# Oviductal Sperm Storage in the Ground Skink Scincella laterale Holbrook (Reptilia: Scincidae)

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ABSTRACT The reproductive tracts of female ground skinks, *Scincella laterale*, collected at various times throughout their reproductive cycle were examined by light and transmission electron microscopy. Examination of the tracts revealed that sperm are retained in the posterior vagina after mating but prior to the ovulation of oocytes. The sperm are not sequestered in specialized glands but occur in scattered clusters in the lumen or among the deep, narrow rugae. The simple columnar lining of the vagina consists mostly of ciliated cells interspersed with occasional secretory cells. After ovulation, as indicated by the presence of eggs in the uterus, sperm are not found in the vagina. No sperm or sperm storage tubules occur in the infundibulum, the characteristic location for sperm storage in scleroglossid squamates that have been studied. Our results are a further indication that too few species have been examined to construct a rigorous phylogenetic hypothesis about the occurrence of sperm storage tubules in lizards. *J. Exp. Zool.* 301A:599–611, 2004. © 2004 Wiley-Liss, Inc.

### **INTRODUCTION**

Literature on female sperm storage in reptiles, including lizards (Sauria), was recently reviewed by Sever and Hamlett (2002). Female sperm storage tubules in lizards were first reported in three species of *Chameleo* (Chamaeleonidae) by Saint Girons ('62) and in Anolis carolinensis (Polychrotidae) by Fox ('63). To date, sperm storage has been studied in representatives of only nine of the 26 families of lizards with data on just 33 of the 4713 species of lizards (total number of lizard species taken from the European Molecular Biology Laboratory Reptile Database, 2003). Microscopic techniques are necessary to study sperm storage tubules and their interactions with sperm, and most previous studies have utilized only light microscopy. Ultrastructural studies are limited to scanning electron microscopy of the sperm storage tubule area of A. carolinensis (Conner and Crews, '80) and several species in the Gekkonidae (Girling et al., '97, '98), scanning and transmission electron microscopy of the sperm storage tubules of the gecko Acanthodactylus scutellatus (Bou-Resli et al., '81), and transmission electron microscopy on sperm receptacles of Anolis sagrei (Sever and Hamlett, 2002). Sperm storage tubules were described earlier in snakes (Fox, '56), but the only ultrastructural studies on snakes are transmission electron microscopy on *Thamnophis sirtalis* (Hoffman and Wimsatt, '72), scanning electron microscopy on *Diadophis punctatus* (Perkins and Palmer, '96), and scanning and transmission electron microscopy on *Seminatrix pygaea* (Sever and Ryan, '99).

Based upon an analysis of the literature that included all light and electron microscopy studies, Sever and Hamlett (2002) proposed a working hypothesis that the ancestral condition in Squamata (Sauria + Serpentes) is possession of sperm storage tubules in the posterior infundibulum (named the tuba or uterine tube by some authors) of the oviduct. Two clades, Iguania and Scleroglossa, represent the basal split in squamate phylogeny (Pough et al., '98). Infundibular sperm storage tubules have been retained in the snakes and scleroglossid lizards that have been studied, whereas in the iguanid lizards, vaginal sperm

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Species	Sperm storage	Level of study	Reference
Hemiergis peronii	Oviduct	$\mathrm{L}\mathrm{M}^1$	Smyth and Smith (1968)
Eumeces egregius	Posterior oviduct; No SSTs <sup>2</sup>	$\operatorname{LM}$	Schafer and Roeding (1973)
Scincidae, general	Infundibulum, SSTs	$\mathbf{L}\mathbf{M}$	St. Girons (1973)
Mabuya scincoides	No storage	$\mathbf{L}\mathbf{M}$	Sarkar and Shivanandappa (1989)
Tiliqua scincoides	Area not specified	Gross	Hoser and Simpson (1997)

TABLE 1. Previous literature on sperm storage in female skinks

<sup>1</sup>Light microscopy.

<sup>2</sup>Sperm storage tubules.

storage tubules have evolved secondarily (Sever and Hamlett, 2002).

