

**SYMPOSIUM OF THE 6TH WORLD CONGRESS OF HERPETOLOGY
REPTILE REPRODUCTION: FROM GENES TO ECOLOGY**

MICHAEL B. THOMPSON, SYMPOSIUM GUEST EDITOR

*School of Biological Sciences (A08), The University of Sydney, New South Wales 2006, Australia
e-mail: Michael.Thompson@bio.usyd.edu.au*

In Memory of
Rajkumar (Raju) Shivappa Radder



With the permission of his family, we dedicated the symposium to the memory of Raju Radder (pictured above), an extraordinary young herpetologist who was registered to participate in the symposium but who suffered a fatal heart attack on 31 May 2008, just 2½ months short of the Congress. Raju had focused his attentions on many aspects of reproduction in reptiles and his contributions to the literature were significant and expanding rapidly (Shanbhag et al. 2008). It was his innovation and dedication that led us to ensure that Raju was part of our symposium and his death touched us all deeply. It is for these reasons that we dedicated the symposium, and these written contributions, to Raju's memory.

REPRODUCTION IN REPTILES FROM GENES TO ECOLOGY: A RETROSPECTIVE AND PROSPECTIVE VISION

MICHAEL B. THOMPSON^{1,2}, DANIEL G. BLACKBURN³, SCOTT L. PARKER⁴

¹*School of Biological Sciences (A08), The University of Sydney, New South Wales 2006, Australia*

²*Corresponding Author: e-mail: Michael.Thompson@bio.usyd.edu.au*

³*Department of Biology and Electron Microscopy Facility, Trinity College, Hartford, Connecticut 06106, USA*

⁴*School of Biological Sciences (A08), The University of Sydney, New South Wales 2006, Australia*

Abstract.—The 6th World Congress of Herpetology (WCH), held in Manaus, Brazil in 2008, provided an excellent venue for a broad, integrative symposium on reproduction in reptiles. This symposium brought together researchers from throughout the world who are working on diverse reptilian species. The symposium's title "Reproduction in Reptiles from Genes to Ecology," captures the methodological breadth of contemporary research as well as its integrative nature. This special issue of *Herpetological Conservation and Biology* presents a series of papers from contributors to that symposium. In this introduction to the special issue, we offer an evolutionary overview of reptilian reproduction and summarize the nature, characteristics, and implications of current research efforts, as represented in the WCH symposium.

Key Words.—behavioral ecology; chelonians; fetal nutrition; lizards; placentation; reproduction; sex determination; viviparity

INTRODUCTION

Reptile reproduction is an area of active research with the potential to answer basic functional and evolutionary questions in biology. Reptiles are extraordinarily diverse in terms of their reproduction, arguably more so than any other group of terrestrial vertebrates. Although research on reptilian reproduction once was viewed as significant for what it might reveal about mammals (Giacomini 1891; Kerr 1919; Harrison and Weekes 1925), the subject has long been viewed as worthy of study in its own right. Contemporary research in this field is inherently integrative, draws on diverse methodologies, and is firmly based within an evolutionary framework (Blackburn 2000).

The 6th World Congress of Herpetology (WCH) held in Manaus, Brazil in July of 2008, provided a venue for an international symposium entitled "Reproduction in Reptiles from Genes to Ecology." A cross-disciplinary symposium on reproduction in reptiles was especially timely because of recent developments in research on reproduction, which is now being unified by molecular biology and ecology. The aim of the symposium was to bring people together to stimulate new ideas and research collaborations. The World Congress of Herpetology allowed a much wider range of fields to be represented than is possible at most conferences, where the focus is generally discipline-based. In fact, in terms of its extraordinary disciplinary breadth and its phylogenetic focus, this symposium was the first of its kind. The symposium attracted biologists from throughout the world; authors of contributed papers came from 11 countries and six continents. As a result,

the WCH reproduction symposium succeeded as a valuable forum for the exchange of information, ideas, and viewpoints, as well as for the development of new collaborations. In fact, several new research collaborations were established at the symposium between individuals who had not previously met. The location of this symposium in Manaus, on the edge of the world's largest rainforest, was fitting, given the participants' shared concerns over issues of conservation and biodiversity.

