

Sex-Determining Mechanisms in Squamate Reptiles

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ABSTRACT Two modes of sex determination occur in squamates: genotypic sex determination (GSD) and temperature-dependent sex determination (TSD). Within each of these two major modes, there are many different variants, or mechanisms. Male heterogamety, female heterogamety, multiple sex chromosome systems, and homomorphic sex chromosome systems are all types of GSD found in squamates. Two patterns of TSD have been reported.

Only three snakes have been investigated for their sex-determining mechanisms, each having GSD, although incubation temperature does cause differential mortality and affects post-hatching physiology. Less than 50 lizard species have been investigated, but there is considerable diversity in the sex-determining mechanisms reported thus far. Apparently, TSD (and/or GSD) has evolved multiple times within a given taxon. Presently, both GSD and TSD are found in the Agamidae, Eublepharidae, and Gekkonidae, and possibly in the Iguanidae, Lacertidae, and Varanidae as well. Only GSD has been reported for the Scincidae and Teiidae.

Correlations within the Eublepharidae suggest an adaptive explanation for the evolution of sex-determining mechanisms; a shift from male-larger dimorphism to female-larger (or no sexual size dimorphism) is accompanied by a shift in sex-determining mode. These shifts are in agreement with similar correlations observed in turtles. © 1994 Wiley-Liss, Inc.

The sex-determining mechanisms of squamate reptiles are poorly known, relative to the large number of species in the group (2,267 snakes, 3,307 lizards [Duellman, '79]). Only 16% of these species have been karyotyped (Olmo, '86), and the effects of multi-temperature incubations have been examined in only a few species. Even so, there is considerable diversity in the sex-determining mechanisms reported thus far (note: here we use *sex-determining mechanism* as defined by Bull ['83]).

Two major modes of sex determination occur in squamates: genotypic sex determination (GSD) and temperature-dependent sex determination (TSD). Within each of these two modes, there are different variants, or mechanisms. Two types of TSD have been reported in lizards (Ewert and Nelson, '91): Pattern Ib (also designated as Pattern FM [Lang and Andrews, this issue]), in which females are produced at cool incubation temperatures and males are produced at warm temperatures, and Pattern II (also designated as Pattern FMF [Lang and Andrews, this issue]), where females are produced predominantly at both cool and warm temperatures, and males are produced predominantly at intermediate temperatures.

Male heterogamety, female heterogamety, multiple sex chromosome systems, and homomorphic sex chromosome systems are all patterns of GSD found in squamates (Gorman, '73; Olmo, '86). Several different mechanisms may occur within a given family, genus, species, or population (see Discussion).

Here we summarize the published data on squamates and present new data for 22 species. Sex-determining mechanisms of all lizard species currently investigated are presented in Table 1. Results for the new data are listed in Appendix A; methods are summarized in Appendices B and C.

SNAKES

Only three snakes have been examined for their sex-determining pattern by comparisons among different incubation temperatures. The small number of species examined may reflect the frequency of heteromorphic sex chromosomes (HSCs) in the group (126 of 294 species karyotyped [Olmo, '86]). GSD is expected for any species with HSCs, but may or may not be present in species lacking them (Bull, '80). Of the three species studied, only *Pituophis melanoleucus* has been karyotyped (re-

TABLE 1. Summary of sex-determining mode for the species discussed in this paper¹