One of the scleroglossid families is Scincidae, a cosmopolitan family of some 1289 species, the largest family of lizards (European Molecular Biology Laboratory Reptile Database, 2003). Only five previous studies have been concerned with sperm storage in female skinks (Table 1). Smyth and Smith ('68) reported that sperm can be found in stained smears of the oviducts of some females of the Australian skink, Hermiergis peronii, from May to November; ovulation occurs in October and November when the males are not producing sperm. The exact location of the sperm in the oviduct of *H. peronii* was not indicated. Schafer and Roeding ('73) reported sperm in the vagina of the American skink, *Eumeces egregius*, but did not find any specialized storage glands and did not present a detailed histological description or any illustrations. In his masterful review of reptile sperm storage, Saint Girons ('73) included the Scincidae among those families of lizards in which infundibular sperm storage glands occur, but offered no further details. Sarkar and Shivanandappa ('89) reported that sperm storage tubules, and presumably sperm storage, are absent in the Indian skink Mabuya carinata. Hoser and Simpson ('97) reported that an isolated Tiliqua scincoides produced a second litter of 12 young a year after producing a litter of 23 young, but provided no data on the location of sperm storage. Based upon this scanty literature, Sever and Hamlett (2002) followed the noted histologist Saint Girons and assumed that sperm storage tubules in the infundibulum are characteristic of skinks, which fits with the phylogenetic placement of Scincidae in the Scleroglossa.

In this study, we examined the oviduct of the ground skink, *Scincella laterale* (Say), throughout its active season in the Piedmont of South Carolina to investigate the possibility of sperm

storage, using light and transmission electron microscopy. Ground skinks are found over much of the eastern and southern United States from the Atlantic coast to central Kansas and Oklahoma, and from southern New York to Florida and the Gulf coast of Texas (Conant and Collins, '98). Although the reproductive biology of *S. laterale* has been studied extensively in some areas, including Texas (Lewis, '51) and Kansas (Fitch, '70; Fitch and Greene, '65), the species has received little attention in South Carolina. Female sperm storage has not been reported previously in *S. laterale*, but based upon the hypothesis of Sever and Hamlett (2002), we predicted that we would find infundibular sperm storage tubules.

## **MATERIALS AND METHODS**

Reproductive tracts were examined from 20 female skinks collected in six sample periods: April 2001, May 2001, October 2001, March 2002, June 2002, and August, 2002. All specimens were collected on the U.S. Department of Energy's Savannah River Site in Aiken County, South Carolina. Specimens were sacrificed on the dates indicated in Table 2, within a week of their capture. Specimens were euthanized by exposure to ether, a procedure approved by the Animal Care and Use Committee of Saint Mary's College. After death, snout-vent length (SVL) was measured from the tip of the snout to the posterior end of the cloacal orifice. Carcasses of all specimens were preserved in 10% neutral buffered formalin and are housed in the research collections at Saint Mary's College.

The abdomen was slit midventrally from the cloacal orifice anteriorly to the stomach region. The number of enlarged vitellogenic follicles in the ovaries or the number of eggs in the uteri was counted, and their longest diameter measured with dial calipers to the nearest 0.01 mm. For

#### SKINK SPERM STORAGE

Date	SVL (mm)	Location	$N^1$	Diameter (mm)	Sperm
29 Mar	44.3	Ovary	3	3.6, 3.8 (2)	0
29 Mar	45.8	Ovary	3	2.9 (2), 3.2	+vagina
29 Mar	47.0	Ovary	3	3.6, 3.8, 3.2	0
20 Apr	36.4	Ovary	2	1.9, 2.1	+vagina
20 Apr	42.7	Oviduct	2	8.8,9.1	0
20 Apr	43.4	Oviduct	3	5.3, 6.4 (2)	0
11 May	39.3	Oviduct	2	9.2 (2)	0
11 May	40.9	Ovary	3	2.2, 2.6 (2)	+ vagina
11 May	45.3	Ovary	4	3.2 (2), 3.6 (2)	+ vagina
19 June	37.4	Oviduct	2	8.1, 8.9	0
19 June	43.1	Rt oviduct	1	8.5	0
		Lt ovary	1	3.0	0
19 June	45.8	Oviduct	2	9.0 (2)	0
19 June	48.9	Oviduct	4	7.9, 8.0, 8.1, 8.3	0
13 Aug	40.8	Ovary	*	0.7 - 0.8	0
13 Aug	41.0	Ovary	*	0.7	0
13 Aug	42.0	Ovary	*	0.7 - 1.3	0
13 Aug	42.9	Ovary	*	0.7—0.8	0
29 Oct	42.4	Ovary	4	0.08 - 0.1	0
29 Oct	44.9	Ovary	4	0.1	0
29 Oct	46.2	Ovary	4	0.05 - 0.1	0

TABLE 2. Specimens examined

\*Count unreliable; numerous unyolked follicles.

