Review

Maternally derived egg yolk steroid hormones and sex determination: Review of a paradox in reptiles

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During the past decade, maternally derived steroid hormones in the egg yolk of oviparous vertebrates have been the focus of attention for their possible role in sex determination and hence, information on the consequences of maternal egg yolk steroids on sex determination has accumulated rapidly in reptiles and birds. Until recently, the observations were dominated by the idea that yolk steroids of maternal origin play an important role in sex determination of oviparous vertebrates. However, more recent studies have cast significant doubt on the above conclusion. These studies suggest instead that steroids may be present in the yolk simply as the byproduct of passive uptake during yolk formation or observed correlations might reflect embryonically produced rather than maternally derived steroids. Thus, the objective of the present review is (i) to provide an overview of such paradoxical observations on the role of maternal yolk steroids in sex determination of reptiles, (ii) to identify and provide brief explanations for the observed paradoxical results, and (iii) to provide some future research directions.

1. Introduction

In most animal species, sex is one of the primary axes of morphological and behavioural variation within a population. Whether an individual develops as a male or a female is one of the most important "decisions" that a developing embryo must make – but how is this decision made? Not surprisingly, the factors that determine an embryo's sex have been the subject of scientific debate for more than 3000 years (reviewed by Mittwoch 2000). A variety of sex-determining mechanisms (SDMs) operate in vertebrates. Among these, genotypic sex determination (GSD) and environmental sex determination (ESD) are two major types of SDMs. More specifically, sex determination has so far proven to be the result of one of the following mechanisms or sometimes the combination of two or more mechanisms (reviewed by Sarre *et al* 2004; Manolakou *et al* 2006): (i) Environmental

action on the embryo at a crucial stage of development. This process is also known as environmental sex determination and many environmental factors have the capacity to affect sex independent of the genetic composition of the zygote; (ii) genetic action, when at least one specific gene is considered to be the central regulator in a cascade of events leading to the determination of sexual phenotype. This mechanism is already known to apply in the case of several animals (e.g. insects, worms and amphibians). However, it is a proposed regulatory mechanism for several species (for detailed discussion *see* Manolakou *et al* 2006); and (iii) the presence of distinct sex chromosomes or gonosomes. The identical allosome pair may be present in both males (e.g. birds) and females (mammals).

Remarkably, recent studies have shown that numerous non-genetic factors (both environmental and physiological) can also influence offspring sex ratio even in species with

GSD. These non-genetic factors include prey abundance, rainfall, season, habitat quality, year of study, clutch sequence, parental condition, clutch size, diet, feeding regimen, incubation temperature, maternal age, dominance status, mate quality, sexual attractiveness, position of the embryo in the uterus, crowding, etc. (reviewed by Kraak and Pen 2002; Pike and Petrie 2003; Sheldon and West 2004; West *et al* 2005; Manolakou *et al* 2006). But what factors interact with sex-specific genetic features to influence the sex of offspring? A factor common to all the preceding stages when sex manipulation could occur is their intimate relationship with circulating maternal hormones. Maternal allocation of steroid hormones to eggs offers a plausible candidate in this respect in oviparous vertebrates because (i) all steroid hormones are lipophilic and therefore highly yolk-soluble, and readily cross biological membranes. Thus, steroids can easily pass from the mother into the yolk during vitellogenesis and mothers could potentially control allocation to each egg (Rutstein *et al* 2005). (ii) Eggs of birds and reptiles often contain significant amounts of maternally derived steroid hormones (Schwabl 1993; Janzen *et al* 1998; Elf 2003, 2004). (iii) Experimental application of excess hormone to eggshells of birds and reptiles can switch genetically determined sex (Wade *et al* 1997; Freedberg *et al* 2006; Radder and Shine 2007). (iv) Maternal allocation seems feasible on physiological grounds, because of widespread transfer of nutrients, gases, water and steroid hormones from pregnant mothers to their developing embryos (Janzen *et al* 1998, 2002; Rutstein *et al* 2005).

