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Annual dynamics of sperm production and storage in the Mediterranean Gecko, *Hemidactylus turcicus*, in the southeastern United States

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Abstract. The Mediterranean Gecko, *Hemidactylus turcicus*, is an invasive species found in warmer regions throughout the world, including the southern United States. In Louisiana, *H. turcicus* appears to be free of competition and has been rapidly expanding its range in the past several decades. However, in Florida and Texas, *H. turcicus* is heavily out-competed by closely related competitors that do not occur in Louisiana, including *H. frenatus*, of which a substantial amount of reproductive morphology and ecology is known. Although the reproductive cycle of *H. turcicus* has been studied in Florida and Louisiana, little is known about the seasonal variation of their oviduct and if they are capable of storing sperm, which can have substantial implications for reproductive competition. We analysed sperm production, storage and seasonal variation in the testes and oviducts of *H. turcicus* using light and electron microscopy. Previous studies found that female *H. frenatus* store sperm in the uterine-infundibular region for up to 36 weeks, and that their oviducts are active year-round. In *H. turcicus*, we found that sperm are stored in the uterine-infundibular region of the oviduct, and sperm are stored from May through August. *Hemidactylus turcicus* has one breeding season per year, producing three to four clutches between May and August, but between September and February minimal activity occurs in the oviduct. Additionally, male *H. turcicus* are producing sperm throughout the entire reproductive season, but whether females utilize stored sperm to fertilize eggs or if they mate continually throughout the spring and summer is unknown.

Keywords: Gekkonidae, histology, lizard, oviducts, reproductive morphology, ultrastructure.

Introduction

As its name implies, the Mediterranean Gecko is native to coastal areas in southern Europe, western Asia, and northern Africa, but the species is invasive and occurs in semi-tropical to tropical areas throughout the world, including the southeastern United States (Conant and Collins, 1998). They are nocturnal and often found on the outer walls of human habitations (Rose and Barbour, 1968). *Hemidactylus turcicus* is the only gecko found in Louisiana, and in Louisiana this species appears to be free of competition with other lizards and has expanded its range from four to 30 parishes in less than two decades (Meshaka et al., 2006). This rapid expansion has been attributed to human-mediated dispersal and building construction (Meshaka et al., 2006), since this species is often found associated with human habitation. Similarly, *H. turcicus* was prevalent in other states such as Florida and Texas until recently. Despite the prosperity of *H. turcicus* in Louisiana, in Florida and Texas they are being eliminated by closely related competitors (*H. frenatus*, *H. garnotii*, and *H. mabouia*; Meshaka, 1995). Meshaka (1995) proposed that this decline in abundance is related to reproductive competition, because *H. frenatus*, *H. garnotti*, and *H. mabouia* have all been seen to have year-round reproductive cycles in the southeastern United States, whereas *H. turcicus* has seasonal reproductive cycles, producing clutches during a restricted timeframe every year and thus producing less offspring than the competing Hemidactyline species (Meshaka et al., 2006). Furthermore, given the propensity for Hemidactyline species to expand their distributions with human development, it seems counter

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intuitive that only *H. turcicus* should be located Louisiana, in an area so near to *H. frenatus*, *H. garnotti*, and *H. mabouia* dominated regions (Florida and Texas).

Analysis of the reproductive cycle of the Common House Gecko, *Hemidactylus frenatus*, showed year round reproduction in Florida, with reduced reproduction between September and February (Murphy-Walker and Haley, 1996). Egg productions are separated by 3-5 weeks during March through August, and females have an average of seven viable clutches during that time period. Sperm is known to be stored for at least 36 weeks between the uterine and infundibular regions of the oviduct (the "transition zone"), where sperm storage tubules (Ssts) are located, and residual sperm have been found in the anterior vaginal region. Although sperm were observed in the anterior vaginal region of *H. frenatus*, this is not a site for long-term sperm storage, as sperm were infrequently located there (Murphy-Walker and Haley, 1996). Yamamoto and Ota (2006) found evidence of *H. frenatus* in the Ryukyu Archipelago (Japan) producing clutches from a single mating (and thus utilization of stored sperm) for up to one year. This difference in length of sperm storage may be due to climatological and environmental differences of the two regions where *H. frenatus* were analysed (southeastern United States and Japan).

In comparison, *H. turcicus* has been observed to have a reproductive season from April until August, and females produced only 2 to 3 clutches per season (Rose and Barbour, 1968). Although seasonal reproduction has been observed in *H. turcicus*, nothing is known about the annual cycle of sperm storage during the reproductive season, and whether the limiting factor causing seasonal reproduction is the result of variation in oviductal activity or if males have alternating cycles of sperm production, as has been observed in North American colubrid snakes (Aldridge, pers. comm.), is unknown. Alternating cycles would indicate that offspring produced would be limited to the stored sperm from few matings at only the very beginning of the reproductive season.

Furthermore, a previous study on *H. turcicus* concluded that *H. turcicus* does not store sperm (Girling et al., 1998). However, this conclusion resulted from observing one May specimen that had only residual sperm in the vaginal region of the oviduct, indicating recent copulation. Alternatively, in the sister species *H. frenatus*, Murphy-Walker and Haley (1996) found sperm stored in the posterior infundibulum regardless of ovarian condition, and that sperm can be stored for up to 36 weeks. Girling et al. (1998) did not address that *H. turcicus* has one known 4 to 5 month reproductive season, and that May is early in the reproductive season; whereas *H. frenatus* is reproductively active year-round in the same region. Because *H. turcicus* is not reproductively active year-round, sperm may not be located in the sperm storage tubules (Ssts) early in the breeding season, and sperm located in the vaginal region of the May specimen may have been the result of recent copulation. If *H. turcicus* store sperm for less time than its competitors, including *H. frenatus*, or is not as efficient at sperm storage, then *H. turcicus* may not be able to produce as many offspring and are likely less able to compete with the number of offspring produced by its competitors.