<sup>1</sup>Number of eggs or follicles.

electron microscopy, the left oviduct was removed and fixed in a 1:1 solution of 2.5% glutaraldehyde and 3.7% formaldehyde in cacodylate buffer at pH 7.2. 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, Port Washington, PA). Plastic sections were cut with an RMC MT7 ultramicrotome (Research and Manufacturing Co., Tucson, AZ) and DiATOME (Biel, Switzerland) diamond knives. Semi-thin sections (0.5-1 µm) for light microscopy were placed on microscope slides and stained with toluidine blue. Ultrathin sections (70 nm) for transmission electron microscopy collected were on uncoated copper grids and stained with solutions of uranyl acetate and lead citrate. Ultra-thin sections were viewed with a Hitachi H-300 transmission electron microscope (Nissei Sangyo America, Mountain View. CA).

The right oviduct was prepared by the standard paraffin method for light microscopy. These reproductive tissues were fixed in 10% neutral buffered formalin, rinsed in water, dehydrated in ethanol, cleared in toluene, and embedded in paraffin. Paraffin sections (10  $\mu$ m) were cut with

a rotary microtome and affixed to albuminized slides. Alternate slides were stained with hematoxylin-eosin (for general cytology), alcian blue 8GX at pH 2.5 (for primarily carboxylated glycosaminoglycans) followed by the periodic acid-Schiff's procedure (PAS, for neutral carbohydrates and sialic acids), and bromophenol blue (for proteins). Procedures followed Dawes ('79) and Kiernan ('90).

### RESULTS

Scincella laterale in South Carolina have a distinct breeding season that starts after hibernation in late winter or spring and extends into early summer. In March, females have large follicles in the ovary, and in April and May, some females still have large ovarian follicles whereas others have ovulated and contain large eggs in the uteri of the oviducts (Table 2, Fig. 1). All of the females collected in June have ovulated, at least into one oviduct. One of the three females collected in March contained oviductal sperm, indicating that mating commences after hibernation. Only females that have not ovulated contain sperm in the oviducts in April and May. In August and October, females possess small ovarian follicles and lack eggs and sperm in the oviducts.



Fig. 1. Reproductive tracts of female *Scincella laterale*. A: 45.8 mm SVL collected in March with enlarged follicles in the ovary. B: 43.4 mm SVL female collected in April with eggs in the uterus.

The uterine portion of the oviduct in postovulatory females is stretched smooth by the eggs that increase in largest diameter from about 3.5 mm at ovulation to 8-9 mm at the time of oviposition (Table 2; Fig. 1, 2A). Smaller follicles remain in the ovary while the larger eggs develop in the uterus, and corpora lutea are observed in the ovary (Fig. 2A). In preovulatory females, the uterine walls are thick and highly folded (Figs. 1, 2B). The posterior portion of the infundibulum is glandular (Fig. 2B), but sperm are never found in this region, where sperm storage tubules are known to occur in some other scleroglossid squamates. Instead, sperm occur only in the vagina of preovulatory females (Fig. 1). The vagina throughout its length is characterized by rugae, but the posterior vagina possesses narrow clefts or "crypts" in the rugae that are lacking in those of the anterior vagina (Fig. 2C,D). Sperm are found only in the posterior region of the vagina where the crypts occur.

# The posterior vagina of preovulatory females during the breeding season

Sperm occur in the lumen of the posterior vagina, in between rugae, and in the crypts (Fig. 3A,B). The orientation of sperm in the lumen and in between rugae is disorderly, but sperm in the crypts usually appear to have their nuclei aligned and oriented into the crypts (Fig. 3C,D). The vaginal lining consists of ciliated simple columnar epithelial cells interspersed with occasional secretory cells (Figs. 3C,D, 4A). The bases of the crypts, however, consist strictly of secretory cells (Fig. 3C,D). Sperm in the lumen of the vagina are often seen mixed in secretory product (Fig. 4A,B), although this is not apparent in the crypts. Whether mixed with secretory product or not, these sperm appear normal in cytology (Figs. 3D, 4B).

The secretion is a mucoid substance that gives positive tests for PAS (neutral carbohydrates and sialic acids) and alcian blue 8GX at pH 2.5 (primarily carboxylated glycosaminoglycans), but not bromophenol blue (proteins). The release of the product is apparently a merocrine process, and some membrane fusion between vacuoles is often evident prior to release (Fig. 4C). Synthetic organelles (endoplasmic reticulum, Golgi bodies) are not prominent in the cytoplasm. Intercellular canaliculi are very narrow and have tight junctions extending inward from the luminal borders (Fig. 4C). The ciliated cells are characterized by elongate, densely staining mitochondria in the apical cytoplasm (Fig. 4D). Both ciliated and secretory cells have irregular, euchromatic nuclei with prominent nucleoli (Figs. 3–5).