Reports of sex differences in yolk hormone levels suggest some kind of functional link between maternally derived yolk hormones and offspring sex; i.e. either hormones determine sex, or mothers allocate different amounts of steroid hormones to eggs destined to carry male vs female embryos (Bogart 1987; Adkins-Regan *et al* 1995; Gil *et al* 1999; Petrie *et al* 2001; Lovern and Wade 2002, 2003b; Correa *et al* 2005; Pike and Petrie 2005, 2006). Interestingly, however, most recent studies dispute the validity of earlier reports of sex differences in yolk steroid concentrations between male and female eggs in reptiles as well as in birds (Eising *et al* 2003; St Juliana *et al* 2004; Pilz *et al* 2005; Gil *et al* 2006; Kratochvil *et al* 2006; Radder *et al* 2007; Warner *et al* 2007). These studies cast significant doubt on the previous general conclusion that reproducing females strategically modify yolk steroid levels relative to offspring sex, and suggest instead that the observed correlations might reflect embryonically produced rather than maternally derived steroids or that steroids may be present in the yolk simply as the byproduct of passive uptake during yolk formation. Clearly, there is a need for a comprehensive review of these conflicting observations on a wide variety of animals from different lineages and with different modes of sex determination. Thus, the main objectives of the present review are (i) to provide an overview of some paradoxical observations on the role of maternal yolk steroids in sex determination of reptiles, (ii) to provide a brief explanation for the observed contradictions, and (iii) to identify some future research directions.

2. Why reptiles for the present review?

Reptiles are a familiar group of vertebrates, having existed for more than 300 million years. These animals reached their zenith during the Jurassic and Cretaceous periods, but today they are represented by only four orders (turtles, crocodilians, squamates and sphenodontians). Reptiles offer exciting model systems with which to explore questions about the mechanism(s) that switch developmental processes among alternative trajectories (such as "male" vs "female") and the interplay between genetic factors and environmental influences. This is because reptiles are known to exhibit an extraordinary variety of sex-determining mechanisms and some of the most complex sex chromosome structures observed in vertebrates (reviewed by Modi and Crews 2005). For example, in many reptiles (most turtles and an increasing number of lizards) sex is determined by the temperature at which eggs are incubated (commonly known as "temperature-dependent sex determination" or TSD). Usually, species that display TSD do not have sex chromosomes (but *see below*). By contrast, several turtles, some lizards and all snakes studied so far exhibit genetic sex determination (GSD). Interestingly, unlike birds and mammals, GSD reptiles exhibit both male and female heterogamety. A few species of turtles and some lizards have male heterogamety (XY males and XX females), whereas other turtles, a few lizards and all snakes have female heterogamety (ZZ males and ZW females). Further, a few select species of lizards and one turtle species exhibit remarkable multimodal sex determination (TSD as well as GSD along with the influence of other non-genetic factors; Servan *et al* 1989; Shine *et al* 2002; Ezaz *et al* 2005; Quinn *et al* 2007). According to Modi and Crews (2005), the ZW chromosomes of snakes reveal increased differentiation as one progresses from the phylogenetically primitive boids to the more advanced viperids. The origin of heteromorphic XY sex chromosomes in two species of turtles is thought to have occurred independently, and these chromosomes appear as autosomes in other species of TSD turtles (reviewed by Modi and Crews 2005). Thus, the variability observed in reptilian chromosomes suggests that sex chromosomes and sex-determining systems have evolved independently in different lineages. Also, a better understanding of sex determination in reptiles could contribute significantly to understanding basic evolutionary biology and molecular mechanisms behind the sex determination of higher vertebrates including our own species, as reptiles are direct

ancestors to birds and mammals (more details are in Modi and Crews 2005). The above unique features have made reptiles an excellent and ideal group with which to test the hypotheses related to sex determination in vertebrates. Previous reviews on the topic of maternal egg-yolk steroids and sex determination in reptiles have dealt only with TSD species (Elf 2003, 2004). The present review is the first comprehensive account of reptiles with different modes of sex determination.

3. Maternal yolk steroids and sex determination in reptiles

In the majority of the studies conducted on reptiles, two principal sex steroid hormones (testosterone [T] and 17*ß* oestradiol [E_2]) have been quantified. These studies report measurable quantities of both of these steroid hormones in the egg yolk (table 1). Additionally, yolk dihydrotestosterone (DHT), androstenedione, progesterone and corticosterone levels have also been reported for a few species (Warner *et al* 2007; Radder *et al* 2007). However, it is the levels of T and E_2 in the egg yolk that are of major interest in the majority of such studies. Data are available for 18 species with diverse modes of sex determination (table 1). Among these, 13 species exhibit TSD, whereas 4 species exhibit genetic sex determination with different types of sex chromosomes (table 1). The remaining one species (*Bassiana duperreyi*) is known to exhibit complex multimodal sex determination (Shine *et al* 2002; Radder *et al* 2007). Overall, the data presented in table 1 represent 5 species of lizards, one crocodilian and 12 turtle species.

