Influence of climatic gradient on spermatogenesis timing of *Trapelus lessonae* (Sauria, Agamidae) in the Zagros Mountains, Iran

FARHANG TORKI

FTEHCR (Farhang Torki Ecology and Herpetology Center for Research), 68319-16589, P.O. Box 68315-139, Nourabad City, Lorestan Province, Iran. torkifarhang@yahoo.com

ABSTRACT - Spermatogenesis is a complicated process with various factors that influence and control it. I collected a number of male specimens of *Trapelus lessonae* in three latitudes (during biological activity) that were different in climate. I removed testes for histological survey. H and E staining techniques were used. The results of screening showed three phases of spermatogenesis during biological activity for three different latitudes. Spermatogenesis timing differed in the three latitudes. Timing of spermatogenesis differed in low elevation populations and began earlier than in higher elevation populations.

SPERMATOGENESIS is a complicated process with a range of factors that influence it. Molecular research indicates that spermatogenesis, and its associated programme for controlling gene expression, commences during changes from the germinal layer into spermatozoid (De Kretser, 1993; Sarge et al., 1995) and these influences vary across taxa (Phillips et al., 1987). Factors that affect the process can be divided into two groups - exogenous and endogenous. Both of these have a direct and indirect affect on spermatogenesis (Culler & Culler, 1977; Duvall et al., 1982; Licht, 1984). Examples of exogenous factors that can be named as environmental factors include temperature (Bona-Gallo & Licht, 1983; Gavaud, 1991), photoperiod (Mendoca & Licht, 1986; Whittier et al., 1987; Beaupre et al., 1997), dryness and moisture of environment (Shine, 1985; Vitt & Congdon, 1978). Examples of endogenous effects include steroidal hormones, nervous system (e.g., Tokarz et al., 1998; Rhen & Crews, 2002) and fat resource (Diaz et al., 1994; Castilla & Bauwens, 1990; Torki, 2006, 2007a,b). There are likely to be other factors that also affect spermatogenesis that are still to be discovered (Phillips et al., 1987).

In temperate-zone lizards with reproductive cycles influenced by climatic season, the male testicular cycle is divided into two well-defined phases: (a) the regenerative phase that occurs in the spring and is characterised by sustained sperm production, and (b) the degenerative phase that begins in late summer, where a break in spermatogenesis is observed (Fitch, 1970; Lofts, 1987; Castilla & Bauwens, 1990). Many studies have shown the effects of latitude on the reproductive ecology of animals (Rising, 1987; Young, 1994; Armbruster et al., 2001).

This study investigated the influence of climate gradient as an exogenous factor on spermatogenesis of *Trapelus lessonae* (Sauria, Agamidae) from the western Iranian plateau, on the western slope of the Zagros Mountains. The study site was located in Lorestan province between mid-Zagros (northern and eastern Lorestan) and southern Zagros (southern Lorestan).

Based on its climate Lorestan province is aptly named ‘little Iran’ (Torki, 2010), and has three different climates across three latitudes. These climates are as follows: (1) cool-temperate in northern Lorestan, geographic position approximately 34°05’N, 47°55’E, 1900 m ASL, 466.4 mm annual rainfall, (2) temperate climate in mid-Lorestan, geographic position approximately 33°29’N, 48°22’E, 1100 m ASL, 363.5 mm annual rainfall, (3) warm temperate in southern Lorestan, geographic position approximately 33°13’N, 47°49’E, 650 m and 337.4 mm annual rainfall.

Based on personal observations, biological activity of lizards in these three latitudes was crudely divided as follows; in the northern latitude, lizards hibernate for five months, from October to February (Torki, 2006, 2007b), in the mid-latitude, lizards hibernate for three months, from November to January, and finally in the southern latitude, lizards hibernate for two months, from December to January.

MATERIALS AND METHODS

Male specimens of *Trapelus lessonae* were collected across all aforementioned latitudes during normal diurnal activity. In the first latitude, 30 specimens were caught; in the second latitude, 33 specimens, and third latitude 42 specimens. Lizards were euthanized and testes of each specimen were removed during each month. Testes were fixed in 96% ethanol, cleared in xylene and embedded in paraffin. Histological sections were cut at 5-7 µm, in haematoxylin followed by an eosin counterstain (H&E). The sections were then examined under light microscopy. To determine spermatogenesis timing and effects of geographic variation, a Tukey HSD test was used.

