Reproductive Biology of *Sceloporus consobrinus* (Phrynosomatidae): Male Germ Cell Development and Reproductive Cycle Comparisons within Spiny Lizards

**JUSTIN L. RHEUBERT,**1,2 **KATHERINE TOUZINSKY,**3 **DAVID M. SEVER,**4 **ROBERT D. ALDRIDGE,**1 **ANTHONY J. WILMES,**1 **DUSTIN S. SIEGEL,**5 and **KEVIN M. GRIBBINS**3,3

1Department of Biology, Saint Louis University, 3507 Laclede Avenue, St. Louis, Missouri 63103 USA
2Department of Biology, Wittenberg University, PO Box 720, Springfield, Ohio 45504 USA
3Department of Biological Sciences, Southeastern Louisiana University, SLU 10736 Hammond, Louisiana 70402 USA
4Department of Biology, Southeast Missouri State University, Cape Girardeau, Missouri 63701 USA

**ABSTRACT**—Reproductive cycles of lizards have long been studied in both field and laboratory scenarios. However, comparisons of spermatogenic cycles and germ cell development strategies in different populations across a large geographic range have yet to be explored. The purpose of this study is to A) describe the spermatogenic cycle and germ cell development strategy of a population of *Sceloporus consobrinus* in southeast Louisiana, B) compare this cycle to a more northern population of this species, and C) compare the reproductive cycles of species within *Sceloporus* (*N* = 21). In *S. consobrinus* from Louisiana, recrudescence begins in the fall (October and November), and the peak of spermatogenesis is reached the following spring/summer (May, June, July). This spermatogenic cycle is similar to that of a more northern population of *S. consobrinus* from Missouri. Within the genus *Sceloporus*, there are two seasonal patterns of spermatogenesis: initiation of spermatogenesis in the summer/fall and initiation of spermatogenesis in the spring. In both summer/fall and spring spermatogenic patterns, spermogenesis occurs in the spring and may continue into the summer. The seasonal timing of recrudescence is an extremely plastic trait that has evolved multiple times throughout the *Sceloporus* clade. However, there appears to be an association of summer/fall and spring recrudescence with latitude. Tropical populations have a higher frequency of spring recrudescence and temperate populations have a higher frequency of summer/fall recrudescence.

Gribbins and Gist (2003) described a novel germ cell development strategy exhibited during spermatogenesis in reptiles (squamates, testudines, crocodylians). Although testicular architecture in reptiles resembles that of other amniotes (Leblond and Clermont, 1952; Russell et al., 1990; Kumar, 1995), germ cells mature and migrate toward centralized lumina within seminiferous tubules as large cohorts similar to that found in amniotes (but lacking the cyst/lobe testicular organization of amniotes; Lofts, 1964). This “atypical” germ cell development strategy for an amniotic testis was termed “temporal,” because spermatogenic events are separated temporally (for review, see Gribbins, 2011). In the “typical” type of germ cell development exhibited by mammals and birds, the seminiferous tubules can be divided along their peripheral axis into sections, which have specific germ cell generations observed together in consistent sequential cellular associations. The number of stages present is also species specific. This spatial organization allows germ cells to be released into the lumen in waves along the length of each seminiferous tubule (Russell et al., 1990). Consequently, this has been termed the spatial germ cell development strategy (for review, see Gribbins, 2011).

Recent work on a variety of reptiles (Squamata, Gribbins et al., 2008; Rheubert et al., 2009a; Testudines, Gribbins et al., 2003; Crocodylia, Gribbins et al., 2006; Wang et al., 2008) suggests that the temporal germ cell development strategy is common among reptiles. Rheubert et al. (2009b) have proposed two hypotheses for the evolution of the germ cell development strategy in reptiles: 1) retention of the anamniote germ cell development strategy in basal amniotes and modern reptiles and convergence of the spatial development in birds and mammals; or 2) spatial development evolved in early amniotes with reversals in crocodilians, lepidosaurians, and chelonians. The former hypothesis leads to a more parsimonious solution when optimized to the evolutionary history of vertebrates (Rheubert et al., 2009b).

