Zoology in the Middle East, 2016
http://dx.doi.org/10.1080/09397140.2017.1269396

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Male reproductive seasonality of the Snake-eyed Lizard, *Ophisops elegans* Ménétriés, 1832, from Lebanon (Reptilia: Lacertidae)

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(Received 7 July 2016; accepted 27 November 2016)

We studied the male reproductive cycle in a population of *Ophisops elegans* from Mount Sannine, Lebanon, by histological analysis. Testicular histology showed active spermatogenesis in spring, followed by a testicular regression at the end of summer and a subsequent recrudescence in autumn. Monthly variations in the epididymis, the ductus deferens and the sexual segment of the kidney were in synchrony with the testicular cycle. They were hypertrophied as spermatogenetic activity increased and atrophied as spermatogenetic activity decreased. Males of *O. elegans* showed a vernal type of spermatogenesis with a close relationship between the evolution of the seminiferous tubules and the secondary sexual characters.

Keywords: Spermiogenesis; epididymis; vas deferens; sexual segment of kidney; vernal cycle

Introduction

The Snake-eyed Lizard, *Ophisops elegans* Ménétriés, 1832 (Figure 4), is abundant and widespread in Lebanon (Hraoui-Bloquet, Sadek, Sindaco, & Venchi, 2002). Phylogenetic analyses showed that Lebanese populations belong to a subclade that comprises *O. elegans* lizards from South-East Turkey, Syria, and Jordan (Kyriazi et al., 2008). Previous studies on reproductive cycles of Mediterranean lacertids focused primarily on the variation in the testes and the epididymis of the male based on external morphology and histological examinations. In general, two types of spermatogenesis occur in Mediterranean lacertids. The first is a vernal type where spermatogenesis takes place mainly in spring after hibernation. This type occurs in some lizards from arid regions such as *Acanthodactylus* and *Mesalina* species (Bons & Saint Girons, 1982; Carretero, 2006). The second type is a mixed type spermatogenesis characterised by two periods of spermatogenetic activity, in spring-summer and in summer-autumn. Most mesic species such as *Podarcis, Psammodromus,* and *Phoenicolacerta* display this pattern with the presence of spermatids and spermatozoa in the seminiferous tubules in autumn and early winter (Angelini, Brizzi, & Barone, 1979; Carretero & Llorente, 1997; Hraoui-Bloquet, 1985, Rizk & Nassar, 2015). However, only the spring-summer activity serves reproductive purposes and males become fertile only when spermatozoa are released from the testis and pass to the epididymis (Depeiges & Dufaure, 1981; Depeiges & Dacheux, 1985; Carretero, Ribeiro, Barbosa, Sá-Sousa, & Harris, 2006). Several studies have investigated the seasonal variations in the epididymis (Saint Girons, 1963; Joly & Saint Girons, 1975; Dufaure & Saint Girons, 1984). Few studies exist on the ductus deferens and the sexual segment of the kidney in Lacertidae. In this paper, we studied the male

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reproductive seasonality of a population of *O. elegans* from Lebanon and determined its reproductive cycle based on histological examinations of gonadal stages and the evolution of the testicular ducts and the sexual segment of the kidney.

Material and Methods

Our sample consisted of 31 males of *O. elegans* collected from April to October 1985 in Mahrouka (33°56'N, 35°50'E; at around 2000 m asl), Mount Sannine, Lebanon. At this altitude, *O. elegans* hibernates from the beginning of November to the end of March. Snout-vent Length (SVL) of each specimen was measured immediately after capture with a caliper to the nearest 0.01 mm. Lizards were anaesthetised, dissected and male reproductive organs (right testis with attached epididymis, the ductus deferens and the right kidney) were excised and fixed in Bouin's solution. They were dehydrated in increasing concentrations of ethanol and kept in butanol until paraffin embedding using standard protocol. They were sectioned with a rotary microtome at 5 µm and stained using Meyer's hematoxylin and eosin. For each male, we recorded its reproductive condition according to three stages. (i) Spermiogenesis, spermatozoa line the lumina of the seminiferous tubules; several rows of metamorphosing spermatids are present. (ii) Regression, seminiferous tubules contain spermatogonia and Sertoli cells. (iii) Recrudescence, marked by a proliferation of spermatogonia and the presence of primary spermatocytes. The smallest reproductively active male was determined by spermiogenesis in progress. Epididymis and ductus deferens slides were examined for the presence of sperm in their lumen. The development of the sexual segment of the kidney was determined according to the criteria of Saint Girons (1972). For each slide, at least five measurements of the diameter of the seminiferous tubules, the diameters and epithelial heights of the epididymis, ductus deferens and the sexual segment of the kidney were recorded with a calibrated ocular micrometer.

