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## **MINI-REVIEW:**

# SEX DETERMINATION AND SEXUAL DIFFERENTIATION IN REPTILES

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## ABSTRACT

Sex determination and sexual differentiation in vertebrates have received a great deal of attention in the past few decades. The current view is that gonochorism (separate sexes) in the earliest vertebrates involved environmental control of sex determination and that genotypic sex determination evolved later (*Intersexuality in the Animal Kingdom*, 438, 1975). Concurrent with the establishment of the latter was the gradual evolution of sex chromosome heteromorphism. Reptiles are excellent models for investigations focusing on sex determination and sexual differentiation because they exhibit: (a) both genotypic and environmentally-determined sex determination, (b) various degrees of sex chromosome heteromorphism, (c) both male and female heterogamety (the former also occurs in mammals, the latter in birds), and (d) variations from the typical 1:1 sex ratio predicted by Fisher (*The Genetical Theory of Natural Selection*, 1930). This paper will review patterns of sex determination for the various groups of reptiles and will briefly discuss physiological mechanisms that may be regulating sexual differentiation.

## GENOTYPIC VS. ENVIRONMENTAL SEX DETERMINATION

Genotypic sex determination (GSD) refers to a system in which the sex of an offspring is normally irreversibly fixed by its own (or its parent's) genotype. Conversely, environmentally-dependent sex determination (ESD) is a system in which an offspring's sex is determined by the environment it encounters at some time after fertilization. The dichotomy between GSD and ESD is not necessarily absolute, in that an organism may have a genotypic mechanism that operates under some conditions but is subject to environmental control under others.

## GENOTYPIC SEX DETERMINATION IN REPTILES

Squatmata: Snakes. Snakes are the only group of reptiles in which GSD is ubiquitous. However, few members of this group have been critically examined for the presence of ESD. Regardless of whether ESD is ever found in this group, the study of sex determination in snakes is of interest because it provides a suggestive evolutionary series of sex chromosome heteromorphisms. Snakes are grouped phylogenetically according to skeletal characters. The boids possess the ancesteral skeletal condition, the colubrids are derived from the ancestral type, and the viperids are derived from the colubrid condition. Sex chromosomes follow a similar pattern: boids are generally homomorphic (ancestral), colubrids differ only in centromere position, and viperids show female heterogamety. Despite the varying degrees of heteromorphism, the sex chromosomes apparently are

derivatives of a single genotypic sex-determining mechanism ancestral to all snakes.

Besides the suggestive pattern for the evolution of sex chromosome heterogamety, snakes also pose a perplexing problem for those interested in the evolution of sex ratios. Fisher (*ob. cit.*) predicted that the primary sex ratio within a population should evolve to 1:1. However, some species of snake produce offsprings with highly skewed sex ratios (Copeia 1985, 649). The skewing may either be in favour of males or females depending on the species. At present there is no conclusive data indicating the reason(s) for this unexpected finding.

Squamata: Lizards. Unlike the snakes, sex chromosome heteromorphisms which occur in at least seven families of lizards have multiple origins. Except for the family Pygopodidae, the number of species with distingishable sex chromosomes is a minority of the species that have been karyotyped. The evidence indicates that heterogamety evolved recently in some of species with sex chromosomes. For example, only two species of Gekkonids are known to possess heteromorphism, and in each case, the heteromorphism is not even observed throughout the species' range (Evolution and Reproduction, 55, 1977; Chromosoma 54, 75, 1976). Even though heteromorphism is rare in this group, GSD is apparently the most prevalent method of sex determination (only two species are known to exhibit ESD).

**Chelonia.** Genotypic sex determination is uncommon in turtles and is apparently of recent origin as both types of sex determination may be observed for different species within the same Family (Copeia 1985, 784). Only three species are known to possess heteromorphic sex chromosomes and in each case it is the male which is heterogametic. **Crocodilia.** All species of Crocodilia have been karyotyped and no sex chromosome heteromorphism has been observed (Cytogenetics 9, 81, 1970). Unlike other reptiles, this group has no microchromosomes so the possibility of overlooking a heteromorphism is unlikely. Available data indicate that crocodilians group probably do not exhibit GSD.