Publication of this symposium in a special issue of *Herpetological Conservation and Biology* (HCB) is highly appropriate, given this journal's breadth of subject matter and its wide distribution. We are grateful to the editors of HCB for the opportunity to present these contributions in a journal accessible to anyone in the world with a computer and an internet connection. In this introduction, we summarize salient features of reptilian reproduction from an evolutionary perspective, outline the nature and significance of research in this field as represented by this journal issue, and consider implications for future study. We hope that presentation of this body of work to an international audience will help stimulate and inform further investigation by revealing significant questions that are being addressed and others that remain unanswered.

Reptilian reproductive features.—Reptiles are an extraordinarily diverse group of animals that occupy a central position in the vertebrate phylogeny (Pough et al. 2009). With over 8700 living species (TIGR Reptile Database. Available from <http://www.reptile-database.org/> [Accessed 15 August 2009]), reptiles are more speciose than most other major chordate groups

Herpetological Conservation and Biology

Symposia of the 6th World Congress of Herpetology

including mammals, lissamphibians, chondrichthyans, sarcopterygians, and agnathans. In fact, among terrestrial chordates, they are exceeded in numbers of species only by birds, which in a cladistic sense are (of course) a subgroup of "Reptilia" (Norell and Xu 2005).

Reptiles first arose in the Paleozoic, and the two major reproductive innovations of amniotes, internal fertilization and the amniotic egg, originated in that time frame. Each of the major reptilian clades, chelonians, crocodylians, sphenodontids, and squamates, plus many extinct groups, diverged in the Mesozoic (Tudge 2000). Such major extant subgroups as lizards, snakes, amphisbaenians, cryptodires, and pleurodires as well as various reptilian families also are Mesozoic in origin (Carroll 1988). Thus the diversity of reptiles reflects over 200 million years of independent evolution of major reptilian lineages, most of which have undergone extensive diversification.

Most reptiles share basic reproductive and developmental features that reflect their common ancestry (Stewart 1997; Blackburn 1998; Pough et al. 2009). Their plesiomorphic features include: (a) sexual reproduction with internal fertilization; (b) a terrestrial egg with an eggshell that inhibits dehydration; (c) a complement of three fetal membranes (chorioallantois, yolk sac, and amnion) that facilitate egg development on land; (d) lecithotrophic nutrition (in which the yolk provides nutrients for embryonic development); (e) production of fully formed offspring that lack a free-living metamorphic stage; and (f) physiological mechanisms for control of gonadal and oviductal functions.

Reptilian reproductive diversity is manifested in several variable features. One is mode of sex determination; reptilian species can exhibit temperature dependent sex determination (TSD), male heterogamety, or female heterogamety (Bull 1980; Janzen and Paukstis 2001). A second variable feature is mode of reproduction. Although most reptiles lay eggs, about 20% of the squamates are viviparous, a pattern that has evolved convergently in many separate lineages (Blackburn 1985, 1999; Shine 1985). A third such feature is mode of embryonic nutrition. Whereas oviparous and most viviparous species are lecithotrophic (Stewart and Thompson 2000; Thompson and Speake 2003), some clades of scincid lizards are highly placentotrophic (Blackburn and Vitt 1992; Thompson et al. 2000; Flemming and Branch 2001; Flemming and Blackburn 2003; Ramírez-Pinilla 2006). A fourth feature, one related to embryonic nutritional mode, is type of placentation. All viviparous squamates have placentae that accomplish gas exchange and water transfer (Yaron 1985; Blackburn 1993; Stewart 1993; Thompson et al. 2004). However, in placentotrophic skinks, the placental membranes also are specialized for

maternal-fetal nutrient transfer (for reviews see Blackburn 1993; Thompson and Speake 2006). Likewise, even relatively lecithotrophic species show specializations for nutrient transfer and other physiological exchange (e.g. Blackburn et al. 2002; Adams et al. 2007). In addition to the reproductive features listed above, reptilian species also vary in numerous aspects of reproductive anatomy, physiology, behavior, and ecology (for a brief literature summary see Blackburn 2000).