We used the statistical software SPSS 20.0® in statistical analyses. All variables were log-transformed and tested for normality. Regression residuals between all variables and SVL were calculated to standardise for size.

Results

The smallest reproductive male measured 43 mm SVL and was collected in May, and therefore only males with SVL ≥ 43 mm in SVL were considered to be adults. The mean SVL was 48.87±2.86 mm, range 43–54 mm. The hibernation period of *O. elegans* lasts about 5 months, from November to the end of March. The testes showed active spermatogenesis in all histological sections between April and July (Figure 1). During this period, seminiferous tubule diameters were at their maximum. Monthly variations of the diameter of the seminiferous tubules (ANCOVA $F_{(6, 39)}$=1041, p<0.001) reflected the changes in the germinal epithelium, with the largest diameters being from April to July with active spermiogenesis. There was a testicular regression in August and September associated with a significant decrease in the seminiferous tubule diameter (Figure 2). Their epithelia were formed essentially by spermatogonia and Sertoli cells. October showed a recrudescence period with the presence of spermatogonia and abundant spermatocytes in October. Seasonal variations in diameter and epithelial height of the epididymis (ANCOVA $F_{(6, 39)}$=255, p<0.001 for diameter and ANCOVA $F_{(6, 39)}$=103.58, p<0.001 for epithelial height) and the ductus deferens (ANCOVA $F_{(6, 39)}$=556.20, p<0.001 for diameter and ANCOVA $F_{(6, 39)}$=20.40, p<0.001 for epithelial height) were found throughout the reproductive period. The sexual segment of the kidney also varied seasonally (ANCOVA $F_{(6, 39)}$=240.35, p<0.001 for diameter and ANCOVA $F_{(6, 39)}$=55.32, p<0.001 for epithelial height). The diameters and epithelial heights of the ductus epididymis, the ductus deferens and the sexual segment of the kidney varied monthly in synchrony with the testicular cycle. They were hypertrophied during the

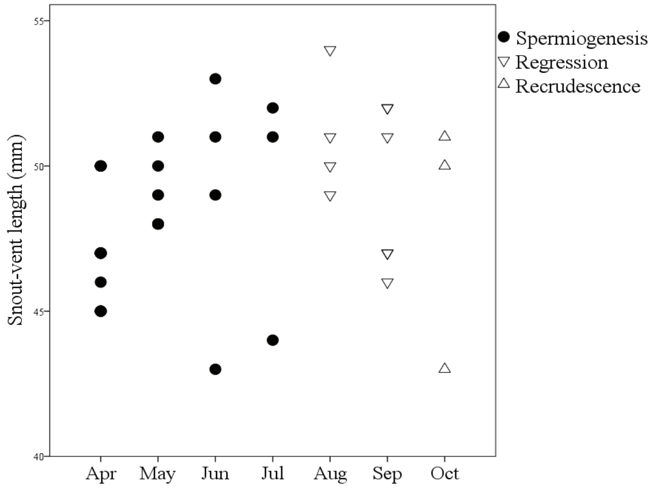

Figure 1. Male reproductive cycle of *Ophisops elegans* in Lebanon.

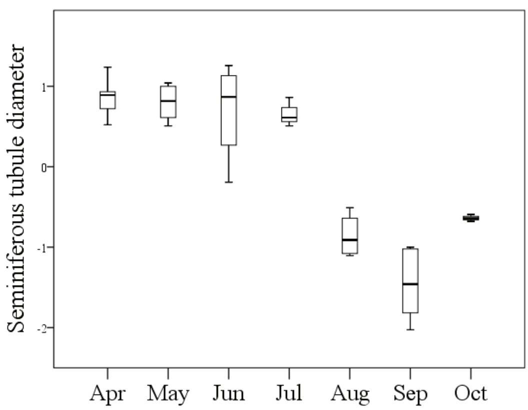

Figure 2. Monthly variation of the seminiferous tubules diameter. The diameters of the seminiferous tubules have been standardised to the lizard size using regression residuals of log-transformed variables. Box lengths represent the interquartile range; Horizontal lines within the boxes represent the median values and the whiskers represent the smallest and largest sample values (minimum - maximum).

