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Research Article

THE SPERMATOGENIC CYCLE OF THE GREEN-BELLIED LIZARD, DAREVSKIA CHLOROGASTER (SAURIA: LACERTIDAE) IN NORTHERN IRAN

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ABSTRACT
Spermatogenic cycle in the Green-bellied lizard, *Darevskia chlorogaster*, was studied in forests of Sari County in Mazandaran Province, northern Iran. Sampling took place periodically every 15 days during the activity period of this species, from 5 April to 20 October 2012. In total, 59 adult males were captured by hand. Testes were removed and processed for histological and morphometric studies. The results show that testes consist of 74.45 seminiferous tubules in average. The mean diameters of seminiferous tubules, lumen, tunica albuginea and germinal layer are 350.14, 162, 17.69 and 64.10 microns, respectively. Spermatogenesis begins in mid March to early April, reaching its peak in late May and early June, and ends between early to mid August. The maximum level of sperm production occurred in early June. The minimum diameter, weight and volume of testes were observed in early August. Four phases were observed during the spermatogenesis of this species: active, transitional, inactive and regenerative. Spermatogenesis of *D. chlorogaster* in Iran is seasonal and the type of spermatogenic cycle is associated.

Keywords: *Spermatogenesis, Lizard, Lacertidae, Darevskia chlorogaster, Iran*

INTRODUCTION

Lizards show two type of spermatogenesis: continuous (aseasonal) and alternate (seasonal). In the continuous type, spermatogenesis is year-round and spermatozoa are found in the lumen of the seminiferous tubules all year. In contrast, the alternate type of spermatogenesis occurs during a well defined period in which spermatozoa are not found in the lumen of the seminiferous tubules (Castilla and Bauwens, 1990; Torki and Gharzi, 2008). Continuous spermatogenesis occurs in tropical regions (Hernandez-Gallegos, 2002). Alternate spermatogenesis occurs in non-tropical regions, especially in temperate zones (Castilla and Bauwens, 1990). In temperate-zones, the male testicular cycle is divided into two well-defined phases as follows: (a) the *regenerative phase* that occurs in the spring and is characterized by sustained sperm production, and (b) the *degenerative phase*, which begins in late summer, when a break in spermatogenesis is observed (Castilla and Bauwens, 1990). Likewise, tropical species in seasonal habitats also display, if less pronounced, a regenerative phase during the wet (reproductive) season.

The spermatogenesis of some lizards in the Iranian plateau, especially in the Zagros Mountains and the west of Iran, has been described and shows alternating spermatogenesis (Heidari and Gharzi, 2006; Gharzi and Torki, 2006a, 2006b; Rastegar-Pouyani and Torki, 2007; Torki, 2006, 2007a, 2007b; Torki and Rastegar-Pouyani, 2006; Torki and Gharzi, 2008).

The Green-bellied lizard, *Darevskia chlorogaster* (Boulenger 1908), has been distributed from southern and southwestern coasts of the Caspian Sea in Iran and Azerbaijan Republic. It is found in Hyrcanian forest on the lower slops of the Alborz Mountains and in the Kopet Dagh (Anderson, 1999). It occurs east as far as the Atrak River of northern Khorasan, from slightly below sea level on the Caspian coast to 900-1500 m on the Atrak (Anderson, 1999).

D. chlorogaster is a forest species; easily ascending tree trunks and rock walls to exploit patches of sunlight in shadowy areas they inhibit (Mertens, 1957). This species is a characteristic species of forests of lowlands and low mountain belt to 600-70 m elevation. The vegetation of the area was characterized by

lianas, grapes, ivy, giant ferns, and trees such as *Parrotia persica*, *Quercus castaneaefolia*, *Acer insigne*, *Ficus carica*, *Gleditschia caspia*, and *Albizzia julibrissin* (Sobolevsky, 1929).
D. chlorogaster have not been studied yet and here we describe the spermatogenic cycle of *D. chlorogaster* in northern Iran for the first time.