The temporal germ cell development strategy leads to discrete episodes of spermatogenesis in reptiles. Previous research has shown that lizards exhibit prenuptial spermatogenesis (sperm are produced immediately prior to the mating season). However, it has been noted that there are variations in lizard spermatogenic cycles (Tinkle, 1969; Adolph and Porter, 1993; Villagrán-Santa Cruz et al., 2009). In some species, recrudescence (the onset of mitotic divisions of germ cells) begins in the summer/fall, whereas in other species, recrudescence begins in the spring (Licht, 1972). However, the starting time of recrudescence appears to be fixed within a species independent of elevation and latitude (Table 1). This was investigated most thoroughly by Angelini and Picariello (1975) who developed the terms thermorigostatic and thermopsicrorigostatic to provide more descriptive terminology explaining the variation observed in reproductive strategies. Also, they showed a relationship between spermatogenic cycle and effective temperature, a measure of temperateness developed by Bailey (1960).

Tinkle (1969) and Tinkle et al. (1970) concluded that natural histories evolve in direct correlation to reproductive effort (using clutch size as a measurement of reproductive effort). Tinkle (1969) alluded to the idea that early maturing species that have multiple broods and have small clutch sizes may have an extended breeding period and that those species typically occur in tropical climates. Although temperature apparently plays a major role in the spermatogenic cycle, few studies have compared reproductive cycles from various geographic regions in lizard models. Three distinct reproductive strategies have been described within the genus *Sceloporus*: 1) spring spermatogenesis; 2) summer/fall spermatogenesis; and 3) continuous spermatogenesis. In all of these cycles, spermogenesis occurs immediately prior to mating; thus, all three cycles are prenuptial by definition.

2Corresponding Author. E-mail: justin.rheubert00@gmail.com

DOI: 10.1670/12-156
Sceloporus consobrinus (Leaché and Reeder, 2002) from Missouri exhibits fall spermatogenesis with recrudescence beginning in September and testicular regression beginning in June and July (Matter, 1987). McKinney and Marion (1985) found that there was variation in testicular cycles within the geographic range of what was formerly Sceloporus undulatus but has since been divided into S. consobrinus (Missouri) and S. undulatus (Alabama) (sensu Leaché and Reeder, 2002; Leaché, 2009). McKinney and Marion (1985) illustrated that testicular recrudescence occurred at similar times, but regression began earlier in the Alabama population (May to July) than in the more northern Missouri population (June and July).

Some species of spiny lizards, such as Sceloporus occidentalis (Goldberg, 1974), start recrudescence in the fall months prior to winter hibernation and spermatogenesis is arrested until spermiogenesis begins in the spring. This reproductive cycle is what Saint Girons (1982) referred to as mixed spermatogenesis. Others, such as Sceloporus horridus, exhibit a spring recrudescence with spermiogenesis beginning immediately after proliferation and meiosis (Valdés-González and Ramírez-Bautista, 2002). Continuous spermatogenesis has only been observed in what Saint Girons (1982) referred to as mixed spermatogenesis.

In the current study, we examined the reproductive cycles of male lizards from the same species across a latitudinal gradient. The purpose of this study is threefold: 1) to investigate the spermatogenic cycle of a southern population of S. consobrinus while examining the germ cell development strategy in comparison with other vertebrate models; 2) to test the hypothesis that spermatogenic cycles of S. consobrinus vary across a latitudinal gradient; and 3) to examine spermatogenic cycles of lizards within the Sceloporus genus within a phylogenetic context.

**Materials and Methods**

Adult male *S. s. consobrinus* were collected monthly in southeastern Louisiana from Sandy Hollow Nature Reserve (30°49.10'N, 90°24.45'W) near Amite, Tangipahoa Parish from August to November 2008 and March to August 2009. Three collection attempts per month were made for *S. consobrinus* between December 2008 and February 2009, but no individuals were observed. Specimens were euthanized with a 0.2-cc intracoelomic injection of sodium pentobarbital within 24 h of capture and reproductive tracts (testis, testicular ducts, kidneys, cloaca) were removed and preserved in Trump’s fixative (EMS, Hatfield, PA) as approved by the Institute of Animal Care and Use Committee and Southeastern Louisiana University where euthanizations and dissections were performed.

