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SEX DETERMINATION IN REPTILES

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ABSTRACT

Two factors in reptile sex determination have been studied: (1) the presence or absence of heteromorphic sex chromosomes, and (2) the influence of temperature. Recognizable sex chromosomes are common in snakes and lizards, but are apparently rare in turtles and absent in crocodilians and the tuatara. Temperature-dependent sex determination (TSD) is common in turtles and has been reported in two lizards and alligators; however, data on TSD are available for few non-turtle species. Present findings on TSD suggest that (1) temperature actually determines sex rather than simply causing differential mortality, and (2) temperature controls sex determination in nature as well as in the laboratory. Only one study, however, has convincingly demonstrated the latter. Sex determination by nest temperature is proposed to interfere with the evolution of sex chromosomes and live-bearing (ovoviviparity); a negative correlation should thus be observed between TSD and sex chromosomes/live-bearing. Present evidence is consistent with these predictions. Possible selective advantages and disadvantages of the different sex-determined mechanisms are discussed, and an attempt is made to deduce their ancestries.

INTRODUCTION

THE BIOLOGY of sex determination and sex differentiation, particularly in mammals, birds, amphibians, and fish, has been studied extensively (see reviews by Bacci, 1965; Mittwoch, 1967, 1973; Ohno, 1967, 1979; Vorontsov, 1973; White, 1973; Reinboth, 1975). Findings indicate that (1) hermaphroditism occurs in a few fish, (2) gonochorism (separate sexes) with genotypic sex determination is the norm in the other vertebrates, and (3) heteromorphic sex chromosomes are common in mammals and birds, less common in reptiles, and are uncommon in amphibians and fishes. The prevailing view is that the most remote ancestors of vertebrates were hermaphroditic, that the earliest origins of gonochorism involved environmental control of sex determination, and that genotypic sex determination was established later, with the gradual evolution of sex chromosome heteromorphism (Mittwoch, 1975; Ohno, 1967; Witschi, 1929a). Witschi (1959) attempted to date the origin of genotypic sex determination in tetrapod verte-

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brates and proposed that it was established 150 million years ago.

Until recently, sex-determining mechanisms were largely unknown in reptiles, but sex chromosomes have now been discovered in many lizards and snakes (Vorontsov, 1973; Gorman, 1973; King, 1977). These sex chromosome systems include male and female heterogamety and represent different degrees of sex-chromosome heteromorphism (Ohno, 1967). These findings suggest that sex determination in reptiles is controlled by genotype and is therefore similar to that in most other vertebrates, except that sex-chromosome heteromorphism is intermediate in degree between that in amphibians and that in mammals and birds. In contrast, recent work on some other reptiles indicates that sex differentiation is controlled instead by incubation temperature during embryogenesis (Charnier, 1966; Pieau, 1975a; Yntema, 1976, 1979; Bull and Vogt, 1979). The usual pattern observed is that low temperatures produce one sex, high temperatures the other; no genotypic effect is evident. Although most studies have been confined to the laboratory, one field study suggests that nest temperatures also control sex determination. This situation differs radically from the sex-determining mechanisms supposed to occur in most vertebrates; such "epigamic" or "environmental" sex determination has previously been considered to exist mainly in nematodes and marine worms, although environmental control of sex-change is known in some hermaphrodites (Bacci, 1965; Robertson, 1972; Charnov and Bull, 1977). Furthermore, this mechanism appears to be widespread in reptiles and may therefore have arisen early in the reptilian lineage. The objectives of this article are (1) to review and present data in regard to genotypic and temperature-dependent sex determination in reptiles, and (2) to provide a theoretical framework for understanding the evolution and ancestry of each mechanism.

DEFINITIONS

A variety of sex-determining mechanisms is known in animals, and the accompanying terminology is often confusing. In this paper, *genotypic* sex determination refers to a genetic system in which the sex of an offspring is normally irreversibly fixed by its own (or its parent's) genotype. This contrasts with *environmental* (epigamic) sex determination, in which an offspring's sex is determined by the environment it encounters as a juvenile (following Bacci, 1965; Vorontsov, 1973; Charnov and Bull, 1977). Temperature-dependent sex determination is therefore a special case of environmental sex determination. The distinction between environmental and genotypic sex determination is not absolute, because individuals living in a heterogeneous environment may have a genotypic mechanism that operates under some conditions but is subject to environmental control under other conditions. The terms temperature-dependent sex determination and genotypic sex determination will be abbreviated TSD and GSD, since they will be used repeatedly.

Male and *female heterogamety* are the two most common types of genotypic sex determination. They are denoted XY♂/XX♀, and ZZ♂/ZW♀, respectively. The X and Y (Z and W) are the regions of the genome which segregate according to sex, and may consist of entire chromosomes, chromosome segments, or merely single loci. The term *sex chromosomes* is used here in a restricted sense to indicate cases in which the X and Y are heteromorphic, i.e. are cytologically distinguishable. The X and Y are not always cytologically distinguishable, but if they are, then usually the Y is heterochromatic, lethal in YY genotypes, and different in size or shape from the X (Darlington, 1937; Berg, 1942; White 1973). The process which leads to these X-Y differences is known as *sex-chromosome differentiation.*

In some cases more than one chromosome assorts according to sex (e.g., X_1X_2 X_1X_2 ♀/ X_1X_2 Y ♂). These multiple sex chromosome systems are thought to arise by fusion of an autosome with a sex chromosome, and they therefore do not cause any fundamental change in the sex-determining mechanism. This paper therefore does not distinguish between the simple and the multiple sex-chromosome systems.

GENOTYPIC SEX DETERMINATION IN REPTILES: EVIDENCE FROM SEX CHROMOSOMES

It has been difficult to assess the presence or absence of genotypic sex determination in reptiles, because two of the experimental tech-

niques used with other animals are not practical to use with reptiles (namely, breeding experimentally sex-reversed individuals, and detection of sex-linked markers). Reptiles are easy to study cytologically, however, and data are often available indicating the presence or absence of heteromorphic sex chromosomes. Sex chromosomes are therefore used here as the indicator of genotypic sex determination. A limitation of this approach is that species with genotypic sex determination will escape detection if the sex chromosomes are not visibly distinct. However, the study of sex chromosomes offers several advantages. Their presence indicates not only which sex is heterogametic, but also (1) the part of the genome which carries the sex determiner, and (2) the degree of differentiation between the X and Y (a rough index of how long they have been determining sex, cf. Ohno, 1967). When sex-chromosome data for a group of closely related species are compared, they thus indicate the recent evolutionary history of the sex-determining mechanism.

Before 1960, no case of heteromorphic sex chromosomes was known with certainty in reptiles, and the existence of such in the class was actually doubted (Matthey and van Brink, 1957; van Brink, 1959). This doubt was due partly to the fact that heteromorphic sex chromosomes had been detected only in groups in which they were ubiquitous (mammals, birds, insects), and it was therefore assumed that sex-chromosome heteromorphism was a characteristic of high levels in the taxonomic hierarchy. This assumption proved to be incorrect, since sex chromosomes have subsequently been discovered in many reptiles, along with homomorphism in many other species. With the exception of one example in turtles, all known cases of sex chromosomes in reptiles are found in the squamates.

