Germinal bed condition in a polyautochronic single-clutched lizard, *Bassiana duperreyi* (Scincidae)

Rajkumar Radder, Richard Shine*

Abstract. In lizards, the eggs are produced within small regions of the ovary known as germinal beds (GBs); previous literature suggests that the number of GBs per ovary may be linked to life-history traits such as clutch size and breeding frequency. In the oviparous montane Australian skink *Bassiana duperreyi*, females produce a single clutch of 3 to 11 eggs each year. Our histological examination of ovaries of this species revealed a single GB per ovary. In combination with previous studies, our results suggest that scincid lizards may offer ideal model systems to explore the functional link between ovarian morphology and life-history traits in lizards.

All female reptiles possess a pair of ovaries attached to the dorsal body wall, but the massive interspecific variation in clutch sizes among lizards (Fitch, 1970) is reflected in significant variation in ovarian morphology. The number of eggs that an ovary can produce per clutch is controlled by (1) the number of germinal beds (GBs) per ovary, (2) the total number of follicles per GB, and (3) the proportion of follicles that become vitellogenic (Jones et al., 1982). The germinal bed is a small region on the dorsal ovarian surface consisting of dividing oogonia, naked oocytes and primordial follicles (Jones et al., 1982; Klosterman, 1983; Shanbhag, 2002; Gúmez and Ramìrez-Pinilla, 2004). Oogonia and oocytes are restricted to the GB, and growing follicles emanate from it. The number of GBs per ovary ranges from one to six among the lizard species studied to date (Jones et al., 1982; Jones and Summers, 1984; Shanbhag and Prasad, 1993; Uribe et al., 1995; Shanbhag et al., 1998; Amey and Whittier, 2000; Stewart and Florian, 2000; Sica et al., 2001).

Previous studies not only have revealed substantial interspecific variation in GB number within lizards, but also suggest broad patterns. For example, most species with allochronic reproduction (ovulating a single egg from each ovary alternatively) and monoautochronic reproduction (ovulating a single egg from both ovaries simultaneously) seem to exhibit only one GB per ovary. However, some lizards that ovulate only a single egg from each ovary, nonetheless possess two GBs per ovary (Wilhoft, 1963) or possess one GB but ovulate many eggs per ovary (Jones et al., 1982). The diversity is even greater among polyautochronic lizards (those that ovulate several eggs simultaneously from both ovaries: Jones et al., 1982). Lizards with high fecundity (large clutches and/or multiple clutches within a single breeding season) usually exhibit two GBs, while those producing smaller clutches possess only one GB (Jones et al., 1982).

Although such correlations between ovarian morphology and fecundity are encouraging (for example, they might allow prediction of life-history traits from the gonads of preserved museum specimens), the available data display a strong phylogenetic bias and hence, generalisations are impossible. Most previous research on lizards has been based on species belonging to the families Iguanidae, Agamidae and Gekkonidae. The more speciose Scincidae has attracted less attention, with information on GB number for only seven species of skinks. Even among this small sample, we lack information on number of ovulations per annum for two of the species. The remaining scincid species exhibit two to six germinal

School of Biological Sciences A08, University of Sydney, NSW 2006, Australia

*Corresponding author; e-mail: rics@bio.usyd.edu.au

beds (Fitch, 1954; Wilhoft, 1963; Jones et al., 1982; Gúmez and Ramìrez-Pinilla, 2004). Interestingly, many oviparous skinks are polyautochronic but ovulate once annually with small and variable clutch sizes (∼3-10 eggs: Greer, 1989). To the best of our knowledge, there are no published reports concerning the number of germinal beds in such polyautochronic lizards. The present study provides data on such a species.

The three-lined skink *Bassiana duperreyi* is a medium-sized terrestrial lizard (to 80 mm snout-vent length) that is abundant in cool-climate habitats in south-eastern Australia. These lizards are oviparous, with females laying a single clutch of 3 to 11 eggs in early summer each year (Pengilley, 1972). We obtained previtellogenic ovaries from five female *B. duperreyi* (3 months old), hatched from eggs collected in the Brindabella Range near Canberra, Australian Capital Territory. These ovaries were preserved in Bouin's fluid, dehydrated in a graded series of ethanol and embedded in paraffin. The paraffin tissue blocks were sectioned at 5 μm. The sections were stained with hematoxylin-eosin and observed under a light microscope to determine the number of germinal beds in each ovary.

