Reproduction of the Salamander *Siren intermedia* Le Conte With Especial Reference to Oviducal Anatomy and Mode of Fertilization

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ABSTRACT Reproduction was studied in a South Carolina population of the paedomorphic salamander *Siren intermedia* with emphasis on anatomy of the female oviduct. The oviduct forms 67–79% of the snout-vent length in this elongate species and can be divided into three portions. The atrium, 7–13% of oviducal length, is the narrow anteriormost portion, with the ostial opening immediately caudad of the transverse septum. The ampulla, 63–75% of oviducal length, is the highly convoluted, middle portion in which gelatinous coverings are added to the eggs during their passage. Hypertrophy of the oviducal glands in the ampulla causes the ampulla to increase in diameter during the ovipository season. The secretion of the eosinophilic oviducal glands is intensely positive following staining with the periodic acid-Schiff procedure and does not react with alcian blue at pH 2.5. This staining reaction, coupled with the presence of abundant rough endoplasmic reticulum and Golgi complexes, indicates that the secretion contains a glycoprotein. The ovisac, 16–25% of oviducal length, is the most posterior portion of the oviduct and holds up to 10–11 eggs prior to oviposition. Oviducal glands similar to those in the ampulla are absent in the ovisac. Oviposition in female sirens occurs during February–April in this population, and male spermatiation is concurrent. Entire oviducts were sectioned from three females collected during the ovipository season and from two collected prior to the breeding season, and sperm were not found in the oviducts of these specimens. Thus no evidence was found for internal fertilization or sperm storage in the oviducts of sirens. © 1996 Wiley-Liss, Inc.

The Sirenidae consists of two species each of *Siren* (*S. intermedia* and *S. lacertina*) and *Pseudobranchus* (*P. striatus* and *P. axanthus*) (see Moler and Kezer, '93). Members of the family are restricted to the southeastern United States and northern Mexico (Duellman and Trueb, '86). The species are elongate, aquatic perennibranchs that lack a pelvic girdle and hindlimbs (Martof, '74). Within the order Caudata, the Sirenidae is the only member of the suborder Sirenoidea (Duellman and Trueb, '86), although some have questioned whether sirens are salamanders at all and proposed placing sirens in their own order, Trachystoma, within the subclass Lissamphibia (Cope, 1889; Goin and Goin, '62).

The mating behavior of sirens has never been observed (Martof, '74). In females of most species of salamanders, ova are fertilized internally by sperm released from cloacal glands (spermathecae) as the eggs pass through the cloaca during oviposition (Boiszieu and Joly, '75). Cloacal glands, which function in the production of spermatophores (males) and the storage of sperm (females), are lacking in the Sirenidae (Seyer, '91b), implying external fertilization. Although Noble and Marshall ('32) and Godley ('83) stated that *Siren intermedia* oviposits their entire clutch in large nests of 206–555 eggs, other studies on *S. intermedia* (Noble and Richards, '32) and *Pseudobranchus striatus* (Goin and Goin, '62) reported eggs laid in
small clusters over a period of days. In *P. striatus*, Goin and Goin (‘62) found it “difficult to see how such eggs could be fertilized after they are laid” and suggested that internal fertilization occurs within the family.

If internal fertilization occurs in sirens, the oviduct could be the site of sperm storage and/or fertilization. Although the male urogenital system has been studied in *Siren intermedia* (Willet, ‘65), the only description of the female urogenital system is a brief account by Vaillant (1863) on *S. lacertina* (which actually may have been *S. intermedia*, see Noble and Richards, ’32). In this study, we examine the male and female reproductive cycles of *Siren intermedia* from South Carolina, with especial emphasis on the anatomy of the oviduct.

**MATERIALS AND METHODS**

*Siren intermedia* were collected using minnow traps in a swampy drainage below the spillway of Risher Pond, on the U. S. Department of Energy’s Savannah River Site (Aiken County, SC). Collections were made on 12–14 January, 16 February, 24–28 February, 6–7 March, 7 April, 1 May, 23–27 June, 14 July, 18 September, and 27 October 1994. Some specimens were sacrificed soon after capture, whereas others were maintained in the laboratory for various periods of time prior to sacrifice. Specimens maintained in the lab were kept in 25 x 75 x 30 cm glass aquaria, supplied with local well water and a liberal diet of bloodworms (Chironomidae). Specimens in aquaria were monitored daily to ascertain whether oviposition occurred. In addition to *S. intermedia*, two *S. lacertina* were captured 6–7 March 1994 and maintained in the laboratory.