Testicular tissues were allowed to fix for at least 24 h then dehydrated with an increasing concentration of ethanol solutions. Tissues were then infiltrated with a 1 : 2 solution of Spurr’s plastic (EMS, Hatfield, PA) as approved by the Institute of Animal Care and Use Committee and Southeastern Louisiana University where euthanizations and dissections were performed.

Slides were examined using a Zeiss compound light microscope (Carl Zeiss Microimaging, Inc., Thornwood, NY) to determine germ cell morphologies and spermatogenic stages. Photographs were taken with an attached SPOT digital camera and 5.0 software (Diagnostic System Laboratories, Webster, TX). Composite micrographs were constructed and analyzed using Adobe Photoshop CS5 (Adobe Systems, San Jose, CA).

**Table 1. Reproductive data for Sceloporus.** Data obtained from other sources is marked with an asterisk (*). Parity: V = viviparous; O = oviparous; Clutches: S = single, M = multiple clutches per year, na = information not available; Latitude = °N; Elevation in meters.

<table>
<thead>
<tr>
<th>Species</th>
<th>Parity mode</th>
<th>Clutches</th>
<th>Recrudescence</th>
<th>Latitude</th>
<th>Elevation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. bicanthalis</em></td>
<td>V</td>
<td>na</td>
<td>Continuous</td>
<td>37</td>
<td>1,500</td>
<td>Hernández-Gallegos et al. (2002)</td>
</tr>
<tr>
<td><em>S. galoriciae</em></td>
<td>O</td>
<td>S</td>
<td>Spring</td>
<td>17</td>
<td>300</td>
<td>Lemos-Espinal et al. (1999)</td>
</tr>
<tr>
<td><em>S. graciosiss</em></td>
<td>O</td>
<td>na</td>
<td>Fall</td>
<td>40</td>
<td>1,300*</td>
<td>Woodbury and Woodbury (1945)</td>
</tr>
<tr>
<td><em>S. grammicus</em></td>
<td>V</td>
<td>na</td>
<td>Spring</td>
<td>19</td>
<td>2,294</td>
<td>Jiménez-Cruz et al. (2005)</td>
</tr>
<tr>
<td><em>S. horridus</em></td>
<td>O</td>
<td>M</td>
<td>Spring</td>
<td>18</td>
<td>1,500</td>
<td>Valdés-González and Ramírez-Bautista (2002)</td>
</tr>
<tr>
<td><em>S. jarrovi</em></td>
<td>V</td>
<td>na</td>
<td>Spring</td>
<td>31</td>
<td>6,500</td>
<td>Goldberg (1971)</td>
</tr>
<tr>
<td><em>S. megister</em></td>
<td>O</td>
<td>M*</td>
<td>Spring</td>
<td>35</td>
<td>150*</td>
<td>Vitt and Ohmart (1974)</td>
</tr>
<tr>
<td><em>S. malachiticus</em></td>
<td>V</td>
<td>S</td>
<td>Spring</td>
<td>9</td>
<td>800–3,200</td>
<td>Marion and Sexton (1971)</td>
</tr>
<tr>
<td><em>S. melanorhinus</em></td>
<td>O</td>
<td>S</td>
<td>Spring</td>
<td>19</td>
<td>10–584</td>
<td>Ramírez-Bautista et al. (2006b)</td>
</tr>
<tr>
<td><em>S. mucronatus</em></td>
<td>O</td>
<td>S</td>
<td>Spring</td>
<td>19</td>
<td>3,200–3,400</td>
<td>Méndez de la Cruz et al. (1988)</td>
</tr>
<tr>
<td><em>S. occidentalis</em></td>
<td>O</td>
<td>S</td>
<td>Fall</td>
<td>34</td>
<td>1,584</td>
<td>Goldberg (1974)</td>
</tr>
<tr>
<td><em>S. orcutti</em></td>
<td>O</td>
<td>S</td>
<td>na</td>
<td>33</td>
<td>244*</td>