Interestingly, among the 13 TSD species, significant sex differences in yolk steroid levels at oviposition were detected in 7 species (table 1). Five species did not show any sex-specific differences in the yolk steroid levels between eggs destined to become sons or daughters, or any relationship with offspring sex ratios (table 1) and in one TSD species (*Trachemys scripta*) the evidence is contradictory (table 1). In 3 out of 4 GSD species, there is no association between offspring sex and maternal yolk steroid levels in egg yolk at oviposition (table 1). In the fourth species (*Anolis carolinensis)* significant sex-specific variation in yolk steroids levels (especially in yolk T levels) has been reported. The maternal yolk steroid profile in eggs at oviposition is available for only one species of skink that remarkably exhibits multiple modes of sex determination. This skink also shows no significant difference in principal sex steroids levels (i.e. T, E_2 or DHT) between eggs destined to become male or female (table 1). Further, the T:E_2 ratio (a proposed link between steroid ratio and mode of sex determination) also exhibits inconsistent patterns among the reptiles studied so far (table 1). Out of 9 TSD species (for which the ratio can be derived), 7 species show a T:

E_2 ratio >1 and the remaining 2 species have a ratio <1 (table 1). Among the GSD species, one has a ratio <1 and the remaining species has a ratio >1 (table 1).

4. Why is the evidence paradoxical in reptiles?

Although early research produced conflicting results as to whether or not maternal steroids are transferred to embryos via egg yolk (Altmann and Hutton 1938; Hertelendy and Common 1965), more recent work using radioactively labelled steroids and steroid radioimmunoassays makes it very clear that maternally derived steroids are common yolk constituents not only in turtles, alligators and lizards but also in birds and fishes (Arcos 1972; Conley *et al* 1997; McCormic 1998; Bowden *et al* 2000; Elf and Fivizzani 2002; Lovern and Wade 2003a,b; Elf 2003, 2004). However, the functional role of maternally derived yolk steroids and their effects (if any) on sex determination remain controversial despite sustained research spanning over a decade. Why is the evidence concerning yolk steroids and sex determination so conflicting in reptiles? Below is a brief summary of the potential reasons for this paradox.

In birds, the inconsistency of results alluding to the role of yolk steroids on sex determination is basically attributed to sampling (yolk) errors. For example, some empirical demonstrations of sexually dimorphic sex steroid concentrations in birds (Petrie *et al* 2001) relied on sampling eggs after the embryonic gonads had differentiated and thus, may have been actively involved in producing or sequestering steroids (Pilz *et al* 2005). In support of this interpretation, later studies demonstrated no sex differences in yolk steroid concentrations of avian eggs at the time of laying (e.g. chicken eggs, Müller *et al* 2002; Eising *et al* 2003; quail eggs*,* Pilz *et al* 2005; swallow eggs, Gil *et al* 2006). By day 10 of incubation, male embryos of these species produce more T whereas female embryos produce more E_2 (Pilz *et al* 2005). The sexes also differ in steroid sequestration, with female embryos metabolizing androgens from the yolk more rapidly than males, whereas males sequester/metabolize E_2 from the yolk more rapidly than females (Pilz *et al* 2005). However, the inconsistency of yolk sampling is not the major cause for the observed paradoxical results in reptiles because: (i) in the majority of studies (in both GSD and TSD reptiles) yolk was sampled immediately after oviposition; and (ii) in reptiles, fertilized eggs usually contain embryos at early stages of development. Eggs of turtles are invariably oviposited at the gastrula stage (~ stage 4–5 of development), crocodilians oviposit when embryos are at the neurula stage (somewhat more advanced than those of turtles; stage 10–14) and the majority of lizards oviposit at developmental stage 30 (Shine 1983; Andrews and Mathies 2000; Harlow 2004). During the above stages of embryonic development at oviposition, in all cases, there is no trace of

Table 1. Summary of yolk sex steroid hormone levels, T:E_2 ratio, mode of sex determination and the role of yolk steroid hormones in sex determination of reptiles