Consistent with reports of most previous studies, we did not detect any intraspecific (among-female) variation in the number of GBs per ovary within our study species (Jones et al., 1982). In gross histology, the *B. duperreyi* ovary exhibited an ovarian wall formed by the ovarian epithelium and tunica albuginea. The ovarian surface epithelium consisted of simple squamous epithelium cells. The tunica albuginea was composed of thin fibrous connective tissue. The ovary itself consisted of follicles at different stages of development and atretic follicles (fig. 1). Over the dorsal surface of the ovary, near the ovarian hilum, there was one GB (fig. 1). This germinal bed was in contact with ovarian epithelium, and formed a continuous band surrounding the hilum. The GB consisted of germinal cells as well as nongerminal cells. The germinal cells were of two distinct types: (1) oogonia, with a large globoid interface nucleus, a prominent nucleolus and lightly stained cytosol, and (2) germinal cells with a round ovoid nucleus that contained chromosomes in meiotic prophase I (especially in diplotene), and a lightly stained cytoplasm. These primary oocytes (the first stage of follicular development) were surrounded by somatic epithelial cells that contained darker cytosol (fig. 1). When the primary oocytes eventually leave the GB, they are surrounded by epithelial cells and enveloped by connective tissue. The oocytes remain adjacent to the GB.

As is the case for many other behavioural and ecological traits (e.g., O'Connor and Shine, 2003), the cosmopolitan lizard family Scincidae appears to encompass a remarkable diversity in ovarian morphology. Unlike other skinks that have been studied to date, the polyautochronic *B. duperreyi* exhibits only a single GB per ovary. In previously-studied skinks, the number of GBs per ovary covers a broad range: four to six small scattered GBs in *Eumeces fasciatus* and *E. copei* (Jones et al., 1982), two in *Leiolopisma metallicum* (Jones et al., 1982), *Scincella lateralis* (Jones et al., 1973), *Carlia rhomboidalis* (Wilhoft, 1963) and *Mabuya longicaudata* (Jones et al., 1982), and either one or two in the viviparous *Mabuya mabouya* (Gúmez and Ramìrez-Pinilla, 2004). Further, scincid lizards appear to exhibit several unique patterns with respect to GBs. For example, *Eumeces* has by far the highest recorded number of GBs per ovary among lizards, and *C. rhomboidalis* possesses a perplexing condition whereby it has two GBs per ovary but ovulates only a single egg per ovary.

Our observations on *B. duperreyi* extend the known diversity of ovarian traits within the Scincidae. Clutch size in *B. duperreyi* ranges from 3 to 11 eggs and these skinks produce a single clutch each year. Thus, each ovary produces an average of about 1 to 4 eggs. Interestingly, most other polyautochronic lizards that ovulate a variable number of eggs (range 1 to 16) exhibit two GBs per ovary. Thus, the overall pattern in oviparous lizards appears to be that two GBs are associated with high fecundity (large and/or multiple clutches per season), whereas species with one GB per ovary display low, fixed clutch sizes (i.e., one egg

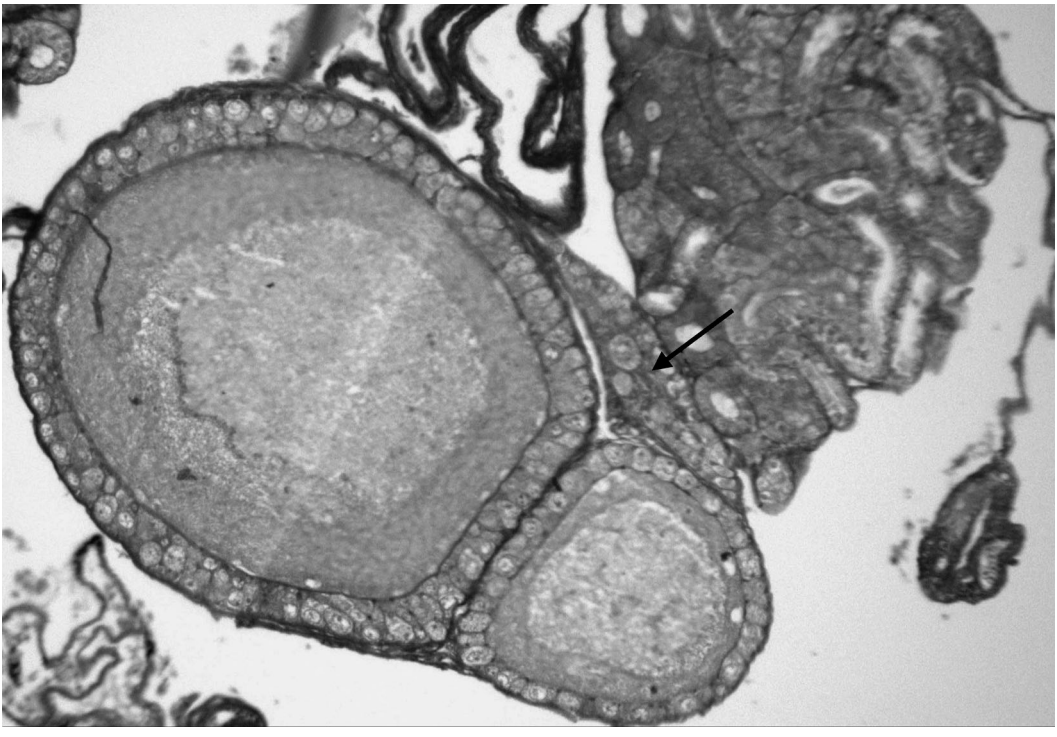

Figure 1. Longitudinal section through an ovary of the scincid lizard *Bassiana duperreyi* showing the germinal bed (GB, arrow). Note the contact of GB with ovarian epithelium and the presence of oogonia inside the GB.