<td>Mayhew (1963)</td>
</tr>
<tr>
<td><em>S. poinsetti</em></td>
<td>V</td>
<td>S</td>
<td>Fall</td>
<td>31</td>
<td>na</td>
<td>Ballinger (1973)</td>
</tr>
<tr>
<td><em>S. pyrocephalus</em></td>
<td>O</td>
<td>M</td>
<td>Spring</td>
<td>18</td>
<td>550</td>
<td>Ramírez-Bautista et al. (2002)</td>
</tr>
<tr>
<td><em>S. scalaris</em></td>
<td>O</td>
<td>S</td>
<td>Fall</td>
<td>31</td>
<td>2,600</td>
<td>Newlin (1976)</td>
</tr>
<tr>
<td><em>S. spinosus</em></td>
<td>O</td>
<td>M*</td>
<td>Fall</td>
<td>19</td>
<td>500</td>
<td>Ramírez-Bautista et al. (2012)</td>
</tr>
<tr>
<td><em>S. undulatus</em></td>
<td>O</td>
<td>M</td>
<td>Fall</td>
<td>33</td>
<td>300*</td>
<td>McKinney and Marion (1985)</td>
</tr>
<tr>
<td><em>S. utiformis</em></td>
<td>O</td>
<td>na</td>
<td>Spring</td>
<td>19</td>
<td>120*</td>
<td>Atland (1941)</td>
</tr>
<tr>
<td><em>S. variabilis</em></td>
<td>O</td>
<td>na</td>
<td>Fall</td>
<td>19</td>
<td>1,000</td>
<td>Ramírez-Bautista and Gutiérrez-Mayén (2003)</td>
</tr>
<tr>
<td><em>S. woodi</em></td>
<td>O</td>
<td>M</td>
<td>na</td>
<td>29</td>
<td>na</td>
<td>Jackson and Telford (1974)</td>
</tr>
</tbody>
</table>
(fall recrudescence, spring recrudescence, continuous), parity mode, latitude, and elevation. Only reproductive studies that included an entire annual cycle or could state definitively the onset of recrudescence and regression were used. Reproductive cycles were divided into fall, spring, and continuous cycles. Fall reproductive cycles show evidence of recrudescence during the fall months and exhibit a short 1–2-month hiatus following testicular regression, spring reproductive cycles show evidence of recrudescence during the spring months with typically a 4–6-month hiatus following testicular regression, and continuous reproductive cycles show no evidence of testicular regression throughout the year. Data gathered from the literature were coded based on latitude, parity mode, and spermatogenic cycle. For phylogenetic analyses, the region inhabited was divided into temperate and tropical regions based on 23.5° N latitude as a dividing line. Data were optimized onto two pre-existing phylogenetic hypotheses proposed by Wiens and Reeder (1997) and Leaché (2010) using a strict parsimony optimization in Mesquite version 2.75 (Maddison and Maddison, 2011). These two phylogenetic hypotheses were left unrooted because of conflicting results posed by out-group determination.

RESULTS

Testicular Structure and Germ Cell Morphology.—The testes are suspended by the mesorchium within the pleuroperitoneal coelom postero-dorsal to the liver. Deep to the visceral pleuroperitoneum, the testes are covered by the tunica albuginea (Fig. 1, Ta). Seminiferous tubules (Fig. 1, St) lie juxtapositioned to one another and are lined with a seminiferous epithelium, containing developing germ cells and Sertoli cells, surrounding a centralized lumen.