Specimens were sacrificed by immersion in 10% MS-222, and snout-vent length (SVL) was measured from the tip of the snout to the posterior end of the vent. Tissues were excised from freshly killed specimens and fixed for preparation by paraffin infiltration for light microscopy (LM), or for embedding in epoxy resin for semithin (LM) or ultrathin sections for transmission electron microscopy (TEM). The fixatives were 10% neutral-buffered formalin for LM, and a 1:1 solution of 2.5% glutaraldehyde in Millonig’s phosphate buffer at pH 7.4 and 3.7% formaldehyde-buffered to pH 7.2 with monobasic and dibasic phosphate for TEM. Testes and associated urinary structures were removed from males, and oviducts were dissected from females. For LM, the entire oviduct was prepared for sectioning, except for 1 mm blocks taken from the proximal and distal ends and from locations just anterior and posterior to the middle 10–40 mm of the oviduct, which were prepared for TEM.

For LM, the tissue was rinsed in water after fixation, dehydrated in ethanol, cleared in Histosol (National Diagnostics, Manville, NJ), and embedded in paraffin. Sections (10 μm) were cut with a rotary microtome, affixed to albuminized slides, and alternate slides were stained with hematoxylin-eosin (HE, for general cytology) or alcan blue at pH 2.5 (AB, for carboxylated glycosaminoglycans) followed by the periodic acid-Schiff procedure (PAS, for “neutral” carbohydrates such as glucose, galactose, mannose, and sialic acids). These staining procedures followed Kiernan (’90).

After initial fixation for TEM, tissues were rinsed in Millonig’s buffer, postfixed in 2% osmium tetroxide, dehydrated in ethanol, cleared in propylene oxide, and embedded in an epoxy plastic resin (EMBED-812; Electron Microscopy Sciences, Fort Washington, PA). Semithin sections (0.5–1.0 μm) for light microscopy were cut with glass knives, placed on microscope slides, and stained with toluidine blue. Ultrathin (70 nm) sections for TEM were collected on uncoated copper grids and stained with solutions of uranyl acetate and lead citrate. Plastic sections were cut with RMC XL1000 and RMC MT7 ultramicrotomes and viewed with a Hitachi H-300 transmission electron microscope.

Stages of spermatogenesis were identified using the criteria established in *Ambystoma* by Armstrong (‘89) and Uribe et al. (‘94). Oocytes in ovarian follicles were considered mature and preovulatory when they were darkly pigmented and >2.0 mm in diameter (Noble and Marshall, ’32). Oocytes were measured using an ocular micrometer in a dissecting microscope.

**RESULTS**

Seasonal changes in trapping success of males and females of *Siren intermedia* were found. The male:female ratio by month was: January 5:6; February 0:5; March 3:2; April 0:3; May 5:1; June 7:0; July 4:0; September 4:1; October 5:0. Thus females were collected commonly only during January–April.

The reproductive system of male *Siren intermedia* matched the description by Willet (‘65). In females, we found that the oviduct is
Fig. 1. Gross anatomy of the oviduct of *Siren intermedia*. A. Drawing of the oviduct of a 176 mm SVL female collected 6–7 March and sacrificed 10 March 1994, showing the three major regions and relationships to some other major viscera. B. Photograph of the oviduct of a 119 mm SVL female collected 16 February and sacrificed 18 February, showing eggs in the ovisac.

The oviduct is divided into three distinct portions (Fig. 1A): (1) an anterior *atrium* into which eggs pass from the coelom, (2) a middle *ampulla* in which gelatinous coverings are secreted onto the eggs, and (3) a posterior *ovisac* in which the eggs are held prior to oviposition (Fig. 1B). These portions of the reproductive tract are described anatomically after consideration of the male and female reproductive cycles.