A thorough analysis of reproduction in *H. turcicus* is required to answer questions regarding reproductive competition within the *Hemidactylus* complex in the southeastern United States. This study describes oviductal sperm storage and seasonal variation in female *H. turcicus* over the course of one year by analysis using various histological methods: light microscopy, scanning, and transmission electron microscopy. Furthermore, this study aims to compare the cycle of sperm storage and production of both female and male *H. turcicus* using light microscopy. The results on male *H. turcicus* are preliminary, and a more detailed analysis is in progress. We hypothesize that reproductive competition between the closely re-

lated species (*H. turcicus* and *H. frenatus*) is a key component to the peculiar distribution of these two species in the southeastern United States, and that female and male *H. turcicus* from the southeastern United States have alternating sperm storage/production cycles (respectively), which would indicate functional sperm storage for female *H. turcicus*.

Materials and methods

Animal collection

Four female specimens and one male specimen per month were collected between March 2006 and March 2008 from southeastern Louisiana (Hammond, Ponchatoula, and Baton Rouge) for analysis (5 specimens per month). Specimens were collected under permits issued by the Louisiana Department of Wildlife and Fisheries and protocols were approved by Southeastern Louisiana University's Institutional Animal Care and Use Committee.

Two female oviducts and one male testis per month were analysed using light microscopy, and the two additional female oviducts were analysed using transmission and scanning electron microscopy. Specimens were sacrificed within a day of collection using sodium pentobarbital, and were fixed in Trump's solution (2.5% gluteraldehyde and 2.5% formaldehyde in 0.1 M sodium cocodylate buffer at pH 7.4) for both light microscopy and electron microscopy.

Light microscopy

Tissue was rinsed in water, dehydrated in a graded series of ethanol, cleared in toluene, and embedded in paraffin. Sections (10 μm thick) were cut with a rotary microtome, and fixed onto slides using albumin. Alternate slides were stained for general cytology using hematoxylin-eosin, alcian blue for carboxylated glycosaminoglycans followed by periodic acid-Schiff's (PAS) procedure for neutral carbohydrates and sialic acids, and bromophenol blue for proteins. These procedures were utilized by Sever and Hopkins (2004) for their analysis of sperm storage in *Scincella laterale*, following protocols of Kiernan (1990).

Slides were analysed using a Leica DM2000 microscope. Photographs were taken using a Leica DF420 digital camera (Leica Microsystems, Wetzlar, Germany). Images were subsequently edited using Adobe Photoshop 7.0 and Adobe Photoshop CS (Adobe Systems Inc., San Jose, CA).

Transmission electron microscopy

Tissues were rinsed in distilled-deionized water and post-fixed in 2% osmium tetroxide, prior to dehydration in a graded series of ethanol. Tissues were cleared in propylene oxide and polymerized in an epoxy resin (EmBed 812, Electron Microscopy Sciences, Hatfield, PA). Subsequently, the tissues were cut with an RMC MT7 ultramicrotome and DiATOME diamond knives. Ultra-thin sections (70 nm) were placed on copper grids and stained with solutions of uranyl acetate and lead citrate. Sections were examined at 80 kV with a JEOL 100 transmission electron microscope, and photographs taken with a L3C CCD camera system (Scientific Instruments and Applications, Duluth, GA). These procedures, sans photograph methodology, were utilized by Sever and Hopkins (2004) for their analysis of sperm storage in *Scincella laterale*, following procedures of Dawes (1979).

Scanning electron microscopy

Tissues were fixed and dehydration followed the procedures for TEM. Tissues were critical-point-dried with a Denton DP-1 and were sputter-coated with a gold-palladium alloy using a Denton Desk IV (Denton Vacuum, Moorestown, NJ). Tissues were examined at 15 kV with a Phillips XL20 scanning electron microscope. These scanning electron microscopy methods follow the procedures used by Sever and Siegel (2006) in their study of sperm aggregations in *Plethodon cinereus*.

Results

Reproductive cycle

Female *H. turcicus* have one 4 to 5 month breeding season (table 1). Vitellogenesis begins to occur between March and April. Mating begins between late April and early to mid-May. Oviposition occurs between mid-May and late August. Gravid females with developing ovar-

Table 1. Chronology of events associated with *Hemidactylus turcicus* reproduction in southeastern Louisiana. Female events are **bolded**, male events are *italicized*. VT, vitellogenesis; MT, mating; ES, egg shelling; OP, oviposition; SS, sperm storage; FS, follicular stasis; SG, spermiogenesis; SP, spermiation.

	VT	**MT**	**ES**	**OP**	**SS**	**FS**	*SG*	*SP*
Mar	X						X	X
Apr	X	X					X	X
May	X	X	X	X	X		X	X
Jun	X	?	X	X	X		X	X
Jul	X	?	X	X	X		X	X
Aug	X	?	X	X	X		X	X
Sep						X		X
Oct						X		
Nov						X	X	
Dec						X	X	
Jan						X	X	
Feb						X	X	

ian follicles were found throughout the summer, indicating continual production of eggs throughout the reproductive season. Thus, between 3 and 4 clutches are likely produced per year. Females from September through February appear to be inactive, with small ovarian follicles found throughout these times (table 2). The lack of change in size of these follicles indicates follicular stasis during this time frame.

Spermiogenesis is well underway in December and is still observed in the testes of a male sacrificed in June (fig. 1; tables 1, 3). Although a few sperm were found in the proximal efferent ducts of males collected in January and February, spermiation increases greatly in March and sperm persist in the proximal efferent ducts (ductuli efferentes, ductuli epididymides, and ductus epididymis) through early September. No sperm were found in the proximal efferent ducts of males collected from October, November and December. Distal portions of the ductus deferens were not examined.

Table 2. Specimen information for female specimens used in light microscopy. Fol = follicles. Snout-vent length (SVL), largest follicle (Largest Fol), and mean follicle (Mean Fol) sizes are all in mm. # Fols indicates total number of follicles per ovary. For presence of sperm (Sperm), 0 = none, + = scarce, ++ = abundant.

