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Reproduction in Reptiles, from Genes to Ecology: A Retrospective and Prospective Vision

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

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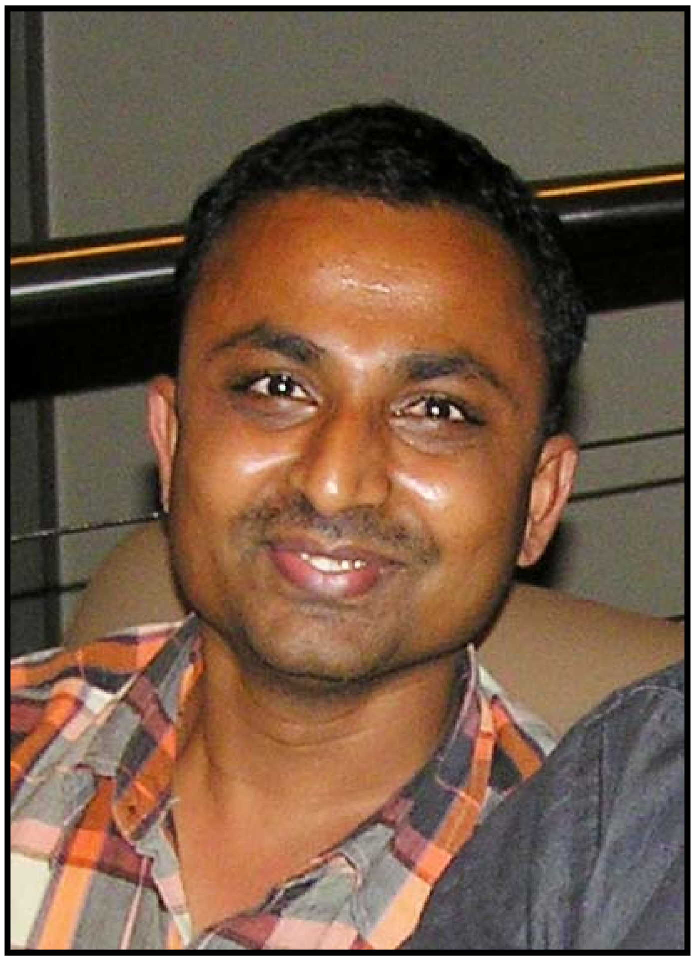
In Memory of
Rajkumar (Raju) Shivappa Radder



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.

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

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***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 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, crocodilians, 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 Ecay, 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 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 artifactual, 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 crocodilians) 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 crocodilians (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.

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