Our observations on *Siren intermedia* and the related *S. lacertina* indicate that rela-
tively few follicles are ovulated at one time. Thus eggs apparently are laid in small groups. An ovisac of *S. intermedia* probably cannot hold many more than 10–11 eggs, as observed in two individuals (Table 1). In the laboratory, eggs were laid separately or in clumps of 2 or 3 eggs, and the eggs adhered tightly to the substrate. Still, the egg-laying season may be relatively short. A captive 349 mm SVL *S. lacertina* laid 120 eggs, which were removed between 15–17 March, and 72 more eggs by 21 March. The latter eggs remained in the enclosure with others oviposited later to see if any of the eggs were fertile; on 7 April, 113 eggs were removed, none fertile. This siren did not deposit any additional eggs prior to its sacrifice on 19 May.

**Reproductive cycles**

**Males**

Testicular lobules from specimens collected in January were filled largely with spermatids indicating the imminent onset of spermiation. No males were present in the February sample, but mature spermatozoa were abundant in the testes and vasa efferentia of males collected in March, and some spermatozoa were present in a specimen examined on 4 May. In late June, testicular lobules were filled with primary spermatogonia. In a male from the September collection, proliferation of the spermatogonia was evident, and lumina of the testicular lobules were lined with secondary spermatogonia. Thus maturation and transfer of sperm into the reproductive ducts occurred between January and March.

**Females**

Six females (117–182 mm SVL) collected and sacrificed in January had 83–179 (mean = 140.3, SD = 25.6) pigmented oocytes >2.0 mm in diameter (2.6–2.9 mm mean diameters; Table 1) in their ovaries. These probably represented full clutch complements. In three females (115–156 mm SVL) collected and sacrificed in February, some oviposition apparently had occurred. The ovaries contained 25, 66, and 68 follicles, and the mean oocyte diameter for each female was 2.6–2.7 mm. In addition, oviducal oocytes (eggs) occurred in the ovisacs of two of the specimens. In the 156 mm individual, five eggs were in the right ovisac and 11 in the left; in a 119 mm female, 10 eggs were in the right and eight in the left ovisac (Fig. 1B; Table 1).

A 144 mm SVL female collected on 24 February oviposited five infertile eggs prior to her sacrifice on 10 March. At that time, only 35 oocytes (2.8 mm mean diameter) remained in the ovaries, whereas the right and left ovisacs each contained five eggs. In 162 mm and 176 mm SVL females collected and sacrificed in March, the ovaries contained 36 (2.7 mm mean diameter) and 16

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1All measurements are in mm; oocytes Jan–Apr refers to pigmented oocytes > 2.0 mm in diameter.
2Snout-vent length.
3Length of oviduct/SVL.
4AT/OD, length of atrium/length of oviduct; AM/OD, length of ampulla/length of oviduct; OS/OD, length of ovisac/length of oviduct.
5OV, number oocytes in ovary; ROS, number oocytes in right ovisac; LOS, number oocytes in left ovisac; DIAM, mean diameter of oocytes.
6Oviposited five infertile eggs in captivity.
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(2.4 mm mean diameter) pigmented ovarian oocytes, respectively.

In three specimens (150–166 mm SVL) collected and sacrificed in April, 8–20 ovarian oocytes > 2.0 mm were found; these oocytes generally were smaller (2.1–2.4 mm mean diameter) than those of females collected from January–March and probably represent follicles undergoing atresia. The specimen (122 mm SVL) collected and sacrificed in May had only six pigmented, atretic oocytes (1.5 mm mean diameter) that were embedded deeply in ovarian tissue.

Finally, a specimen collected and sacrificed in September contained ~ 225 pigmented and unpigmented ovarian oocytes 1.0 mm in mean diameter. This specimen apparently was in the act of yolking oocytes for the forthcoming breeding season.

In summary, these specimens indicate that the height of the ovipository season for females was February and March, although some oviposition may commence in late January and the season could extend into early April. This time period corresponds with the period when males undergo spermiation.

**Oviducal anatomy**

Gross anatomy

The oviduct is long, 67–79% of SVL (mean = 73%, SD = 3.3). The anterior end is proximal to the posterior border of the transverse septum, and the caudal end joins the dorsal roof of the anterior end of the cloaca. A mesentery attaches the oviducts to the dorsal body wall and forms attachments with: (1) adjacent digestive viscera, (2) the lungs, which extend along almost the entire length of the oviducts, and (3) the ovaries, which occur medial to the posterior end of the oviducts. The oviducts are divided into three regions (Fig. 1A).

