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Eutherian-like reproductive specializations in a viviparous reptile

(placenta/embryology/convergent evolution/reproductive strategy/lizard)

DANIEL G. BLACKBURN*, LAURIE J. VITT†, AND CAROL A. BEUCHAT‡

*Department of Anatomy, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853; †Biology Department, University of California, Los Angeles, CA 90024; and ‡Department of Physiology, College of Medicine, University of Arizona, Tucson, AZ 85724

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ABSTRACT The viviparous Brazilian scincid lizard *Mabuya heathi* exhibits a suite of reproductive specializations widely believed to be confined to the eutherian mammals. This skink ovulates the smallest known reptilian egg (≈ 1.0 mm in diameter). Placental transport accounts for >99% of the dry mass of the periparturient fetus, representing a degree of placentotrophy proportionately greater than that reported in any other non-mammalian vertebrate. Placental morphology and the timing of fetal growth implicate the chorioallantoic placenta in maternal–fetal nutrient transfer. The yolk sac placenta regresses prior to any major increase in embryonic dry mass. Precocious gonadal maturation and postponement of reproductive investment until well after ovulation enables females to become pregnant at 3–4 months of age, long before attainment of full adult body size.

Reptile viviparity typically is regarded as a simple phenomenon in which eggs are retained in the maternal oviducts and hatch *in utero* before birth, a pattern sometimes termed “ovoviviparity” (1–3). Although morphologically specialized placentas have been described for a few squamate reptiles (4–6), quantitative data on placental transport are limited to water and small amounts of inorganic ions, amino acids, and nitrogenous wastes (7–10). Eutherian mammals, in which nutrients are supplied by means of a placenta (“placentotrophy”), contrast markedly with reptiles, in which the large yolk-rich egg usually is assumed to contain sufficient energy for embryonic development (“lecithotrophy”) (6, 11).

We present evidence that *Mabuya heathi*, a Brazilian scincid lizard, exhibits a suite of reproductive characteristics virtually unknown outside of the eutherian mammals, representing an extreme among known reptilian adaptations for viviparity. Specializations of *M. heathi* include the following: (i) ovulation of an egg smaller than that known for any other reptile; (ii) a prolonged (8–12 months) gestation; (iii) formation of a chorioallantoic placenta with a close association between fetal and maternal tissues; (iv) development of a transitory yolk sac placenta; (v) a postovulatory supply of virtually all of the nutrients for development; and (vi) a degree of placentotrophy greater (relative to ovum size) than has been demonstrated in any other non-mammalian vertebrate. This species also possesses other unique adaptations, including the ability of females to become pregnant while at a “juvenile-like” body size and subsequently to grow large enough to accommodate the developing fetuses prior to major embryonic growth.

MATERIALS AND METHODS

Specimens of *M. heathi* were collected (by L.J.V.) near Exu, Pernambuco, Brazil, from March 1977 through February 1978. Upon sacrifice of each specimen, relevant morpho-

metric and meristic data were collected (12) and reproductive tracts were excised and placed in 10% formalin. Individual ovum–embryo samples were removed, rolled dry on paper towels, and weighed. Further studies of the preserved material were conducted in 1981–1983. Representative samples were oven dried at 60°C to a constant mass and weighed to determine preserved dry mass. Morphology was examined through microdissection with a Wild M8 binocular dissection microscope with a photographic attachment. Extraembryonic membranes were carefully peeled from adjacent tissues and conventionally processed for scanning electron microscopy by counterfixing in OsO₄, washing in sodium cacodylate buffer, dehydrating in acetone, critical point drying, and coating with gold in a Balzer sputter coater. Specimens were viewed by means of an AMR 1000 A scanning electron microscope.

RESULTS

M. heathi breeds seasonally and produces one litter per year. In our sample, litter size ranged from 2 to 9 ($\bar{x} = 5.03 \pm 0.12$; $n = 131$) and was positively correlated with female snout-vent length (SVL) ($r = 0.52$; $F_{1, 129} = 47.7$; $P < 0.0001$; brood size = $-1.339 + 0.088 \times \text{SVL}$). Gestation lasted 8–12 months, with ovulation and fertilization occurring from October to January and parturition occurring the following fall (September to October) (Table 1). Our sample suggests that all females in the population become pregnant during each reproductive season, including those born only 3–4 months previously. These females are born at about 31 mm SVL, reach sexual maturity while still at a small body size (45–52 mm SVL), and only subsequently grow large enough (68–92 mm SVL) to accommodate the developing fetuses.