Species	Sex-determining mode
Agamidae	
<i>Agama agama</i>	TSD ²
<i>A. caucasia</i>	TSD, Pattern II? ³
<i>A. (Stellio) stellio</i>	TSD?? ⁴
<i>Pogona vitticeps</i>	GSD
Chamaeleontidae	
<i>Chamaeleo pardalis</i>	GSD? ⁵
<i>Rhampholeon k. kerstenii</i>	? ⁶
Eublepharidae	
<i>Coleonyx brevis</i>	GSD?
<i>C. variegatus</i>	GSD
<i>Eublepharis macularius</i>	TSD, Pattern II
<i>Hemitheconyx caudicinctus</i>	TSD, Pattern II
Gekkonidae	
<i>Gekko japonicus</i>	TSD, Pattern II
<i>Phelsuma abbotti</i>	TSD ⁷
<i>P. cepediana</i>	GSD?? ⁸
<i>P. dubia</i>	TSD?
<i>P. g. guimbeau</i>	ESD
<i>P. laticauda</i>	TSD?
<i>P. guentheri</i>	ESD
<i>P. lineata prusila</i>	TSD?
<i>P. madagascariensis grandis</i>	TSD, Pattern II
<i>P. m. madagascariensis</i>	TSD?
<i>P. m. kochi</i>	?
<i>P. ornata</i>	GSD??
<i>P. sundbergi</i>	GSD?
<i>Tarentola angustimentalis</i>	TSD??
<i>T. annularis</i>	TSD??
<i>T. boettgeri hierrensis</i>	TSD
<i>T. delalandii</i>	TSD??
<i>T. gomerensis</i>	TSD??
<i>T. mauritanica</i>	TSD
Iguanidae	
Anolinae	
<i>Anolis carolinensis</i>	GSD
Basiliscinae	
<i>Basiliscus plumifrons</i>	GSD?
Crotaphytinae	
<i>Crotaphytus collaris</i>	GSD?
Iguaninae	
<i>Brachylophus fasciatus</i>	?
<i>Cyclura</i> sp.	?
<i>Dipsosaurus dorsalis</i>	GSD?; TSD, Pattern II? ⁹
Sceloporinae	
<i>Sceloporus jarrovi</i>	GSD
<i>S. occidentalis</i>	GSD?
<i>S. undulatus consobrinus</i>	GSD
<i>S. u. garmani</i>	GSD?
<i>S. u. hyacinthinus</i>	GSD
Lacertidae	
<i>Lacerta viridis</i>	GSD
<i>Podarcis muralis</i>	GSD
<i>P. pityusensis</i>	TSD??
<i>P. hispanica</i>	?
Scincidae	
<i>Eumeces fasciatus</i>	GSD
<i>E. obsoletus</i>	GSD?
Teiidae	
<i>Cnemidophorus inornatus</i>	GSD?

<i>C. uniparens</i>	GSD? ¹⁰
<i>Kentropyx striatus</i>	?
Varanidae	
<i>Varanus niloticus</i>	?
<i>V. salvator</i>	TSD?

¹Species are listed by family. ESD, environmental sex determination; GSD, genotypic sex determination; TSD, temperature-dependent sex determination.

²Has TSD; pattern has not been established.

³Has TSD, probably Pattern II (= FMF), but more data are needed to establish pattern with certainty.

⁴TSD is suggested, but many more data are needed to support the claim.

⁵Probably has GSD, but more data are needed for certainty.

⁶Data are equally consistent with GSD or TSD.

⁷Probably has TSD, but more data are needed for certainty.

⁸GSD is suggested, but many more data are needed to support the claim.

⁹See text.

¹⁰Parthenogenetic.

viewed in Gorman, '73). Sex chromosomes were not found, but a male was examined, and only female heterogamety is known in snakes (Gorman, '73).

Females of a live-bearing snake, *Nerodia fasciata*, were collected just prior to or shortly after ovulation and placed in one of three temperature-controlled environments (Osgood, '80). Although there were slight sex ratio skews at all temperatures, there were no significant differences between treatments, and none of the sex ratios were significantly different from 1:1.

A reanalysis of Osgood's ('80) data for individual brood sex ratios found a difference among incubation temperatures (Dunlap and Lang, '90). Mean sex ratio/brood was 0.61 for 21.2–22.2°C and 0.45 for 29.4–30.6°C. Both the means and the distribution of brood sex ratios were significantly different between high and low incubation temperatures. However, the possibility of differential mortality cannot be ruled out completely.

Eggs of *Lampropeltis triangulum andesiana* were incubated at three temperatures (25.6, 26.7, and 27.8°C) (Hammack, '89). Statistics were not provided, but an analysis of the raw data showed that sex ratios were not statistically different from 1:1 nor from each other (chi-square, $P = 0.72$).

Temperature affected sex ratios in the pine snake, *Pituophis melanoleucus*, through differential mortality of the sexes (Burger and Zappalorti, '88). Males had higher embryonic mortality at low incubation temperatures and females at high ones. In addition, snakes from eggs incubated at low temperatures had significantly more morphological abnormalities and differed in some post-hatching physiological parameters (Burger et al., '87).

LIZARDS

TSD is now known in several lizards, with both Pattern Ib (= FM) and Pattern II (= FMF) having been reported. Each putative Pattern Ib study found females at one or two low temperatures and mostly males at one or two warmer temperatures. But separation of other patterns from Pattern II requires an examination of most of the full range of viable incubation temperatures for the species (Ewert and Nelson, '91). Examination of temperatures warmer than those used in initial reports have led to the demonstration that putative Ib patterns were actually Pattern II both in a lizard (Viets et al., '93) and in two crocodylians (Lang and Andrews, this issue). None of the "Pattern Ib" species cited below were so examined and should thus be regarded as tentative. This caveat is especially important when only two temperatures have been tested or when precise data are not published.