The atrium is the anterior 7–13% (mean = 10%, SD = 1.0) of oviductal length. We do not use the more traditional term “infundibulum” for this region because the anterior end of the siren oviduct is not funnel-shaped. The atrium is straight and relatively narrow compared to the middle region of the oviduct.

The ampulla is the middle 63–75% (mean = 69%, SD = 3.0) of the oviduct. This glandular region is highly convoluted in specimens collected from all seasons and becomes greatly hypertrophied during the ovipository season. It is in this section of the oviduct that outer gelatinous capsules are added to the follicles.

The ovisac is the posterior 16–25% (mean = 20%, SD = 2.0) of the oviduct. Eggs are held in the ovisac prior to oviposition; in three of our specimens a single ovisac held 5–11 eggs. Unlike the “ovisac” of some frogs, in which gelatinous egg coverings are secreted (Salthe, ’63), the ovisac of sirens is simply a holding area. The ovisac is narrow and rugose outside of the ovipository season but expanded and smooth-walled internally while holding eggs.

Histology and cytology

The entire oviducts of five females were sectioned, and all lacked sperm. Specimens included a 119 mm SVL female collected and sacrificed in February with only 66 mature oocytes remaining in her ovaries and 18 eggs in her ovisac, and 162 mm and 176 mm SVL females collected and sacrificed in March that contained only 36 and 16 mature ovarian oocytes remaining, respectively (Table 1). These data indicate that these females were in the ovipository and presumed mating period. The other two females, a 139 mm SVL individual collected and sacrificed in January, and a 150 mm SVL female collected and sacrificed in September, represent individuals sacrificed outside of the presumed mating and ovipository period.

The oviduct is lined with simple, ciliated epithelium that varies from columnar to cuboidal when a particular region is empty or inactive but becomes squamous when the region is hypertrophied or contains follicles. Oviducal glands occur in the atrium and the ampulla (Fig. 2A–C). These glands, when actively secreting, are strongly PAS+ and hypertrophy so that they occupy most of the connective tissue of the tunica propria. Although the atrium and ampulla both have oviducal glands, the atrium is narrower and less convoluted. Oviducal glands are lacking in the ovisac (Fig. 2D).

Superficial to the basal lamina of the oviducal epithelium is a thin connective tissue layer, the tunica propria, in which collagen fibers are abundant. The basal portion of the tunica propria contains numerous enlarged capillaries, characterized by a diameter sufficient to hold several red blood cells in any particular section and by walls composed of simple squamous endothelium. Superficial to the tunica propria is a sheet of longitudinal smooth muscle, the tunica muscularis, which is only one or two layers thick in the atrium and ampulla but many layers thick in the
Fig. 2  *Siren intermedia*. Sagittal paraffin sections of the oviduct stained with alcian blue at pH 2.5 and the periodic acid-Schiff procedure, showing development of oviducal glands (Og) during the breeding season. A. Atrium of a 162 mm SVL specimen collected 6–7 March and sacrificed 10 March. B. Ampulla of specimen used in A. C. Ampulla of a 119 mm SVL individual collected 16 February and sacrificed 18 February. D. Junction between the ampulla and atrium of the specimen used in A. Lu, lumen of oviduct; Sm, smooth muscle; Vp, visceral pleuroperitoneum.
ovisac. Superficial to the muscularis is the visceral pleuroperitoneum (Fig. 2).

Seasonal differences occur in the histology and histochemistry of the oviducts. No diagnostic staining was done on the specimens collected and sacrificed in January. The oviducal glands, however, are eosinophilic and appear to be full of secretory material. In specimens collected and sacrificed in February and March, which were in the process of laying eggs (the ovaries contained only 16-68 mature oocytes and the ovisacs of some specimens contained eggs), the epithelial lining of the atrium is AB+, and a mix of AB+ and PAS+ material occurs throughout the cytoplasm. The ampulla is greatly hypertrophied. The oviducal glands of the ampulla are intensely PAS+, and an AB+ reaction is limited to the mucosal epithelium. Both the atrium and ampulla lack eggs, and the inner lining is rugose. In specimens collected in March, the areas of PAS+ activity in the atrial and ampullar epithelium are not as uniform as in oviducts of females collected in February, perhaps indicating depletion of the secretory product (Fig. 2B, C). The epithelial cytoplasm appears more basophilic in March specimens than in February specimens.