Premitotic cells, spermatogonia, lie within the basal compartment of the seminiferous epithelium. Spermatogonia A (Fig. 2, SpA) contain dispersed chromatin and are ovoid in shape. Spermatogonia A divide mitotically to give rise to spermatogonia B (Fig. 2, SpB), which also contain dispersed chromatin but usually show a more round morphology. Both spermatogonial types are present within the seminiferous epithelium of all months sampled during this study. Spermatogonia B divide mitotically and decrease in size as they transform into pre-leptotene spermatocytes (Prophase I of meiosis; Fig. 2, PL).

These spermatocytes have well-defined nuclear membranes and prominent nuclei. Also, they are the smallest of the dividing germ cells. During the first prophase the chromatin becomes more filamentous and darker staining in leptotene spermatocytes (Fig. 2, LP). The nucleus begins to increase in size, and cytoplasmic volume decreases as meiotic cells progress during development. Chromatin fibers thicken further in zygotene cells (Fig. 2, ZY) and as the chromosomes condense, larger areas of nucleoplasm form in pachytene spermatocytes (Fig. 2, PA). The chromatin fibers finish condensation and reside just below the nuclear membrane in diplotene cells (Fig. 2, DI), and the nuclear membrane begins to degenerate. The fully condensed chromosomes line up at the metaphasal plate in metaphase I of meiosis (Fig. 2, M1). After completion of the first nuclear and cytokinesis events, the secondary spermatocytes (Fig. 2, SS) are much smaller in cell size than the preceding primary spermatocytes. The second meiotic division (Fig. 2, M2) follows the same general patterns as meiosis I with the chromosomal amount and cell size halved when compared to primary spermatocytes.

After completion of meiosis II the germ cells begin the stepwise differentiation into mature spermatozoa that can be broken into seven steps based on the terminology of Russell et al. (1990) for mammalian species. Step 1 spermatids (Fig. 2, S1) mark the beginning of spermiogenesis with a well-defined nucleus and the presence of an acrosomal vesicle. Step 2 spermatids (Fig. 2, S2) have acrosomal vesicles that make contact with the nuclei causing a slight indentation in the apex of each nucleus. During the Step 3 spermatid stage (Fig. 2, S3), the acrosomal granule becomes visible within the acrosomal vesicle near the nuclear membrane. The acrosomal vesicle is fully developed in Step 4 spermatids (Fig. 2, S4), and the germ cell begins elongation. Elongation continues as the acrosomal vesicle flattens the apex of the nucleus in Step 5 spermatids (Fig. 2, S5). During this time, the chromatin condenses, giving the nucleus a darker staining appearance. In Step 6 spermatids (Fig. 2, S6), the nucleus is almost homogenous and the flagellum is clearly visible. The nucleus is fully elongated and homogenous in Step 7 spermatids (Fig. 2, S7), giving the spermatid its filiform shape just prior to shedding into the lumen as a mature spermatozoon (Fig. 2, MS).

Fig. 1. Cross-section of the testis in Sceloporus consobrinus showing the seminiferous tubules (St) and surrounding tunica albuginea (Ta). Scale = 100 μm.