Month	SVL	Sperm	Egg Size	# Fols	Largest Fol	Mean Fol
Jan	52.2	0	–	3	1.1	0.7
Jan	56.5	0	–	5	1.7	0.8
Feb	53.1	0	–	3	1.6	1.2
Feb	55.4	0	–	3	1.8	1.5
Mar	49.1	0	–	5	1.3	0.9
Mar	56.1	0	–	3	1.6	1.2
Apr	50.8	0	–	4	1.6	1.2
Apr	54.7	0	–	4	2.0	1.4
May	55.1	++	5	4	1.5	1.0
May	54.8	++	8.4	4	1.7	1.1
June	56.0	++	–	5	1.8	1.2
June	51.7	++	–	6	1.5	1.0
July	57.5	0	–	0	–	–
July	53.8	++	9	4	1.6	1.0
Aug	53.9	++	–	2	2.8	1.8
Aug	55.6	+	7	4	1.4	0.9
Sept	56.7	0	–	4	1.5	0.9
Sept	55.9	+	–	5	1.6	1.0
Oct	52.7	0	–	3	0.5	0.3
Oct	55.3	0	–	4	1.4	1.1
Oct	56.2	0	–	4	1.5	1.1
Nov	52.6	0	–	2	1.4	1.3
Nov	54.9	+	–	2	1.3	1.1
Dec	56.3	0	–	2	1.3	1.2

Table 3. Specimen information for male specimens used in light microscopy. SVL is in mm. For presence of sperm, Sp-T indicates amount of sperm in testes, Sp-ED indicates amount in the proximal efferent ducts; 0 = none, + = scarce, ++ = moderate amounts, + + + = abundant, + + ++ = copious amounts.

Month	SVL	Sp-T	Sp-ED
Jan	50.6	++	+
Feb	55.0	++	+
Mar	48.2	+ + +	+ + +
Apr	56.8	+ + +	+ + ++
May	54.2	+ + ++	+ + ++
June	56.8	+ + ++	+ + ++
July	48.9	+ + ++	+ + ++
Aug	54.8	+ + +	+ + +
Sept	54.2	+	++
Oct	53.1	0	0
Nov	54.0	0	0
Dec	52.6	+	0

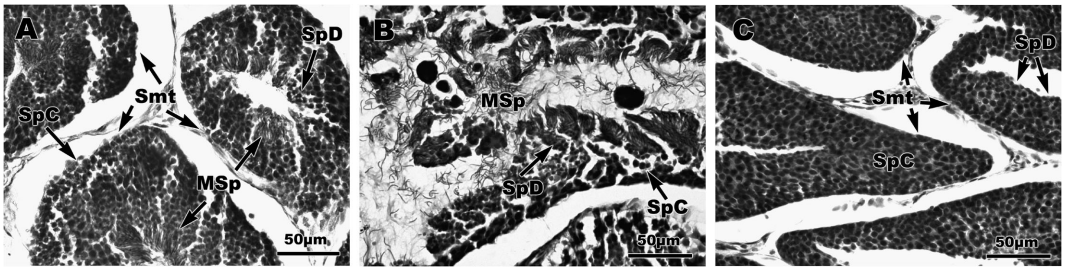

Figure 1. Amount of sperm present in male *Hemidactylus turcicus* testes from January, June, and November. A. Mature sperm present in the testis in a male collected in January prior to the female reproductively active times. B. Testis of a male collected in June showing abundant mature sperm during the reproductive season. C. Absence of spermiogenesis and mature sperm in a testis of a male collected in November during the inactive time of the year. MSp, mature sperm; Smt, seminiferous tubule; SpD, spermatid; SpC, spermatocyte.

Female sperm storage

Sperm were located in the posterior infundibulum of the oviduct in sperm storage tubules (Ssts), and were embedded deep in the glands and oriented parallel to one another (fig. 2). No sperm was found in more posterior regions of the oviduct, likely because no specimens were collected immediately after mating. Sperm stained basophilic in hematoxylin, alcian blue positive, and slightly bromphenol blue positive (fig. 2a-c). With transmission electron microscopy, no sperm were found in the lumen of Ssts and were not embedded in the epithelium (fig. 2d). Scanning electron microscopy provided an overview of the spacing of numerous gland orifices (fig. 2e) and the parallel arrangement of sperm (fig. 2f).

Large aggregations of sperm were found embedded deep within the sperm storage tubules between the months of May and August and were found both with the presence of enlarged ovarian follicles and with eggs *in utero*, which were found to occur simultaneously in some specimens (table 2). Few sperm were found in September and November, and such sperm were scarce and likely residual.

Oviductal histology

The oviduct of *H. turcicus* consists of three layers: the inner mucosa (the epithelium and lamina propria), the middle muscularis, and the outer visceral pleuroperitoneum. The middle muscularis of the anterior infundibulum consists only of circular fibers, and the muscularis becomes thicker toward the posterior infundibulum and uterine region. The vagina has both circular and longitudinal layers and has the thickest middle muscularis in the oviduct. The epithelium of the inner mucosa throughout the oviduct is comprised of columnar cells, and contains ciliated cells alternating with non-ciliated secretory cells. Simple tubular glands first appear anteriorly in the mid infundibulum and occur throughout the rest of the posterior infundibulum and uterus; the vagina lacks tubular glands. The glands in the posterior infundibulum are branched and acinar, and resemble sperm storage tubules reported in other squamates. The oviductal epithelium and associated tubular glands share the same characteristics of alternating ciliated and secretory cells.

