Ultrastructure of the Reproductive System of the Black Swamp Snake (*Seminatrix pygaea*). IV. Occurrence of an Ampulla Ductus Deferentis

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**ABSTRACT** The distal end of the ductus deferens of *Seminatrix pygaea* (Cope) differs from more proximal portions of the tube by possessing a highly fluctuated epithelium. Similar morphology has been reported in this area in one lizard and some mammals and the region has been designated in these taxa as the ampulla ductus deferentis. In this study, light and transmission electron microscopy were used to study the posterior ductus deferens in *S. pygaea* and compare the histology in this region to more proximal regions of the duct and to descriptions of the ampulla of other species. *Seminatrix pygaea* stores sperm throughout the ductus deferens during the entire year. The epithelium of the ductus deferens is not secretory, but the presence of numerous small apical vesicles indicates a role in fluid absorption. In addition to the highly folded epithelium, clusters of sperm nuclei are more intimately associated with the apical ampullary epithelium than elsewhere in the ductus deferens. No evidence of phagocytosis of sperm was found. In contrast, the ampulla of mammals and presumably the lizard *Calotes versicolor* is glandular and phagocytic. The common character shared by the squamates and mammals with specializations in this area is the folded epithelium, and whether this is due to shared ancestry with amniotes or is homoplastic is unresolved. J. Morphol. 262:714–730, 2004.

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**KEY WORDS:** Reptilia; Squamata; Serpentes; *Seminatrix*; reproduction; ductus deferens; histology; ultrastructure

In many mammals, the distal end of the ductus deferens differs histologically from more proximal portions and is called the ampulla. In contrast to the rest of the ductus deferens, the epithelium of the ampulla is deeply folded and tortuous outpocketings that arise between the folds extend into the surrounding muscularis and possess secretory cells (Setchell et al., 1994). The mammalian ampulla is a multifaceted organ, morphologically similar to the seminal vesicles (Riva et al., 1982), and has been variously implicated in maturation, nourishment, storage, and phagocytosis of sperm (Cooper and Hamilton, 1977; Bergerson et al., 1994).

In squamate reptiles, the ductus deferens is usually described as rather uniform in structure along its length and is the acknowledged organ of sperm storage (Fox, 1952). In the agamid lizard *Calotes versicolor*, however, Akbarsha and Meeran (1995) noted that the terminal end of the ductus deferens is markedly rugose and swollen during the breeding season. These authors claimed that this region was the same as the ampulla ductus deferentis of mammals. The observations of Akbarsha and Meeran (1995) were limited to gross morphology and light microscopy.

Contrary to Akbarsha and Meeran (1995), the question of whether an ampulla occurs in the urogenital ductus of squamates had been addressed previously. Van den Broek (1933: 88), describing the urogenital ducts of lizards, stated, “Kurz vor der Einmündung besitzt der Ductus deferens eine Ampulle ductus deferentis. Bei brünstigen Tieren soll diese Ampulle prall mit Spermien gefüllt sein.” In the next paragraph, van den Broek turns his attention to snakes and reported, “Auf Grund der Abbildungen von Martin St. Ange verzeichnet Hoffmann bei Schlangen eine Ampulla ductus deferens; sie soll während der Brunstzeit mit Spermien, in der Zwischenzeit mit Harn angefüllt sein.” Volsøe (1944: 67), however, stated that van den Broek was incorrect concerning the location of an ampulla at the distal end of the ductus deferens. Volsøe wrote, “The error is due to a misinterpretation made by Hoffman (1890) of a figure in Martin St. Ange (1856). It is the ureter which has an ampulla. In the mating period this ampulla is filled with spermatozoa...” In *Vipera berus*, Volsøe found that the ductus deferens tapers near its distal end and joins with the ureter before entering the urodaeum of the cloaca. This common tube is considered part of the ureter by Volsøe and is the location of the “ampulla.” W. Fox (1952) and H. Fox (1977) followed Volsøe’s interpretation that the ureter, not the ductus deferens, possesses an ampulla.

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Published online in Wiley InterScience (www.interscience.wiley.com)
DOI: 10.1002/jmor.10272
In this article, the ductus deferens of the Black Swamp Snake, *Seminatrix pygaea*, is described using light and electron microscopy, with special attention to differentiation of the distal end of the duct. I am unaware of any previous ultrastructural studies on the ductus deferens of a squamate. From seminiferous tubules of the testis, *S. pygaea* possesses efferent ducts (designated the ductuli efferentes and ductuli epididymidis by Volske, 1944) that lead into the epididymis, from which sperm pass into the ductus deferens. The ultrastructure of these ducts will be the topic of a subsequent report.