active period from April to July and atrophied during the rest of the reproductive season. High values of the epididymal and deferens tubule diameters were observed from April to July (Scheffé post hoc test, $P < 0.05$). Their lumen was filled with sperm and secretion granules. Both tubules underwent a decrease in diameter during the inactive period of reproduction from August to October (Figure 3A, C). Significant differences were found in epithelial heights of epididymis between the active and inactive period (Scheffé post hoc test, $P<0.05$), but no significant differences were found in the epithelial heights of the deferens tubules (Scheffé post hoc test, $P>0.05$) (Figure 3B-D). Measurements of the sexual segment of the kidney revealed significant differences between the active and inactive period (Scheffé post hoc test, $P<0.001$). They were hypertrophied with secretory active epithelial cells during the active period and completely regressed and mucous during the inactive period (Figure 3E-F).

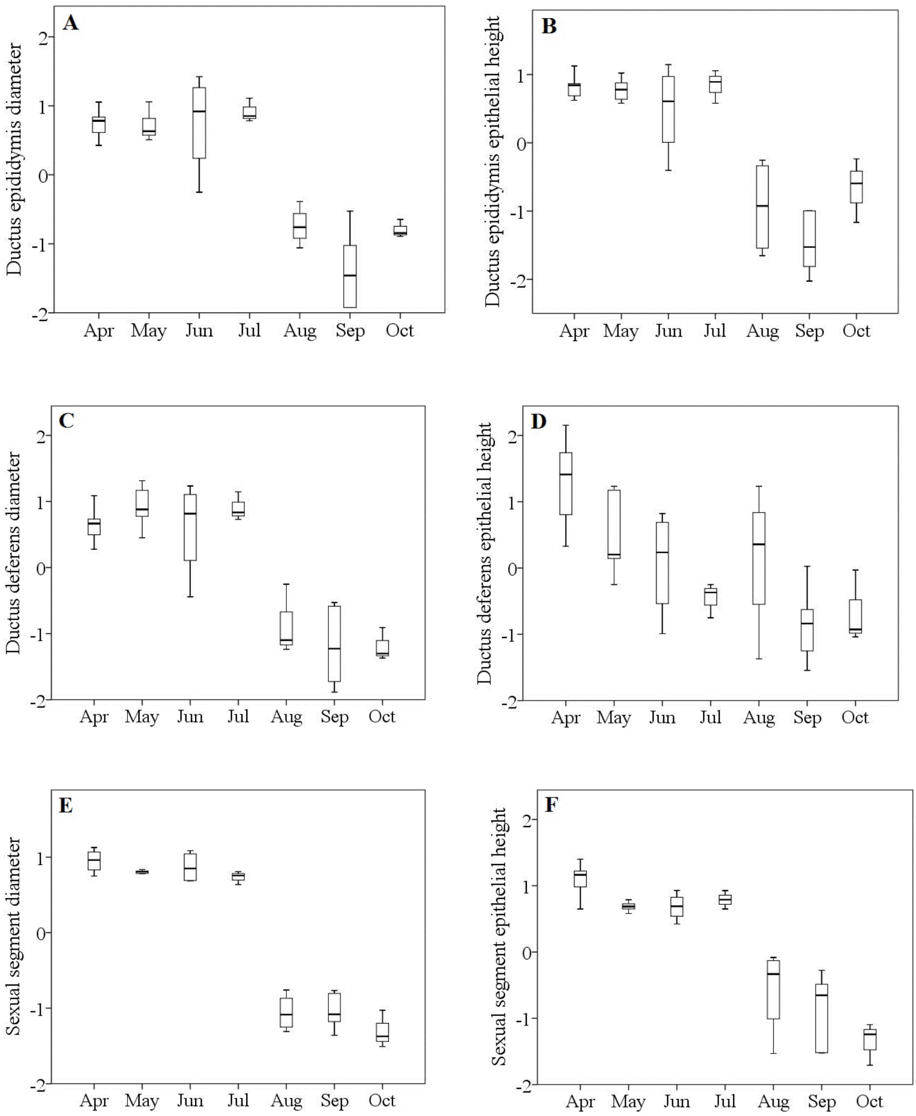

Figure 3. Monthly variation of the diameter and epithelial height of ductus epididymis (A, B), ductus deferens (C, D), and sexual segment of the kidney (E, F), in males of *O. elegans*. Y axes show the regression residuals between the diameters and epithelial heights of the epididymis, ductus deferens and the sexual segment of the kidney and the SVL (both log-transformed). Box lengths represent the interquartile range; horizontal lines within the boxes represent the median values and the whiskers represent the smallest and largest sample values (minimum - maximum).