Occasionally, sperm are found embedded in the cytoplasm of secretory cells (Fig. 5). These sperm or parts of sperm are embedded directly in the secretory cells (Fig. 5A), associated with clear vacuoles (Fig. 5C), or embedded in secretory vacuoles (Fig. 5B, D). In one instance, a group of embedded sperm nuclei is adjacent to a Golgi apparatus, and next to a degenerating cell and a cell with numerous small dense bodies that perhaps are primary lysosomes (Fig. 5B). The



Fig. 2. Light micrographs of paraffin sections through the oviduct of *Scincella laterale*. A: Ovary and adjacent uterine region containing a large yolked egg of a 37.4 mm SVL female collected in June. B: Infundibulum and anterior uterus of a 42.7 mm SVL female collected in April with enlarged ovarian follicles and no eggs in the uterus C: Anterior vagina of same

specimen as B, showing lack of sperm and crypts in the rugae. D: Posterior vagina of the same specimen as C, showing presence of sperm and crypts in the rugae. Sections stained with hematoxylin and eosin. Cl, corpus luteum; Cr, crypts; Fo, developing follicles; In, infundibulum; Lu, lumen; Ov, ovary; Ru, rugae; Sp, sperm; Ut, uterus; Ye, yolked egg.



Fig. 3. 45.3 mm SVL female *Scincella laterale* collected in May with sperm in the posterior vagina, no eggs in the uterus, and enlarged ovarian follicles. A: Light micrograph of an epoxy section. B-D: Transmission electron-micrographs showing (B) an overview of a region of the posterior vagina,

(C) sperm crypt in the vaginal mucosa, and (D) orientation of sperm nuclei in a crypt. Ci, cilia; Cr, crypt; Lp, lamina propria; Mv, microvilli; Nu, epitheial cell nucleus; Ru, rugae; Sn, sperm nucleus; Sp, sperm; Sv, secretory vacuole.



Fig. 4. Transmission electron-micrographs through the posterior vagina of a 36.4 mm SVL *Scincella laterale* collected in April with enlarged ovarian follicles, no eggs in the uterus, and sperm in the posterior vagina. A: Sperm associated with ciliated and secretory cells and secretory material in the lumen. B: Portions of sperm cells associated with luminal secretory material. C: Apical cytoplasm of a secretory cell.

**D**: Apical cytoplasm of a ciliated cell. Ax, axoneme; Bb, basal bodies; Ci, cilia; Lu, lumen; Mi, mitochondria; Mpt, middle piece of the tail; Mv, microvilli; No, nucleolus; Nu, epithelial cell nucleus; Ppt, principle piece of the tail; Se, secretory material; Sn, sperm nuclei; Spse, sperm in secretory material in the lumen; Sv, secretory vacuoles; Tj, tight junctions.



Fig. 5. Transmission electron-micrographs through the posterior vagina of a 45.3 mm SVL *Scincella laterale* collected in May with enlarged ovarian follicles, no eggs in the uterus, and sperm in the posterior vagina. A: Sperm in a crypt and embedded in crypt epithelium. **B**, **C**, **D**: Sperm embedded in

epithelial cells. Ci, cilia; Cr, cypt; Db, electron dense bodies; Dc, degenerating epithelial cell; Es, embedded sperm; Go, Golgi apparatus; Mi, mitochondria; Mpt, middle piece of the tail; No, nucleolus; Nu, epithelial cell nucleus; Sn, sperm nuclei; Sv, secretory vacuoles; Va, vacuoles.

embedded portions of sperm cells generally appear normal in cytology.

# The posterior vagina of postovulatory females in the breeding season

The vaginal crypt area of females with eggs in the uterus is very similar to that of preovulatory females except for the lack of sperm (Fig. 6). The euchromatic nuclei are again elongate and often irregular and possess prominent nucleoli (Fig. 6A). The crypts are well-formed (Fig. 6A), secretory material is present in the lumen of the vagina (Fig. 6B), and large secretory vacuoles are numerous apically in the secretory cells (Fig. 6C). Some nonciliated cells, however, lack apical secretory vacuoles, and are characterized by electron-dense mitochondria, occasional Golgi bodies, numerous small vesicles, and narrow intercellular canaliculi with numerous junctional complexes (Fig. 6D). The mitochondria of ciliated cells are more oval and electron-lucid (Fig. 6C).