MATERIALS AND METHODS

Study area: The locality was Zaree Forest (54˚ 7 E, 36˚ 32 N), in Mazandaran Province in northern Iran, located in southern coasts of Caspian Sea. They were on logs and tree trunks of the forest floor. The climate of this area is wet and temperate (between 1.6˚C and 22.5˚C).
Sampling: All specimens were collected periodically every 15 days during the activity period of this species from 5 April to 20 October 2012. *D. chlorogaster* is a diurnal lizard (Figure 1) and sampling occurred during day light from 8:00 AM to 14:00 PM. In total, 59 sexually mature males were captured by hand, usually from forest.

Figure 1: *D. chlorogaster* in Zaree Forest in Mazandaran Province of Iran *(Photos by A. Choopani)*

Methods: The animals were treated in accordance with the guidelines of the local ethics committee. Collecting permits and legal requirements were prepared by Department of Environment of Mazandaran Province of Iran (letter number: 13505). The specimens were transferred alive to the zoology laboratory of Islamic Azad University, Damghan Branch, Damghan, Iran. The following measurements were taken: SVL (snoutvent length), TL (tail length), HL (head length), RTL (length of right testis), LTL (length of left testis), RTW (width of right testis), LTW (width of left testis), RTWe (weight of right testis), LTWe (weight of left testis), RTV (volume of right testis), LTV (volume of left testis), RHL (length of right hemipenis), LHL (length of left hemipenis), RHW (width of right hemipenis), LHW (width of left hemipenis), TD (diameter of tunica albuginea), SVN (number of seminal vesicles), SVD (diameter of seminal vesicle), GLD (diameter of germinal layer), LD (diameter of lumen), SgN (number of spermatogonia), SpN (number of spermatocytes), StN (number of spermatids), SN (number of sperms), and gonadal index (GI = gonadal weight/body weight × 100). Length, width and diameter were measured by dial caliper with an accuracy of 0.02 mm. Weight was measured by scale with an accuracy of 0.001 g. Volume of the testis, was calculated by using the formula for the volume of an ellipsoid: $4/3\ \pi$ ab2, where a = ½ the longest axis, and b = ½ the shortest axis. Gonads, once removed, were measured for metric and meristic studies. The weight, diameter (length and width) and volume were measured for the right and left testis separately. Since there was no significant difference between the macroscopic characters of the left

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and right testis we only used measurements for left testes. After fixing the testes in 10% formalin, tissues were dehydrated, cleared in xylene, infiltrated with paraffin, embedded and sectioned and stained with haematoxylin and eosin following standard histological protocols. The sections were studied by light microscopy at 100× and 400× magnification. Photographs were prepared by digital camera. Data were analyzed by SPSS 17 software, ANOVA and paired t-test ($P > 0.05$).

RESULTS

The mean body weight, SVL, LCD and HL of males were 4.386 g, 58.24 mm, 114.28 mm and 14.24 mm, respectively. The smallest SVL was 51.70 mm and the largest one was 65.80 mm (range = 14.10 and SD = 2.863).

Testes are oval-shaped and white. They consist of 40-90 seminiferous tubules surrounded by the tunica albuginea. Thin connective tissue septa from tunica as well as fibrocytes are found between the tubules.

The descriptive statistics of characters in *D. chlorogaster* are shown in Table 1. The mean diameter of the germinal layer is 64.10 microns and changes from 107 micron in early April to 22 micron in early August. The minimum diameter, weight and volume of testes were observed on late July.