Squamata: Snakes

A suggestive evolutionary series of sex-chromosome heteromorphisms occurs in the snakes, the degree of Z-W differentiation ranging from apparent homomorphism to major differences in the sizes of the Z and W chromosomes (Kobel, 1962, 1967; Becak, Becak, Nazareth, and Ohno, 1964; Ohno, 1967; Gorman, 1973). Snakes are grouped phylogenetically according to skeletal characters (Romer, 1956). Boas and pythons possess the ancestral skeletal condition, colubrids are morphologically derived from the ancestral type, and vipers are derived from the colubrid condition. Sex chromosomes follow a similar pattern. Most boids show chromosomal homomorphism, most colubrids show female heterogamety, often with the Z and W equal in size but different in centromere position, and viperids show female heterogamety with the Z and W unequal in size (Fig. 1; Kobel, 1962, 1967; Becak et al., 1964; Ohno, 1967). In the one known example of heteromorphism in a boid, the Z and W chromosomes differ by an inversion (Mengden and Stock, in press). The same basic karyotype is generally preserved across these different families, and wherever sex chromosome heteromorphism is observed, it is in the fourth largest chromosome pair (see Fig. 1). The Z chromosome is the same size and usually the same shape in different snakes, but the W varies between and even within species (Ohno, 1967;

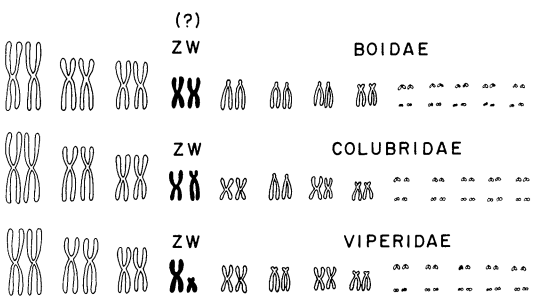

FIG. 1. SCHEMATIC REPRESENTATION OF SEX CHROMOSOMES IN SNAKES

(Redrawn from Becak et al., 1964). The top karyotype is generally representative of female boids (boas and pythons), the middle karyotype of female colubrids (common, non-venomous temperate species), and the lower karyotype of female viperids (e.g., rattlesnakes). Karyotypes in these families generally are remarkably similar, and demonstrate a progressive evolution of sex chromosome heteromorphism in the fourth largest pair. At the time when the boids differentiated, sex chromosome heteromorphism was not evident; it is still not evident in many boids today. Later, when the colubrids became distinct, heteromorphism had evolved to a recognizable state, the Z and W maintaining the same size but differing in centromere position. Subsequently in the viper lineage, the W became reduced in size. (Becak et al., 1964; Ohno, 1967.)

Gorman, 1973; Baker, Mengden, and Bull, 1972; Vorontsov, 1973). It appears that no obvious sex-chromosome differentiation had yet occurred when the boids diverged from the main lineage, but differentiation had progressed to a heteromorphic state by the time the colubrids became distinct. An even further derivation of sex-chromosome heteromorphism subsequently evolved in the proto-viperid lineage, and has evolved in some colubrids as well. Despite the different degrees of heteromorphism, however, the sex chromosomes seem to be derivatives of a single genotypic sex-determining mechanism ancestral to all snakes.

Squamata: Lizards

Sex-chromosome heteromorphism is observed in seven families of lizards, but these examples occur sporadically and indicate multiple origins rather than a single ancestral type, as in snakes (Gorman, 1973; King, 1977). The sex chromosomes of lizards contrast with those of snakes in three ways. (1) In all but one family, the majority of species studied lack heteromorphism. (2) The sex chromosomes are not highly differentiated in at least two independent cases. (3) The heterogametic sex varies within two infraorders. (1) The number of species with sex chromosomes and the number of species karyotyped are given for 12 families of lizards (Table 1, after King, 1977). Except for the Australian family Pygopodidae, the number of species with sex chromosomes is a minority of the species karyotyped. The Gekkonidae are especially interesting: heteromorphism is known in 2 of 54 species, and in each of these two cases, the heteromorphism is not even observed throughout the species' range (King and Rofe, 1976; King, 1977). The karyotypes of lizards are not comparable across families, so it is not known if all heteromorphisms in lizards involve the same chromosome segment, as seems to be true in snakes. Although not indicated in the table, the sex chromosomes of lizards are often microchromosomes and could be overlooked in many species. Therefore, these figures represent minimum estimates for the incidence of sex chromosome heteromorphism. (2) Detailed studies of sex chromosomes in a whiptail lizard (Family Teiidae) indicate that the X and Y are not highly differentiated

TABLE 1

Sex chromosomes in lizards

The table adopts the taxonomy of Romer (1956); families are not included if fewer than 5 species have been karyotyped. Adapted from King (1977), with Lacertidae modified according to data of Bhatnagar and Yoniss (1976).

TAXON	SSC/SK*	HETEROGAMETIC SEX
Infraorder Gekkota		
Fam. Gekkonidae	2/54	♀
Pygopodidae	5/6	♂
Infraorder Iguania		
Fam. Iguanidae	45/145	♂
Agamidae	0/19	–
Chamaeleontidae	0/36	–
Infraorder Scincomorpha		
Fam. Xantusiidae	0/10	–
Teiidae	1/46	♂
Lacertidae	4/33	♀
Scincidae	3/35	♂
Infraorder Anguimorpha		
Fam. Anguidae	0/12	–
Infraorder Platynota		
Fam. Varanidae	4/18	♀
Infraorder Amphisbaenia		
Fam. Amphisbaenidae	0/28	–

* No. of species with sex chromosomes/No. of species karyotyped.

there (Bull, 1978). The X and Y recombine along most of their lengths, and the short differential segments are dissimilar in staining characteristics but nonetheless pair during late pachytene. The sex chromosomes of one gekko (Family Gekkonidae) may also be relatively undifferentiated. The W is larger than the Z, and consists of a euchromatic portion that is apparently equivalent to the Z plus an additional heterochromatic arm (King, 1977). (3) The heterogametic sex varies within and among infraorders of lizards (Table 1). If the groupings by infraorder represent ancestral lineages, then male or female heterogamety must have at least three separate origins, if it be assumed that either type is ancestral.

The above evidence suggests that sex-chromosome heteromorphism has been independently derived many times in lizards and that some examples have evolved recently (Gorman, 1973; King, 1977). Two interpretations of this variation are (1) that these cases

represent multiple origins of GSD, or (2) that these cases all stem from a single origin of GSD, and that only the heteromorphisms are independently derived. This latter possibility deserves explanation, for it is not immediately clear how both male and female hererogamety could be manifestations of a single system. Examples from other animals, though, illustrate that the heterogametic sex may change from male to female or the reverse even though the same GSD is maintained throughout the process. The change involves a transition through a multi-locus or multi-allelic genotypic sex-determining mechanism (Bull and Charnov, 1977). Therefore, additional evidence is desirable before it is concluded which interpretation of the sex-chromosome variation in lizards is more likely to be true.

Chelonia

The only documented occurrence of sex chromosomes in turtles is that of male heterogamety in one genus (two species) of the mud turtle family (Kinosternidae, Bull, Moon, and Legler, 1974; Sites, Bickham, and Haiduk, 1979). Again, the sex chromosomes seem to be of recent origin. The X and Y differ only in a terminal heterochromatic knob, and perhaps a nucleolar organizer. The heteromorphism was not observed in a male of a different genus in the same subfamily (Bull et al., 1974).