#### TEMPERATURE-DEPENDENT SEX DETERMINATION IN REPTILES

A special type of ESD is temperature-dependent sex determination (TSD). TSD is probably the ancestral form of sex determination for all groups of reptiles (Quart. Rev. Biol. 55, 3, 1980). The first report of TSD in reptiles was made in 1967 by Charnier (Soc. Biol. Quest. Af. 160, 620); however this phenomenon was brought to the attention of the scientific community largely as the result of research performed by C. Pieau and C. L. Yntema. Data indicate that temperature is in fact controlling sexual differentiation of the embryonic gonad rather than causing differential mortality of one sex (Science 206, 1186, 1979).

Not surprisingly, the vast majority of experiments examining ESD in reptiles have focused on the effects of temperature. This narrow focus may be unfortunate because other epigamic factors that may also be affecting sex determination are currently being overlooked (e.g. water potential of the substrate has been shown to influence sex determination in one species (J. Exp. Zool. 226, 467, 1983)). Therefore, while the discussion of ESD in this paper will focus on the effects of temperature, readers are cautioned that temperature may not be the sole environmental factor influencing sex determination.

Patterns for TSD. One surprising observation in the patterns of TSD among groups of reptiles is that maleproducing and female-producing temperatures in lizards and crocodilians are the reverse of those found in turtles. Temperatures of 25-27°C produce females in lizards and crododilians, whereas, the same temperatures produce males in turtles. Whether this is an actual reversal of the sex determining mechanism or simply a phase shift in the response to temperature has yet to be determined. Current data indicate that TSD is ubiquitous in crocodilians, frequent in turtles, rare in lizards, and absent in snakes (Table 1). At present, information is not available for the type(s) of sex determination in the amphisbaenids and the tuatara.

In species with TSD, the biases of sex ratios are dramatic. Over a 1-2°C range the sex ratio will go from almost all male offspring to all female. The temperature at which this shift in sex ratio occurs (the threshold temperature) is species-specific but one threshold generally is found between 28° and 30°C. Previously, it was assumed that there was a single threshold temperature for each species but findings presented by Yntema (J. Morphol. 150, 453, 1976) on snapping turtles (*Chelydra serpentina*) demonstrated that two threshold temperatures were present (*i.e.* females are produced both at high and low temperatures). This spurred the search for a second threshold in other

Crocodilia Alligatoridae + Crocodylidae +	D
Alligatoridae + Crocodylidae +	
Crocodylidae +	
Chalania	
Chelonia	
Chelidae –	
Chelonidae +	
Chelydridae +	
Dermochelyidae +	
Emydidae +/-	-
Kinosternidae +/-	
Trionychidae –	
Squamata (lizards)	
Agamidae 1 s	5
Gekkonidae I s	5
Iguanidae –	
Lacertidae –	
Squamata (snakes) All -	

TABLE I: Temperature-dependent sex determination (TSD) in reptiles.

species. Currently, several species have now been found which exhibit two thresholds. We must await critical examination of more species to determine if two thresholds are the normal pattern rather than the exception. One of the problems encountered in this search is that the second threshold temperature, if present, is usually at the extreme range of survivability for incubation at constant temperatures. However this should not dissuade researchers from seeking a second threshold as temperatures within natural nests are often variable and at least occasionally are in the range which would cause mortality if conditions remained constant.

The discrepancy between thermal profiles found in the field and those used in the laboratory has been used to support the argument that TSD is simply a laboratory artifact which does not apply to natural conditions. However, data have now been presented which demonstrate that findings in the laboratory regarding the occurrence and pattern of TSD correlate to those obtained in the field (Ecology 66, 1115, 1985).

Another area of concern for researchers is the permanence of temperature-induced sex (*i.e.* does sex of an individual change as the organism matures?). This question was probably raised as a result of studies involving the influence of steroid hormones on sex determination (see below). Recent work indicates that sex reversal probably does not occur after hatching in species that exhibit TSD (J. J. Bull, in press).