Table 1: Descriptive statistics of characters in *D. chlorogaster*

	n	Minimum	Maximum	Mean		Std.	Variance
characters				Statistic	Std. Error	Deviation	
HV	59	7.18	9.85	8.8836	0.09206	0.70710	0.500
Weight	59	3.056	6.128	4.38668	0.095420	0.732936	0.537
SVL	59	51.70	65.80	58.2415	0.37283	2.86378	8.201
LCD	59	82.76	144.25	114.280	2.01304	15.46249	239.089
HL	59	12.11	16.35	14.2446	0.10712	0.82278	0.677
RTL	59	2.63	5.06	3.8815	0.08449	0.64899	0.421
LTL	59	2.27	5.45	3.7278	0.09011	0.69213	0.479
RTW	59	1.43	3.83	2.6994	0.07088	0.54443	0.296
LTW	59	1.78	4.28	2.7271	0.07563	0.58095	0.338
RTWe	59	0.004	0.039	0.02036	0.001160	0.008909	0.000
LTWe	59	0.004	0.040	0.01968	0.001212	0.009312	0.000
RTV	59	3.30	36.60	16.2598	1.07083	8.22518	67.654
LTV	59	3.73	51.18	16.2825	1.24456	9.55964	91.387
RHL	59	2.25	5.63	3.4993	0.09727	0.74718	0.558
LHL	59	2.18	6.66	3.5092	0.11083	0.85129	0.725
RHW	59	0.96	2.82	1.6931	0.05467	0.41994	0.176
LHW	59	0.85	2.78	1.6810	0.05841	0.44866	0.201
GI	59	0.24	1.63	0.9120	0.04420	0.33950	0.115
TD	59	10.00	30.00	17.6949	0.59525	4.57223	20.905
SVN	59	44.00	91.00	74.4576	1.78806	13.73433	188.632
SVD	59	235.00	422.00	350.14	6.34197	48.41361	2373.0
GLD	59	22.00	107.00	64.1017	2.24971	17.28034	298.610
LD	59	96.00	245.00	162.00	5.04075	38.71870	1499.0
SgN	59	38.00	201.00	101.44	3.82036	29.34472	861.113
SpN	59	75.00	322.00	208.27	6.99281	53.71278	2885.0
StN	59	36.00	206.00	113.36	3.82070	29.34736	861.268
SN	59	0.00	265.00	123.9153	13.73487	105.49952	11130.148

The Sertoli cells, spermatogonia and spermatocytes are observed in the germinal layer of the seminiferous tubules after hibernation from March to October. The spermatids are also observed in the germinal layer of the seminiferous tubules from March to October but their number is reduced markedly after August. The spermatozoids are observed in the lumen of tubules from April to mid August and there are no spermatozoids after mid August. Interstitial cells occur in all months. Large numbers of mature sperms are present in the tubules throughout spring (Figure 2). In July, seminiferous epithelial heights and tubule diameters increase. Spermatids become abundant during late May. Early spermiogenesis begins in late March and accelerates during this period. Spermiogenesis continues from April to August. Tubule diameters and epithelial heights reach maximum sizes during this period. By mid August the germinal epithelium has been reduced. Early regression begins in late July and early September with the germinal layer exhausted and reduced to one to three layers, which are situated in the connective tissue between the seminiferous tubules containing numerous capillaries. Sertoli cells generally have one or two nucleoli and are restricted to the outer one or two rows of cells during spermiogenesis. During the non-reproductive season, spermatogonia, spermatocytes and spermatids are present in the tubules, but mature sperm are not observed (Figure 3).