Seasonal variation histology

We identified three distinct regions of the *H. turcicus* oviduct: the infundibulum, uterus, and vagina. Pre-ovulatory females prior to mating were seen to have relatively uniform regions of the oviduct, with the entire oviduct being PAS+ for neutral carbohydrates and sialic acids and BB+ for proteins, and sometimes possessing AB+ material (primarily carboxylated glycosaminoglycans) in the uterus (figs 3a, 4a). Alternatively, mated females that had already produced at least one clutch had oviducts that are neither strongly PAS+ nor BB+, although the Ssts in the posterior infundibulum are AB+ (figs 3b, 4b). Post-oviposition, inactive oviducts are not strongly PAS+, AB+, or BB+ in any region of the oviduct (figs 3c; 4c), and during the inactive period the oviduct reduces in size (fig. 3d). The oviduct of a November specimen (fig. 3d) is notably smaller than any of the other oviducts, but the specimen was 54.9 mm SVL, which is larger than depicted April and June specimens (54.7 and 51.7 mm SVL, respectively), and only slightly smaller than the September specimen (56.7 mm SVL).

Ultrastructure

Ultrastructural analysis was conducted on 12 specimens. During the reproductive season, active oviducts have large amounts of secretory vacuoles and secretory products (fig. 5). The infundibulum has two abundant types of secretory products during the breeding season: glycoproteins, which are characterized by a core of electron dense proteins in a vacuole containing polysaccharides (Sever and Hopkins, 2005; fig. 5a); and mucoidal substances, which have less electron dense material that is dif-

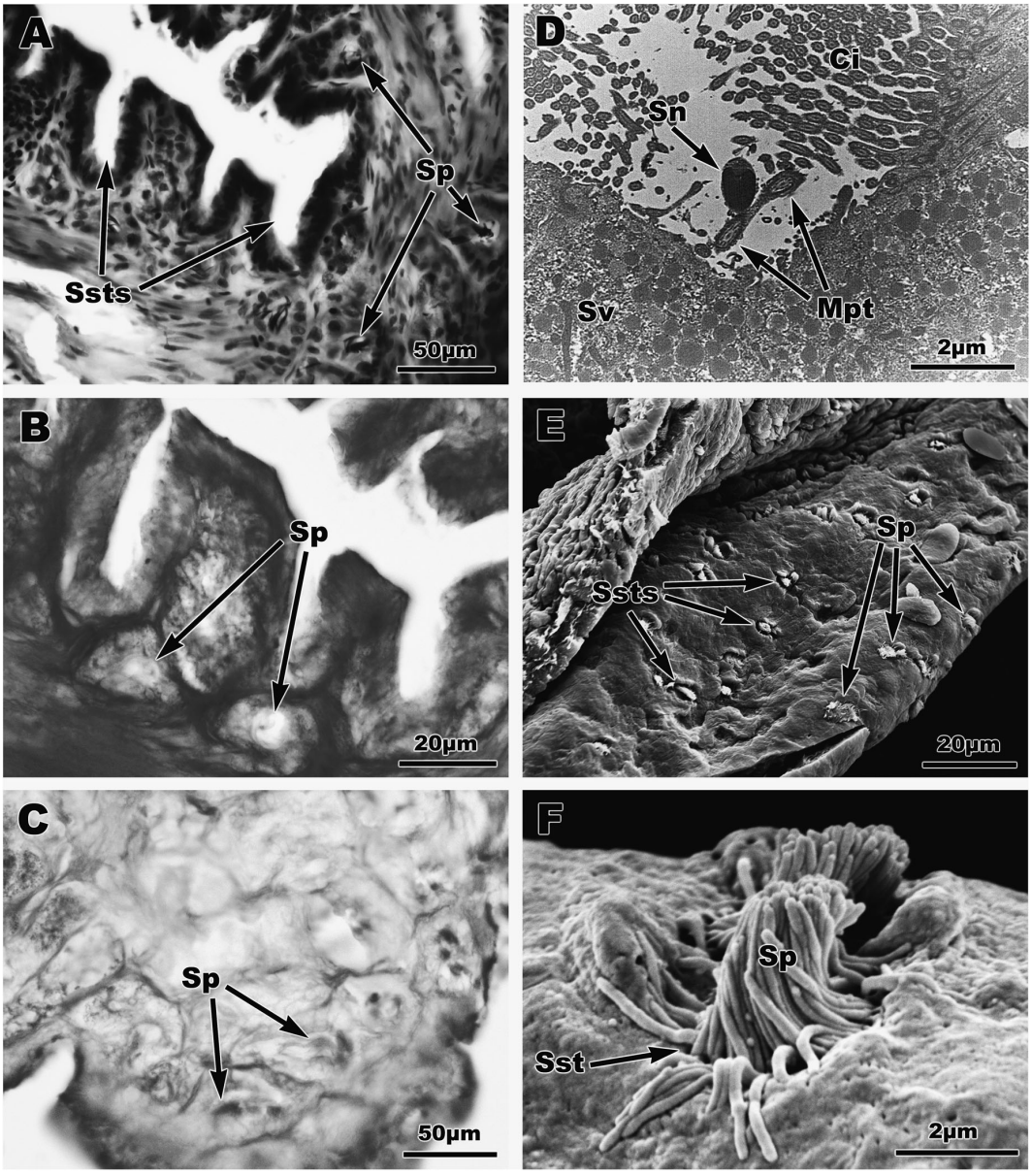

Figure 2. Histochemistry and ultrastructure of stored sperm in the *Hemidactylus turcicus* oviduct. A. Sperm in oviductal sperm storage tubules of a post-ovulatory female from May stained with hematoxylin-eosin for general cytology. B. Sperm in oviductal sperm storage tubules of a post-ovulatory female from May stained with periodic-acid Schiff's procedure and stained with alcian blue at pH 2.5; sperm are slightly AB+. C. Oviductal sperm storage tubules of a post-ovulatory female from May stained with bromophenol blue; sperm are slightly BB+. D. Transmission electron micrograph of sperm in the lumen of a sperm storage tubule from a post-ovulatory female from June. E. Scanning electron micrograph of the posterior infundibulum of a post-ovulatory female from June, depicting abundant amounts of sperm located in the sperm storage tubules. F. Higher magnification of (E), depicting the organization of sperm into parallel arrays. Mpt, middle piece of tail; Sn, sperm nucleus; Sp, sperm; Ssts, sperm storage tubules; Sv, secretory vacuoles.