Fig. 2. Germ cell types found within the seminiferous epithelium of Sceloporus consobrinus. Spermatogonia A (SpA), Spermatogonia B (SpB), Pre-leptotene (PL), Leptotene (LP), Zygote (ZY), Pachytene (PA), Diplotote (DI), Meiosis I (M1), Secondary spermatocyte (SS), Meiosis II (M2), Step 1 spermatid (S1), Step 2 spermatid (S2), Step 3 spermatid (S3), Step 4 spermatid (S4), Step 5 spermatid (S5), Step 6 spermatid (S6), Step 7 spermatid (S7), mature spermatozoon (MS). Scale = 10 μm.
Sceloporus consobrinus Germ Cell Cycle.—The testes of *S. consobrinus* are spermatogenically active during March. Spermatogonial cells (Fig. 3A, Sp) are observed in close proximity to the basal lamina (Fig. 3A, black arrowhead). Meiotic cells (Fig. 3A, Me) and spermiogenic cells (Fig. 3A, Ss) are seen within the seminiferous epithelium. During April, spermatogonial cells (Fig. 3B, Sp) line the basement membrane and meiotic cells (Fig. 3B, Me), and spermiogenic cells (Fig. 3B, Ss) are found within the germinal epithelium. In May, spermatogonial cells (Fig. 3C, Sp) again are found juxtapositioned to the basement membrane (Fig. 3C, black arrowhead). During this time, meiotic cells (Fig. 3C, Me) make up the majority of the seminiferous epithelium although a few spermiogenic cells (Fig. 3C, Ss) are observed. Mature sperm (Fig. 3C, Ms) are found in the May seminiferous lumina from April spermatids, which have completed spermiogenesis and have been spermiated. June is characterized by an increase in elongating spermatids and by large amounts of mature spermatozoa (Fig. 3D, Ms) within the lumen. June is marked by increases in elongating spermatids and by large amounts of mature spermatozoa (Fig. 3D, Ms) within the lumen. The numbers of meiotic cells (Fig. 4A, Me) and spermiogenic cells (Fig. 4A, Ss) have decreased by July. Although mature spermatozoa (Fig. 4A, Ms) are still found within the lumen, the amount seems to have decreased substantially (though no statistical analyses were performed). During August (Fig. 4B) and September (Fig. 4C), spermatogonial cells (Fig. 4B, Sp) and a few remnant deteriorating germ cells are present within the germinal epithelium, and the lumen is completely absent or has decreased in size. In September, the number of spermatogonial cells filling the epithelium has increased indicating testicular recrudescence. By October, the majority of germ cells have entered the meiotic phase (Fig. 4D, Me); however, no mature spermatozoa are observed within the lumen. In November testes, individual variation of spermatogenic activity is observed between different specimens. Individuals caught on the same day exhibit differing stages within the seminiferous epithelium with one individual containing the early stages of meiotic cells (Fig. 5A, Me), one individual showing the later stages of meiotic cells (Fig. 5B, Me), and one individual that has even begun spermiogenesis (Fig. 5C, Ss).

**Seasonal Activity and Germ Cell Development Strategy.**—Spermatogenic recrudescence in *S. consobrinus* is marked by the beginning of spermatogonial proliferation in September (Fig. 6A, *S. consobrinus*). By November, spermiogenesis and spermatiation were observed in one individual. Spermatogenesis begins in fall and continues through midsummer of the following year. A short testicular quiescent period was observed in August.

---

Fig. 3. Cross-section of seminiferous tubules of *Sceloporus consobrinus* during A) March, B) April, C) May, and D) June. Germ cell types include spermatogonia (Sp), meiotic cells (Me), spermiogenic cells (Ss), and mature spermatozoa (Ms). Basal lamina (black arrowhead). Scale = 50 µm.
Although all germ cell types can be found within the seminiferous epithelium during the spermiogenically active months, a large prolific event is observed in the fall with the primary spermatocytes transforming to spermiogenic cells in the spring. Spermiogenesis is completed by June, and a large spermiation event is observed during June and July. This pattern of testicular cycle represents a temporal release of germ cells within an amniotically structured testis.

Sceloporus Reproductive Cycles.—Variation can be observed within the reproductive cycles of the Sceloporus genus (Fig. 6A,B top panel) with fall (Fig. 6A) and spring (Fig. 6B) cycles dominating (continuous is only observed in one species, S. bicanthalis; Hernández-Gallegos et al., 2002, and potentially a population of S. variabilis; Ramírez-Bautista et al., 2006a). However, factoring out individual variation (which was shown previously in this paper) and annual variation (James, 1991; Castilla et al., 1992) and accounting for arbitrary delineations for months (i.e., difference between 31 August and 1 September), and giving a ± 1-month variance (Fig. 6A; B middle panel), no monthly differences can be observed (Fig. 6A,B) within a species. However, three major trends appear within Sceloporus spermatogenic cycles: 1) fall spermatogenesis (Fig. 6A); 2) spring spermatogenesis (Fig. 6B); and 3) continuous spermatogenesis (not shown). The two populations of S. consobrinus (Missouri and Louisiana) exhibit fall spermatogenesis further suggesting spermatogenic cycles are fixed within a species.