The specimens of *Seminatrix pygaea* came from the same collections in South Carolina that have been used in three previous studies on reproductive anatomy of this viviparous natricine snake (Sever and Ryan, 1999; Sever et al., 2000, 2002). Other aspects of the reproductive cycle of *S. pygaea* were described by Seigel et al. (1995).

In males of *Seminatrix pygaea*, the ductus deferens is known to possess sperm throughout the year, although spermatogenesis is not continuous (Sever et al., 2002). The testis is inactive in March, but spermatogenesis is under way in samples from May. The efferent testicular ducts lack sperm in March and May, indicating that sperm contained in the epididymis and vas deferens are from the spermatogenic cycle of the previous year or possibly even an earlier spermatogenic cycle; the duration of sperm storage in the ductus deferens is unknown. Spermation begins in June and July. In October, spermiation is nearly complete, and the efferent ducts contain much sperm. The sexual segment of the kidney, responsible for the secretion of seminal fluid, follows a cycle similar to that of spermatogenesis, with release of secretory material initiated in March. Matting apparently occurs in late spring, the only period in which sperm have been found in oviducts of females (Sever and Ryan, 1999).

### MATERIALS AND METHODS

*Seminatrix pygaea* is a small (20–40 cm snout–vent length as adults), highly aquatic snake that is limited to the southern Atlantic coastal plain of the United States (Dorcas et al., 1998). All specimens used in this study were collected at Ellenton Bay, located on the Department of Energy’s Savannah River Site in Aiken County, South Carolina. This “Carolina bay” is freshwater, 10 ha, shallow (2 m maximum depth), and relatively permanent, located on the Department of Energy’s Savannah River Site in Aiken County, South Carolina. This “Carolina bay” is freshwater, 10 ha, shallow (2 m maximum depth), and relatively permanent.

Collections were made during four periods in 1998 (10 May, 7 June, 22–24 July, and 29 September to 2 October), and one period in 1999 (17–22 March). Snakes were collected in unbaited minnow traps and from under coverboards alongside the bay. Specimens were sacrificed within a week of capture (Table 1).

Specimens were killed by a lethal injection (3–5 ml) of Nembutal (Abbott Laboratories, North Chicago, IL). This procedure was approved by the Animal Care and Use Committee of Saint Mary’s College, Notre Dame, Indiana, where the injections were done. After death, snout–vent length (SVL) was measured from the tip of the snout to the posterior end of the cloacal orifice. The urogenital organs were removed and prepared for light microscopy (LM) and transmission electron microscopy (TEM). Carcasses of all specimens were preserved in neutral buffered formalin (NBF) and will be deposited in the collections of the University of Michigan Museum of Zoology, Ann Arbor, Michigan.

For LM examination, tissues were initially fixed in NBF, rinsed in water, dehydrated in ethanol, cleared in toluene, and embedded in paraffin or glycol methacrylate (JB-4 Plus, Electron Microscopy Sciences, Port Washington, PA) plastic resin. Paraffin sections (10 μm) were stained with toluidine blue, and ultrathin sections (70 nm) for TEM were placed on uncoated copper grids and stained with uranyl acetate and lead citrate. Alternate paraffin slides from each specimen were stained with hematoxylin-eosin (general histology), brilliant indocyanine 6B (BB, for proteins), and Alcian blue 8GX at pH 2.5 (AB, for primarily carboxylated glycosaminoglycans) followed by the periodic acid-Schiff’s procedure (PAS, for neutral carbohydrates and sialic acids). Sections (2 μm) from tissues embedded in JB4 were stained with methylene blue and basic fuschin. Procedures followed Dawes (1979), Humason (1979), and Kiernan (1990).

Tissue for TEM was trimmed into 1-mm blocks and fixed in a 1:1 solution of 2.5% glutaraldehyde in Millonig’s phosphate buffer and 3.7% formaldehyde buffered to pH 7.2 with monobasic and dibasic phosphate. After initial fixation, tissues were rinsed in distilled-deionized water, postfixed in 2% osmium tetroxide, dehydrated through a graded series of ethanol, cleared in propylene oxide, and polymerized in an epoxy resin (Embed 812, Electron Microscopy Sciences). Plastic sections were cut with an RMC MT7 ultramicrotome (Research and Manufacturing, Tucson, AZ). Semithin sections (500 nm) for LM were stained with toluidine blue, and ultrathin sections (70 nm) for TEM were placed on uncoated copper grids and stained with uranyl acetate and lead citrate. TEM observations were made with a Hitachi H-300 (Nissei Sangyo America, Mountain View, CA).