Sensitive periods. An important consideration in the study of TSD is the sensitive period during which temperature affects sex determination. The results obtained from these inquiries are rather perplexing in that the sensitive period apparently varies among taxa and between sexes within the same species (Quart. Rev. Biol. 55, 3, 1980). Some of the confusion regarding the former is undoubtedly the result of different staging criteria used to describe embryological development among various groups of reptiles. For example,

temperature affects sex determination in turtles between stages 17-19 inclusive, while the sensitive period is reported to be between stages 37-39 for lizards. The impression is that sex is determined in turtles earlier in embryogeny than it is in lizards. However, there are only 25 stages described for turtle embryogeny (J. Morphol. 125, 219, 1968); thus apparent variations in the sensitive period for sex determination between turtles and lizards are difficult to interpret. The observation that within a species the sensitive period for male induction may not coincide with the period for female induction may be attributable to the fact that embryological stages are based on gross morphological characters the occurrence of which becomes less and less dependent upon temperature as development progresses (J. Morphol. 125, 219, 1968). Sexual differentiation however is due to physiological processes which are probably temperature sensitive throughout development. Thus two embryos may be at the same morphological stage of development but vary substantially in their physiological stage.

**Constraints on TSD in nature.** There are at least three circumstances that exclude the occurrence of TSD in nature: (a) the presence of heteromorphic sex chromosomes, (b) live-bearing, and (c) brooding. These constraints are due to theoretical considerations and are consistent with the available data (for a more detailed discussion see Quart. Rev. Biol. 55, 3, 1980).

## MECHANISMS OF GONADAL DIFFERENTIATION

The physiology of gonad determination has not been resolved for any vertebrate. The sequence of events leading to gonadal differentiation apparently follows a similar pattern for all gonochoristic vertebrates: germ line cells migrate to the area where the somatic portion of the gonad is developing and then either move into the cortical (centre) or remain in the medullary (outer) regions of gonad. If the cells migrate into the cortex, the gonad develops into a testis, if they remain in the medullary region an ovary develops. At this time the major candidates for the primary inducer of gonadal differentiation are H-Y antigen and steroid hormones.

Early studies on sex determination. Early physiological studies of vertebrate sex determination addressed the possible role of steroid hormones on gonadogenesis. By administering exogneous steroids to larvae and embryos of various species complete or partial gonadal reversal was noted in all vertebrate classes, but functional sex reversals were restricted to anamniotes (for review see Neuroendocrinology of Reproduction: Physiology and Behavior, 159, 1981). The failure of steriods to produce functional sex reversals in amniotes coupled with the discovery that testes developed in XY mammals with the mutation 'testicular feminization' (an aberrancy of androgen receptors that prevents incorporation of testosterone into cells) fostered the search for an alternative primary inducer of gonad determination. With the discovery of a substance called H-Y antigen many researchers felt that the search had ended.

H-Y Antigen. The heterogametic sex of birds (female) and mammals (males) have been shown to be H-Y antigen positive. This finding is so consistent within these groups that H-Y antigen has been proposed as the primary inducer of sex in all vertebrates. H-Y antigen has also been noted in reptiles which exhibit TSD, with approximately 50 per cent of the hatchlings being H-Y antigen positive and an equal percentage being negative for this antigen. These percentages hold regardless of incubation temperature (and therefore the sex of individuals examined; Differentiation 22, 73, 1982). This finding has lead researchers to propose that there is a weak genetic sex determining mechanism in organism that have TSD which is simply over-ridden by temperature. However, the above results indicate that H-Y antigen is not the primary inducer of gonadal differentiation in species that exhibit TSD. In addition, recent findings have cast doubt as to the central role of H-Y antigen in gonadal differentiation in species exhibiting GSD as well (e.g. Nature 320, 170, 1986).