Contemporary research on reptiles.—The main focal points of contemporary research do not lie with the shared, ancestral features of reptiles, but rather with manifestations of their evolutionary diversification. As shown by contributions to this symposium volume, several reproductive phenomena currently are receiving attention from laboratories around the world. One is sex determination, which is explored herein with regard to functional mechanisms, evolution, and ecological implications (Doody and Moore 2010; Nelson et al. 2010). Second are the linked phenomena of placentation and fetal nutrition. Among contributions to this symposium volume are discussions of placental structure, function, and development (Blackburn and Flemming, this volume; Leal and Ramirez-Pinilla, this volume; Murphy, this volume), specializations for maternal-fetal nutrient transfer (Biazik et al., this volume), and mechanisms of maternal calcium provision to embryos (Herbert et al., this volume; Stewart and Eday, this volume). A third phenomenon is squamate viviparity, which is being studied extensively in terms of functional mechanisms (including endocrinological and immunological aspects; Parker et al., this volume; Paulesu et al., this volume) and evolutionary and ecological characteristics (Cree and Hare, this volume). Other contributions more broadly explore patterns of reproductive evolution within a genus or family. Finally, multiple papers deal with ecological or behavioral aspects of reproduction, including thermal biology, nesting ecology, mate choice, competition and assisted reproduction (Molinia et al. 2010).

Despite the diversity of phenomena under investigation, contemporary research on reptile reproduction has several overarching attributes. One obvious attribute is its multidisciplinary nature; it draws on methods and principles of anatomy, physiology, endocrinology, biochemistry, immunology, genetics, molecular biology, ethology, ecology, conservation biology, and evolutionary biology. Papers and authors represented in this volume cannot easily be classified by their subdisciplines or techniques; rather, they draw upon whatever methods and concepts are appropriate for answering a given set of questions. The contributions use both descriptive and experimental approaches and

Reproduction in Reptiles from Genes to Ecology

Thompson et al.—Introduction

encompass empirical as well as theoretical aspects. Likewise, some also consider applied aspects, such as (to use one example) the projected implications of climate change on conservation in species with temperature dependent sex determination.

A second and related attribute of modern research is its integrative nature. “Integrative biology” is the term of the decade, and for its defining characteristics, we can turn to descriptions in a recent review by Prof. Marvalee Wake (2008): integrative biology is “both an attitude about the scientific process and a description of a way of doing science.” Its “principles emphasize not just multidisciplinary but also transdisciplinary research.” Our symposium and this volume are firmly aimed at doing what Wake (2008) described as: “Integration facilitates the generation of new hypotheses and new questions because representatives with an array of expertise communicate with one another about general but complex issues.”

Other attributes of modern research also are evident from contributions to this symposium volume. One attribute is the use of model taxa, e.g., to explore such features as embryonic diapause, modes of sex determination, mechanisms of placental transport, and patterns of reproductive evolution. While the concept of model species can be misapplied (Blackburn 2006), the fact remains that reptiles include ideal taxa for investigation of complex phenomena and patterns. A second attribute of modern research is its technological sophistication. Contributions to this volume draw on electron and light microscopy, confocal microscopy, gel electrophoresis, immunocytochemistry, molecular analyses, assisted breeding and radioimmunoassay technology. Of course, technological sophistication is not required for quality research, since excellent research continues to be conducted with more traditional methods, but its use herein is appropriate and illuminating. Another attribute is that current research is phylogenetically-driven. The revolution spawned by cladistics continues and is manifested by the use of cladograms and phylogenies to direct reproductive research and interpret resultant data.

Somewhat less evident from this volume is the phylogenetic diversity of current research, since the contributions to this volume primarily are focused on lizards and sphenodontids. This taxonomic focus is somewhat artificial, because some of our participants have done considerable research on snake reproduction (e.g., Blackburn et al. 2002; Stewart and Brasch 2003), and given that chelonians (and to a lesser extent crocodylians) have received attention in the reproductive literature (e.g., Schwanz and Janzen 2008; Whitehead et al. 1992).