RESULTS AND DISCUSSION

Based on Tukey HSD test ($\alpha = 0.05$), spermatogenesis timing for the three latitudes varied as follows: Latitude (1) was divided into three phases (a) from March to May, phase (b) during June and July, phase (c) during August and September. Latitude (2) was divided into three phases, (a) from February to April, phase (b) during May and June, and phase (c) from July to October. Finally, latitude 3 was divided into three phases, (a) from February to April, phase (b) during June, and phase (c) from July to November. In all latitudes, during phase (a) spermatozoa were found in the lumen of seminiferous tubules with primary and secondary spermatocytes (Fig. 1). During phase (b), in most specimens, spermatozoa were found in the lumen of seminiferous tubules with primary and secondary spermatocytes. Finally, during phase (c) lumen of seminiferous tubules were found, but without spermatozoa or spermatocytes.

Usually spermatogenesis timing is related to environmental conditions (Duvall et al., 1982; Whittier et al., 1987). Timing of spermatogenesis activity has previously been described by the author (Torki, 2006, 2007 a, b). In this experiment, spermatozoa in the lumen of seminiferous tubules have been found. Therefore, spermatogenesis activity in this study occurred primarily during phase (a) because during this phase spermatozoa were found in the lumen of seminiferous tubules and also on primary and secondary spermatocytes. Based on these results, active spermatogenesis in the three latitudes occurred during two different times as follows: during March to May in the first latitude and during February to April in the second and third latitudes. Phase (b), named by the author as a 'transitional phase' (Torki, 2006, 2007a, b), had a duration of two months in the first and second latitudes but only one month in the third latitude. Inactive phases during biological activity in the first latitude were shorter (two months) than in the other latitudes (five months).

Spermatogenesis occurs in reptiles at different times **(**Sherbrooke, 1975; Torki, 2006, 2007a, b**)** across temperate regions. In reptiles, it is often observed in spring and early summer (Fitch, 1970; Lofts, 1987; Castilla & Bauwens, 1990). In some temperate regions, spermatogenesis in lizards occurs after hibernation (Fitch, 1970; Lofts, 1987; Castilla & Bauwens, 1990). In the tropics, spermatogenesis can occur continuously (especially in the ITCZ region = Inter Tropical Convergence Zone) (e.g., Sherbrooke, 1975; Hernandez-Gallegos et al., 2002; Torki, 2006, 2007a). However, in some tropical regions, spermatogenesis is not continuous and alternation of spermatogenesis occurs (Wilhoft & Reiter, 1965; Marion & Sexton, 1971). The effects of elevation in spermatogenesis timing in one tropical region have been shown to be zero (Hernandez-Gallegos et al., 2002) but in this study of a temperate species spermatogenesis timing is clearly related to elevation of the sampled lizard; that in turn could be related to temperature and other climatic conditions. At low elevations that have high temperatures, spermatogenesis commenced earlier in populations of *T. lessonae* than at higher elevations which have lower temperatures.

Lizards of temperate regions in other studies presented two periods in spermatogenesis with a degeneration and regeneration period (e.g., Lofts, 1987; Castilla & Bauwens, 1990**)**. In the Zagros Mountains the degeneration period in autumn and winter (or hibernation period) occurred and regeneration started during spring and lasted across summertime biological activity (Torki, 2006, 2007a, b). The duration of the two periods therefore suggests that spermatogenesis is related to climate condition and hibernation timing for a number of lizards in the region (e.g., Diaz et al., 1994; Huang, 1997; Torki, 2007b**)**. The results herein confirm