# The posterior vagina outside of the breeding season

In females collected in late summer and fall, with small ovarian follicles and no eggs or sperm in the oviducts, the nuclei of the vaginal epithelial cells are still euchromatic, often irregular, and possess prominent nucleoli, but they appear to be more electron dense. In contrast to females from the breeding season, the vaginal epithelium of females from outside of the breeding season possess wider intercellular canaliculi (Fig. 7B), fewer secretory vacuoles (Figs. 7A–C), and more abundant Golgi complexes (Fig. 7C,D). Mitochondria are dense but less elongate than in the breeding season (Fig. 7C,D).

# DISCUSSION

Fitch ('70) stated that at the northern end of the range of *Scincella laterale*, as in northeastern Kansas, egg-laying extends from some time in April into July, and that two broods per season may be typical, with as little as 22 days (cf., Anderson, '65) between clutches. Lewis ('51) found that on the Gulf Coast of Texas egg-laying may extend from early April through the first half of August, and Fitch ('70) proposed that as many as five clutches per female seem possible in that region. We do not know how many clutches a female *S. laterale* from our sample could produce annually, but eggs of ovipository size were first observed in our sample from 20 April and last

observed in the sample from 19 June. However, we did not have any samples between 29 March and 20 April or between 19 June and 13 August. Regardless, using the developmental time of 3–5 weeks between ovulation and oviposition suggested by Fitch and Greene ('65) and Fitch ('70), more than one clutch could be laid by females in our population. Because sperm are absent in females with eggs in the uterus, fertilization of a later clutch would require additional matings.

The only other transmission electron microscopy studies on sperm storage structures in lizards are on the Indian gecko, *Acanthodactylus scutellatus*, by Bou-Resli et al. ('81) and on *Anolis sagrei* by Sever and Hamlett (2002). The major differences and similarities found in ultrastructure of sperm storage tubules are summarized in Table 3 and discussed below.

Bou-Resli et al. ('81) also used the term "crypts" to describe the invaginations in which sperm storage occurred, although the crypts in *Acanthodactylus scutellatus* appear to be relatively deeper and contain more secretory activity than the crypts we found in *Scincella laterale*. Also, the crypts in *A. scutellatus* are "irregularly distributed invaginations in the folds of the infundibulum" (Bou-Resli et al., '81) rather than occurring in the vagina. The openings into the crypts of *A. scutellatus* are surrounded by cilia, and cilia become less numerous distally. In the more shallow crypts of *S. laterale*, the basal cells are always secretory and non-ciliated.

During storage in female Acanthodactylus scutellatus, sperm are oriented with their heads clustered in bundles towards the blind end of the crypts, as we observed in Scincella laterale. Bou-Resli et al. ('81) also observed sperm embedded in the cytoplasm as well as in the intercellular spaces. Our observations on embedded sperm in S. laterale are consistent with those of Bou-Resli et al. ('81), who found that degradation of such sperm did not occur. Although synthetic organelles are rarely observed, large numbers of oval, electron-dense granules occur in the sperm storage tubule cytoplasm in A. scutellatus, as opposed to the electron-lucid vacuoles noted in S. laterale. Blood capillaries frequently occur close to the crypts of A. scutellatus, a characteristic not noted for S. laterale. During the time of ovulation and oviposition in A. scutellatus, sperm were observed in the oviduct with their heads coming out first while the tail is still immersed in the crypt. We did not find sperm in the oviducts of any female S. laterale that had oviductal eggs or had



Fig. 6. Transmission electron-micrographs through the posterior vagina of a 39.3 mm SVL *Scincella laterale* collected in May with eggs in the uterus and no sperm in the vagina. A: Overview of the vaginal epithelium and sperm crypts. **B**, **C**: Crypts devoid of sperm. **D**: Apical epithelium of a secretory

cell lacking vacuoles. Ci, cilia; Cr, crypt; Ds, desmosome; Go, Golgi apparatus; Ic, intercellular canaliculi; Mi, mitochondria; Mv, microvilli; No, nucleolus; Nu, epithelial nucleus; Se, secretory product; Sv, secretory vacuoles; Tj, tight junction; Ve, vesicles.