The evidence that sex chromosomes are absent in other turtles is strong but not absolute (Gorman, 1973). Most turtles have large diploid numbers (ca. 50), over half of which are microchromosomes, so heteromorphism in the small chromosomes might easily be overlooked. However, detailed studies of the Emydidae (pond turtles, Bickham and Baker, 1976), Kinosternidae (Sites, Bickham, Haiduk, and Iverson, 1979), and Chelidae and Pelomedusidae (side-necked turltes, Bull and Legler, unpub.) have failed to detect heteromorphisms even when staining techniques of high resolution have been used.

Crocodilia

All species of Crocodilia have been karyotyped, and both sexes have been studied in the majority of them (Cohen and Gans, 1970). No sex chromosome heteromorphism has been observed. Unlike the karyotypes of other reptiles, those of crocodiles have no small microchromosomes, so overlooking a heteromorphism is less likely than with other reptiles.

Rhyncocephalia

The single living member of this order is the tuatara (*Sphenodon punctatus*). Chromosomes of one male and one female were studied by Wylie, Veale, and Sands (1968), who found no sex-chromosome heteromorphism.

To summarize, sex-chromosome heteromorphism has been detected in the karyotypes of many snakes and lizards, and in one genus of turtle. Sex chromosomes are apparently absent in the tuatara, in crocodilians, in most turtles, and in many snakes and lizards, Genotypic sex determination is likely present in the species with heteromorphic sex chromosomes, but it may or may not be present in those lacking heteromorphic sex chromosomes. The data suggest that the evolutionary trend has been from chromosomal homomorphism toward heteromorphism, because homomorphism seems to be the ancestral condition of all orders, and in some lizards the sex chromosomes are unique to the genus or species. The variation in sex-chromosome systems indicates that the sex-determining mechanisms have been in some sense unstable, but the data do not strongly discriminate between single-origin and multiple-origin hypotheses for GSD in reptiles.

TEMPERATURE-DEPENDENT SEX DETERMINATION

Laboratory studies over the last ten years have indicated that in some reptiles the temperature at which eggs are incubated affects the sex ratio of hatchlings (defined as the proportion of males) (Table 2). This effect has been observed in five families of turtles and two of lizards, but the majority of reptilian families have not been studied. Also, T. Joanen (unpub.) has found that incubation temperature affects sex ratios in alligators; the details are here withheld, as the study is yet incomplete. Not all reptiles show a response of sex ratio to incubation temperature. Hatchling sex ratios were unaffected by temperature in one lizard species, one snake species, and one turtle species (see Table 2).

TABLE 2

Reptiles tested for temperature-dependent sex determination with data on sex chromosomes and live-bearing

TAXON	SEX TSD	SEX CHROMOSOMES?	LIVE-BEARING?	REFERENCE
Chelonia				
Fam. Emydidae (9)	+	−(F)	−(O)	Bull and Vogt, 1979; Pieau, 1971-1978; Vogt and Bull, unpub.
Testudinidae	+	−(F)	−	Pieau, 1971, 1975b
Chelydridae (2)	+	−(F)	−	Yntema, 1976, 1979; Bull, Houseal, Vogt, unpub.
Cheloniidae	+	−(F)	−	Yntema and Mrosovsky, 1979
Kinosternidae	+	−(SF)	−	Bull, Houseal, Vogt, unpub.
Trionychidae	−	−(F)	−	Bull and Vogt, 1979
Squamata				
Lizards				
Fam. Agamidae	+	−(F)	+(F)	Charnier, 1966
Gekkonidae	+	−(SF)*	−(SF)	Wagner, Appendix I
Lacertidae	−	+(G)	+(G)	Raynaud and Pieau, 1972
Snakes				
Fam. Colubridae	−	+(G)	+(S)	Osgood, Appendix II
Crocodilia				
Fam. Alligatoridae	+	−(O)	−(O)	Joanen, unpub.

* Only two species have been studied, and only males for these.

Except where indicated, only one species has been studied for TSD in each family. One or more species have been studied for sex chromosomes. Symbols: +, presence, −, absence; the brackets following each + or − indicate the taxonomic level at which the association is true. S, species. G, genus. SF, subfamily. F, family. O, order. The reference in respect to live-bearing is Fitch (1970). The references relating to sex chromosomes are to be found in the previous section of this paper.

In species with temperature-dependent sex determination (TSD), the biases of sex ratio are dramatic (Fig. 2). Usually, low temperatures (22°-27°C) produce one sex, higher temperatures (30° and above) produce the other, and an intermediate 1°-2° range permits both. The temperature at which the shift in sex ratio occurs (the threshold temperature) differs among species, but it usually lies between 27°C and 31°C. A surprising observation is that the male-producing and female-producing temperatures in lizards are the reverse of those found in turtles. Low temperatures produce females in lizards and males in turtles.

There are some indications of variation upon this simple pattern. (1) In the snapping turtle (*Chelydra*), both extreme warm and extreme cool temperatures produce females (see Fig. 2C, and Yntema, 1976, 1979). (2) In the alligator snapping turtle (*Macroclemmys*) and the mud turtle (*Kinosternon*), only females develop at 31°C, but some females also develop at 25°C (40%, 21%, respectively; Bull, Houseal, and Vogt, unpub.). Possibly these two species are like most other turtles but possess a lower or less steep threshold, but they might also be exhibiting a low-temperature effect, as in *Chelydra*. However, *Chelydra* does not hatch at the extreme cool temperatures (20°C); the female-producing effect is demonstrated by incubating the eggs at 20°C only temporarily, and otherwise shifting them to 26°C (Yntema, 1979). Thus, a low-temperature effect may be more widespread than suggested by experiments using continuous incubation at one temperature (as in most examples of Fig. 2).