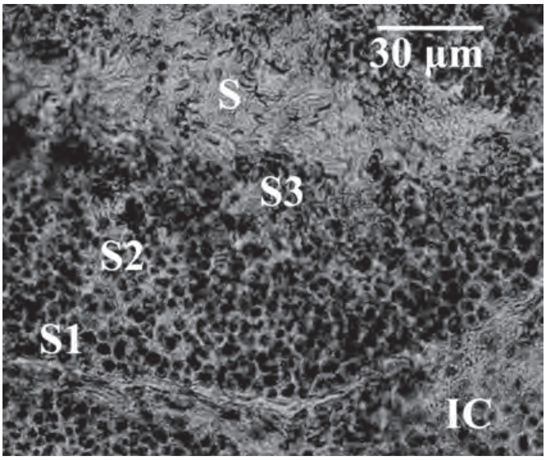

Figure 1. Seminiferous section during spermatogenesis active in phase (a) in *Trapelus lessonae*. S1: first Spermatocyte; S2: secondary Spermatocyte; S3: spermatid; S: Spermatozoa; IC: Interstitial cells.

this notion for *Trapelus lessonae* because duration of degeneration from cold climate (or first latitude) is shorter (seven months) than in the warmer, third climate (ten months). As winter temperatures in temperate regions are lower than spring months, some lizards undergo a hibernation phase where biological activities are dormant (Costanzo et al., 1995; 1988; Costanzo & Lee, 1995). Hibernation periods for poikilotherm lizards have an important role in survival because during hibernation lizards consume less energy and all of their remaining energy is stored for winter survival as well as for renewing the reproductive system (Costanzo et al., 1988; Storey et al., 1988; Grenot et al., 1996). During hibernation, lizards' body fat reserves are the only source to renew the testis volume and production of spermatocytes (Castilla & Bauwens, 1990; Heideman, 1995; Sharma & Shanbhag, 1992; Wapstra & Swain, 2001). This period for lizards inhabiting warmer, temperate climates like the third latitude, is shorter (two months) than cool temperatures or first latitude (five months).

The entire reproductive system is renewed during hibernation periods, and as a result, hibernation period has been called regeneration period for hibernating lizards. This strategy is the result of natural selection during evolution in each hibernating taxon (Storey and Storey, 1985; Costanzo, 1985; Ultsch, 1989; Litzgus et al., 1999; Torki, 2006, 2007a, b). This study confirms the influence of climate gradient to spermatogenesis timing in *Trapelus lessonae*, in the western Iranian plateau.

ACKNOWLEDGEMENTS

I wish to thank Todd Lewis (Editor) and Steven C. Anderson (University of the Pacific, Stockton, California, USA) for editing my manuscript. The FTEHCR is an independent institution supported solely by a bequest from the father of Farhang Torki. It is not supported by any other institute or organisation in the Islamic republic of Iran.

REFERENCES

Armbruster, P., Bradshaw, W.E., Ruegg, K. & Holzapfel, C.M. (2001). Geographic variation and the evolution of reproductive allocation in the pitcher-plant mosquito, *Wyeomyia smithii*. *Evolution* **55** (2), 439-444.

Beaupre, E.C., Tressler, C.J., Beaupr, S.J., Morgan. J.L.M., Bottje, W.G. & Kirby. J.D. (1997). Determination of testis temperature rhythms and effects of constant light on testicular function in the domestic fowl (*Gallus domesticus*). *Biol. Reprod.* **56**, 1570-1575.

Bona-Gallo, A. & Licht, P. (1983). Effect of temperature on sexual receptivity and ovarian recrudescence in the garter snake, *Thamnophis sirtalis parietalis*. *Herpetologica* **39**, 173-182.

Castilla, A.M. & Bauwens, D. (1990). Reproductive and fat body cycles of the lizard, *Lacerta lepida*, in central Spain. *J. Herpetol.* **24**, 261-266.

Costanzo, J.P. (1985). The bioenergetics of hibernation in the eastern garter snake *Thamnophis sirtalis sirtalis*. *Physiol. Zool*. **58**, 682-692.

Costanzo, J.P., Claussen, D.L. & Lee, R.E. (1988). Natural freeze tolerance in a reptile. *Cryo-Lett* **9**, 380-385.

Costanzo, J.P., Grenot, C. & Lee, R.E. (1995). Supercooling and freeze tolerance promote winter survival of the European common Lizard, *Lacerta vivipara*. *J. Comp. Physiol. B* **165**, 238-244.

Costanzo, J.P. & Lee, R.E. (1995). Supercooling and ice nucleation in vertebrate ectotherms.