Aspects of reptilian reproduction on which information is notably scarce relate to the shared,

ancestral features of reptiles mentioned above. Consider the amniote egg: its evolution is widely viewed as one of the key events in vertebrate history (Romer 1959; Smith 1959; cf. Skulan 2000) because it liberated amniotes from aquatic reproduction. Nevertheless, we lack certain information that might allow reconstruction of that evolutionary event; notably, we know very little about the fetal membranes of chelonians, sphenodontids, and crocodylians (Stewart 1997), and most of what we know about them in squamates is based on viviparous forms. Another relevant feature is eggshell composition and functional properties. These features have been studied in a number of reptilian species (e.g., Packard et al. 1982; Osborn and Thompson 2005; for reviews see Packard and DeMarco 1991; Thompson and Speake 2004). However, very few attempts have been made to explain eggshells in broad evolutionary terms; in fact two (Aoki 1993; Kohring 1995) have concluded that eggshells are not ancestral for reptiles.

In summary, research on reproduction in reptiles helps explain the extraordinary diversity of living forms and has the potential to offer insight into major evolutionary events and complex biological phenomena. Such research also provides information critical to maintenance of natural and captive populations, issues that are of vital concern under the growing threats of habitat devastation and anthropogenic climate change. The present symposium volume offers a sampling of some of the best of contemporary research, while outlining directions for future study and its promise in explaining the functional and evolutionary diversity of an extraordinary group of animals.

With the permission of his family, we dedicated the symposium to the memory of Rajkumar (Raju) Shivappa Radder, an extraordinary young herpetologist who was registered to participate in the symposium but who suffered a fatal heart attack on 31 May 2008, just 2½ months short of the Congress. Raju had focused his attentions on many aspects of reproduction in reptiles and his contributions to the literature were significant and expanding rapidly (Shanbhag et al. 2008). It was his innovation and dedication that led us to ensure that Raju was part of your symposium and his death touched us all deeply. It is for these reasons that we dedicated the symposium, and these written contributions, to Raju’s memory.

LITERATURE CITED

- Adams, S.M., S. Liu, S.M. Jones, M.B. Thompson, and C.R. Murphy. 2007. Uterine changes during placentation in the viviparous skink *Eulamprus tympanum*. *Journal of Morphology* 268:385–400.

Herpetological Conservation and Biology
Symposia of the 6th World Congress of Herpetology