Cytologically, oviducal glands are simple invaginations of epithelial cells lining pores that open into the oviducal lumen. During the breeding season, elongate microvilli are present along the luminal border of glands (Fig. 3A), although areas also occur that lack microvilli (Fig. 3B). Several types of secretory products are present. One type of secretory vacuole is large (often 2-3 μm in diameter), moderately electron dense, and fills much of the cytoplasm (Figs. 3, 4A), exclusive of the basal nucleus. Mucous droplets can be found interspersed among these vacuoles in some areas (Fig. 3B).

In other regions, smaller (1-1.5 μm), more electron-dense secretory vacuoles also occur (Fig. 4A), perhaps representing concentrations of the secretory product. Associated with the production of secretory vacuoles are extensive profiles of rough endoplasmic reticulum (Rer) and Golgi complexes with associated condensing vacuoles (Fig. 4B). The combination of a PAS+ reaction with organelles involved in peptide synthesis (Rer) indicates that the secretions contain neutral carbohydrates conjugated with proteins (i.e., glycoproteins; Kiernan, '90).

The ovisac has a thick muscularis and lacks oviducal glands (Fig. 5). When filled with eggs, the ovisacs are widened and therefore smooth-walled internally. Areas of ciliated epithelium occur among cells that lack cilia (Fig. 5A, B). Although oviducal glands are lacking, the cytoplasm contains vacuoles filled with a flocculent material (Fig. 5B, C) that probably is responsible for the PAS+ reaction of the epithelium. The cytoplasm surrounding these vacuoles contains numerous mitochondria with tubular cristae and free ribosomes (Fig. 5C). Also, the intercellular canaliculi are convoluted more in the ovisac than in other portions of the oviduct.

In the specimen collected and sacrificed in September, the atrium is not greatly hypertrophied. A weak AB+ reaction occurs around the luminal border, but a PAS+ reaction is limited to the nuclei in the epithelium. In the ampulla, scattered AB+ areas occur deep to the luminal border, but an AB+ reaction does not occur superficially and oviducal glands are not well developed (Fig. 6A). Cilia are not numerous (Fig. 6B, C) and are most highly developed in the posterior portion of the ampulla. Clusters of lysosomes are numerous in many cells in the ampulla (Fig. 6D), and perhaps these lysosomes are involved in degradation of secretory product left from the previous breeding season. Finally, intercellular spaces deep to the apical junctional complexes are wider (Fig. 6C) than in specimens collected during the breeding season.