Discussion

Males of *Ophisops elegans* showed a seasonal testicular cycle with a vernal type spermatogenesis. Seasonal variation in reproduction is common among lizards inhabiting seasonal climates (Saint Girons, 1984; Carretero, 2006). Environmental variations and/or genetic factors modify the pattern of spermatogenesis by influencing the timing

Figure 4. Snake-eyed Lizard, *Ophisops elegans*, in Lebanon (Mount Sannine at around 2000 m).

of the reproductive activity (Carretero, 2006). Our studied population begins its cycle one month later than in Iran, but both populations enter hibernation in the same month (Torki, 2007). The sympatric lizard *Trachylepis vittata* begins and ends its male cycle one month earlier than that of *O. elegans*, and the time duration of the male cycle of *Lacerta media* is identical to *O. elegans* males (Hraoui-Bloquet, Sadek, & Sabeh, 1999; Nassar & Hraoui-Bloquet, 2014). The beginning of spermiogenesis occurs in autumn in two other sympatric lizards, *Phoenicolacerta laevis* (Hraoui-Bloquet, 1985) and *Phoenicolacerta kulzeri* (Rizk & Nassar, 2015). The timing of reproductive activity was reported to vary between different species (Hraoui-Bloquet, 1985; Pérez-Quintero, 1996; Hraoui-Bloquet et al., 1999; Carretero et al., 2006) and between different populations of a species (Carretero & Llorente, 1997; Roig, Carretero, & Llorente, 2000). By contrast, reproductive and ecological characteristics may be similar between different lacertid lizards (Pérez-Mellado, Valakos, Guerrero, & Gil-Costa, 1993).

Monthly variations in the testicular ducts were closely associated with spermatogenic activity of the testis. As long as spermatozoa were released within the testicular ducts, they remained enlarged during all the active reproductive period. Their diameter attained the highest values. They became small as spermatogenic activity ceased in the testis. Males of *O. elegans* showed a pseudostratified epithelium of their epididymides with large secretory granules at their apical surface in the active reproductive period. The ultrastructural description of the epididymis in *Zootoca vivipara* revealed the presence of two cell types, the secretory principal cells and basal cells (Depeiges & Dufaure, 1981; Depeiges & Dacheux, 1985). Secretory granules of the principal cells are large in the active reproductive period, and occupied the apical cytoplasm of the principal cells

(Depeiges & Dufaure, 1981; Mesure, Chevalier, Depeiges, Faure, & Dufaure, 1991). According to a classification based on the presence and the diameter of the secretory granules in squamates, Dufaure and Saint Girons (1984) reported that only type 1 secretory granules (ranging in size from 10 µm to 12 µm) were found in Lacertidae. The contents of secretory granules were discharged into the lumen of the epididymis where they mixed with sperm and contributed to the maturation of spermatozoa (Depeiges & Dufaure, 1981; Dufaure & Saint Girons, *1984)*. However, variations in the epithelium height of the ductus deferens were not significant. This might be explained by the fact that secretory granules were too small in the epithelial cells of the ductus deferens and did not affect the stratification of the epithelium. In addition to the simultaneous changes in the testes and in the testicular ducts that occur during the reproductive season of *O. elegans*, important modifications occur also in synchrony in the sexual segment of the kidney. The tubules become enlarged and produce granular secretion in the active reproductive period. The granular secretory activity switched to mucoidal secretion during the inactive reproductive period. An asynchronous switch at an individual level was reported in *Podarcis muralis* where some males showed involution of their seminiferous tubules with hypertrophied secondary sexual characters (Joly & Saint Girons; 1975). Our results showed a regression of secondary sexual characters in *O. elegans* at the same time in the seminiferous tubules and vice versa, a close relationship between the evolution of the seminiferous tubules and the secondary sexual characters.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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