- Aoki, R. 1993. The multiple origins of the eggshell in amniote evolution. *Journal of Fossil Research* 27:9–43.
- Biazik, J.M., M.B. Thompson, and C.R. Murphy. 2010. Paracellular and transcellular transport across the squamate uterine epithelium. *Herpetological Conservation and Biology* 5:257–262.
- Blackburn, D.G. 1985. Evolutionary origins of viviparity in the Reptilia. II. Serpentes, Amphisbaenia, and Ichthyosauria. *Amphibia-Reptilia* 5:259–291.
- Blackburn, D.G. 1993. Chorioallantoic placentation in squamate reptiles: structure, function, development, and evolution. *Journal of Experimental Zoology* 266:414–430.
- Blackburn, D.G. 1998. Structure, function, and evolution of the oviducts of squamate reptiles, with special reference to viviparity and placentation. *Journal of Experimental Zoology* 282:560–617.
- Blackburn, D.G. 1999. Are viviparity and egg-guarding evolutionarily labile? *Herpetologica* 55:556–573.
- Blackburn, D.G. 2000. Reptilian viviparity: past research, future directions, and appropriate models. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology* 127:391–409.
- Blackburn, D.G. 2006. Squamate reptiles as model organisms for the evolution of viviparity. *Herpetological Monographs* 20:131–146.
- Blackburn, D.G., and A.F. Flemming. 2010. Reproductive specializations in a viviparous African skink and its implications for evolution and biological conservation. *Herpetological Conservation and Biology* 5:263–270.
- Blackburn, D.G., J.R. Stewart, D.C. Baxter, and L.H. Hoffman. 2002. Placentation in garter snakes: Scanning EM of the placental membranes of *Thamnophis ordinoides* and *T. sirtalis*. *Journal of Morphology* 252:263–275.
- Blackburn, D.G., and L.J. Vitt. 1992. Reproduction in South American lizards of the genus *Mabuya*. Pp. 150–164 *In* *Reproductive Biology of South American Vertebrates: Aquatic and Terrestrial*. Hamlett, W.C. (Ed.). Springer-Verlag, New York, New York, USA.
- Bull, J.J. 1980. Sex determination in reptiles. *Quarterly Review of Biology* 55:3–21.
- Carroll, R.L. 1988. *Vertebrate Paleontology and Evolution*. W.H. Freeman and Co., New York, New York, USA.
- Cree, A., and K.M. Hare. 2010. Thermal regimes for successful gestation in two viviparous, cool-climate lizards: a prerequisite for studies on offspring phenotypes. *Herpetological Conservation and Biology* 5:271–282.
- Doody, J.S., and J.A. Moore. 2010. Clinal variation in maternal traits influencing fitness of reptilian embryos. *Herpetological Conservation and Biology* 5:283–289.
- Flemming, A.F., and D.G. Blackburn. 2003. Evolution of placentotrophy in viviparous African and South American lizards. *Journal of Experimental Zoology* 299A:33–47.
- Flemming, A.F., and W.R. Branch. 2001. Extraordinary case of matrotrophy in the African skink *Eumecia anchietae*. *Journal of Morphology* 247:264–287.
- Giacomini, E. 1891. Materiali per la storia dello sviluppo del *Seps chalcides* (Cuv.) Bonap. *Monitore Zoologica Italiano* 2:179–192, 198–211.
- Harrison, L., and C.H. Weekes. 1925. On the occurrence of placentation in the scincid lizard, *Lygosoma entrecasteauxi*. *Proceedings of the Linnaean Society of New South Wales* 50:472–486.
- Herbert, J.F., C.R. Murphy, and M.B. Thompson. 2010. Calcium transport across the uterus in two species of *Pseudemoia* with complex placentae. *Herpetological Conservation and Biology* 5:290–296.
- Janzen, F.J., and G.L. Paukstis. 2001. Environmental sex determination in reptiles: ecology, evolution, and experimental design. *Quarterly Review of Biology* 66:149–179.
- Kerr, J.G. 1919. *Text-book of Embryology*. Macmillan, London, Great Britain.
- Kohring, R.R. 1995. Reflections on the origin of the amniote egg in the light of reproductive strategies and shell structure. *Historical Biology* 10:259–275.
- Leal F., and M.P. Ramírez-Pinilla. 2010. Evolution and development of the extraembryonic membranes in lizards: heterochronies and placentotrophy. *Herpetological Conservation and Biology* 5:297–310.
- Molinia, F.C., T. Bell, G. Norbury, A. Cree, and D.M. Gleeson. 2010. Assisted breeding of skinks or how to teach a lizard old tricks! *Herpetological Conservation and Biology* 5:311–319.
- Murphy, C.R. 2010. Comparative placentation, epithelial plasticity and the ‘efficient barrier hypothesis.’ *Herpetological Conservation and Biology* 5:320–323.
- Nelson, N.J., J.A. Moore, S. Pillai, and S.N. Keall. 2010. Thermosensitive period for sex determination in Tuatara. *Herpetological Conservation and Biology* 5:324–329.
- Norell, M.A., and X. Xu. 2005. Feathered dinosaurs. *Annual Review of Earth and Planetary Sciences* 33:277–299.
- Osborne, L., and M.B. Thompson. 2005. Chemical composition and structure of the eggshell of three oviparous lizards. *Copeia* 2005:683–692.
- Packard, M.J., and V.G. DeMarco. 1991. Eggshell structure and formation in eggs of oviparous reptiles. Pp. 53–70 *In* *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Deeming, D.C. and M.W.J. Ferguson (Eds.). Cambridge University Press, Cambridge, England.
- Packard, M.J., G.C. Packard, and T.J. Boardman. 1982. Structure of eggshells and water relations of reptilian eggs. *Herpetologica* 38:136–155.