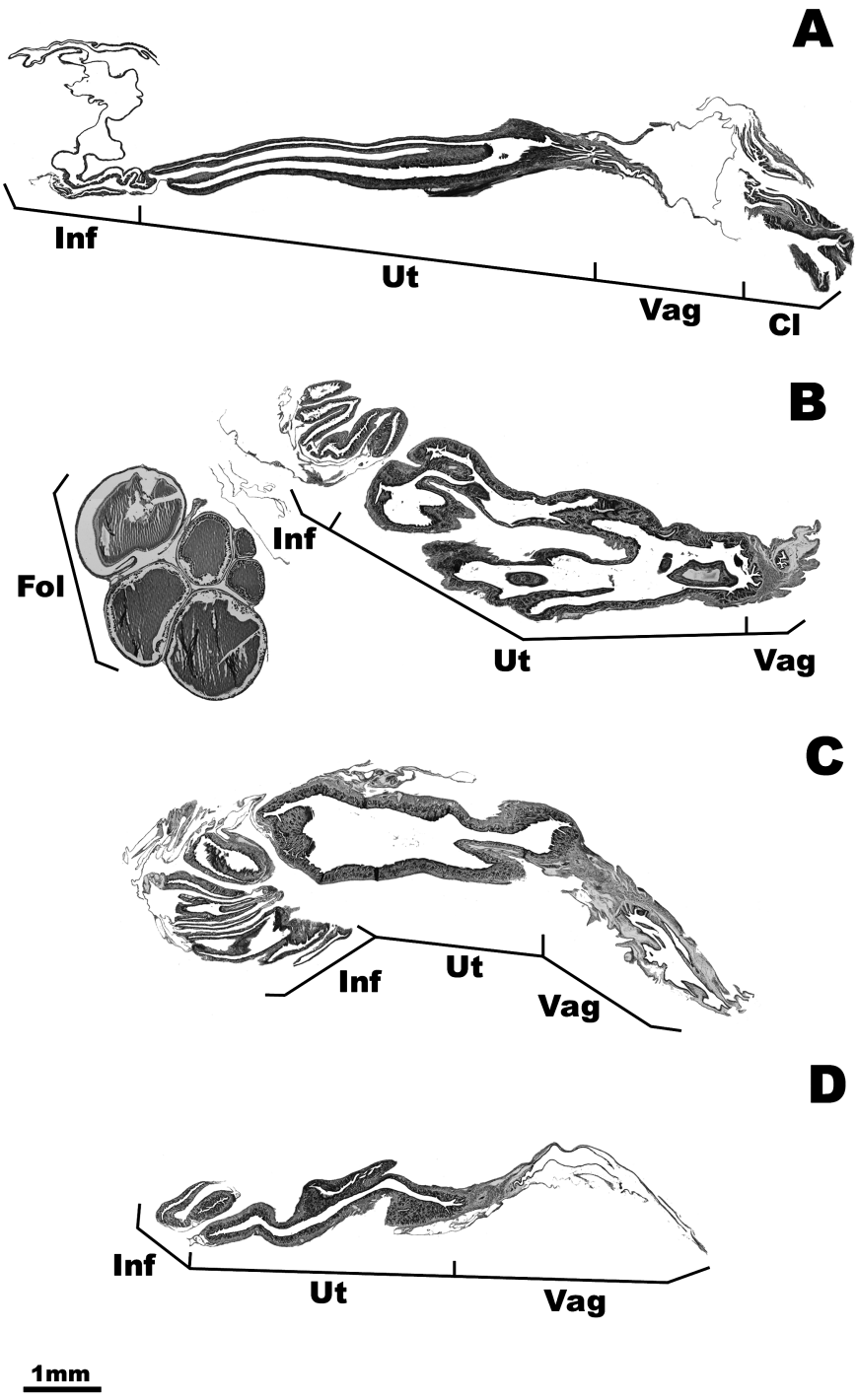

Figure 3. Seasonal variation of the *Hemidactylus turcicus* oviduct using hematoxylin-eosin stain for general cytology. A. Pre-ovulatory April oviduct, which is stretched and exhibits relatively basophilic secretions throughout the oviduct. B. Post-ovipostory June specimen which has scant glandular secretions and is developing another clutch of ovarian follicles. C. Post-reproductive oviduct from September, which is still stretched, and lacks glandular secretions and developing ovarian follicles. D. Inactive oviduct from a November specimen, which is substantially reduced in size from the September female, although both were similar in body size. Inf, infundibulum; Cl, cloaca; Fol, ovarian follicle; Ut, uterus; Vag, vagina.

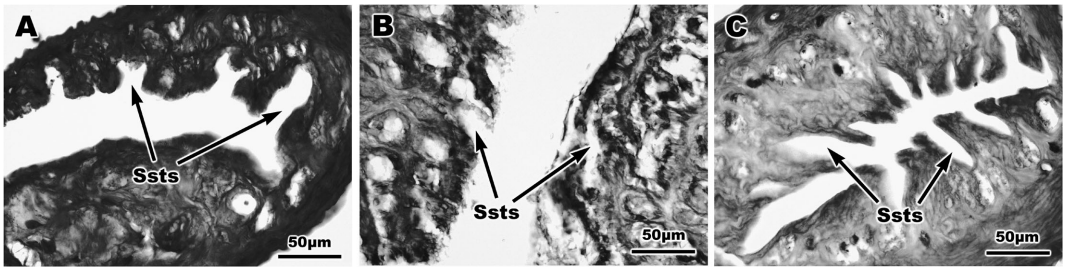

Figure 4. Seasonal variation in the sperm storage tubules of female *Hemidactylus turcicus* using PAS/AB stain. A. Pre-ovulatory sample from April staining strongly PAS+ throughout the Ssts. B. Post-ovipository sample from June with strongly AB+ linings of the Ssts. C. Inactive oviduct of a November specimen with only the sperm storage tubule epithelium staining slightly PAS+. Ssts, sperm storage tubules.

fused uniformly across the vacuole (fig. 5b). However, secretions located in the uterus and vagina are primarily mucoidal substances (fig. 5c-f). The uterine epithelium is especially active in the production of secretory material with Golgi complexes and rough endoplasmic reticulum associated with condensing vacuoles (fig. 5d).