M. heathi ovulates eggs with an average diameter of 1.0 mm (range = 0.9–1.2 mm) (Fig. 1A) and an average dry mass of 0.40 mg (range = 0.22–0.68 mg). Near-term fetuses (Fig. 1B) average 31 mm SVL and 154 mg of dry mass, representing a developmental dry mass increase of 38,400%.

More than 99% of the increase in embryonic dry mass occurs during the 6 months following formation of the chorioallantois (Table 1). The most rapid mass increase occurs in the last 3 months of pregnancy, during which the chorioallantoic placenta is morphologically specialized. During this period, the allantois contacts the entire inner surface of the chorion, vascularizing it by means of the accompanying umbilical vessels. Although a shell membrane separates embryonic and maternal tissues very early in development, this shell membrane is no longer visible by mid-gestation. Thus, the chorion and uterus appear to be in direct contact, with the result that uterine vessels leave their impression on the outer surface of the chorion (Fig. 1C). At the embryonic pole of the egg, an ovoid placentome develops. This is a highly vascularized and thickened area of the chorion that attaches

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Abbreviation: SVL, snout-vent length.

Table 1. Timing of reproduction and development in *M. heathi*

Month	Event	% of near-term dry mass
Sept.–Oct.	Parturition, ovulation (all but first-year females)	<1
Nov.–Dec.	Ovulation (all females), yolk sac formation, water uptake	<1
Jan.	Ovulation (first-year females), yolk sac formation, water uptake	<1
Feb.–April	Allantois forms and expands	<1
May–June	Allantois expands, chorioallantois is established	<1
July	Yolk sac loses contact with chorion, placentome forms	1–11
Aug.	Rapid development	10–11
Sept.	Development completed, growth continues, parturition of some	10–97
Oct.	Growth continues, yolk sac resorbed, parturition	59–100

to the oviduct wall adjacent to the major uterine vessels (Fig. 1D). At this site, an amorphous material accumulates, which appears to be similar in consistency to material within the uterine glands. The quantity of this material increases during the rapid growth phase but disappears prior to parturition.

Formation of the yolk sac occurs soon after ovulation. The eggs quickly swell with fluid to reach 3–4 times their original diameter (Fig. 1E). From May until the time of its pre-parturient resorption, the yolk sac is thick-walled and well vascularized (Fig. 1F), and its dry weight can exceed 6 times that of the newly ovulated egg. During the period of rapid embryonic growth, the yolk sac is excluded from the inner surface of the chorion by the allantois.

DISCUSSION

The large gestational increase in dry mass of the reproductive product in *M. heathi* indicates a substantial postovulatory supply of nutrients by the mother. Two structures are available for maternal–fetal nutrient exchange in viviparous reptiles and mammals, the yolk sac placenta and the chorio-

allantoic placenta (5, 6, 13). The chorioallantoic placenta is implicated in nutrient transfer in *M. heathi* for three major reasons: (i) virtually all of the increase in dry mass of the egg–embryo complex occurs subsequent to establishment of the chorioallantois; (ii) the chorioallantoic placenta is morphologically specialized during the period of heightened maternal–fetal nutrient transfer; (iii) the yolk sac lacks contact with the wall of the chorion during most of this period of nutrient transfer. The chorioallantoic placentome appears grossly similar to literature descriptions of those believed to function in nutrient transfer in distantly related skinks (4, 5, 13, 14), and we postulate that the placentome is a major site of nutrient transfer in *M. heathi*. This hypothesis is supported by morphological features as well as the observation that the placentome is the site of accumulation of material apparently derived from maternal uterine glands.

The yolk sac placenta of *M. heathi* may function in water uptake early in gestation, for absorption of fluid occurs soon after its formation. Such uptake may facilitate liquification of the yolk as a prerequisite for its utilization by the embryo (5, 15). Whether the yolk sac plays a role in nutrient uptake or waste storage in *M. heathi* is unknown.

A few features require confirmation through study of fresh or optimally fixed material. For example, it is possible that formalin fixation affected our measurements of the dry mass of the embryonic tissues. Formalin can slightly alter wet mass of vertebrate tissues (16, 17), most likely through osmotic exchange of water (18). Although most of the functional inferences we have drawn are well supported by morphology, their verification awaits histochemical and physiological investigation. Likewise, ultrastructural examination is necessary to substantiate the absence of the shell membrane during late gestation (see ref. 7).