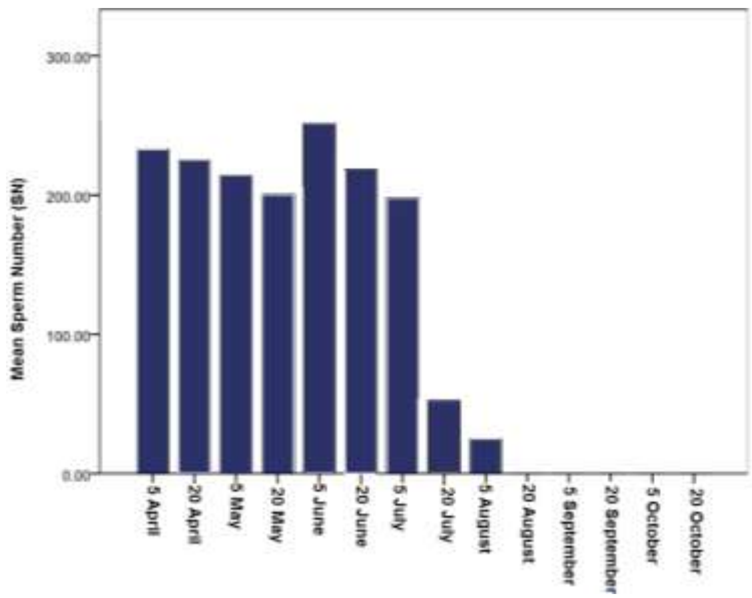

Figure 2: Mean of sperm numbers of *D. chlorogaster* from April to October 2012

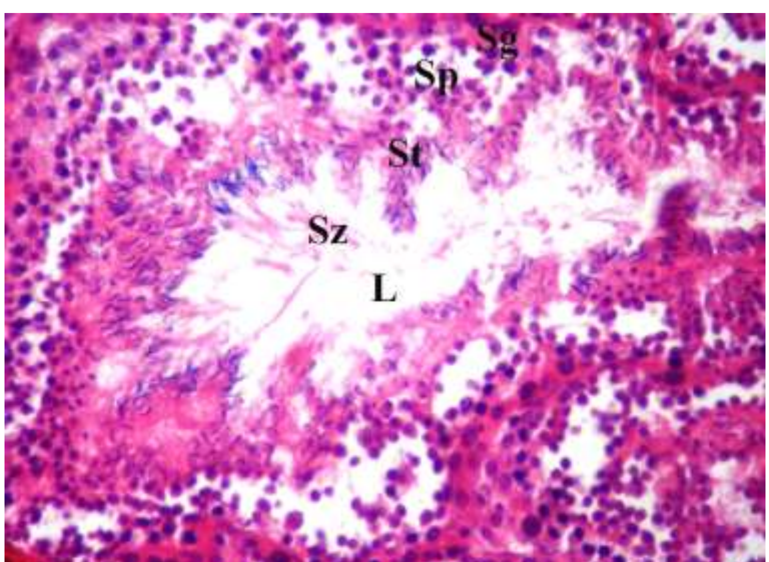

Figure 3: The active phase of spermatogenesis in *D. chlorogaster* (5 June 2012). L (lumen), Sg (spermatogonia), Sp (spermatocytes), St (spermatids) and Sz (spermatozoa) *(Photos by A. Choopani)*

The maximum amount of gonadal index (GI) was observed in late April and decreased from late July to mid August because the body and testis weight were reduced as a result of the high reproductive activity in spring. The germinal layer diameter of seminiferous tubules is reduced from April to August, which demonstrates the active phase of spermatogenesis and differentiation of the germinal cells to the sperms. The germinal layer diameter is increased from September to October, which shows the regenerative phase. The maximum level of sperm production is on early June.

GI was increased from early September to late October because the body and testes weights increased on account of fat storage and the lack of reproductive activity.

Testicular histology shows that spermatogenesis begins in early April, reaching its peak in late May and early June, and ends between early to mid August.

