinodontid, is significantly different. In *Fundulus,* testis structure is lobular (Matthews, 1938; Pickford et al., 1972); the cells lining the cysts in which sperm develop are known as lobule boundary cells. These are located within the basement membrane of the lobule (Grier and Linton, 1976). Because of a similar function, the phagocytosis of residual bodies, cells lining the cysts in the testes of *Oryzias* and *Fundulus* must be considered homologous. They should therefore be called "lobule boundary cells", or "Sertoli cells", because these names are established in the literature regarding testis structure in teleosts.

It has been previously supposed that sperm embedded within lobule boundary cell cytoplasm degenerate in *O. latipes* (Gresik et al., 1973). However, the present study has failed to uncover evidence of this unless the sperm had been phagocytized along with residual bodies and are incorporated into the resulting digestive vacuoles. The flagella of individual sperm embedded within lobule boundary cells project into the germinal cyst lumen in the related cyprinodontids (Grier and Linton, 1976). In the poeciliid teleosts, sperm normally become embedded in Sertoli cell cytoplasm (homolog of lobule boundary cells) only to be released into the sperm ducts (Billard, 1969; Hurk et al., 1974; Grier, 1975a) at the time of spermiation. There appears to be no reason to assume that the small number of sperm that become embedded in lobule boundary cells of *O. latipes,* or in cyprinodontids, degenerate. They are probably released when sperm are voided from the cyst into the testis duct system.

Intercellular bridges provide the morphological basis for synchronous germ

cell divisions and maturation (see Fawcett et al., 1959; Fawcett, 1961; Dym and Fawcett, 1971). Therefore, they must provide a means by which cytoplasmic components can be transferred between cells. While this has never been demonstrated for cells destined to form sperm, cytoplasmic bridges are requisites for material flow from insect nurse cells to oocytes as demonstrated in the autoradiographic study of Bier (1963). Furthermore, intercellular connections established between in vitro phagocytes provide a means for material transport between cells (Aronson, 1963; Yokomuro and Nozima, 1972). The presence of intercellular bridges between spermatogonia, spermatocytes and spermatids in the teleost *O. latipes* lends credence to the presumption that all the germ cells within a cyst remain interconnected throughout meiosis and form a continuous syncytium through which materials flow from one "cell" to another affording nutrient exchange and synchronous development. The germ cells probably do not separate until the residual bodies are cast off toward the end of spermiogenesis. These contentions are supported by the observation of extensive intercellular connections between synchronously developing germ cells in mammals (Dym and Fawcett, 1971; Moens and Go, 1972) and persistent intercellular connections between residual bodies (Dym and Fawcett, 1971).

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