Diameters of representative sections through the middle and distal (ampulla) areas of the ductus deferens of LM (both paraffin and JB4) and TEM thick sections were measured using an ocular micrometer. Measurement was through the lumen and epithelium. No attempt was made to do a detailed morphometric analysis because the histological techniques used do not provide sufficient data for statistical treatment. For example, the ampullary region is short, and in preparation of a tissue block for ultrathin sectioning the number of semithin sections taken is limited. Rather, data on duct diameters were collected to provide some indication of the magnitude of variation that was present be-
between proximal and distal portions of the duct and between seasons.

RESULTS

Like other snakes, the ductus deferentia of Seminatrix pygaea show bilateral asymmetry in length, with the right ductus deferens being some 30% longer than the left (Fig. 1). As with other size data, a sufficient sample of snakes to conduct a morphometric study on variation in length of the ductus deferentia was not available, but the individual illustrated in Figure 1 can serve as an example. This specimen, collected 16 May, has an SVL of 194 mm, and from the distal end of the testis the right ductus deferens is 74.0 mm long (38.1% of body length) and the left is 50.6 mm long (26.1% of body length). The superficial layer of each ductus deferens is a transparent serous membrane that connects to the dorsal body wall by a mesentery that is continuous from the mesorchium to the cloaca. Volsøe (1944) considers the mesentery of the ductus deferens to be a “pseudomesentery” because the duct is actually retroperitoneal and suspended into a lymphatic space. This observation by Volsøe was difficult to confirm from my observations of gross anatomy and histology. If true, however, the outer covering of the ductus deferens is not pleuroperitoneum and therefore will be referred to here simply as the “serosa.” The serosa is relatively straight and does not vary much in width throughout its length, being 0.6–0.8 mm dia in the specimen illustrated in Figure 1. Within the serous sheath, the epithelial portion of the ductus deferens and its surrounding white sheath of smooth muscle and connective tissue is laterally coiled, 0.2–0.4 mm dia, and the coils pass the width of the serosal sheath (Fig. 2). In the distal 3.5 mm of the ductus deferens, the coils of the tube still exist but are no longer visible through the serosa (Fig. 2A). This is the area designated presumptively here as the ampulla. The width of the epithelial portion of the duct of the ampulla is similar to that of more proximal parts of the ductus deferens, although most often the ampulla is slightly narrower (Table 1). The walls of the ampulla and the ureter do not join until the tubes reach the anterior wall of the cloaca (Fig. 2B).

Paraffin sections of the ductus deferens from none of the samples gave a strong positive reaction to PAS, AB, or BB. Thus, no cycle of secretory activity was found using these stains and reactions.

Below, the histology and cytology of the ductus deferens and the ampulla is described from specimens collected, as indicated in Materials and Methods and Table 1, in March, May, June, July, and October. In March: 1) snakes have just emerged from hibernation; 2) the ductus deferentia contain...
sperm that were transported from the testis beginning the previous October, and perhaps sperm from a prior spermatogenic cycle; and 3) secretions of the sexual segment of the kidney are released into the ureters (see Introduction; Sever et al., 2003).

March

Ductus deferens (Figs. 3, 4). As mentioned above, the ductus deferens consists of the inner epithelial lining and outer tissue layers. The epithelium is not folded, except where the tube bends in its coiling, where a major fold results (Fig. 3A). No definitive lamina propria occurs superficial to the epithelium. Instead, a thin muscularis composed of circular smooth muscle is tightly applied to the epithelium (Fig. 3B). Superficial to the muscularis is loose connective tissue, which contains numerous blood vessels, and the outermost layer is the squamous mesothelium (Fig. 3B).