Optimization onto the phylogenetic hypothesis proposed by Wiens and Reeder (1997) resolved the ancestral character states for Sceloporus as spring spermatogenic cycle, inhabiting tropical areas, and oviparous. The same holds true for the Leaché (2010) hypothesis except that spermatogenic cycle is unresolved. The phylogenetic hypothesis proposed by Wiens and Reeder (1997) resulted in optimizations for spermatogenic mode, region inhabited, and parity mode as more parsimonious with 7, 6, and 3 steps, respectively, compared to 7, 7, and 4 steps for Leaché (2010). From these results, it is clear that spermatogenic cycle provides little information in terms of evolutionary history of Sceloporus, because a fall spermatogenic cycle has evolved numerous times independently within Sceloporus on both the Wiens and Reeder (1997) and Leaché (2010) topologies.

Several species of Sceloporus have migrated (immigrated or extended) into temperate regions from tropical regions. From tree to tree comparison (compare Fig. 7A or D to Fig. 7B or E, respectively), it appears as though the fall spermatogenic cycle is linked to species that have moved into temperate regions. The only exceptions are Sceloporus jarrovi (spring and temperate), S.
**DISCUSSION**

The testicular organization of *S. consobrinus* is consistent with reports on amniotic vertebrates (i.e., seminiferous tubules lined with a germinal epithelium in which germ cells develop). Spermatogenesis begins in September with proliferation of the spermatogonia. Spermatogenesis is first observed in November although the majority of spermiogenesis and spermiation largely occurs in June and July (spermiation is observed prior to this and sperm are found in the testicular ducts as early as March; JLR, unpubl. data). This model of germ cell development, in which germ cells develop in tandem with one another and are released in large episodic events, is consistent with what has been observed in all reptiles studied to date and some primates (Luetjens et al., 2005; Gribbins et al., 2008; Wang et al., 2008; Rheubert et al., 2009b). This method differs from the typical mammalian model in which germ cells are arranged in developmental order along the peripheral axis of a seminiferous tubule (Russell et al., 1990).

Two predominate spermatogenic cycles can be observed within genus *Sceloporus*: a fall recrudescence or spring recrudescence (see Fig 6). Whether a spring or fall spermatogenic cycle is observed, all lizards exhibit prenuptial spermatogenesis (Aldridge et al., unpubl. data). Saint Girons (1982) used the term “mixed” spermatogenesis to describe the fall spermatogenic cycle. However, a review of the literature and terminology suggests this is a confusing and nondefinitive term. Angelini and Picariello (1975) used the term thermorigostatic (spermatogenesis with stasis during the warmest and coldest months of the year), as a more accurate description. However, neither this nor other terms proposed by Angelini and Picariello (1975) has been adopted; therefore, we use the terms spring and fall to describe recrudescence.

*Sceloporus consobrinus* from various latitudinal regions exhibit similar spermatogenic cycles. Although these cycles are indistinguishable from one another because of variation (see Fig. 6), data collected in southeastern Louisiana more closely resembles the Missouri population examined by McKinney and Marion (1985) and Matter (1987) with regression starting later in the year (July and August). This finding is interesting as Leaché (2009) found evidence (nuclear DNA) that *S. consobrinus* is paraphyletic, and this population shares a more recent common ancestor with *S. undulatus*. However, mitochondrial DNA supports *S. consobrinus* being monophyletic. Furthermore, this differs from the findings of Villagrán-Santa Cruz et al. (2009), who investigated populations of *S. miliaris* at different...
elevations and found that the timing of peak spermatogenesis differed (although recrudescence and quiescence did not). This suggests that correlations between spermatogenic variables and latitude (or potentially climate or elevation) at small (i.e., populational) scales vary and have implications for evolutionary constraints. This evolutionary restriction may have dramatic effects in terms of climate change as Sinervo et al. (2010) have predicted.