M. heathi and probably certain other New World *Mabuya* (12) represent a previously unrecognized extreme in reptile reproduction. The ovulated egg of *M. heathi* is far smaller than the eggs of any other reptile genus known (see Table 2). Extensive data on viviparous lizards and snakes (22, 23) suggest that the 8- to 12-month gestation length of *M. heathi* is among the longest known for reptiles. Even more remarkable is the enormous gestational increase in embryonic dry mass, conclusive evidence for substantial maternal–fetal nu-

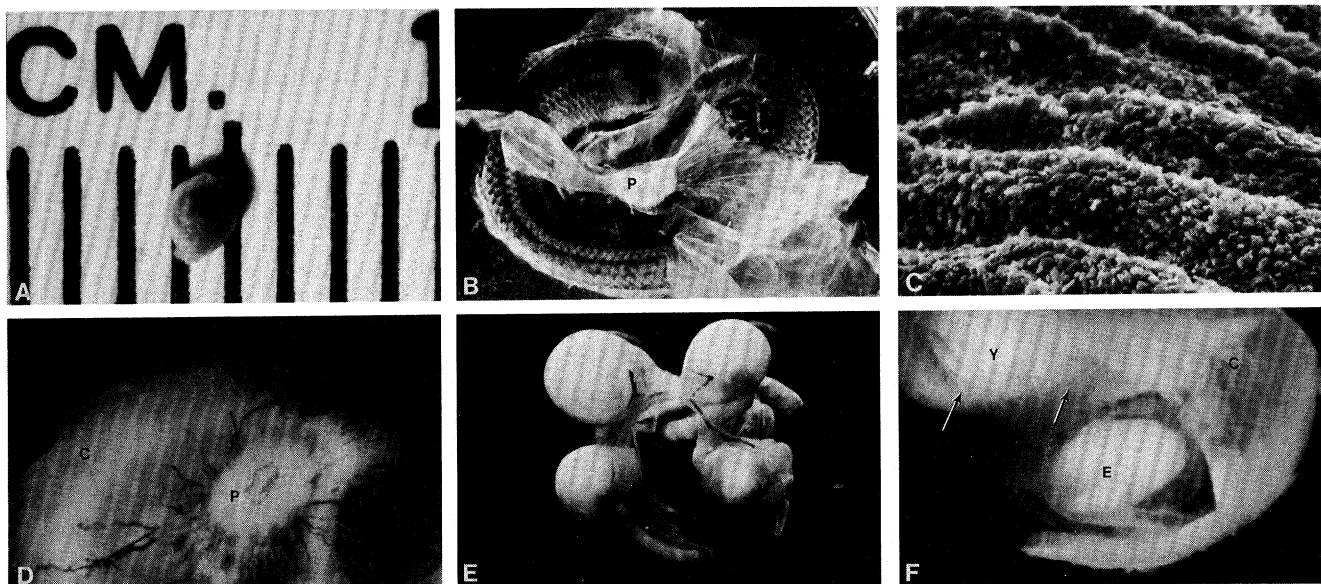


FIG. 1. Development and placentation of *M. heathi*. (A) Newly ovulated ovum, partially enclosed in oviductal tissue. ($\times 7$.) (B) Near-term fetus. ($\times 2.25$.) (C) Scanning electron micrograph of outer surface of chorion, late gestation, showing surface ridges. ($\times 117$.) (D) Chorioallantoic placentome, late gestation, with uterine tissue removed. ($\times 5.48$.) (E) Ova in uterine oviduct, early gestation, showing swelling due to fluid uptake. ($\times 3.45$.) (F) Yolk sac, mid-gestation; arrows indicate vasculature. ($\times 14$.) C, chorion; E, embryo; P, placentome; Y, yolk sac.

Table 2. Volumes of newly ovulated ova of some viviparous reptiles

Species	Ovum volume, mm ³	Source or ref.
<i>M. heathi</i>	0.52	This work
<i>Chalcides chalcides</i>	12	14
<i>Sceloporus aeneus bicanthalis</i>	159*	19
<i>Hoplodactylus maculatus</i>	231	20
<i>Egernia whitei</i>	524	21
<i>Thamnophis sirtalis</i>	567	†
<i>Enhydryis dussumieri</i>	2356	15
<i>Egernia cunninghami</i>	4189	21

Volumes were calculated from dimensions given in the cited sources by using the formula for the volume of an ellipsoid, $V = 4\pi a^2 b/3$, in which V = volume and a and b represent the larger and smaller radii, respectively (19).