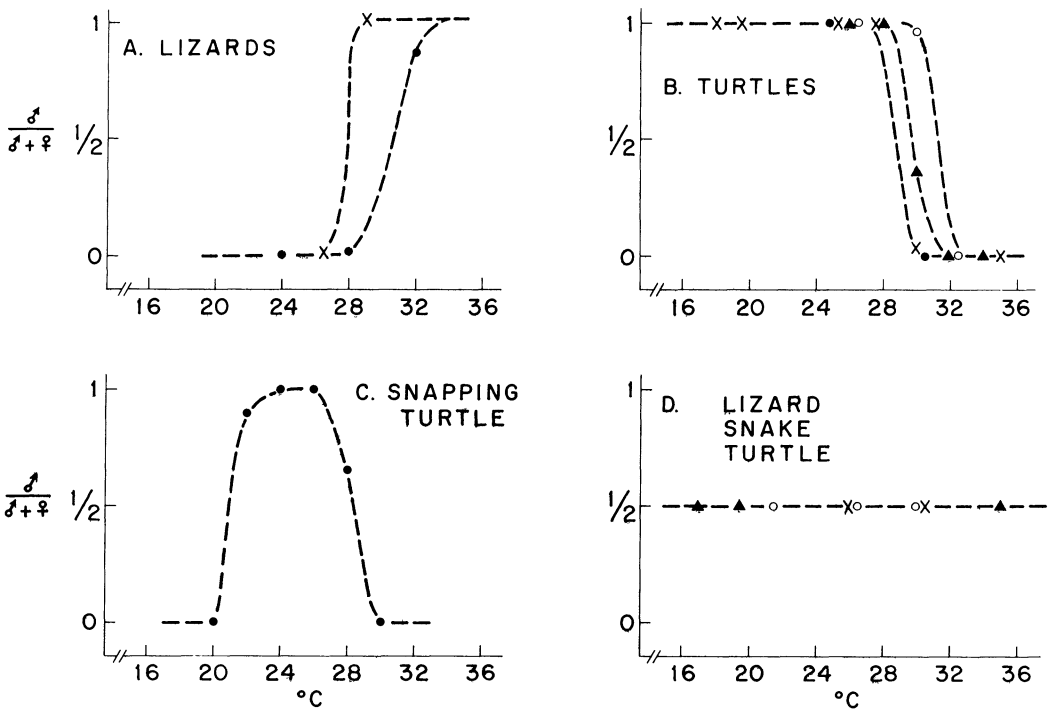

FIG. 2. RESPONSES IN SEX RATIO TO INCUBATION TEMPERATURE IN REPTILES

Dashed lines indicate hypothetical responses based on the data points shown. A. Lizards: solid circles, *Eublepharis macularius* (F. Gekkonidae); crosses, *Agama agama* (F. Agamidae). B. Turtles: crosses, *Emys orbicularis* (F. Emydidae); open circles, *Testudo graeca* (F. Testudinidae); triangles, *Caretta caretta* (F. Cheloniidae); solid circles, *Graptemys* (3 spp.) and *Chrysemys picta* (F. Emydidae). C. Snapping Turtle: *Chelydra serpentina* (F. Chelydridae). D. Lizard, Snake, Turtle: triangles, *Lacerta viridis* (F. Lacertidae); open circles, *Natrix fasciata* (F. Colubridae); crosses, *Trionyx spiniferus* (F. Trionychidae). The sex ratios of ½ in (D) are based on totals of males and females which do not differ significantly from ½. [References and additional data are given in Table 2.]

Vogt and Bull (unpub.) have studied the threshold temperature in seven species (three genera) of emydid turtles from three localities. Surprisingly, turtles in Alabama and those in Wisconsin produce nearly all males at 28°C and nearly all females at 30°C (one Wisconsin species produces only 90% females at 30°C). However, two species showed a greater tendency to produce females at 28°-29°C in populations from Tennessee than in populations from Wisconsin. A preliminary conclusion from these results is that the threshold temperature seems generally to be conserved (although not absolutely so) among closely related species.

Whether temperature controls sex differentiation or causes differential mortality was not known until recently. Some workers simply assumed that temperature controls sex differentiation, and did not consider the alternative possibility of differential mortality, or they failed to report the proportion of eggs which failed to hatch. Recent data indicate that differential mortality is indeed unlikely to be the explanation for some species (snapping turtles, Yntema, 1979; four emydids, Bull and Vogt, 1979; a lizard, Wagner, Appendix I), and no definite evidence has suggested that differential mortality does occur. Therefore, the hypothesis that temperature controls sex determination may be correct for all of the sex-ratio biases.

The developmental stages during which sex determination is sensitive to temperature have been investigated extensively in snapping turtles (Yntema, 1979) and map turtles (Bull and Vogt, unpub.). These studies use the following procedure and rationale. Eggs are incubated at

a male-producing temperature for the first x stages of the developmental period and are then shifted to a female-producing temperature for the remainder of development (or vice versa). The hatchlings may be all male, all female, or some of each, depending upon the stage at which the embryos were shifted. When results from different experiments are compared, it is seen that temperatures up to a certain developmental stage and those beyond a subsequent stage have no effect on the sex ratio, but that temperatures between these two stages do. The sensitive period is defined as the interval between two such developmental stages.

The studies on map turtles and snapping turtles show that stages in approximately the middle third of development are most sensitive to temperature. The results are interesting but difficult to interpret. First, it seems that they depend somewhat upon the temperatures used in the experiments. For example, 25°C and 28°C are both male-producing when used throughout development in map turtles, but when combined with female-producing temperatures, 28°C has less effect than 25°C. Therefore, a different sensitive period may be obtained in experiments with 28°C and 31°C than in experiments with 25°C and 31°C. When 25°C and 30.5°C are used with map turtles, the sensitive period is between developmental stages 15 and 22 (using Yntema's 1968 classification of stages). Male determination can occur early in the period; nearly all embryos become male if they are incubated through stage 17 at 25°C. Also, an isolated pulse of 25°C over a 3- to 4-stage interval during this period can have a substantial male-inducing effect. Female determination is not fixed until later in the period: nearly all embryos become female if they are incubated to stage 23 at 30.5°C, but incubation through stage 18 at this temperature has no apparent female-determining influence. An isolated pulse of 31°C over 3 to 4 stages in the sensitive period has little female-producing effect. Therefore, male determination is more easily induced than female determination in map turtles.

Results from snapping turtles are similar to these in some ways but different in others (Yntema, 1979). If 30°C and 26°C are used, the sensitive period is among stages 14 to 19, much as in map turtles. However, incubation at 30°C through either half of the sensitive period induces femaleness in most embryos; male determination requires incubation at 26°C for nearly all of the sensitive period. Here, female determination is more easily induced (by 30°C) than male determination. If 20°C and 26°C are used, however, the sensitive period is restricted to stages 14 through 16. Both 20°C and 26°C seem equally matched at determining femaleness and maleness, respectively. Thus, 20°C and 30°C are both female-determining, but they are differentially effective. In view of the complexities of sex determination revealed in these experiments, one can imagine that the manner in which sex is decided in a nest, with short-term and long-term temperature fluctuations, will be difficult to elucidate.

Most laboratory studies do not directly address the question of whether temperature determines sex in nature. Most work has been done with constant temperature incubation, and it is important to know how sex determination operates when incubation temperatures fluctuate, as in nests of some species (Pieau, 1974; Burger, 1976; Bull and Vogt, 1979). The import of this is straightforward. If all embryos have a genetic disposition toward a certain sex but male and female differentiation require different temperatures, then only one sex will occur in constant-temperature experiments. This situation would give the seeming result of environmental sex determination in the laboratory, even though sex determination was genotypic in nests, with fluctuating temperatures. Two laboratory studies have pursued this question by investigating sex determination when incubation temperatures fluctuate on a daily basis. Pieau (1973) incubated eggs of a European pond turtle (*Emys*, Family Emydidae) in the following ways: (A) a daily cycle between 24°C and 30°C, or (B) a daily cycle between 26°C and 31°C. The threshold temperature in this species is 28°-29°C, so the mean temperature of cycle (B) coincides with the threshold. Pieau observed that both sexes developed in cycle (B), whereas males and intersexes developed in cycle (A). His analysis was based on histological sections of embryos, and the ultimate differentiation of the intersexes is unknown. Bull and Vogt (1979) performed a similar experiment with eggs of two species of North American map turtles (*Graptemys*, Family Emydidae). Cycle (A) was a daily fluctuation from 23°C-33°C, and cycle (B) was a daily fluc-

tuation from 20°C-30°C. Although the mean temperatures of these two cycles differ by only 3°C, cycle (A) produced only females, cycle (B) only males, and the proportions hatching were high enough to rule out differential mortality. Analysis was based merely on inspection of gonads under a dissecting microscope, but no indication of intersexes was observed, as might correspond to Pieau's observations. Many of these turtles were kept alive for two or three months, and still there was no indication of intersexes. Thus there appears to be no basic difference between incubation under constant or fluctuating temperature in map turtles, but the interpretation for *Emys* is not certain.