Most of the diameter of the ductus deferens is due to the width of the lumen (Fig. 3A). The lumen is filled with whorls of sperm, some of which are adjacent to the epithelium, but the main masses of sperm are somewhat separated from the epithelium (Fig. 3A,C). The epithelium is pseudostratified and clearly contains two types of cells (Figs. 3C, 4A). Columnar principal cells have central, round nuclei with some peripheral heterochromatin and, often, a prominent nucleolus. The principal cells contain oval mitochondria, numerous small vacuoles, and Golgi complexes are found in the apical cytoplasm of some sections (Fig. 4). Intercellular canaliculi are narrow and possess areas of parallel, straight membranes as well as regions where the plasma mem-

Fig. 2. Seminatrix pygaea. A: Posterior ends of the urogenital ducts in the specimen illustrated in Figure 1A. B: Paraffin section through a 26.1-cm specimen sacrificed 16 May showing sections through the ureter and ductus deferens in the area where their outer coverings begin to merge prior to joining the urodaeum (arrow labeled “B” in Fig. 2A). C: Sagittal JB4 section through the proximal portion of the ductus deferens of a 23.8-cm SVL specimen sacrificed 30 July, showing the relationship of the coils of the ductus deferens to outer tissue layers. amp, ampulla; B, area from which section was taken for Figure 2B; dd, ductus deferens; ep, epithelium of the ductus deferens; kd, kidney; sp, sperm; sr, serosa; ur, ureter.
Fig. 3. Seminatrix pygaea. A: JB4 section through the ductus deferens of a 26.2-cm SVL specimen sacrificed 31 March. B: TEM of tissue layers superficial to the epithelium of the ductus deferens of a 23.3-cm specimen sacrificed 31 March. C: Same specimen as B, showing the epithelium of the ductus deferens in relation to sperm in the lumen. bc, basal cell; ct, connective tissue proper; ep, epithelium of the ductus deferens; lu, lumen; me, mesothelium; mu, muscularis; pc, principal cell; sp, sperm; sr, serosa.
branes highly interdigitate (Fig. 4A). Tight junctions occur at the luminal ends of the intercellular canaliculi. The luminal border of the principle cells is lined with uniformly long microvilli. Cilia are absent. Although sperm nuclei appear especially concentrated in some areas adjacent to the epithelium (Fig. 4B), no overall organization is apparent among the sperm masses in the lumen (Fig. 3C). Sperm nuclei can appear to be in contact with microvilli of the principle cells, but no indication of endocytosis of sperm occurs in these areas (Fig. 4C). Numerous small vesicles occur in the apical cytoplasm, perhaps indicating absorption of luminal substances by endosomes.

The second obvious cell type consists of basal cells that are scattered between the principle cells along the basal lamina (Figs. 3C, 4A). The cytoplasm of the basal cells does not reach the luminal border, accounting for the classification of the epithelium as pseudostratified. Basal cells contain large, irregular nuclei, scant cytoplasm, small vacuoles, and small dark mitochondria.

**Ampulla (Figs. 5, 6).** The layers of the ampulla are similar to the rest of the ductus deferens except that the superficial connective tissue layer is more abundant where this layer joins with the body wall as the duct enters the cloaca (Fig. 5A). The increased thickness of connective tissue proper may account...
Fig. 5. *Seminatrix pygaea*. A,B: JB4 sections through the ampulla of a 26.2-cm specimen sacrificed 31 March. C: TEM section of the ampulla of a 23.2-cm SVL specimen sacrificed 31 March. A: Overview of the ampulla. B,C: Association of sperm with the epithelial projections of the ampulla. bv, blood vessel; ct, connective tissue proper; ep, epithelium of the ductus deferens; ic, intercellular canaliculus; lu, lumen; mi, mitochondria; mu, muscularis; mv, microvilli; nu, nucleus; sp, sperm; sn, sperm nucleus; ve, vesicles.
for the inability to grossly observe the coils of the ampulla in this area.

The lumen of the ampulla in March contains sperm, but not as much as noted in the rest of the ductus deferens. Sperm are concentrated in two areas: a mass occurs in the center of the lumen, and other groups of sperm are closely associated with the epithelium of the ampulla (Fig. 5A,B). Fluid removal during dehydration could account for separation of central and peripheral masses of sperm. The epithelium of the ampulla appears thicker than that of the remainder of the ductus deferens due to the evagination of cells into irregular projections into the lumen (Fig. 5A,B). Crypts occur between the projections and, with LM, the nuclei of bundles of sperm cells appear oriented into these crypts (Fig. 5B). TEM reveals, however, that the sperm are not embedded in the epithelium lining the crypts, although sperm may be closely associated with the microvilli (Figs. 5C, 6A).