DISCUSSION

Reproductive cycle

The number of mature ovarian oocytes we observed in female Siren intermedia prior to oviposition is lower than reported elsewhere. We found 83-179 darkly pigmented oocytes >2.0 mm in diameter in the ovaries of females sacrificed during January. Martof ('73) reported that the average complement of eggs is "about 200" for S. intermedia. The only published egg counts are the reports of Noble and Richards ('32) and Godfrey ('83) on specimens from Florida, and Noble and Marshall ('32) who examined specimens from Arkansas. These observations are somewhat contradictory. Noble and Richards ('32) found 265 eggs from the caudal end of the right oviduct of a female, a number not consistent with our observations. Noble and Richards ('32), however, reported that captive sirens laid small numbers of eggs over a period of several days, which would seem to indicate fewer eggs in the oviduct at any one time. Noble and Marshall ('32) found 299 mature ovarian oocytes.
Fig. 3. *Siren intermedia*. Ultrastructure of the luminal border of oviducal glands from the ampulla of a 119 mm SVL specimen collected 16 February and sacrificed 18 February. A. Apical border possessing microvilli (Mv). B. Apical border lacking microvilli and possessing mucous droplets (Md) in addition to moderately electron-dense secretory vacuoles (Sv). Lu, lumen of oviducal gland; Md, mucous droplet; Mv, microvilli; Sv, moderately dense secretory vacuoles.
Fig. 4. *Siren intermedia* oviduct. Ultrastructure of the ampulla of a 119 mm SVL specimen collected 16 February and sacrificed 18 February. **A.** Relationship of a gland to the epithelium lining the oviducal lumen (Lu). **B.** Organelles involved in production of the glycoprotein secretory products. Cv, condensing vacuoles; Ds, dense secretory vacuoles; Go, Golgi complexes; Lu, lumen of oviduct; Nu, nuclei of oviducal epithelial cells; Rer, rough endoplasmic reticulum; Sv, moderately dense secretory vacuoles.
Fig. 5. *Siren intermedia* ovisac. Ultrastructure of the ovisac of a 119 mm SVL female collected 16 February and sacrificed 18 February. A. Overview of epithelial lining showing ciliated (Ci) and unciliated areas. B. Detail of ciliated border. C. Perinuclear area showing vacuoles of flocculent material (Fm) and associated organelles. Bb, basal bodies; Bl, basal lamina; Cf, collagen fibers; Ci, cilia; Fm, flocculent material in vacuoles; Lu, lumen of oviduct; Mi, mitochondria; Nu, nuclei of oviducal epithelial cells; Ri, ribosomes.
Fig. 6. *Siren intermedia* ampulla in a 150 mm SVL female collected 18 September and sacrificed 20 September. **A.** Histology of a paraffin section stained with hematoxylin-eosin. **B.** Ultrastructure of the epithelial border of the ampulla. **C.** Detail of the apical border. **D.** Detail of the basal portion of the oviducal epithelial showing lysosomes (Ly). Bl, basal lamina; De, spot desmosomes; Ic, intercellular canaliculus; Ld, lipid droplet; Lu, lumen of the oviduct; Mi, mitochondria; Nu, nuclei of oviducal epithelial cells; Og, oviducal glands; Vp, visceral pleuroperitoneum; Zo, zonula occludens.
(139 in the right ovary and 160 in the left) in a *S. intermedia* measuring 396 mm total length, and they observed “nests” of 260 and 555 eggs in shallow hollows in the mud at the bottom of ponds. Godley ('83) reported finding two *S. intermedia* nests in masses of aquatic vegetation in Florida, and both nests were attended by single females. One nest, attended by a 151 mm SVL female contained 206 eggs and the other nest, attended by a 167 mm SVL female, had 362 eggs.

More observations on the number of mature oocytes produced during a reproductive season (and its relationship to body size), mode of oviposition (large nests or small clusters of eggs), and geographic variation in fecundity are needed for *S. intermedia*.

**Oviducal anatomy**

Detailed reports on the anatomy of the oviducts of salamanders are few. McCurdy ('31) found that the oviduct of *Triturus torosus* (Salamandridae) has four parts: an anterior, widened ostial portion, a second, highly convoluted region, a third, widened area where jelly coats of the eggs are added, and a “uterine” portion extending from the posterior end of the kidney to the cloaca. The anterior three divisions have thinly muscular walls, whereas uterine walls are thick. All divisions have villi, simple columnar ciliated epithelium and secretory cells. No mention was made of tubular glands, and some variation exists in the extent of ciliation and abundance of secretory cells.

Rodgers and Risley ('38) studied development of the reproductive system in *Ambystoma tigrinum* (Ambystomatidae). They found two regions, an anterior, convoluted oviducal portion and a posterior, straight uterine part. In the oviducal region, the lamina propria is thin and the epithelium is thick with tubular glands that open into the lumen. The uterus has thinner walls than the oviduct, and the uterine mucosa has columnar epithelium with a lamina propria that is more abundant and less compact than in the oviducal region. From their illustration, it seems that tubular glands are absent in the uterine region.

The oviduct of the salamandrid *Notophthalmus viridescens* is divisible into five regions, designated A–E, that differ in their polysaccharide histochemistry and are involved in secreting jelly coats around the eggs (Humphries, '66). All areas have a PAS+ secretion, and secretions from areas A, B, and D are alcian blue+ as well. Although the jelly coats are necessary for successful sperm penetration during fertilization, the coats block sperm penetration when they become hydrated after expulsion of the fertilized eggs from the cloaca (McLaughlin and Humphries, '78).