from each ovary: Jones et al., 1982; Amey and Whittier, 2000; Shanbhag, 2002). Nonetheless, at least one species with a single GB (*Agama agama*) produces more than one egg (1 to 6 eggs) per ovary, showing that moderately large and variable clutch sizes can be produced from a single GB (Jones et al., 1982). The single germinal bed in this species may have been derived from a merger of the "two GBs per ovary" condition seen in related agamid taxa (e.g., *Calotes versicolor*: Jones et al., 1982). We know too little about ovarian morphology in other scincid lizards to infer any pattern of evolutionary change to produce the situation seen in *B. duperreyi*, but our data suggest that scincids may provide excellent model systems with which to further explore the relationships between life-history traits and female gonadal morphology in lizards. For example, multiple phylogenetic transitions between variable-clutch-size and low-fixed-clutch size reproductive modes within the Scincidae (Shine and Greer, 1991) provide a powerful opportunity to investigate the functional relationship between fecundity and ovarian morphology.

Acknowledgements. This work was supported by grants from The Australian Research Council. We thank Laura, Joanna and Darryl Cameron (School of Medicine, University of Sydney) for providing access to histology facilities.

References

Amey, A.P., Whittier, J.M. (2000): The annual reproductive cycle and sperm storage in the bearded dragon, *Pogona barbata*. Aust. J. Zool. **48**: 411-419.

Fitch, H.S. (1954): Life history and ecology of the five-lined skink, *Eumeces fasciatus*. Univ. Kans. Publ. Mus. Nat. Hist. **8**: 1-156.

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

Greer, A.E. (1989): The Biology and Evolution of Australian Lizards. Surrey Beatty and Sons, Sydney, Australia.

Gúmez, D., Ramírez-Pinilla, M. (2004): Ovarian histology of the placentotrophic *Mabuya mabouya* (Squamata, Scincidae). J. Morphol. **259**: 90-105.

Jones, R., Summers, C. (1984): Compensatory follicular hypertrophy during the ovarian cycle of the house gecko, *Hemidactylus frenatus*. Anat. Rec. **209**: 59-65.

Jones, R., Roth, J., Gerrard, A., Kiely, R. (1973): Endocrine control of clutch size in reptiles. I. Effects of FSH on ovarian follicular size-gradation in *Leiolopisma laterale* and *Anolis carolinensis*. Gen. Comp. Endocrinol. **20**: 190-198.

Jones, R.E., Swain, T., Guillette Jr., L.J., Fitzgerald, K.T. (1982): The comparative anatomy of lizard ovaries, with emphasis on the number of germinal beds. J. Herpetol. **16**: 240-252.

Klosterman, L. (1983): The ultrastructure of germinal beds in the ovary of *Gerrhonotus coeruleus* (Reptilia: Anguidae). J. Morphol. **178**: 247-265.

O'Connor, D., Shine, R. (2003): Lizards in 'nuclear families': a novel reptilian social system in *Egernia saxatilis* (Scincidae). Mol. Ecol. **12**: 743-752.

Pengilley, R. (1972): Systematic relationships and ecology of some lygosomine lizards from southeastern Australia. Unpublished PhD Dissertation. Australian National University, Canberra.

Shanbhag, B.A. (2002): Reproductive biology of Indian reptiles. Proc. Indian Natl. Sci. Acad. **B 68**: 497-528.

Shanbhag, B.A., Prasad, B.S.K. (1993): Follicular dynamics and germinal bed activity during the annual ovarian cycle of the lizard, *Calotes versicolor*. J. Morphol. **216**: 1-7.

Shanbhag, B.A., Subraya, L., Saidapur, S.K. (1998): Pattern of recruitment, growth of developing follicles, and germinal bed activity in the tropical gecko, *Hemidactylus brooki*. J. Herpetol. **32**: 566-572.

Shine, R., Greer, A.E. (1991): Why are clutch sizes more variable in some species than in others? Evolution **45**: 1696-1706.

Sica, S., Fierro, D., Iodice, C., Muoio, R., Filosa, S., Motta, C. (2001): Control of oocyte recruitment: regulative role of follicle cells through the release of a diffusible factor. Mol. Reprod. Dev. **58**: 444-450.

Stewart, J., Florian, J. (2000): Ontogeny of the extraembryonic membranes of the oviparous lizard, *Eumeces fasciatus* (Squamata: Scincidae). J. Morphol. **244**: 81-107.

Uribe, M.D.A., Omana, M.E.M., Quintero, J.E.G., Guillette, L.J. (1995): Seasonal variation in ovarian histology of the viviparous lizard *Sceloporus torquatus torquatus*. J. Morphol. **226**: 103-119.

Wilhoft, D.C. (1963): Reproduction in the tropical Australian skink, *Leiolopisma rhomboidalis*. Am. Mid. Nat. **70**: 442-461.

Received: April 12, 2006. Accepted: April 28, 2006.