Biological Ice Nucleation and Applications. Lee, R.E., Warren, G.J. & Gusta, L.V. (Eds.). Minnesota: Am. Phytopathol. Soc. Press.

Cuellar, H.S. & Cuellar, O. (1977). Evidence for endogenous rhythmicity in the reproductive cycle of the parthenogenetic lizard *Cnemidophorus uniparens* (Reptilia: Teiidae). *Copeia* **1977**, 554- 557.

De Kretser, D. (1993). *Molecular Biology of the Male Reproductive System.* San Diego: Academic Press.

Diaz, J.A., Alonso-Gómes, A.L. & Delgado, M.J. (1994). Seasonal variation of gonadal development, sexual steroids, and lipid reserves in a population of the lizard *Psammodromus algirus*. *J. Herpetol*. **28**, 199-205.

Duvall, D., Guillette, L.J.,Jr. & Jones, R.E. (1982). Environmental control of reptilian reproductive cycles. *Biology of Reptiles.* C. Gans and H. Pough (Eds.). Pp. 201-231. New York: Academic Press.

Fitch, H.S. (1970). Reproductive cycles in lizards and snakes. *Misc. Publ. Mus. Nat. Hist. Univ. Kansas* **52**, 1-247.

Grenot, C., Garcin, L. & Tse´re´-Page`s, H. (1996). Coldhardiness and behavior of the European Common Lizard, *Lacerta vivipara*, from French populations during the winter. *Adaptations to the Cold: Tenth International Hibernation Symposium.* Geiser, F., Hulbert, A.J. & Nicol, S.C. (Eds.). Pp. 115-121. Armidale: University of New England Press.

Gavaud, J. (1991). Role of cryophase temperature and thermophase duration in thermoperiodic regulation of the testicular cycle in the lizard, *Lacerta vivipara*. *J. Exp. Zool.* **260**, 239-246.

Heideman, N.J.L. (1995). The relationship between reproduction, and abdominal fat body and liver condition in *Agama aculeata aculeata* and *Agama planiceps planiceps* (Reptilia: Agamidae) males in Windhoek, Namibia. *J. Arid Environ.* **31**, 105-113.

Hernandez-Gallegos, O., Mendez-de la Cruz, F.R., Villagran-Santa Cruz, M. & Andrews, R.M. (2002). Continuous spermatogenesis in the lizards *Sceloporus bicanthalis* (Sauria: Phrynosomatidae) from high elevation habitat of central Mexico. *Herpetologica* **58** (4), 415-421.

Huang, W.S. (1997). Reproductive cycle of the oviparous lizard *Japlura brevis* (Agamidae: Reptilia) in Taiwan, Republic of China. *J. Herpetol*. **31**, 22-29.

Jacqueline, L.D., Costanzo, J.P., Brooks R.J. & Lee, R.E. Jr. (1999). Phenology and ecology of hibernation in spotted turtles (*Clemmys guttata*) near the northern limit of their range. *Can. J. Zool.* **77**, 1348-1357.

Lofts, B. (1987). Testicular functions. *Hormones and Reproduction in Fishes, Amphibians, and Reptiles*. Norris, D.O. and R.E. Jones (Eds.). Pp. 283-325. New York: Plenum Press.

Marion, K.R. & Sexton, C.J. (1971). The reproductive cycle of *Sceloporus malachiticus* in Costa Rica. *Copeia* **1971**, 517-526.

Mendonça, M.T. & Licht, P. (1986). Photothermal effects on the testicular cycle in the musk turtle, *Sternotherus odoratus*. *J. Exp. Biol.* **239**, 117-130.

Phillips, J.A., Frye, F., Bercovitz, A. Jr., Calle, P., Millar, R., Rivier, J. & Lasley, B.L. (1987). Exogenous GnRH overrides the endogenous annual reproductive rhythm in green iguanas, *Iguana iguana*. *J. Exp. Zool.* **241**, 227-236.

Rising, D.J. (1987). Geographic variation in testis size in savannah sparrows (*Passerculus sandwichensis*). *Wilson Bull.* **99**, 63-72.

Rhen, T. & Crews, D. (2002). Variation in Reproductive Behaviour within a Sex: Neural Systems and Endocrine Activation. *J. Neuroendocrinology* **14**, 517-531.