Greven ('80a) reported that the oviduct of the ovoviviparous salamandrid *Salamandra salamandra* has five regions: an anterior *pars recta*, a middle *pars convoluta* with three divisions, and a posterior *uterus* where the fetuses develop. The regions are distinguished based upon cell types in the epithelial layer. Ciliated epithelium occurs in all regions except the uterus (Greven and Ruterbories, '84). Simple gland cells and tubular glands are found in all areas but are especially numerous in the *pars convoluta*, and products include neutral as well as acid mucosubstances (Greven, '80a).

The uterus in *S. salamandra* produces small amounts of secretion, probably not enough to supply nutritional demands for developing fetuses (Greven, '80b). Indeed, ion flow is generally from the uterine lumen into the uterine mucosa, perhaps regulating the intrauterine milieu appropriate for development of offspring (Greven, '80b). In the viviparous *S. atrofasciata*, however, the anterior end of the uterus, the “zona trophica,” is highly glandular and may produce nutrients for the fetus *in utero* (Greven, '77). In viviparous frogs and caecilians, virtually the entire oviducal epithelium proliferates and secretes nutrient material (Wake, '93).

In summary, the oviducal anatomy of *Siren intermedia* at the gross, histological, and cytological levels seems unique among those described for other salamanders, regardless of mode of fertilization or whether oviducal retention of developing fetuses occurs.

**Implications for internal versus external fertilization**

Sperm were absent from the oviducts of all females examined histologically. For the specimens collected during the ovipository period in which some oviposition apparently had occurred, this finding leaves two possibilities: (1) no oviducal sperm storage/fertilization occurs, or (2) oviducal sperm storage/fertilization does occur, but these individuals had voided all the sperm from previous matings. Salamanders in the suborder Salamandroidea store sperm in specialized cloacal glands, spermathecae, that are lacking in sirens (Sever, '91a, '94). Eggs in oviparous salamandroids are fertilized internally as they
pass through the cloaca during oviposition (Boisseau and Joly, '75). In salamandrid species in which detailed studies on the cytology of sperm storage and fertilization have been done, the presence of abundant spermatozoa in the spermathecae prior to and after oviposition has been documented (Sever, '91c, '92; Sever and Brunette, '93; Sever and Klopfen, '93; Brizzi et al., '95; Sever et al., '95). Thus if oviducal sperm storage and/or fertilization occurred in Siren intermedia, one would expect to find some trace of sperm in the three animals sacrificed during their ovipository period. The absence of oviducal sperm provides support for the traditional view that fertilization in the Sirenidae is external.

Implications for phylogeny

The species of Sirenidae have been considered as either early offshoots from the urodelian lineage, one of the most derived salamander families, or not even salamanders at all (Cope, 1889; Hecht and Edwards, '77; Estes, '81; Milner, '83; Duellman and Trueb, '86; Hillis, '91; Larson, '91; Sever, '91a,b, '94; Larson and Dimmick, '93). The most recent cladistic analysis, based upon a combination of molecular and morphological data, places the Sirenidae basally as the sister group of all other families of salamanders (Larson and Dimmick, '93).

Key characters in the latter analysis are the absence of cloacal glands involved in internal fertilization in the Salamandroidea and thus the presumption of external fertilization within the Sirenidae. Internal fertilization resulting from spermatophore production by males and sperm storage in spermathecae by females is a synapomorphy of all other families of salamanders (Larson and Dimmick, '93). The most recent cladistic analysis, based upon a combination of molecular and morphological data, places the Sirenidae basally as the sister group of all other families of salamanders (Larson and Dimmick, '93).

ACKNOWLEDGMENTS

DMS received support from National Science Foundation Grant DEB 90-24918 and U.S. Department of Energy (D.O.E.) contract DE-AC05-76ORO0-033. LCR was supported by the Undergraduate Research Program of the Savannah River Ecology Laboratory. JDK was supported by contract DE-AC09-76SR00-819 between the University of Georgia and the D.O.E. This paper is publication number seven from the Saint Mary's College Electron Microscopy Facility.

LITERATURE CITED


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