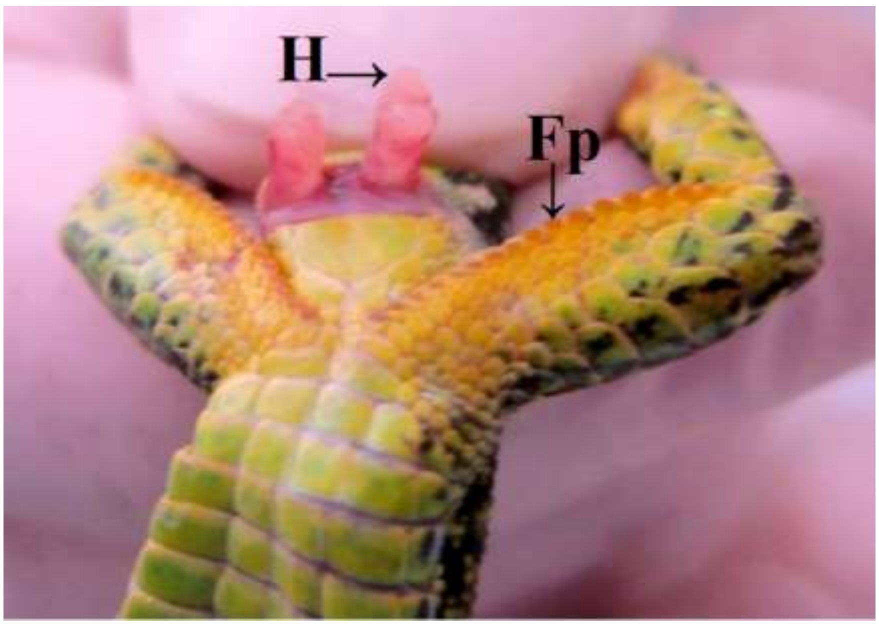

Figure 4: Hemipenis (H) and femoral pores (Fp) in *D. chlorogaster* (Photos by A. Choopani)

One pair of white or light pink hemipenes are located at the base of the cloaca (Figure 4). Hemipenes are characterized by a more or less club-shaped trunk and pedicel portion as opposed to a voluminous apex. The apex is subdivided into two lobes. Their mean length of right and left hemipenes are 3.49 and 3.50 mm, and the mean width of right and left one are 1.69 and 1.68 mm, respectively. There are no significant differences in the testis and hemipenis characters between the left and right side of body (paired t-test, $P > 0.05$ in all cases).

DISCUSSION

Based on a statistical and histological study, we have defined four phases during the activity period in *D. chlorogaster*, active, transitional, inactive and regenerative phases. In the active phase, sperms are found in the lumen of the seminiferous tubules, and this phase occurred during and after the post-hibernation period from March to early August. In the transitional phase, sperms in the lumen of the seminiferous tubules are found in some specimens but not in others, and this phase occurred in August. In the inactive phase, the lumen of the seminiferous tubules in all specimens is without spermatozoa, and this phase occurs during pre-hibernation and extends from late August to October. In the regenerative phase, the diameter of germinal layer increases and extends from late October to late February.

Based on the timing of spermatogenesis activity and body length, three types of activity in the timing of spermatogenesis were observed in the lizards of Iran, as follows: (a) early active spermatogenesis that occurred in the smallest body length; (b) late spermatogenesis that occurred in the greatest body length;

and (c) normal active spermatogenesis that occurred in medium body length (Torki and Rastegar-Pouyani, 2006; Rastegar-Pouyani and Torki, 2010).

Based on their studies, two phases of testicular activity were observed during hibernation, as follows: (1) a relatively silent phase occurred during early hibernation, during which testicular tissue slightly increased, and (2) an activation phase occurred in the late hibernation period during which the testicular tissue greatly increased. Testicular activation in temperate regions starts during the late hibernation period, and this period has an important role in renewing the testicular tissue in hibernating lizards (Rastegar-Pouyani and Torki, 2010).

Studies have also shown that there is no significant difference between the volume of the left and right testis for any species (Todd, 2008).

Anderson (1999) has been recorded SVL= 72 mm for this species but our record for the largest one was 65.80 mm.

However, the timing of spermatogenesis is many lizards are different, but the histological structure in these lizards is similar (Gharzi and Torki, 2006a). Nevertheless, in many lizards the timing of spermatogenesis activity took place following climate conditions (Duvall *et al.*, 1982; Fitch, 1970). Climate conditions and geographic position are the two main factors that strongly regulate spermatogenesis timing in lizards.

On the other hand, geographic variation in the timing of spermatogenesis activity can occur due to a climate gradient or latitude gradient (Gharzi and Torki, 2006b).

The results show that the spermatogenesis of *D. chlorogaster* is seasonal and the type of spermatogenic cycle is associated similar to the other reptiles of this region such as *Cyrtopodion caspium* (Hojati *et al.*, 2013) and *Natrix natrix* (Faghiri *et al.*, 2011) that have been previously studied.