Despite the presence of the luminal projections, the epithelium of the ampulla does not exhibit many cytological differences from that of other portions of the ductus deferens. The epithelium is still pseudostratified with columnar principal cells interspersed with basal cells along the basement membrane (Fig. 6A). Unlike the rest of the ductus deferens, however, two types of principle cells could be discerned with TEM based on the density of the cytoplasm (Fig. 6B). These cell types are designated light cells and dark cells, based on the electron-density of their cytoplasm. These cells are not distinctive with LM. Dark cells are more uncommon. Cytologically, light and dark cells appear similar.

**Fig. 6.** *Seminatrix pygaea.* Same specimen from March used in Figure 5. **A:** Principal cells and a basal cell. **B:** Light and dark cells. **C:** Organelles in the apical cytoplasm. bc, basal cell; ic, intercellular canaliculus; lu, lumen; mi, mitochondria; mf, microfilaments; mu, muscularis; mv, microvilli; no, nucleolus; nu, nucleus; ppt, principle piece of the sperm tail; sn, sperm nucleus; tj, tight junction; ve, vesicles.
Both contain numerous mitochondria and vesicles of various sizes. Intercellular canaliculi are, as in the ductule portion of the ductus deferens, narrow and often tortuous. Nuclei are round and characterized by some peripheral heterochromatin and a prominent nucleolus. Microvilli are uniform in length and cilia are absent.

**May, June, and July**

**Ductus deferens (Fig. 7).** The ductus deferens anterior to the ampulla does not differ significantly among specimens examined from these months. The lumen is packed with sperm up to the edges of the epithelium in most samples (Fig. 7A), although in the July samples groups of sperm could also be found clearly separated from the epithelium (Fig. 7B). As contrasted to the March specimens, the nuclei in the epithelial cells are more heterochromatic, vesicles and microvilli appear relatively smaller, and mitochondria are more electron-dense (Fig. 7C,D). Intercellular canaliculi are still very narrow. In some cells, rough endoplasmic reticulum occurs around the apical border of the nucleus (Fig. 7D).

**Ampulla (Fig. 8).** Sperm are numerous in the lumen (Fig. 8A) and appear to be mixed with a considerable amount of granular substance (Fig. 8B). The crypts are well developed, and sperm are associated with the crypts. Mitochondria are electron-dense, irregular in shape, and appear more numerous in some cells than others. Inclusions occur in some vacuoles, and irregular electron-dense bodies occur in clusters in some cells (Fig. 8C) and cytologically resemble secondary lysosomes described in the ductus deferens of mammals (Kunkelmann and Kuhnel, 1984). In certain areas, light cells have abnormal nuclei and appear to be undergoing degeneration (Fig. 8D).

**October**

**Ductus deferens (Fig. 9).** Like previous samples, the ductus deferens is packed with sperm to the edges of the epithelium (Fig. 9A). The epithelium appears more active than in other months. Mitochondria are numerous and elongate, and profiles of smooth endoplasmic reticulum and rough endoplasmic reticulum fill the cytoplasm (Fig. 9C). Clusters of lipid droplets occur in the basal areas of some cells (Fig. 9D).

**Ampulla (Figs. 10, 11).** The ampulla appears to have fewer sperm than in other months. As in other months, however, clusters of sperm appear intimately associated with the apical ampullary epithelium (Fig. 10A,B). The cytoplasm of the epithelial projections contains numerous small dark mitochondria and large vesicles (Fig. 10C,D). Rough endoplasmic reticulum and Golgi complexes are prevalent (Fig. 11A), and nuclei are euchromatic (Fig. 11B). Electron-dense particles that resemble lysosomes occur in some cells (Fig. 11B), and clusters of vesicular material are found in the lumen adjacent to the epithelium (Fig. 11C).

**DISCUSSION**

The ductus deferens of *Seminatrix pygaea* varies little throughout the year. The epithelium of both the ductule portion and the ampulla is most active in October, when newly formed sperm are entering the efferent ducts. At no time, however, does the epithelium produce large secretory granules that release a product into the lumen by exocytosis or apocrine processes, as reported for the ampulla of mammals (Sirigu et al., 1979; Riva et al., 1982; Kunkelmann and Kuhnel, 1984; Setchell et al., 1994), and implied for the lizard *Calotes versicolor* (Akbarsha and Meehan, 1995). The presence of rough endoplasmic reticulum and Golgi bodies indicates some product synthesis. The products likely include the lysosome-like granules observed in the ampulla. These lysosomes are most likely involved in breaking down intercellular substances and worn-out organelles. Phagocytosis of sperm, as reported for the epithelium of the ductus deferens of mammals (Cooper and Hamilton, 1977) and birds (Aire, 2002a), was not observed. Basal cells seemed inactive and gave no indication of an exogenous origin or an intraepithelial macrophage function, as suggested in some mammals (Yeung et al., 1994; Holschbach and Cooper, 2002).