Fluctuating temperatures may not characterize the nests of all species. Nests of some sea turtles are deep (80 cm) and experience little daily fluctuation in temperature (Mrosovsky and Yntema, in press). The results of constant-temperature laboratory incubation are therefore more relevant to sex determination in sea turtles than to map turtles and perhaps other species with shallow nests (20 cm deep).

Two experiments have studied sex determination in nests (Bull and Vogt 1979; Pieau, 1974). Bull and Vogt collected freshly laid eggs on nesting beaches and reburied them at one of two sites on the same beaches; the sites differed in exposure to the sun. The young that hatched from warm nests (exposed to the sun) were nearly all females, while those from the cooler, shaded nests were all males. The sex ratios in 14 natural (undisturbed) nests of map turtles were consistent with the experimental observations: progeny from each nest tended to be all male or all female. Pieau (1974) also performed an outdoor experiment and obtained nearly all males from his nests. Unfortunately, his results are inconclusive because he initially incubated the eggs in the laboratory at male-producing temperatures, buried the eggs at sites that did not necessarily represent those of the parental population, and used no controls.

A question yet to be thoroughly investigated is whether or not the sex phenotype of hatchlings corresponds to that of adults. No study has raised the hatchlings to adulthood to investigate sex reversal or fertility; the lengthy immature period in these species (5 to 15 years) inhibits such research. Three sets of observations indirectly suggest that hatchling and adult sex phenotypes agree well. (1) Intersex phenotypes are rare among hatchlings in the laboratory and field studies, except in a few of the temperature-shift experiments (Yntema, 1979; Bull and Vogt, unpub). In particular, incubation temperatures which lead to the differentiation of both sexes usually do not cause intermediate degrees of sex differentiation, but rather lead to a clear male-female dichotomy among the hatchlings (Yntema, 1976, 1979; Bull and Vogt, 1979, unpub.). If temperature affected the gonads independently of their ultimate differentiation, this dichotomy would not be expected. (2) There is a well-defined developmental period during which temperature influences sex differentiation, and incubation temperatures of later stages have no effect on sex. Hatchlings raised for three months show progressive gonadal differentiation in the direction expected from incubation temperature. There is no indication of sex reversal; rather, the embryo appears to be committed to a particular phenotype after the temperature-sensitive period (Yntema, 1976; Bull and Vogt, 1979). (3) Dissections of hundreds of specimens suggest that intersex phenotypes are rare in natural populations of turtles (my own observations on chelid turtles; pers. comm. from J. M. Legler for chelid and emydid turtles; pers. comm. from D. Owens for sea turtles). In map turtles hatched and raised in captivity for seven years, secondary sexual characteristics have remained constant from their inception at age two (R. C. Vogt, pers. comm.). It therefore seems likely that hatchling sex phenotype is a reliable indicator of adult sex phenotype.

The above studies collectively suggest that sex can be determined by incubation temperature in many, but not all, reptiles. How often incubation temperature controls sex determination in nature is unknown for most of these species, but the study on map turtles indicates that temperature is the major influence. The demonstration of TSD in the laboratory may not always correspond to strict environmental sex determination in nature, since genotypes may partially control sex determination as well, but it will be surprising if the laboratory demonstration of TSD does not correspond to some natural environmental sex determination, if only under extreme conditions.

The data on TSD complement the data on sex chromosomes and provide a fuller understanding of sex determination in reptiles. Sex

chromosomes indicate that GSD is present in many species, but the lack of sex chromosomes is uninformative. Data on TSD are complementary in two ways. First, if it is shown that nest temperature determines sex, then GSD is not operative. Second, a sex ratio of ½ which is invariant with incubation temperature (as in three species) is suggestive of GSD, although there may be alternative explanations. Thus, the data on TSD facilitate recognition of GSD as well. Although some observations on TSD or sex chromosomes have ambiguous interpretations as they stand alone, it is to be hoped that the data on both, when combined, will associate in consistent patterns that are less ambiguous.

INCOMPATIBILITY BETWEEN HETEROMORPHIC SEX CHROMOSOMES AND TSD IN NATURE

There seem to be two major categories of sex determination in reptiles: TSD and sex chromosomes (GSD). In the preceding discussion, they have been treated independently of each other in order to facilitate the presentation, but inevitably one must consider whether these two categories of sex-determining mechanisms are indeed independent, or instead are mutually exclusive. That is, can a species have both TSD and sex chromosomes? Theoretical considerations suggest that the coexistence is unlikely, and the existing data are consistent with that prediction.

In theory, a negative association between sex chromosomes and environmental sex determination is to be expected, because the evolutionary pathways leading to their coexistence are improbable. This prediction is not simply a claim that genotypic and environmental sex determination cannot coexist. They may coexist in the following way (for a possible example, see below, a discussion of amphibians). Inhabitants of a heterogeneous environment may have a weak sex-determining locus which operates under most environmental conditions, but is overridden under extreme conditions (e.g., an XX zygote becomes female except in unusually cool areas). With random mating, the frequencies of the sex-determining alleles automatically compensate for environmental sex determination and equilibrate to yield a population sex ratio is not ½, then a zygote of the rare sex will, determination coexist (Bull, unpub.). An equilibrium sex ratio of ½ (50% of zygotes become male) is perhaps to be expected, because this is the equilibrium sex ratio for a wide class of situations (Fisher, 1930). If the zygotic sex ratio is not ½, then a zygote of the rare sex will, on average, contribute to more offspring than will a zygote of the common sex. Consequently, genes which overproduce the rare sex will be favored because (1) they are transmitted to the rare sex more often than average, and (2) each individual of the rare sex transmits more alleles than does an individual of the common sex. The increase in frequency of the genes overproducing the rare sex leads to a decrease in the bias in sex ratio, and the equilibrium of ½ is approached. Since a sex ratio of ½ is the equilibrium even when there is a combination of environmental and genotypic sex determination, there is no selection to modify the level of environmental influence, and the two sex-determining modes may coexist.

The coexistence of environmental and genotypic sex determination is selected against only if differentiated sex chromosomes are involved. To illustrate, consider first the case of a species with differentiated sex chromosomes in which environmental sex determination begins to evolve. Male heterogamety is assumed, but the outcome does not depend on the heterogametic sex. The onset of environmental sex determination may occur if the embryonic process which triggers the male or female pathway of differentiation is susceptible in some individuals to a natural environmental stimulus. Some of these susceptible individuals consequently develop contrary to their sex chromosome constitution and become XY females or XX males, while most of the population maintains the usual condition of XY males or XX females. Owing to the small proportion of sex reversals, the XY females usually mate with XY males and produce ¼ YY progeny. Now if the X and Y are differentiated, the Y is likely to have accumulated detrimental genes, and YY genotypes are then subvital (see review by Lucchesi, 1978). Hence a substantial portion of the progeny of XY females do not survive or reproduce, and individuals that do not become XY females, i.e. are not susceptible to environmental sex determination, leave more offspring and increase in frequency. This analysis shows that environmental sex determination is not likely to evolve in populations with sex chromosomes.