REFERENCES

Anderson SC (1999). The lizards of Iran. Society for the Study of Amphibians and Reptiles. Ithaca, New York.

Castilla AM and Bauwens D (1990). Reproductive and fat body cycles of the lizard *Lacerta lepida* in Central Spain. *Journal of Herpetology* **24** 261–266.

Duvall D, Guillette LJ and Jones RE (1982). Environmental control of reptilian reproductive cycles. In: *Biology of Reptiles* edited by Gans C and Plough H (New York: Academic Press) **13** 201–231.

Faghiri A, Shiravi A, Hojati V and Kami HG (2011). Observation on the spermatogenic cycle of the Grass Snake, *Natrix natrix* (Serpentes: Colubridae) in Northern Iran. *Asian Herpetological Research* **2** 55–59.

Fitch HS (1970). Reproductive cycles in lizards and snakes. Miscellaneous Publication of the Museum of Natural History, University of Kansas, 52: 1–247.

Gharzi A and Torki F (2006a). A histological description of testicular tissue in lizards of central Zagros. *Proceedings of the 2nd International Conference of Biology, Tarbiat Modares University, Tehran, Iran,* 194.

Gharzi A and Torki F (2006b). The effects of climate and altitude on reproductive cycle of lizards. *Proceedings of the 2nd International Conference of Biology, Tarbiat Modares University, Tehran, Iran* 192.

Heidari N and Gharzi A (2006). A study of spermatogenesis in two sympatric lizards of *Laudakia* genus (Sauria: Agamidae) in western Iranian Plateau. *Proceedings of the 2nd International Conference of Biology, Tarbiat Modares University, Tehran, Iran* 193.

Hojati V, Parivar K, Rastegar-Pouyani E and Shiravi A (2013). Observations on the spermatogenic cycle of the Caspian Bent-toed Gecko, *Cyrtopodion caspium*, in Iran (Sauria: Gekkonidae). *Zoology in the Middle East* **59** 20–29.

Mertens R (1956). Amphibien und Reptilien aus S.O. Iran. 1954, *Jahreshefte des Vereins für vaterländische Naturkunde in Württemberg* **111** 90–97.

Rastegar-Pouyani N and Torki F (2007). Spermatogenesis without spermiogenesis in *Laudakia caucasia* (Reptilia: Agamidae). *Iranian Journal of Animal Biosystematics* **3**(1) 37–42.

Rastegar-Pouyani N and Torki F (2010). The role of hibernation on testicular cycle and testicular activation during dormancy in nature in hibernating lizards. *Russian Journal of Herpetolgy* **17** 251–254.

Sobolevsky NI (1929). The herpetofauna of Talysh and Lenkoran lowland (zoogeographical monography). *Mem. Zool. Dep. Soc. Natur.* Issue 5 [in Russian].

Todd AC (2008). Using testis size to predict the mating systems of New Zealand geckos. *New Zealand Journal of Zoology* **35** 103–114.

Torki F (2006). Spermatogenesis of the agama *Trapelus lessonae* in the central Zagros Mountains, Iran, *Zoology in the Middle East* **38** 21–28.

Torki F (2007a). The role of hibernation on testicular cycle and testicular activation during dormancy in the hibernated lizard *Trapelus lessonae* (Reptilia: Agamidae) in nature. *Salamandra* **43** 245–248.

Torki F (2007b). Reproduction of the snake-eye lizard, *Ophisops elegans* Ménétriés, 1832 in western Iran (Squamata: Sauria: Lacertidae). *Herpetozoa* **20** 57–66.

Torki F and Gharzi A (2008). Spermatogenesis timing in a population of *Ophisops elegans* (Sauria: Lacertidae), Western Iran. *Asiatic Herpetological Research* **11** 132–135.

Torki F and Rastegar-Pouyani N (2006). Relation between body size and spermatogenesis timing in hibernate lizards. *Proceedings of the 2nd International Conference of Biology, Tarbiat Modares University, Tehran, Iran* 190.