Throughout the active oviduct, mitochondria are abundant, and nuclei of epithelial cells are irregularly shaped and contain electron dense nucleoli (fig. 5). Additionally, nuclei of secretory cells are largely heterochromatic, with large amounts of electron dense material interspersed, whereas nuclei of ciliated cells are more euchromatic and thus possess more uniformity in electron density throughout the nucleus (fig. 5).

Inactive oviducts are characterized by fewer secretory vacuoles throughout the oviduct (fig. 6). Although epithelial nuclei are still abundant and irregularly shaped, their nucleoli are less prominent. Furthermore, mitochondria are less prominent and scarce, and intercellular canuliculi are wider. Golgi complexes occur in high abundance in order to produce the secretions needed during the reproductively active season. Some secretory mucoidal substances are found throughout the oviduct, but the secretory vacuoles are more electron lucent and substantially smaller and less abundant in inactive females (fig. 6).

Discussion

Reproduction in Hemidactylus turcicus

Hemidactylus turcicus exhibited one reproductive season in Louisiana between May and August, with a maximum of 3-4 clutches per year, and stored sperm in the posterior infundibulum for a maximum of 5 months (20 weeks). The seasonal variation observed in the oviduct, including increased abundance and prominence of mitochondria and nucleoli throughout the oviduct, are similar to the seasonal variation observed in the vaginal region of *Scincella laterale* (Scincidae) (Sever and Hopkins, 2004). Infundibular Ssts in *H. turcicus* are consistent among other gekkonids that have been analysed, including *H. frenatus* (Murphy-Walker and Haley, 1996), *Nactus multicarinatus*, and *N. pelagicus* (Eckstut et al., in press).

The length of sperm storage between mating and oviposition of the first clutch is unknown, but both events occur initially from April to May (table 1). Thus, if sperm storage occurs prior to the first ovulation of the season and sperm are subsequently discarded or destroyed, this must be considered short-term sperm storage. In this study we were unable to reject the hypothesis that females and males mate throughout the reproductive season, because males produce sperm throughout the reproductive season. Thus, it is possible that the initial stored sperm become nonfunctional in subsequent fertilizations and *H. turcicus* must re-mate to produce multiple clutches.