Fig. 7. Transmission electron-micrographs through the posterior vagina of a 42.4 mm SVL *Scincella laterale* collected in October with small ovarian follicles, no eggs in the uterus, and no sperm in the posterior vagina. A: Overview of the vaginal epithelium and sperm crypts. B: Border of the oviductal epithelium. C: Luminal border of a secretory cell

and adjacent ciliated cells. **D**: Supranuclear cytoplasm of a secretory cell. Ci, cilia; Cr, crypt; Ds, desmosome; Go, Golgi apparatus; Ic, intercellular canaliculi; Lp, lamina propria; Lu, lumen; Mi, mitochondria; No, nucleolus; Nu, epithelial cell nucleus; Sv, secretory vacuoles; Tj, tight junction.

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Sperm storage tubules	Acanthodactylus scutellatus (Gekkonidae)	Scincella laterale (Scincidae)	Anolis sagrei (Polychrotidae)	
Reference	Bou-Resli et al. (1981)	Present study	Sever and Hamlett (2002)	
Location	Infundibulum	Posterior vagina	Anterior vagina	
Туре	Deep crypts	Shallow crypts	Tubulo-alveolar	
Epithelium	Ciliated pores, secretory distally	Ciliated pores, secretory distally	Ciliated and secretory neck cells, unspecialized bulb	
Secretory vacuoles	Electron-dense	Electron-lucid	Electron-lucid	
Synthetic organelles	Sparse	Sparse	Sparse	
Embedded sperm Sperm present with	Yes	Yes	Yes	
eggs in utero	Yes	No	Yes	
Multiple clutches	Not reported	Possibly	Yes	
Length of storage	4 months	Unknown	Unknown	

TABLE 3. Comparison among lizards studied with transmission electron microscopy

oviposited. Whether crypts simply provide a protective factor (Saint Girons, '73) or a nutritive function (Cuellar, '66) could not be determined in *A. scutellatus* (Bou-Resli et al., '81). As in *S. laterale*, however, the crypts basically represent a continuation of the oviductal lining and appear unspecialized for sperm storage. This condition seems to be the usual situation for sperm storage tubules in reptiles (Gist and Jones, '87; Sever and Hamlett, 2002).

Anolis sagrei, like Scincella laterale, has sperm storage tubules in the vagina (Sever and Hamlett, 2002). However, the sperm storage tubules in A. sagrei are not simple crypts in the posterior vagina, but tubulo-alveolar glands in the anterior vaginal tube (terminology of Fox, '63) or uterovaginal transition area (terminology of Conner and Crews, '80). As opposed to the usual situation reported for reptile sperm storage tubules, the glands in A. sagrei show differentiation from the surrounding oviductal lining. The neck of the glands consists of ciliated cells and non-ciliated secretory cells, but the vacuoles of the secretory cells seem larger than elsewhere in the vagina, especially in specimens that contain the most sperm. The neck region opens into a distal bulb where the epithelial cells lack cilia and any secretory product, and, indeed, appear almost syncytial. Sperm are clustered into bundles or aligned randomly in the bulbs, perhaps depending upon the numerical density of sperm. Embedded portions of sperm cells occur only in secretory cells of the neck region, and these portions resembled those of normal sperm in the lumen.

Unlike *Scincella laterale* in South Carolina, which has a very distinct egg-laying season limited to spring and early summer (Table 2), *Anolis* 

sagrei apparently produces multiple clutches, each consisting of a single egg, over a more extended period, like many other species of Anolis (Conner and Crews, '80). All of the females examined by Sever and Hamlett (2002) were collected in April and had one well-developed egg in one uterus as well as a more recently ovulated egg in the other uterus. These developing eggs obviously had been fertilized, but all of the females also had sperm in the sperm storage tubules of both oviducts, irrespective of the degree of egg development. Thus, perhaps sperm from one mating can be stored efficiently to allow fertilization of multiple clutches in A. sagrei, unlike the situation postulated for S. laterale. Because storage occurs in the distal bulbs of specialized glands in A. sagrei, these sperm are not swept away during oviposition.

Thus, a considerable amount of variation has been found in the ultrastructure of sperm storage in the few lizards that have been examined, and the data from light microscopy on other lizards hint that the full panoply of variation has yet to unfold. With information available on fewer than one-hundredth of all known lizard species, it is certainly premature to construct any robust hypotheses concerning phylogeny of sperm storage in this group. Phylogeny is also likely to be obfuscated by convergence due to similar functional adaptations and perhaps design constraints imposed by the basic structure of the vertebrate oviduct and sperm (Sever and Hamlett, 2002).

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