*Value given directly by the source cited.

†Unpublished data.

trient transfer. In other viviparous reptiles for which quantitative data are available, embryonic dry mass remains constant or decreases during gestation (8, 10, 19, 24). A distantly related Old World skink, *C. chalcides*, may have begun to approach the type of reproductive specializations seen in *M. heathi*. The ovum of *C. chalcides* measures ≈ 2.5 – 3.0 mm in diameter (13, 14), or about 24 times the volume of the ovum of *M. heathi* (Table 2). The developmental dry mass increase of *C. chalcides*, whose chorionic vesicle increases in diameter from 3 mm to 22 mm during the 3-month gestation (14), has not been measured.

One consequence of the reproductive strategy of precocial gonadal maturation and the ovulation of minute ova is that *M. heathi* females are able to utilize their first breeding season by becoming pregnant at 3–4 months of age, long before they have attained full adult body size. During early gestation, these young pregnant females grow rapidly, reaching a size large enough to accommodate the developing fetuses prior to the period of rapid embryonic growth. The adaptive significance of this reproductive pattern is discussed in further detail elsewhere (12).

M. heathi has strongly converged upon a reproductive pattern long believed to be unique to eutherian mammals. Salient features of this pattern include viviparous production of

young, a long gestation period, ovulation of tiny ova, early development of a yolk sac placenta, subsequent formation of a chorioallantoic placenta with a close association between maternal and embryonic tissues, and placental transport of nearly all of the nutrients for development. The ovum size of *M. heathi* lies within the range of mammalian ovum sizes; marsupials and eutherians ovulate eggs ranging between 0.04 mm and 0.24 mm in diameter (13, 25), and monotremes ovulate eggs of 3–4 mm in diameter, which swell with uterine fluids to 15×17 mm prior to oviposition (26). Although considerably less than that of eutherian mammals, the developmental dry mass increase of *M. heathi* embryos is proportionately larger than those of all other placentotrophic vertebrates for which data are available (see Table 3). A greater relative gain in mass is exhibited only by embryos of the sand tiger shark *E. taurus*, a species in which fetal nutrition is accomplished through oophagy and adelphophagy (intra-uterine cannibalism) (28).

Recent analyses (29–32) have firmly established that viviparity has evolved on numerous independent occasions in vertebrate history. Although evolutionary convergence in fetal nutritional adaptations has occurred frequently among these lineages, only New World *Mabuya* are known to have converged strongly upon the eutherian reproductive pattern (33). Study of the iguanid lizard *Sceloporus aeneus* has revealed that in its simplest form, viviparity can evolve at the subspecific level by means of relatively minor morphological and physiological modifications (34). In contrast, *M. heathi* represents the opposite extreme of the continuum of specializations for the live-bearing mode. The diversity of reproductive patterns represented in *Mabuya* (22), as well as the remarkable evolutionary convergence between *M. heathi* and the eutherian mammals with respect to adaptations for fetal nutrition, suggest this skink genus to be ideal for future morphological, physiological, and evolutionary studies of viviparity.

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Table 3. Developmental mass changes of selected viviparous vertebrates

Taxon	Change in mass, %	Nutritional mode*	Source or ref.
Reptilia			
<i>M. heathi</i>	+38,400	P	This work
<i>Pseudechis porphyriacus</i>	0	L (P)	8
<i>Sphenomorphus quoyi</i>	-10	L (P)	24
<i>Nerodia rhombifera</i>	-22	L (P)	10
<i>Notechis scutatus</i>	-35	L	8
<i>S. aeneus bicanthalis</i>	-54	L	19
Amphibia			
<i>Nectophrynoides occidentalis</i>	+14,000–±28,000†	H	27
Osteichthyes			
<i>Clinus superciliosus</i>	+34,400 (?)	H, P	28
<i>Poeciliopsis turneri</i>	+1,780	P	28
<i>Poeciliopsis monacha</i>	-37	L	28
Chondrichthyes			
<i>Eugomphodus taurus</i>	+1,180,000	A, O	28
<i>Mustelus canis</i>	+1,050	P	28
<i>Centrophorus granulosus</i>	-54	H	28

Values refer to dry mass unless otherwise indicated. A, adelphophagy; H, histotrophy; L, lecithotrophy; O, oophagy; P, placentotrophy.

*All are probably lecithotrophic early in development.

†Wet mass; estimating from other vertebrates, the dry mass increase may be about 1/2 of this value.

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