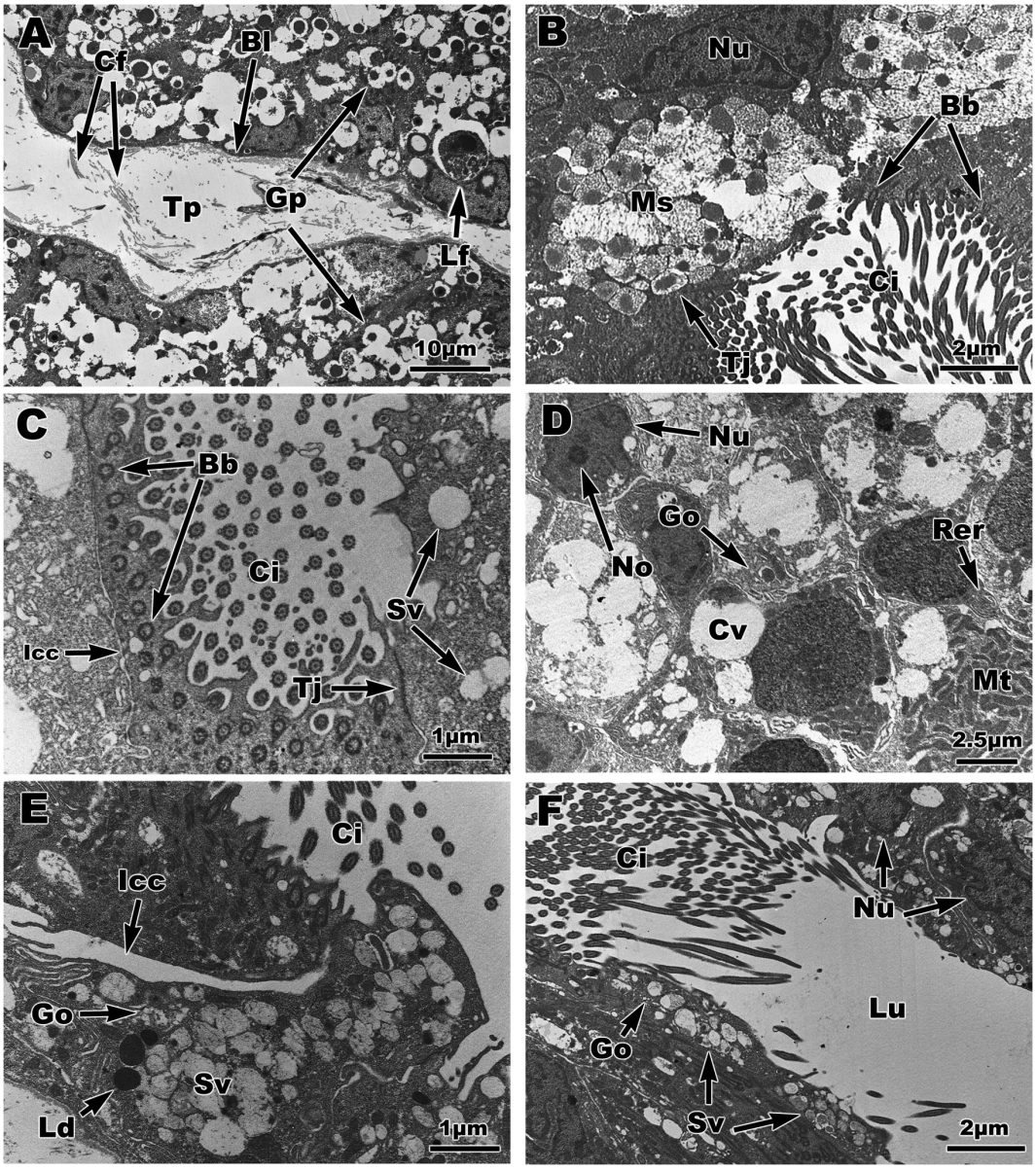

Figure 5. Transmission electron micrographs of an active oviduct of a June specimen. A. Highly secretory region of the infundibulum with abundant amounts of glycoproteins. B. An area of the infundibulum characterized by secretion of mucoidal substances. C. Uterus showing apex of a ciliated cell and a secretory cell with electron lucent vacuoles. D. Uterus illustrating intensive synthetic activity of secretory materials. E. Vagina with relatively wide intercellular canaliculi, and copious mucoidal secretory vacuoles. F. Vagina illustrating the apical nuclei of secretory cells. Bb, basal bodies; Bl, basal lamina; Cf, collagen fibers; Ci, cilia; Cv, condensing vacuole; Go, Golgi bodies; Gp, glycoproteins; Icc, intercellular canaliculi; Ld, lipid droplets; Lf, lipofuscin; Lu, lumen; Ms, mucoidal substance; Mt, mitochondria; No, nucleolus; Nu, nucleus; Rer, rough endoplasmic reticulum; Sv, secretory vacuole; Tj, tight junction; Tp, tunica propria.

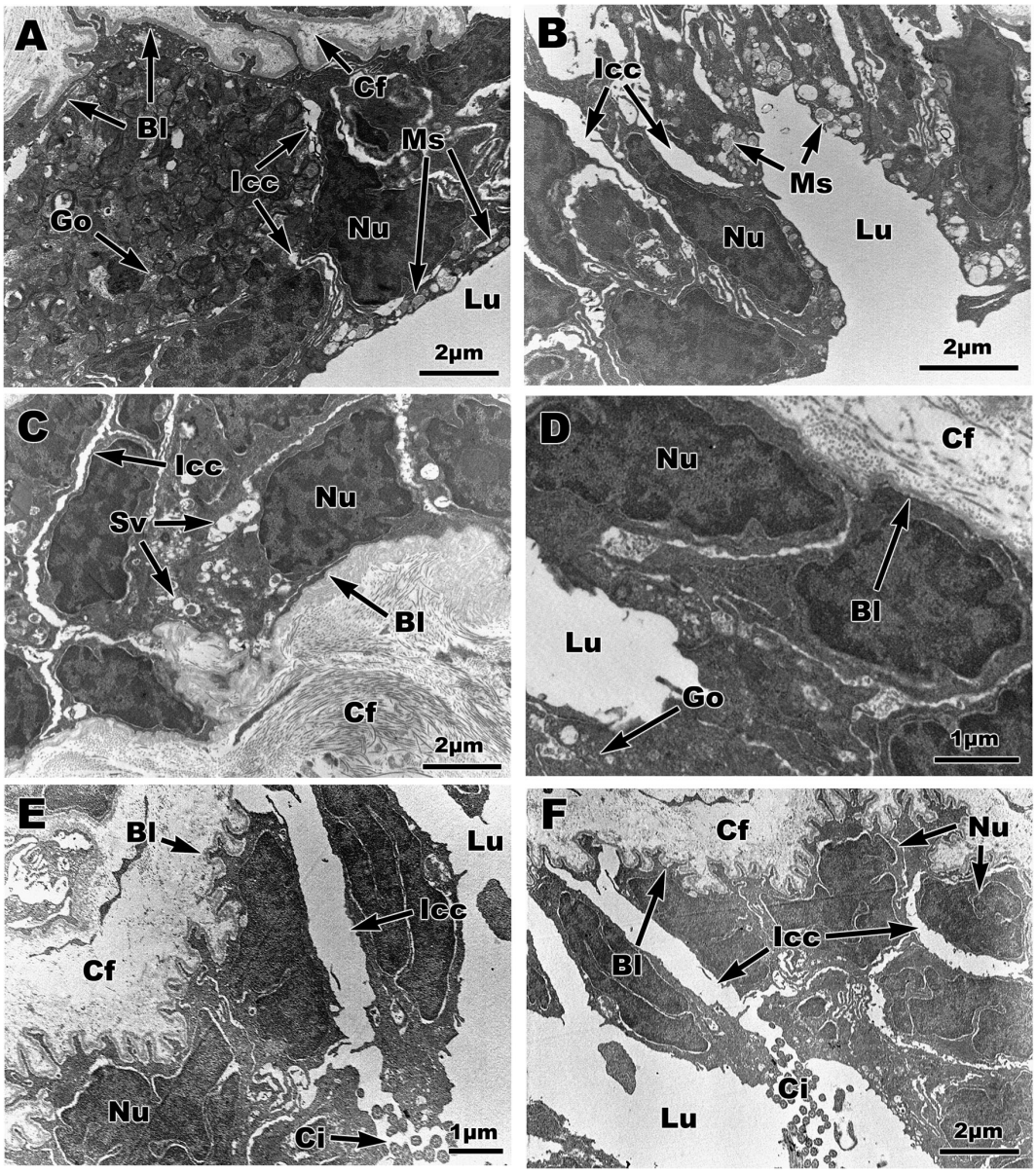

Figure 6. Transmission electron micrographs of an inactive oviduct of a November specimen. A. Infundibulum showing that low amounts of secretory activity are present, all secretions are mucoidal, and Golgi bodies are abundant. B. Infundibulum illustrating that intercellular canaliculi are wider than in active oviducts. C. Uterus in which secretory activity is low, and intercellular canaliculi are wide. D. Uterus with abundant Golgi bodies and irregular heterochromatic nuclei. E. Vagina with exceptionally wide intercellular canaliculi. F. Vagina showing heterochromatic nuclei occupying much of the cytoplasm. Bl, basal lamina; Cf, collagen fibers; Ci, cilia; Go, Golgi bodies; Icc, intercellular canaliculi; Lu, lumen; Ms, mucoidal substance; Mt, mitochondria; Nu, nucleus; Sv, secretory vacuole.