The reverse process is also unlikely. Suppose now that an incipient Y chromosome is present; it affects sex determination but has not differentiated to the point of carrying detrimental alleles. If environmental sex determination is also present, this will again lead to YY genotypes, but in this case they are viable and fertile. (YY genotypes are normal and fertile in some amphibians and fishes; see Mikamo and Witschi, 1963; Kallman, 1970; Collenot, 1975; Kawamura and Nishioka, 1977.) Selection then no longer operates against environmental sex determination, but the presence of fertile YY genotypes prevents deterioration of the Y by selecting against Y chromosomes that do accumulate detrimental alleles and by allowing the Y to recombine (Nei, 1970; Charlesworth, 1978). The Y is then no longer likely to differentiate and to become morphologically distinct from the X chromosome. Therefore, heteromorphic sex chromosomes are not expected to occur together with environmental sex determination, regardless of the evolutionary history of the process.

The evidence which at present bears upon this association suggests that TSD and sex chromosomes do not occur together. (A) Sex chromosomes are unknown in any subfamily (often in any family) in which TSD occurs, but they do occur in two of the three genera which show no response to temperature (see Table 2). (B) Species with sex chromosomes show strict adherence to male or female heterogamety (see references on sex chromosomes). If their natural environment indeed affected sex determination, then XX and XY genotypes would not show a perfect correspondence with sex. Thus it seems at present that TSD and sex chromosomes are mutually exclusive sex-determining mechanisms, although many more examples await testing.

Some qualification of the above prediction is warranted. Heteromorphic sex chromosomes are not likely to occur if environmental sex determination is present, provided that (1) a heteromorphic Y chromosome is deleterious in YY genotypes, and that (2) environmental sex determination leads to YY genotypes. If either of these assumptions proves false, then TSD and sex chromosomes might coexist, and the cytological evidence should indicate which assumption is violated. For example, if (1) is false, then YY individuals will be normal and should be observed. Invalidity of (2) means that only XX is subject to environmental influence, whereas XY is not. Hence XX will be of both sexes in the population, but XY will be only of one sex. An additional qualification concerns the nature of TSD. The theory is based on selection in natural populations. Sex chromosomes may evolve only if TSD is not experienced in nature. However, TSD is usually studied in the laboratory, and there may not be strict correspondence between TSD in the laboratory and the field. Species with sex chromosomes may therefore show TSD in the laboratory, even though it is not experienced in nature.

The actual correspondence between TSD in the laboratory and in nature is a problem that has been raised already, and it was suggested that one solution would be to study TSD in both circumstances. There is, however, an indirect means of addressing the same problem. A species with sex chromosomes that does not experience TSD in nature will show strict adherence to male or female heterogamety, and this situation can be observed cytologically. If such a species shows TSD in the laboratory, then it would be clear that the laboratory results do not extrapolate to nature. If instead, all species with sex chromosomes fail to show TSD in the laboratory, there would be a stronger basis for supposing correspondence between TSD in the laboratory and the field.

SELECTION ON THE SEX-DETERMINING MECHANISM

Knowledge of the selective forces acting upon the sex-determining mechanism may lead to a better understanding of the variation observed in reptiles. The purpose here is to search for a possible redeeming value of TSD and to discuss how these mechanisms may evolve in populations. Whereas above, TSD has been discussed as both a laboratory and a natural phenomenon, the arguments in this section will deal only with TSD as a phenomenon in nature. A principal question is whether sex determination by nest temperature presents a selective advantage that could account for the occurrence of TSD in many reptiles. This problem is addressed more generally by Charnov and Bull (1977), who have considered what circumstances favor any particular form of environmental sex determination. Their model proposes that environmen-

tal sex determination offers advantages by allowing the sex of the embryo to respond to its immediate environment. Suppose, for example, that hatchlings from cold nests can become either good males or substandard females, and that the opposite is true for warm nests. With GSD, each individual has a significant chance of becoming a substandard male or female; individuals with TSD are favored because they can respond to their immediate environment and become the sex which is most benefited. Therefore, if certain temperatures, or conditions associated with nest temperatures, enhance male fitness, more than female fitness, then this model suggests how TSD can be selected for instead of GSD. At present, no evidence for the model is apparent, but the fitness effects may be subtle, and no studies have yet attempted to reveal them.

The Charnov-Bull model describes conditions under which environmental sex determination would be advantageous. The persistence of temperature-dependent sex determination need not imply that it is advantageous, however. It might merely be as beneficial as GSD, or even inferior, but persist for a lack of mutations that would cause GSD to evolve. Both possible hypotheses have some merit. These topics are to be the subject of a theoretical paper (Bull, unpub.), some conclusions from which can be stated here. If male and female fitnesses are independent of nest temperature, then environmental sex determination is no longer advantageous, but neither is it intrinsically disadvantageous. As stated above, in a constant environment the sex ratio equilibrates at ½ regardless of the proportion of genotypic and environmental sex determination, and selection is neutral thereafter. The neutrality of environmental sex determination disappears, however, if the environment fluctuates and thereby causes fluctuations in the population's sex ratio. Genotypic sex determination can spread throughout such a population because of its stabilizing effect on the sex ratio (unless male and female fitnesses vary with nest temperature as mentioned above). Perhaps it can be assumed that if nest temperature determines sex, yearly and long-term climatic fluctuations cause variations in the population's sex ratio. To explain the persistence of TSD on the basis of these models requires either that male or female fitness be a function of nest temperature, or that GSD mutations have not arisen in the past.

Sex-Ratio Adjustment with TSD

Selection should effectively modify the sex ratio if nest temperature controls sex determination. The manner in which the embryo's sex determination responds to temperature is apparently complicated, but conceivably, slight changes in the threshold between male-producing and female-producing temperatures or in the length of exposure to particular temperatures required to induce male (female) differentiation might cause significant changes in the hatchling sex ratio. It is surprising, though, that threshold temperatures are thus far so conservative (see above). The nest site and the timing of nesting also affect the sex ratio of the progeny, and selection may act upon genetic variation of these parameters in the mother. Parameters such as these are likely to be under the control of many genes with small effects and could thus respond rapidly to selection if environmental changes shift the sex ratio from its equilibrium. Although it is theoretically possible to predict this equilibrium, to do so is not practical because it requires a complete knowledge of the fitnesses associated with incubation in nests of different temperatures. In general, the equilibrium is not ½ among the eggs (Bull and Charnov, unpub.), but if the only fitness effect correlated with nest temperature is that of survival in the egg stage, then the equilibrium is at an equal number of male and female hatchlings in the population.

Major environmental changes may cause temporary changes in the population sex ratio through effects on the primary sex ratio. As an example, one can imagine a situation in which new nesting sites such as islands are created, that by virtue of a lack of shading vegetation are predominantly female-producing (in turtles). If these sites are also free of the various predators that depress the hatch at other sites, nesting of a substantial number of females on these new islands could cause a disproportionate number of female offspring in the hatch. A sex-ratio bias would result until selection returned the population to equilibrium. A bizarre evolutionary process which causes similar sex-ratio biases occurs if there is homing, whereby fe-

males lay at the sites at which they were born. This leads to runaway "selection" to produce daughters, because only the females lay eggs and they therefore lay at female-producing sites. Selection for genetic control of the sex ratio will lag behind this process, but will eventually bring the sex ratio near its equilibrium, unless the environment continually changes.