The numerous small vesicles in the cytoplasm of all regions of the ductus deferens indicate that other vesicular transport pathways could be present, including the absorption of luminal fluids into endosomes, as described in the ductuli efferentes and epididymides of birds and mammals (Setchell et al., 1994; Aire, 2002b). Also, vesicles could be transporting substances to the apical plasma membrane, resulting in the attraction of sperm. The intimate association of clusters of sperm nuclei to the ampullary epithelium strongly suggests that these cells could be producing ligands for sperm-associated lectins. The presence of glycoconjugates in the mammalian ampulla is well established, and differences exist between epididymal and ampullary sperm in the binding of a number of FITC-labeled lectins (Bergerson et al., 1994). In isthmic sperm reservoirs in the female pig, Töpfer-Peterson et al. (2002) demonstrated that mannosyl-oligosaccharides exposed by epithelial cells had high affinity for bonding with lectins of sperm nuclei. Sperm adherence in the oviductal isthmus results in prolonged activity.

Esponda and Bedford (1987), however, examined “duct content” from the ductus deferens of three species of snakes in the genus *Nerodia*, and found that although at least one glycoprotein is present, it does not appear to associate with sperm. The surface of the sperm appear to bind certain molecules from secretions after leaving the testis, but these do not...
Fig. 7. *Seminatrix pygaea*. A: JB4 section through the ductus deferens of a 27.9-cm specimen sacrificed 16 May. B: JB4 section through the ductus deferens of a 23.8-cm specimen sacrificed 30 June, showing the association between the epithelium and sperm. C: TEM of principal cells of a 31.2-cm SVL specimen sacrificed 16 May. D: Same specimen as C, showing details of the apical cytoplasm. ax, axoneme; ep, epithelium of the ductus deferens; Go, Golgi complex; ic, intercellular canaliculus; lu, lumen; mi, mitochondria; mu, muscularis; mv, microvilli; no, nucleolus; nu, nucleus; rer, rough endoplasmic reticulum; sp, sperm; tj, tight junction; ve, vesicles.
Fig. 8. *Seminatrix pygaea*. A: JB4 section through the ampulla of a 23.8-cm specimen sacrificed 9 June. B–D: TEM sections through a 31.2-cm specimen sacrificed 16 May. B: Luminal border. C: Lysosomes. D: Abnormal nucleus in a principal cell and adjacent basal area. anu, abnormal nucleus; bc, basal cell; cf, collagen fibers; ct, connective tissue proper; ep, epithelium of the ductus deferens; gr, granular substance; ly, lysosome; mu, muscularis; nu, nucleus; sp, sperm; va, vacuoles.
Fig. 9. Seminatrix pygaea. A: JB4 section through the ductus deferens of a 25.5-cm specimen sacrificed 8 October. B: Same specimen as A, showing association between the epithelium and sperm. C: TEM through the ductus deferens of a 29.0-cm specimen sacrificed 8 October. D: Same specimen as C, showing details of principal cell cytoplasm. bc, basal cell; ep, epithelium of the ductus deferens; Go, Golgi complex; ic, intercellular canaliculus; Id, lipid droplet; mi, mitochondria; mu, muscularis; mv, microvilli; nu, nucleus; ser, smooth endoplasmic reticulum; sp, sperm.
Fig. 10. *Seminatrix pygaea*. Ampulla of specimens sacrificed 8 October. A: JB4 section from a specimen 25.5-cm SVL. B: Same specimen as A, showing association between epithelium and sperm. C: TEM section from a specimen 29.0-cm SVL, showing apical projections and sperm. D: Same specimen as C, showing details of the apical cytoplasm in an area with numerous mitochondria. ep, epithelium of the ductus deferens; Go, Golgi complex; lu, lumen; mi, mitochondria; mu, muscularis; mv, microvilli; rer, rough endoplasmic reticulum; sn, sperm nucleus; sp, sperm; sr, serosa; tj, tight junction; ve, vesicles.
Fig. 11. *Seminatrix pygaea*. TEM of a 29.0-cm SVL specimen sacrificed 8 October. A: Apical cytoplasm. B: Euchromatic nucleus in a principal cell with lysosomes and two types of mitochondria. C: Vesicular inclusions in the lumen. Go, Golgi complex; ic, intercellular canaliculus; lu, lumen; ly, lysosome; mi, mitochondria; mv, microvilli; nu, nucleus; ppt, principle piece of the sperm tail; rer, rough endoplasmic reticulum; ser, smooth endoplasmic reticulum; sn, sperm nucleus; ve, vesicles; vm, vesicular material.
contain con A- or WGA-reactive components that would combine with those lectins associated with testicular sperm. The duct content examined by Esponda and Bedford (1987) was recovered from the most proximal end of the duct, presumably where the sperm enter the ductus deferens. Thus, the secretions they mention are likely epididymal in origin. The epididymis is the only portion of the snake efferent duct system where epithelial secretory granules occur that release a product into the lumen (Fox, 1977; Jones, 1998). No attraction of sperm to the epithelium of the ductus deferens of *Seminatrix pygaea* occurs until the ampulla.