Reproduction in Reptiles from Genes to Ecology
Thompson et al.—Introduction

- Parker, S.L., C.R. Murphy, and M.B. Thompson. 2010. Uterine angiogenesis in squamate reptiles: implications for the evolution of viviparity. *Herpetological Conservation and Biology* 5:330-334.
- Paulesu, L., S. Jantra, F. Ietta, R. Brizzi, A.M. Avanzati, and E. Bigliardi. 2010. Cytokines in vertebrate reproduction. *Herpetological Conservation and Biology* 5:335-340.
- Pough, F.H., C.M. Janis, and J.B. Heiser. 2009. *Vertebrate Life*. 8th Ed. Pearson Education, San Francisco, California, USA.
- Ramírez-Pinilla, M.P. 2006. Placental transfer of nutrients during gestation in an Andean population of the highly matrotrophic lizard genus *Mabuya* (Squamata: Scincidae). *Herpetological Monographs* 20:194-204.
- Romer, A.S. 1959. *The Vertebrate Story*. University of Chicago Press, Chicago, Illinois, USA.
- Schwanz, L.E., and F.J. Janzen. 2008. Climate change and temperature-dependent sex determination: Can individual plasticity in nesting phenology prevent extreme sex ratios? *Physiological and Biochemical Zoology* 81:826-834.
- Shine, R. 1985. The evolution of viviparity in reptiles: an ecological analysis. Pp. 605-694 *In* *Biology of the Reptilia*. Vol. 15. Gans, C., and F. Billet (Eds.). John Wiley & Sons, New York, New York, USA.
- Shanbhag, B.A., S.K. Saidapur, and R. Shine. 2008. Rajukumar Shivappa Radder (1973-2008). *Herpetological Review* 39:261-262.
- Skulan, J. 2000. Has the importance of the amniote egg been overstated? *Zoological Journal of the Linnean Society* 130:235-261.
- Smith, H.W. 1959. *From Fish to Philosopher*. Ciba Pharmaceutical Products, Summit, New Jersey, USA.
- Stewart, J.R. 1993. Yolk sac placentation in reptiles: structural innovation in a fundamental vertebrate nutritional system. *Journal of Experimental Zoology* 266:431-449.
- Stewart, J.R. 1997. Morphology and evolution of the egg of oviparous amniotes. Pp 291-326 *In* *Amniote Origins*. Sumida, S.S., and K.L.M. Martin (Eds.). Academic Press, San Diego, California, USA.
- Stewart, J.R., and K.R. Brasch. 2003. Ultrastructure of the placenta of the natricine snake, *Virginia striatula* (Reptilia: Squamata). *Journal Morphology* 255:177-201.
- Stewart, J.R., and T.W. Eca. 2010. Patterns of maternal provision and embryonic mobilization of calcium in oviparous and viviparous squamate reptiles. *Herpetological Conservation and Biology* 5:341-359.
- Stewart, J.R., and M.B. Thompson. 2000. Evolution of placentation among squamate reptiles: recent research and future directions. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology* 127:411-431.
- Thompson, M.B., and B.K. Speake. 2003. Energy and nutrient utilisation by embryonic reptiles. *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology* 133:529-538.
- Thompson, M.B., and B.K. Speake. 2004. Egg morphology and composition. Pp. 45-74 *In* *Reptilian Incubation: Environment, Evolution and Behaviour*. Deeming, D.C. (Ed.). Nottingham University Press, Nottingham, Great Britain.
- Thompson, M.B., and B.K. Speake. 2006. A review of the evolution of viviparity in lizards: structure, function, and physiology of the placenta. *Journal of Comparative Physiology B* 176:179-189.
- Thompson, M.B., J.R. Stewart, and B.K. Speake. 2000. Comparison of nutrient transport across the placenta of lizards differing in placental complexity. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology* 127:469-479.
- Thompson, M.B., S.M. Adams, J.F. Herbert, J.M. Biazik, and C.R. Murphy. 2004. Placental function in lizards. *International Congress Series* 12785:218-225.
- Tudge, C. 2000. *The Variety of Life*. Oxford University Press, New York, New York, USA.
- Wake, M.H. 2008. Science for the 21st century. *BioScience* 58: 49-353.
- Whitehead, P.J., R.S. Seymour, and G.J.W. Webb. 1992. Energetics of development of embryos of the Australian fresh-water crocodile, *Crocodylus johnstoni* - relation to duration of incubation. *Physiological Zoology* 65:360-378.
- Yaron, Z. 1985. Reptile placentation and gestation: structure, function, and endocrine control. Pp. 527-603 *In* *Biology of the Reptilia*. Vol. 15. Gans, C., and F. Billet (Eds.). John Wiley & Sons, New York, New York, USA.