Interactions with the Life History

It was suggested above that deviations from the equilibrium sex ratio which accompany environmental sex determination are disadvantageous and may favor GSD. This selection is weak, however, unless the sex-ratio deviations are extreme and frequent. Although environmental variation no doubt causes some unavoidable sex-ratio deviations in the case of environmental sex determination, the magnitude of the deviations can be greatly influenced by the life history. Certain kinds of life histories are more prone than others to experience sex-ratio deviations with environmental sex determination, and these will most strongly favor GSD. Environmental sex determination will itself select for life histories that minimize sex-ratio deviations. A life history affects the sex ratio in several ways. (1) The environmental cue directing sex determination is important. The cue must be variable in space, in order to insure the production of both sexes within the range of dispersal (Berg, 1942), and the cue must be constant in time, to avoid sex-ratio fluctuations. (2) The mobility of the species is important. Spatial variance in offspring sex ratio may be greatly reduced among adults in species capable of long-distance dispersal. (3) Finally, the reproductive longevity of adults is important. Yearly extremes in hatchling sex ratio have little effect on the population sex ratio in long-lived species, and male and female excesses tend to cancel. Long-term directional changes in the environmental cue will cause biased population sex ratios, but these occur gradually in long-lived species, and selection begins acting to reverse them in the early phases of the bias.

The life histories of reptiles with TSD are well-matched to fit these requirements. Nest temperature is variable in space, varying with depth and exposure to the sun, but it is relatively constant from year to year in that the climate does not change drastically. The mobility of reptiles allows them to move long distances and thereby to effect panmixia over large areas. The longevity of many reptiles, especially turtles and crocodiles, is upwards of 30 years, and the reproductive life is often more than three-fourths of this (Goin and Goin, 1971, p. 126). This correspondence in reptiles of TSD with "appropriate" life histories is not necessarily surprising. If nest temperature indeed determines sex in these species, then the evolution of life histories that lead to extreme sex-ratio biases may have been prevented. Alternatively, the evolution of "inappropriate" life histories may have led to the evolution of GSD. To this end, it will be useful to consider sex determination in species whose life histories are not appropriate for TSD.

Parental Incubation

Temperature-dependent sex determination may not be compatible with the life history if parents thermally regulate the development of their young. As parental incubation evolves in a population with TSD, there may be an accompanying sex-ratio bias because more embryos experience thermally similar environments, unless parents happen to incubate at a temperature which produces both sexes, or unless different parents incubate at different temperatures. A sex-ratio bias might either prevent parental incubation from spreading throughout the population or might select for GSD.

Live-bearing (ovoviviparity) and brooding are two types of reptilian behavior which involve temperature regulation of the embryos. In live-bearers, pregnant females characteristically "bask" in the sun, presumably to maintain a high or constant body temperature (Packard, Tracy and Roth, 1977; Shine and Bull, 1979). In brooding species, females coil around their eggs and regulate egg temperature by generating heat with muscle contractions (Fitch, 1970; mine is a restricted definition of brooding). The evolution of both behaviors may thus be facilitated by genotypic sex determination. Live-bearing has arisen over thirty times in lizards and snakes and is known in hundreds of species, whereas brooding is known only in pythons (Fitch, 1970; Shine and Bull, 1979). These situations offer ample opportunities to

study the correlation with TSD (the snakes are less interesting, if GSD is indeed ubiquitous in this suborder). Table 2 lists the incidence of live-bearing in the taxa known to have TSD. The associations are much the same as for sex chromosomes, except that some Asian agamid lizards are live-bearing, whereas an African agamid exhibits TSD. (Here again one must consider whether the demonstration of TSD in the laboratory corresponds to the same in nature.)

Temperature-dependent sex determination should not be affected by parental incubation if the incubation occurs outside the temperature-sensitive period of development. A variety of squamates carry eggs only part of the way through development before laying (Tinkle and Gibbons, 1977; Shine and Bull, 1979). If TSD in fact hinders the evolution of live-bearing, there might nonetheless be species with TSD that would carry embryos up to the temperature-sensitive stages prior to laying.

THE ANCESTRY OF SEX DETERMINATION IN REPTILES

The ancestry of sex-determining mechanisms in reptiles is of interest for understanding not only the origins of the mechanisms observed in this class, but also of the origins of the mechanism in birds and mammals, whose lineages stem from early reptiles. Given the multiplicity of sex-chromosome systems and TSD mechanisms in reptiles (see Tables 1, 2, Fig. 2), the uselessness of fossils to study this problem, and the lack of understanding of the physiological and molecular bases for these mechanisms, one cannot at present hope to arrive at definite conclusions about their origins. I limit myself to an overview of sex-determining mechanisms, which may bear upon their ancestries.

Amphibia

The amphibians are used here as a starting point, because they may reflect the stock from which early reptiles emerged. In the species studied, sex determination is controlled by genotype, but only weakly so, and sex chromosomes are not usually observed (White, 1973). Genotypic control is demonstrated by mating of sex-reversed individuals with normal ones (XX · XX or XY · XY matings) and observing sex-ratio biases in the progeny.

Sex-ratio biases are also noted in some species when progeny of normal matings (XX · XY) are raised at extreme temperatures. High temperatures (25°-30°C) produce an excess of males, whereas low temperatures (5°-10°C) produce an excess of females (Witschi, 1929b; Houillon and Dournon, 1978; Pieau, 1975a). In two cases the sex-ratio bias has been shown to result from temperature-induced sex reversal rather than from differential mortality. In the wood frog, if the extreme temperature is applied shortly after the onset of normal sexual differentiation, the histological course of gonad differentiation is observed to reverse in half the larvae (Witschi, 1929b). In a salamander raised at a high temperature, breeding experiments confirmed that a genotypic female had been converted into a male (Houillon and Dournon, 1978). In addition to temperature, factors such as hormones and overripeness of eggs readily affect sex determination in some species (Richards and Nace, 1978; Kawamura and Nishioka, 1977). Some environmental sex determination may even occur naturally, because YY males have been observed in natural populations (Kawamura and Nishioka, 1977).

Modern amphibians, therefore, exhibit components of GSD as well as TSD, and sex determination may have been similar in the ancestors of early reptiles. Possibly the GSD and TSD of amphibians have different physiological bases than the mechanisms of reptiles, and extrapolation from sex determination in one group to that in the other is quite speculative. But regardless of the physiological basis of sex determination, it is puzzling how an amphibian-like mechanism could have prevailed in early reptiles, if the onset of terrestrial development led to increases in incubation temperature such as we now observe in reptiles. Sex determination in amphibians inhabiting warm aquatic environments might provide some clues.

Reptiles

In each of two orders, Squamata and Chelonia, there are species with and without TSD, as well as species with and without heteromorphic sex chromosomes. In crocodilians and the tuatara, heteromorphic sex

chromosomes are seemingly absent, whereas TSD operates in alligators. On the basis of this evidence it seems at least as likely that the ancestral, stem reptiles had TSD as that they had GSD. Furthermore, TSD is sufficiently widespread in turtles to make us argue that it is of remote ancestry in this order. Therefore, although the evidence regarding the ancestry of sex determination in reptiles is equivocal, the strong possibility that TSD is ancestral to this class, or at least of remote ancestry in some orders, requires a reassessment of current ideas about the evolution of vertebrate sex determination.