Species	SDM/ mode of SDM	Yolk steroid levels (ng/g)		T:E_2 ratio	Sex differences in yolk steroids at oviposition/role in sex determination	Source*
		T	E_2			
Crocodile						
Alligator mississippiensis	TSD (H)	2.3 ± 0.18	19.0 ± 1.3	> 1.0	Yes	1, 2
Lizards						
Anolis carolinensis	GSD (XX/XY)	NR	NR	--	Yes	3
Amphibolurus muricatus	TSD (H)	0.10 ± 0.01	ND	--	No	4**
Bassiana duperreyi	TSD/GSD (XX/XY)	0.31 ± 0.06	0.42 ± 0.06	>1.0	No	5
Eublepharis macularius	TSD (H)	0.15 ± 0.03	0.55 ± 0.06	>1.0	Yes	2
Paroedura picta	GSD (XX/XY or ZZ /ZW)***	1.48 ± 0.06	0.39 ± 0.02	<0.1	No	6
Turtles						
Apalone mutica mutica	GSD (H)	NR	0.27 ± 0.04	--	No	7
Apalone spinifera hartwegi	GSD (H)	NR	0.69 ± 0.01	--	No	7
Chelydra serpentina	TSD (H)	2.56 ± 0.09	2.78 ± 0.09	~ 1.0	No	1, 2, 8
Chelydra serpentina serpentina	TSD (H)	NR	2.12 ± 0.20	--	Yes	7
Chrysemys picta	TSD (H)	0.68 ± 0.04	0.89 ± 0.06	>1.0	Yes	1, 2
Chrysemys picta bellii	TSD (H)	NR	1.86 ± 0.20	--	No	7
Chrysemys picta marginata	TSD (H)	1.62 ± 0.10	1.23 ± 0.42	<1.0	No	9
Emydoidea blandingii	TSD (H)	1.15 ± 0.09	0.99 ± 0.12	<1.0	Yes	2
Graptemys ouachitensis	TSD (H)	0.15 ± 0.01	2.66 ± 0.18	>1.0	Yes	2
Graptemys pseudogeographica	TSD (H)	0.19 ± 1.91	1.91 ± 0.156	>1.0	Yes	2
Trachemys scripta	TSD (H)	0.67 ± 0.17	2.81 ± 0.70	>0.1	Yes/No	1,2,7
Trachemys scripta elegans	TSD (H)	NR	0.87 ± 0.05	--	No	7, 9

* To reduce literature citations, for many species, data available in reviews are referenced to the reviews rather than the original publications.

The role of yolk steroids in sex determination was determined based on interclutch variation and correlations in some turtles.

(GSD, genotypic sex determination; SDM, sex determination mechanism; TSD, temperature-dependent sex determination; XX/XY refers to genetic sex determination with male heterogamety; H, homomorphic chromosomes; ND, hormone level not detected in the yolk; NR, mean and SE are not reported in original publication; T:E_2 ratio, testosterone:oestradiol ratio based on the mean values).

Source

(1) Elf (2003). **(2)** Elf (2004). **(3)** Lovern and Wade (2003). **(4)** Warner *et al* (2007). **(5)** Radder *et al* (2007). **(6)** Kratochvil *et al* (2006). **(7)** Janzen *et al* (1998). **(8)** St. Juliana *et al* (2004). **(9)** Bowden *et al* (2001).

** Personal communication. *** Sex determination mode is GSD but sex chromosomes are not described yet (Blumberg *et al* (2002). Kratochvil *et al* (2006); personal communication with Kratochvil L).

the gonadal–adrenal–mesonephric kidney (GAM) complex. The GAM complex is thought to be the source of steroid biosynthesis in developing embryos during the later stages of development (Elf 2004; Doddamani 2006; Radder *et al* 2007). Also, during the early stages of development the reptilian GAM is quiescent and does not produce any significant quantity of steroid hormones (Lovern and Wade 2003a; Elf 2004; Doddamani 2006; Radder *et al* 2007). Thus, yolk-sampling inconsistencies may not be a major cause of the conflicting results in reptiles as they are in birds.