Although previous data suggest that spermatogenic cycles are influenced by certain abiotic factors, such influence may not hold true in nature. Previous research has shown that photoperiod (Licht, 1972; Marion, 1982), rainfall (Saint Girons, 1982), and temperature (Licht, 1966) can effect spermatogenic cycles in laboratory settings. However, populations inhabiting different areas with different climatic influences exhibit similar spermatogenic cycles. Furthermore, our present findings suggest that species inhabiting similar geographic regions (even in sympatry) exhibit differences in spermatogenic cycles. Our data suggest that spermatogenic cycles are plastic and have evolved multiple times (Fig. 7) with no single driving force.

All Sceloporus examined in this study that exhibit fall recrudescence are associated with higher latitudes, but this is not the case for spring cycles (all spring cycles are not always associated with lower latitudes). It may be that all cycles originated in the spring and then, for some species, evolved to fall cycles as more temperate regions were inhabited (e.g., Sceloporus orcutti, S. jarrovi, and Sceloporus magister). The evolution of extended spermatogenic cycles in more temperate areas seems evident in S. bicanthalis (which exhibits continuous spermatogenesis), because this species inhabits a temperate region within a tropical latitude (elevation 1,850–4,250 m). However, deeper investigations are needed to understand why all high-elevation species have not evolved a longer spermatogenic cycle. Tinkle (1969) suggested that the tropics provide long periods favorable for reproduction. However, if this were the case for spermatogenesis, why is the spermatogenic cycle extended in the temperate regions where conditions favorable for reproduction are not extended? Smith and Hall (1974) stated that cold-induced species have protracted reproductive cycles, which is clearly not the case with male spermatogenic cycles. It may be possible that spermatogenic cycles evolved to begin proliferation earlier such that mature spermatozoa are present for the first mating opportunities in the spring or that sexual selection pressures are more prominent in temperate species as suggested by Gribbins et al. (2011).

The notion that climate is the sole deciding factor in the timing of spermatogenesis seems obsolete in light of new data. Historical researchers such as Volsøe (1944), Licht (1966), and Angelini and Picariello (1975) have published works on spermatogenic cycles alluding to climate being the major driving force, and Licht (1972) went as far to say, “Of all the environmental factors, temperature appears to be the single most important and widespread.” However, these researchers lacked sample size and likely looked at extreme cases and found correlations that were not tested rigorously. With the accumulation of data over the past 30 years, more in-depth investiga-
Fig. 7. Character optimization of spermatogenic cycle, region inhabited, and parity mode across the molecular and morphological hypothesis proposed by Wiens and Reeder (1997) and the purely molecular hypothesis proposed by Leaché (2010).
tions can be used to falsify the assumption of climate being the major or only driving force for spermatogenesis. An in-depth analysis into a single genus or species (e.g., S. consobrinus) that occupies a variety of climates (although similar in respect to squamates as a whole) has shown that there are exceptions to this generalization.

It appears that, although an extreme change in a single abiotic factor can influence spermatogenesis, a suite of characters within the organism’s niche play a large role in the timing of spermatogenesis, similar to what Shine and Berry (1978) suggested for the evolution of viviparity. Even though “outliers” are observed showing exceptions to the generalizations, more data on various reproductive strategies (including female strategies for which data are lacking) may lead to results that could potentially give researchers insights into how life-history strategies are arranged and what mechanisms underlie variation in reproductive strategies.

Acknowledgments.—The authors thank C. Murray, J. Lee, and J. Babin for their assistance with collection of specimens. JML expresses gratitude to O. Hernandez-Gallegos for his input and discussions during the preparation of the manuscript. Collections and tissue preparation also received support from National Science Foundation grant DEB-0809831 to DMS and competitive research grants from Wittenberg University.

Literature Cited


McKinney, R. B., and K. R. Marion. 1985. Plasma androgens and their association with the reproductive cycle of the male fence lizard,


Accepted: 25 March 2013.