Squamates lack seminal vesicles and other accessory sex glands along the efferent ducts except for the epididymis. The source of seminal fluid in squamates is the sexual segment of the kidney (Regaud and Policard, 1903; Saint Girons, 1972; Kühnel and Krisch, 1974; Sever et al., 2002). The secretions from the renal sexual segment apparently mix with sperm after the merger of the ureter and ampulla of the ductus deferens, which in the Black Swamp Snake apparently occurs in the urodaeum. Weil (1984) suggested that renal sexual segment secretions may contribute to sperm transport and capacitation in the fall and to sexual behavior in the spring. Perhaps secretions from the renal sexual segment also influence orientation of sperm further proximally in the ampulla. Obviously, this is a topic ripe for further investigations!

Another question that remains to be answered is whether the most distal portion of the ductus deferens of *Seminatrix pygaea*, or of the lizard *Calotes versicolor*, is homologous to the ampulla ductus deferentis of mammals. Turtles (Chelonia), crocodilians (Crocodilia), and birds (Aves) lack any known modification of the posterior ductus deferens, but recent phylogenetic hypotheses indicate a closer sister-group relationship between mammals and squamates than between mammals and other amniote groups (Hedges and Poling, 1999). The comparison of neontological morphological data on the ductus deferens of extant squamates and mammals may help determine whether similarities among these taxa in the “ampullary area” are the result of common ancestry or result from convergence.

Considerable variation in the ductus deferens may occur among squamates and among mammals. Fox (1952) noted that, although the ductus deferens appears to be nonsecretory in snakes, this may not be equally true in lizards. The careful illustrations of Volsøe (1944) definitely show that the ductus deferens and the ureter in *Vipera berus* merge prior to the cloaca, and the ampulla occurs in the portion after the merger. No morphological descriptions of the ampulla of *Vipera* were presented besides the observations that the region is somewhat widened and the ductus deferens opens between two transverse folds. Why the ampulla in this case should be considered the distal end of the ureter (as do Volsøe, 1944; W. Fox, 1952; H. Fox, 1977) rather than the distal end of a “urogenital duct” is unclear, but the fact remains that some interesting variations may occur among squamates. These differences may prove to be related to reproductive adaptations and be phylogenetically relevant.

In Table 2, I have compared some characteristics of the ampulla of mammals with the morphology found in the posterior end of the ductus deferens in *Seminatrix pygaea* and *Calotes versicolor*. The mammalian condition is more specialized than that of the two reptiles that have been studied. Also, only observations from light microscopy are available for *C.*

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<th><em>Seminatrix pygaea</em></th>
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<td>Golgi, RER</td>
<td>Yes</td>
<td>No data</td>
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</tr>
<tr>
<td>Lysoosomes</td>
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<tr>
<td>Intracellular inclusions</td>
<td>Yes</td>
<td>No data</td>
<td>No</td>
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<tr>
<td>Elaborations of basal plasma membranes</td>
<td>Yes</td>
<td>No data</td>
<td>No</td>
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<tr>
<td>Filaments in basal cells</td>
<td>Yes</td>
<td>No data</td>
<td>No</td>
</tr>
<tr>
<td>Phagocytosis of sperm</td>
<td>Yes</td>
<td>Proposed</td>
<td>No</td>
</tr>
<tr>
<td>Attraction of sperm</td>
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<td>No data</td>
<td>Yes</td>
</tr>
<tr>
<td>Sperm storage</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sperm nourishment</td>
<td>Yes</td>
<td>No data</td>
<td>No data</td>
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<tr>
<td>Sperm maturation</td>
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<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>Lamina propria</td>
<td>Present</td>
<td>Present</td>
<td>Indistinct</td>
</tr>
<tr>
<td>Muscularis</td>
<td>3 layers</td>
<td>Thick</td>
<td>1 layer</td>
</tr>
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</table>