Some recent studies have attempted to link interspecific variation in the $T:E_2$ ratio, as well as absolute steroid levels, with modes of sex determination (Lovern and Wade 2003a; Kratochvil *et al* 2006; Radder *et al* 2007). According to these studies, GSD species are reported to have a $T:E_2$ ratio >1, and experimental manipulations of yolk steroid levels in these species suggest that T may be more effective than E_2 in affecting early development (Schwabl 1996; Lipar and Ketterson 2000; Lovern and Wade 2003a). In contrast, $T:E_2$ ratios <1 have been reported in several TSD species, and experimental manipulations of yolk steroids suggest that E_2 plays an important role in sex determination (ref. in Lovern and Wade 2003a; Radder *et al* 2007). Additionally, a complex pattern of steroid ratio is evident in a species with multiple modes of sex determination (*B. duperreyi*, Radder *et al* 2007). However, a review of $T:E_2$ ratio data in both TSD and GSD species failed to provide any robust general pattern, as reported in section 3 above.

Lovern and Wade (2003b) demonstrated sex-specific variation of yolk steroids in a lizard, the green anole *A. carolinensis*. This is the only study that strongly demonstrates sex-specific allocation of yolk steroids in GSD reptiles studied so far (table 1). However, *A. carolinensis* differs from most other reptiles in producing only a single egg at a given time. The left and right ovaries are used in alternation, and ovulation occurs frequently (at an interval of 7–14 days: Smith *et al* 1973; Andrews 1985). This unusual reproductive mode would allow reproducing female anoles to allocate different yolk hormones to different eggs. In contrast, most reptiles ovulate egg(s) from each ovary simultaneously during the breeding season. Moreover, unlike birds or *A. carolinensis,* most reptiles yolk all follicles for a given clutch simultaneously, and hence follicles are exposed to the same maternal hormonal environment (Bowden *et al* 2002; Janzen *et al* 2002). This type of reproduction is a major physiological constraint to sex-specific maternal allocation of steroids because it would require a reproducing female to somehow produce different quantities of androgens as well as oestrogens for different eggs without compromising her own reproductive physiology. Successful reproduction is a fine-tuned balance between various sex steroids, and especially between androgens and oestrogens (Whittier and Tokarz 1992). Because sex steroid production is under the control of hypothalamic and pituitary hormones, mechanisms for selective local enhancement of specific hormone levels for differential sex steroid allocation are difficult to visualize.

Reports of physiological constraint for sex-specific resource allocation, including steroid hormones, to simultaneously developing follicles are available in birds also (for a detailed discussion refer Young and Badyaev 2004; Badyaev *et al* 2006a, b). Additionally, data from both reptiles and birds do not show unequivocally that levels of a single hormone can be regulated independently in plasma and yolk (Müller *et al* 2007). For instance, at the time of laying, E_2 and T levels are often similar (reviewed by Müller *et al* 2007). Thus, the lack of sex-specific yolk steroid allocation in the majority of studies on GSD and TSD species may reflect physiological constraints. Yet, in sequentially ovulating birds it is proposed that sex-specific oocyte growth, yolk partitioning, and spatial and temporal clustering of ova are some of the possible mechanisms by which mothers can bias sex-specific resource allocation (Young and Badyaev 2004; Badyaev *et al* 2006a, b). However, the above mechanisms are species and population specific (Badyaev *et al* 2006a, b). To the best of my knowledge there is no such information on any of the simultaneously ovulating oviparous reptiles at present, and thus, this area of research warrants further investigation.

Some previous studies on reptiles have relied upon experimental manipulations (applying exogenous steroids directly to eggs or implanting hormone capsules to experimentally enhance steroid levels in mothers and eggs) and indirect relationships (between maternal plasma levels and sex ratios, or correlating interclutch variations to variation in offspring sex ratios; Bowden *et al* 2000; Kratochvil *et al* 2006) rather than quantifying naturally occurring levels of yolk steroids to explore correlations between yolk steroid levels and sex ratios of the offspring that hatch from those eggs. Such indirect correlations and experimental manipulations may not reflect the natural situations. The most important source of steroid hormones (other than corticosterone) secreted to the eggs is probably the follicular wall rather than the maternal plasma (reviewed by Müller *et al* 2007). Thus, a better way to study the transfer of steroid hormones may be direct stimulation of steroid hormone production in the follicles using trophic hormones (such as follicle-stimulating hormone [FSH]). Such trophic hormone stimulation will enable us to investigate how synchronously hormone releases into the plasma and eggs, and to study the factors that modulate the release into plasma versus eggs (reviewed by Müller *et al* 2007). Another way to study the consequences of maternal yolk steroids on sex ratios is to quantify naturally occurring hormones in the egg yolk at oviposition and correlate that with the offspring sex that hatches from the same eggs. Interestingly, recent studies with reptiles that followed the latter approach (correlating naturally occurring yolk steroids to sex of the offspring) consistently demonstrated that yolk steroid hormones do not determine offspring sex and are not allocated in a sex-specific way in either GSD or TSD species (St Juliana *et al* 2004; Radder *et al* 2007; Warner *et al* 2007).