The data on sex chromosomes bear somewhat upon this issue. In lizards, snakes, and turtles, the observed sex chromosomes appear to be recent evolutions. This trend toward increasing heteromorphism could reflect a trend toward stronger genotypic control of sex determination. If one assumes that sex determination in early reptiles was environmental, then sex chromosomes would not have evolved at this time (recalling the preceding arguments). The subsequent evolution of GSD could then have been followed by the appearance of sex chromosomes. However, if one assumes that GSD is ancestral, it would be puzzling that sex chromosomes did not evolve in those ancestors but have evolved, on many occasions, more recently. The weakness of this argument is that the evolution of sex chromosomes may require more than just GSD, and the lack of ancient sex-chromosome systems may simply reflect an absence of these other requirements rather than an absence of GSD.

Mammals and Birds

Mammals and birds are both derived from early reptilian stocks. In contrast to modern reptiles, the mammals and most birds are characterized by highly uniform sex chromosome systems. The same X chromosome is preserved throughout at least the marsupial and placental mammals, as suggested by the similarity of X-linked traits and the karyotypic similarity of the X chromosome in different mammals (Ohno, 1967; Pathak and Stock, 1974; see VandeBerg and Cooper, 1978, for contrary evidence in respect to monotremes). Karyological evidence from birds also suggests uniformity of the Z in this lineage (Ohno, 1967; Ray-Chaudhuri, 1973) although heteromorphic sex chromosomes may be lacking in one group (Tagaki and Sasaki, 1974). Therefore, the common ancestor to these surviving mammals very likely had GSD with sex chromosomes, and birds may also have a remote ancestry of GSD. This might be taken as evidence for the occurrence of GSD in the earliest reptiles (although the bird and mammal systems are clearly independently evolved), except that birds and mammals both thermoregulate the development of their young (Drent, 1975; Whittow, 1970, 1973). For reasons discussed above, this condition may preclude TSD from operating, and thereby introduces a bias in the data. TSD could not have persisted into recent times in mammals or birds even if it had been present in their ancestors. The evolution of GSD may even have been a direct consequence of selection imposed by parental incubation. The current lack of information on the ancestry of parental incubation in mammals and birds (Whittow, 1970, 1973) precludes ruling out this possibility, so the data on sex determination in birds and mammals must be viewed with caution. One possibility is to look for remnants of TSD in birds, but the narrow thermal tolerances of avian embryos (Drent, 1975) complicate investigation of a wide range of temperatures. Lutz-Ostertag (1966) attempted such investigation in the quail by incubating eggs at 2°C above normal. The high temperature caused retention of the Müllerian duct (oviduct) in males but had no effect on the gonads. Thus, at present, the ancestry of sex-determining mechanisms in reptiles is on all accounts unresolved.

CONCLUSIONS AND IMPLICATIONS

Reptiles present an impressive variety of sex-determining mechanisms, from sex chromosomes to temperature-dependent sex determination (TSD). The work on sex chromosomes is comprehensive, encompassing enough species for a general pattern of evolution to become apparent: sex chromosome heteromorphism seems to have evolved recently in reptiles, not being ancestral to any orders. The work on TSD is less comprehensive. Fewer species have been studied, often without regard to ecological considerations or

the possibility of sex reversal after hatching. More work is needed to fill these gaps. At present, TSD seems to be the natural sex-determining mechanism in some species and tends to be found in species without sex chromosomes.

The phenomenon of TSD poses several interesting problems for future research. With sex determination by nest temperature, the population sex ratio is sensitive to the organism's life history and to its local environment. The season of nesting, the site of nesting, and parental incubation are factors which affect the sex ratio, and these may vary across a species' range. Reptiles are suitable for studies of geographic and interspecific variations in parameters which affect the sex ratio, and such studies would help in understanding the coevolution of the sex ratio, sex determination, reproductive biology, and perhaps biogeography.

An unexplored area for research is the physiology of sex differentiation. The histology and embryology of gonadogenesis has not been thoroughly studied, and such work would be informative in the context of sex differentiation in other vertebrates. In particular, it would be interesting to know the relationship between the H-Y antigen and sex determination. This antigen has been implicated as the primary inducer of male differentiation in mammals (Ohno, 1979; Silvers and Wachtel, 1977; Nagai, Ciccarese, and Ohno, 1979). The same or a similar antigen is also sex-limited in frogs, birds (Wachtel, Koo, and Boyse, 1975), and turtles (Zaborski, Dorizzi, and Pieau, 1979) and may therefore have a sex-determining effect in these vertebrates as well.

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APPENDIX I

By E. WAGNER

*Temperature-dependent sex determination in a gekko lizard**

Eggs of the leopard geckoe, *Eublepharis macularius,* were incubated throughout development at one of several temperatures, and the resulting sex ratios were observed among the young after they began maturing. Sex was diagnosed on the basis of secondary sexual characteristics (anal pores, behavior). The observations were as follows:

	Temperature		
	24°C	27°-29°C	32°-33°C
Males	0	1	14
Females	7	44	2
Died	3	14	2

There is an obvious sex-ratio bias associated with incubation temperature. Two alternative explanations are that (1) sex is determined genotypically and the bias is due to differential mortality, or that (2) incubation temperature determines sex. Assuming that there was no bias in distributing the eggs among different temperatures and that all dead lizards belonged to the rarer sex, the probability of observing these results under hypothesis (1) is less than 10^{-4} (Fisher's exact test, lumping 24°C with 27°-29°C data). For lack of a more plausible alternative, it seems likely that sex can be determined by incubation temperature in this lizard.

* From a talk given to the Society for the Study of Amphibians and Reptiles in June, 1978.

APPENDIX II

By D. OSGOOD

Sex ratio and incubation temperature in a watersnake

In the course of a study (Osgood, 1978), on the North American live-bearing watersnake *Nerodia (Natrix) fasciata,* adult females were collected from the wild just prior to or shortly after ovulation. These females were brought into the laboratory and placed in different controlled-temperature environments until birth of their young. The sex ratios of broods from individual females are recorded below. Each pair of numbers corresponds to the brood of a single, unique female.

21.2°-22.2°C		26.1°-26.7°C		29.4°-30.6°C	
♂	♀	♂	♀	♂	♀
3	0	9	9	1	4
5	3	11	9	10	22
6	3	13	16	10	13
13	14	11	10	8	8
18	17	18	10	9	9
8	8	7	7	18	11
16	15	23	20	8	13
14	11	8	5	2	2
12	10	5	12	16	19
5	2	16	15	12	14
		7	6	6	5
		5	12		
		20	13		
100	83	153	144	100	120

Although the over-all sex ratio at the low temperature is slightly different from that at the high temperature, none of the totals differs significantly from the others in pairwise comparisons (Fisher's exact test). Also, none of the sex ratios is significantly different from 1:1. Thus, temperature seems to have no definite effect on the sex ratio in this species, and there is no reason to reject a hypothesis of genotypic sex determination with a sex ratio of ½.