*Data for mammals from Sirigu et al. (1979), Riva et al. (1982), Kunkelmann and Kuhnel (1984), Bergerson et al. (1994), and Setchell et al. (1994); data for *Calotes variegates* from Akbarsha and Meeran (1995).*
versicolor, so data on many cytological features are missing.

Some of the most interesting differences between the ductus deferens of mammals and that of Seminatrix pygaea are the presence in mammals of a distinct subepithelial lamina propria and three thick layers of smooth muscle comprising the muscularis externa: an inner longitudinal layer, middle circular or oblique layer, and outer longitudinal layer (Setchell et al., 1994). In S. pygaea, a distinct lamina propria does not exist and the muscularis is limited to a narrow band of circular fibers. According to Akbarsha and Meeran (1995), the muscularis of the central portion of the ampulla of Calotes versicolor is “extremely thick” and a distinct lamina propria is present. The muscularis must be responsible for expulsion of sperm during ejaculation, and perhaps the thickness in mammals (and apparently C. versicolor) leads to more forceful ejaculations than one would expect from a duct (like that of Seminatrix pygaea) lined with a thin muscularis. At least, sperm is generally absent in the lower portion of the ductus deferens in mammals after ejaculation (Kunkelmann and Kühnel, 1984) and outside of the breeding season in C. versicolor (Akbarsha and Meeran, 1995), whereas abundant sperm were present in the ampullae of S. pygaea in all specimens sampled.

Surely, depletion of sperm in the ampulla occurs after copulation by snakes, but little data apparently exist on this subject. Observations on several lizards and snakes indicate that the male alternates hemipenes (and apparently ductus deferentia) if additional matings are separated by less than 3 days (Olsson and Madsen, 1998). In Seminatrix pygaea, sperm must percolate down the ductus deferens, like lymph through the weakly muscled lymph vessels, and the muscularis may not have a major role in evacuation of the ductus deferens during copulation. Thus, unless other factors are involved, the voiding evacuation of the ductus deferens during copulation and the muscularis may not have a major role in sperm storage of vari-

able periods may occur, phagocytosis of sperm also has been reported (Cooper and Hamilton, 1977).

In contrast, aside from the observations of numerous clear vacuoles in the apical cytoplasm of the ampullary epithelium in Calotes versicolor, no evidence of glandular activity exists for the squamate ampulla. No evidence of phagocytosis exists, and sperm storage occurs. The only feature that the ampullae of mammals and snakes has in common and causes this area to differ from the rest of the ductus deferens is that the epithelium of the ampulla has irregular narrow folds.

Is this character, irregularity of the epithelium, enough to consider that this condition resulted from common ancestry, or is this character simply convergent, and perhaps a highly fluctuated epithelium in the posterior ductus deferens has arisen independently numerous times? No way exists to answer these questions currently, with the meager data from only one TEM study (the current study) on a squamate (out of some 7,000+ extant taxa) and similar data from a small number of mammals; obviously, more work is necessary. It is clear, however, that the posterior end of the ductus deferens or common urogenital duct can differ from more anterior regions in squamates. Thus, as a working hypothesis, I propose that the name “ampulla ductus deferentis” be used for this area of highly folded epithelium when it occurs in the posterior end of the sperm duct of a squamate.

ACKNOWLEDGMENTS

I thank Travis Ryan for supplying the snakes and Mary Culley, Penelope Kistka, Rebecca Stephenson, and Allyson Treavor for their help in the laboratory. Thanks to W.A. Hamlett and his students for preparation of the JB-4 samples. I thank Viktoria Cox for the drawing in Figure 1 and Kevin M. Gribbins for critical review of an earlier draft of the manuscript.

LITERATURE CITED


Riva A, Testa-Riva F, Usai E, Cossu M. 1982. The ampulla ductus deferentis in man, as viewed by SEM and TEM. Arch Androl 8:157–164.