The variety of methods used to identify sex of the offspring is probably another minor source of concern in some reptilian studies. For example, in some studies offspring sex was confirmed by morphological (e.g. postanal scale dimorphism methods; Lovern and Wade 2003b) or

indirect methods (i.e. measuring representative samples of one or two eggs from each clutch and correlating that with clutch or population sex ratios; Bowden *et al* 2000; St. Juliana *et al* 2004; Kratochvil *et al* 2006). In other cases, yolk was sampled destructively, i.e. by freezing eggs at collection or oviposition and sacrificing the embryos (St Juliana *et al* 2004; Kratochvil *et al* 2006). Also, in some instances, morphological sexing methods were not reconfirmed by gonadal histology or molecular sexing methods (Lovern and Wade 2003b; Kratochvil *et al* 2006). However, one should note here that in many studies sex of the offspring was indeed confirmed by gonadal observations (Janzen *et al* 1998; Bowden *et al* 2000; Radder *et al* 2007). Of particular note, in a recent study on the Australian alpine skink *B. duperreyi,* offspring sex was confirmed and reconfirmed by all available sexing methods (i.e. morphological and histological methods initially, Radder *et al* 2007) and later with molecular sexing methods as well (unpublished observations). Also, in the study by Radder *et al* (2007) instead of relying on a representative sample only, yolk was sampled from all eggs in a given clutch using a non-destructive yolk sampling method (allometric engineering) and importantly, offspring were obtained from those yolk-sampled eggs. Such longitudinal data on yolk steroids from eggs and offspring are necessary to understand sex determination in more detail since it will overcome some of the previous methodological problems and bias associated with many reptilian studies.

5.   Conclusion and future directions

If maternally derived yolk hormones do not determine offspring sex and are not allocated in a sex-specific way, what is the biological role of such hormones? One can only speculate that sex steroids in the yolk may influence other aspects of offspring development in reptiles (Fraipont *et al* 2000; Uller and Olsson 2003; Rhen *et al* 2006), perhaps in ways that either minimize or enhance sibling competition as in birds (Schwabl 1996; Schwabl *et al* 1997; Eising *et al* 2001; Lipar and Ketterson 2000; Navara *et al* 2006). Alternatively, steroids may be present in the yolk simply as the byproduct of hormonal regulation of maternal physiology, due to passive uptake during yolk formation (Janzen *et al* 2002). This second (non-adaptive) explanation fits well with the majority of observations in reptiles; clutches with higher levels of one type of sex steroid (androgens) also exhibit higher levels of other steroids (oestrogens), perhaps reflecting simple variation among reproducing females in overall hormone titres. One should also note here that some bird species differentially allocate yolk steroids to male and female eggs but sex steroids do not influence sex determination (Müller *et al* 2002; Rutstein *et al* 2005), so the functional roles (if any) of sex steroids in yolk remain

unclear. Further, as in birds, it remains possible that other yolk steroids (other than sex steroids) that are not produced by the ovary (e.g. corticosterone) may play an important role in sex determination in reptiles, or that yolk sex steroids are involved but in a more complex way in combination with other factors that have not yet been quantified (Pike and Petrie 2005, 2006).

Where do we go from here? Clearly, current observations on the role of maternal yolk sex steroid hormones on offspring sex determination in reptiles are conflicting. The question, whether mothers can independently regulate plasma and yolk hormones without compromising their own reproductive physiology, and independent single-hormone regulation in plasma as well as yolk, remains unclear and open for further investigations. A better understanding of the hormone-mediated effects on sex determination may be achieved only by understanding the physiological, ecological and endocrinological constraints on both embryos and mothers. Recent rapid developments in non-destructive yolk sampling, molecular sexing and sensitive radioimmunoassay methods for reptiles will overcome some of the methodological problems associated with studying yolk steroids and sex ratios in the past. Integration of the above methods into future research may lead to rapid advances not only in understanding the paradoxes associated with maternal yolk hormones and sex determination, but also in an understanding of the consequences of maternal yolk hormones for offspring growth and development.

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