Implications for competition with Hemidactylus frenatus

In the southeastern United States, evidence for *H. turcicus* being reproductively out-competed by *H. frenatus* results from a shorter reproductive season, length of sperm storage, and number of clutches (and thus overall number of offspring) produced (table 4). However, it should be noted that these data reflect a situation where both species have equal likelihood of producing offspring that survive to reproductive maturity, and at present this hypothesis has not been tested.

Furthermore, in this study we were unable to reject the hypothesis that *H. turcicus* have nonfunctional sperm storage, because the males produce sperm throughout the entire reproductive season, and *H. frenatus* is known to have functional sperm storage. Functional sperm storage (observed in *H. frenatus*) can minimize risks associated with mating ("costs of mating"; Muenchow, 1978) that are unavoidable with nonfunctional sperm storage (hypothesized in *H. turcicus*). Continually mating requires additional investment of energy, because mating requires orchestration of the activities of two organisms, which has been proposed to be energetically inefficient, because energy is expended in locating a mate, courtship (both the physical acts and production involved in courtship displays), and the act of copulation. The pursuit of males can be harmful to the female, by harm occurring during copulation, heightening risks of predation, and increased likelihood of disease transmission (Muenchow, 1978).

Table 4. Comparative sperm storage data on *Hemidactylus turcicus* and *H. frenatus*. *Hemidactylus frenatus* data were collected from Murphy-Walker and Haley (1996), Yamamoto and Ota (2006), and Ota (1994). *indicates climatologically dependent. "Post. Inf." indicates posterior infundibulum.

	H. frenatus	*H. turcicus*
Sperm Storage Length	9-12 months	$\leqslant$4 months
Sst Location	Post. Inf.	Post. Inf.
Reproductive Cycle	Year round*	Spring-summer
# Clutches/Year	6-7 (12-14 eggs)*	3-4 (6-8 eggs)

Furthermore, even if *H. turcicus* does have functional sperm storage, sperm are stored for a maximum of four months, and *H. turcicus* are known to produce a maximum of 3 to 4 clutches in southeastern United States, regardless of geographic location (Meshaka, 1995). Alternate studies have found that *H. frenatus* store sperm anywhere from 9 months (Murphy-Walker and Haley, 1996) to a year (Yamamoto and Ota, 2006), and these two studies were conducted in different regions of the world (southeastern United States and Japan, respectively). Furthermore, year-round reproduction has been reported in tropical *H. frenatus* from Florida and Hawaii (Murphy-Walker and Haley, 1996; Krysco et al., 2003), whereas more northern *H. frenatus* appear to have seasonal reproduction in areas such as Japan, where oviposition occurs between April and September (Ota, 1994).

We propose that a possible reason for this seasonality could be due to cold tolerance of *Hemidactylus* eggs. *Hemidactylus frenatus* eggs have been reported to die at temperatures lower than 18°C (Ota, 1994). We suggest that this may

Table 5. Climate data for Baton Rouge, Louisiana and Key West, Florida. These data were obtained from uk.weather.com for 2007 (Accessed: 9 January 2008). All temperatures – Average maximum (Avg Max), average minimum (Avg Min), and mean – are in degrees Celcius. Temperatures less than or equal to 18°C (the temperature at which *Hemidactylus frenatus* eggs die) are *italicized*. In Baton Rouge, eight months of the year (October-May) had average minimum temperatures of less than or equal to 18°C. Key West had only one month with a minimum average temperature of exactly 18°C.

	Baton Rouge, LA			Key West, FL		
	Avg Max	Avg Min	Mean	Avg Max	Avg Min	Mean
Jan	16	*4*	10	24	*18*	21
Feb	18	*6*	12	24	19	22
Mar	22	*9*	16	26	21	23
Apr	26	*13*	19	28	22	25
May	29	*18*	23	29	24	27
Jun	32	21	27	31	26	28
Jul	33	22	28	32	27	29
Aug	33	22	27	32	26	29
Sep	31	19	26	31	26	28
Oct	27	*13*	20	29	24	27
Nov	22	*9*	15	27	22	24
Dec	17	*6*	12	25	19	22

also be the case with *H. turicus*, as climatalogical data coincide with our recorded reproductive cycle of *H. turcicus* (tables 1, 5). Climatological data from Baton Rouge, Louisiana, one of the primary collection sites for this study, show an average daily minimum temperature of lower than 18°C between the months of October and April, with average daily minimum temperatures of 19°C in September and 18°C in May (table 5). However, even though southeastern Louisiana is more temperate than the Florida Keys (table 5), Meshaka (1995) found no difference in reproductive cycles between *H. turcicus* in Florida, Texas, and Louisiana. Thus, *H. frenatus* may in part be able to out-compete *H. turcicus* by sheer number of offspring: *H. frenatus* with 12-14 offspring per year (Murphy-Walker and Haley, 1996; Yamamoto and Ota, 2006), and *H. turcicus* with 6-8 offspring per year (this study). These results likely have implications for the absence of *H. frenatus* in Louisiana, which may be a result of their inability to have eggs survive in cooler temperatures (Ota, 1994). Although *H. turcicus* does not have oviposition between the months where the average daily minimum temperature is <18°C, every month in Louisiana has had a record minimum temperature of <18°C (table 5). If *H. turcicus* eggs have a slightly higher tolerance to the cold, this may be a reason why *H. turcicus* is found in Louisiana while *H. frenatus* is absent.

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