Steroid hormones and TSD. Recent discoveries by researchers investigating sex determination and environmental effects on embryogenesis in reptiles that exhibit TSD have lead to the resurrection of the idea that steroid hormones may have a central role in gonad determination (at least in this group). This newfound interest is based on the following information. First, steriod hormones can cause complete sex reversal in turtles if the hormones are applied during certain stages of gonadal development. In species with TSD this time corresponds to the sensitive stages for temperature induction of sex (see above); in species with GSD it is apparently somewhat earlier in development. Second, the application of exogenous testosterone (T) can cause a gonad to differentiate into an ovary. This finding may seem puzzling given the traditional view that androgens cause 'maleness'; but testosterone can be aromatized to an estrogen (E). If during certain stages of development, all embryos have the biochemical machinery to convert T to E (the back conversion, E to T, has never been found in organic systems) then differentiation of a gonad into a testis may simply be by default (i.e. a testis is formed if no steroid hormones are present). Note that this explanation would also account for the formation of a testis in organisms with the mutation 'testicular feminization'. A final piece of information concerns a potential source of steroid hormones available to developing embryos. Studies examining the influence of incubation temperature on catabolism of various components in the yolk by embryonic turtles report that at male-producing temperatures (25-28°C), embryos are catabolizing relatively less lipids than embryos at female-producing temperatures (21-23°C and 30-32°C). Because cholesterol (which is a precursor to steroid hormones) is associated with the lipid fraction of the yolk, it may be that embryos at female-producing temperatures are simply converting this cholesterol into steroids thereby inducing the gonad to differentiate into an ovary. While none of the above can be considered conclusive evidence that steriod hormones are the primary inducer of sex in species that exhibit TSD, there is sufficient data to

warrant further investigation as to the possible role of these substances on sexual differentiation.

#### CONCLUSIONS

Although the study of sex determination and, to a lesser extent, sexual differentiation in reptiles have received a great deal of attention during the past decade, more work is needed to fill large gaps in our understanding of these phenomena in reptiles (and other vertebrates as well). For example, reptiles exhibiting TSD are suitable for studies of geographic and interspecific variations in those parameters which affect sex ratios. Such studies would aid in understanding the coevolution of sex determination, reproductive biology, sex ratio, and biogeography. Further areas of interest include: the determination if factors other than temperature influence sex determination; the type(s) of sex determining mechanism(s) found in amphisbeanids and the tuatara; differences in the responses to hormone between species exhibiting TSD and those with GSD; and the search for a species that exhibits both GSD and ESD. This small sample of the issues waiting to be resolved indicates that studies of sex determination and sexual differentiation in reptiles will be fertile areas of inquiry for years to come.

#### ACKNOWLEDGEMENTS

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# EFFECT OF EXOGENOUS TESTOSTERONE ON THE EPIDERMAL GLANDS OF HEMIDACTYLUS FLAVIVIRIDIS

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#### ABSTRACT

The influence of exogenous testosterone on the epidermal glands i.e.  $\beta$ -glands and pre-anal glands, of *Hemidactylus flaviviridis* was studied 15 and 30 days post treatment during breeding and non-breeding phases of gonadal cycle. Parameters like length and breadth of pre-anal glands and their cellular and nuclear dimensions, appearance of further development of  $\beta$ -glands were considered. In general, it was found that the changes observed 30 days post treatment were more obvious, however, 15 days of treatment did influence both the glands studied, to some extent. Also the exogenous hormone showed pronounced effect during non-breeding period than during the breeding period.

#### INTRODUCTION

Sexual dimorphism in terms of holocrine epidermal specialization in gekkonines has been confirmed by Maderson and others (Maderson, 1970; Maderson and Chiu, 1970; Menchel and Maderson, 1971, 1975). It has also been observed by Chauhan (1985) that only males of *Hemidactylus flaviviridis* possess the epidermal glands, i.e. pre-anal glands and ß-glands and females do not possess any of these. It was also observed that pre-anal glands' activity varied with respect to testicular cycle (Chauhan and Chauhan, 1985). The pre-anal glands are holocrine structures which open on

the ventral femoral side through pores, whereas  $\beta$ -glands are nothing but glandular cells developed within the epidermal cell layers.

The effects of sex steroids (chiefly the androgen) on the epidermal glands of different gekkonid lizards have been extensively studied (Maderson and Chiu, 1974; Chiu *et al.*, 1970, 1975 and Maderson *et al.*, 1979). Their findings suggested that only males have pre-anal glands and not females. It was also proposed that the differentiation of epidermal glands involved a synergistic action between androgens and hormones responsible for shedding.