Sarge, K.D., Bray, A.E. & Goodson, M.L. (1995). Altered stress response in testis. *Nature* **374**, 126.

Sharma, R.N. & Shanbhag, B.A. (1992). Testis and abdominal fat body relationship in the garden lizard, *Calotes versicolor. Zool. Anz.* **228**, 31-38.

Sherbrooke, W.C. (1975). Reproductive cycle of a tropical lizard, *Neusticurus ecpleopus* Cope, in Peru. *Biotropica* **7**, 194-207.

Shine, R., (1985). The evolution of viviparity in reptiles: an ecological, analysis. *Biology of the Reptilia*, *Vol. 5.* C. Gans and F. Billett (Eds.). Pp. 605-694. New York: John Wiley & Sons.

Storey, K.B. & Storey, J.M. (1985). Adaptation of metabolism for freeze tolerance in the gray tree frog, *Hyla versicolor*. *Can. J. Zool.* **63**, 49-54.

Storey, K.B., Storey, J.M., Brooks, S.P.J., Churchill, T.A. & Brooks, R.J. (1988). Hatchling turtles survive freezing during winter hibernation. *Proc. Nat. Acad. Sci. USA* **85**, 8350-8354.

Tokarz, R.R., McMann, S., Seitz, L. & John-Alder, H. (1998). Plasma corticosterone and testosterone levels during the annual reproductive cycle of male brown anoles (*Anolis sagrei*). *Physiol. Zool.* **71**, 139-146.

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

Torki, F. (2007a). Reproduction of the snake-eye lizard, *Ophisops elegans* MÉNÉTRIÉS, 1832 in western Iran (Squamata: Sauria: Lacertidae). *Herpetozoa* **20** (1/2), 57-66.

Torki, F. (2007b). The role of hibernation on testicular cycle and testicular activation during dormancy in the hibernating lizard *Trapelus lessonae* (Reptilia; Agamidae) in nature. *Salamandra* **43** (4), 245-248.

Torki, F. (2010). Thirty threatened animals collected and identified in Lorestan province. Iranian Department of Environmental. Lorestan, Iran. (In Farsi) 138 p.

Ultsch, G.R. (1989). Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles, and snakes. *Biol. Rev. Camb. Phil. Soc.* **64**, 435-516.

Wapstra, E. & Roy, S. (2001). Reproductive correlates of abdominal fat body mass in *Niveoscincus ocellatus*, a Skink with an asynchronous reproductive cycle. *J. Herpetol.* **35**, 403-409.

Wilhoft, D.C. & Reiter, E.O. (1965). Sexual cycle of the lizard, *Leiolopisma fuscum*, a tropical Australian skink. *J. Morphol.* **116**, 379-388.

Whittier, J.M., Manson, J.T., Crews, D. & Licht, P. (1987). Role of light and temperature in the regulation of reproduction in the red-sided garter snake, *Thamnophis sirtalis parietalis*. *Can. J. Zool.* **65**, 2090-2097.

Vitt, L.J. & Congdon, J.D. (1978). Body shape, reproductive effort and relative clutch mass in lizards: resolution of a paradox. *Am. Nat.* **l12**, 595-608.

Young, E.B. (1994). Geographic and seasonal patterns of clutch-size variation in house wrens. *The Auk* **111** (3), 545-555.

Acknowledgements

On behalf of the British Herpetological Society the Editors wish to thank the following reviewers and contributors who have greatly enhanced the content of the 2011 volume;

Tim Aplin
John Baker
Trevor Beebee
David Bird
David Bull
Jamie Bowkett
Thomas M. Doherty-Bone
Alex Figueroa
S.R. Ganesh
Chris Gleed-Owen
Melissa Gogliath
Stephen R. Goldberg
Paul B.C. Grant
Roland Griffin
Michelle Haines
Rahbet Haskane
Anne Leonard
Darryn Nash
Dave Price
Gary Powell
Adam Radovanovic
Alex Ramsay
Leonardo B. Ribeiro
Trevor Rose
Ben Tapley
Farhang Torki
Josiah H. Townsend
Simon Townsend
Katy Upton
Larry David Wilson