Agamidae

Agama agama has been frequently cited as having Pattern Ib TSD (Bull, '80, '83; Deeming and Ferguson, '88; Ewert and Nelson, '91), although only two incubation temperatures were employed (Charnier, '66). *Agama caucasia* has TSD (Langerwerf, '83), but pattern was not established. *A. caucasia* was subsequently reported as having Pattern II TSD and *A. stellio* as having Pattern Ib TSD, but sample sizes, sex ratios, or incubation temperatures were not given (Langerwerf, '88).

Janzen and Paukstis ('91b) claimed TSD for *Calotes versicolor*, referenced by a personal communication from J.J. Bull. However, their claim appears to be mistaken acceptance of hearsay (J.J. Bull, personal communication).

Our data indicate that *Pogona vitticeps* has GSD (Appendix A). No significant relationship between incubation temperature and sex ratio was found, and there were no significant differences between temperatures. No HSCs were found when karyotyped (Witten, '83). *P. vitticeps* is the first agamid found to have GSD.

Chamaeleontidae

Chamaeleo pardalis probably has GSD (Appendix A). Incubations (G. Ferguson, unpublished data) at room temperature ($23^{\circ}\text{C} \pm 5.5$) and at 28°C produced sex ratios near 1:1. *C. pardalis* has been karyotyped, but sex chromosomes have not been reported (reviewed in Gorman, '73).

Three male and two female *Rhampholeon k. kerstenii* hatched at 28°C (Leptien, '91), consistent with either GSD or TSD.

Eublepharidae

The Eublepharidae contains seven genera and 17 or 18 species (Grismer, '88). Of the species karyotyped, none possess HSCs (Gorman, '73; Murphy, '74); karyotypes of *Aeluroscalabotes*, *Goniurosaurus*, *Holodactylus*, and *Coleonyx mitratus* have yet to be determined.

The Pattern Ib (= FM) TSD response initially reported for *Eublepharis macularius* (Bull, '87; Wagner, '80) was recently shown to be Pattern II (= FMF) TSD (Viets et al., '93). Additional data (Fig. 1) have not changed the overall pattern appreciably from that reported (Viets, '93; Viets et al., '93) but indicate that 28°C and 35°C do not produce females exclusively. *Hemitheconyx caudicinctus* was shown to exhibit TSD, but a clear pattern was not established (Anderson and Oldham, '88). *H. caudicinctus* has a Pattern II curve that closely resembles that of *E. macularius* (Fig. 1). A highly significant effect was found between incubation temperature and sex ratio (Appendix A).

Our data on *Coleonyx variegatus* are consistent with GSD (Appendix A). Different constant incubation temperatures had no statistically significant effect on sex ratio (Fig. 2). For data from other institutions, total incubation times were accurately recorded, whereas incubation temperatures were slightly ($\pm 0.5^{\circ}\text{C}$) to moderately ($\pm 1.1^{\circ}\text{C}$) variable. Due to the highly significant negative correlation ($P < 0.0001$) between incubation temperature and total incubation time, and because total time was recorded for all lizards, sex ratio data in *C. variegatus* are presented as a function of total incubation time (days to hatching). Estimated constant incubation temperature equivalent

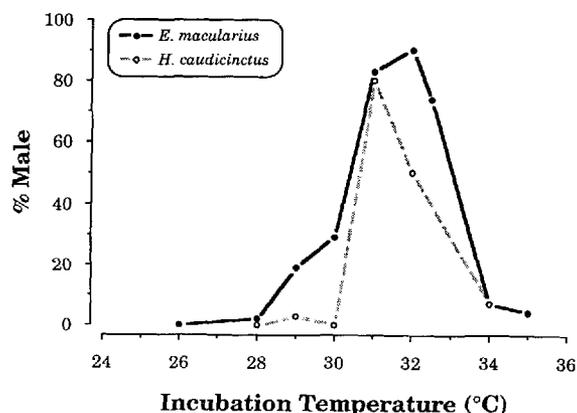


Fig. 1. Hatching sex ratio (expressed as percent male) as a function of various constant incubation temperatures in *Eublepharis macularius* and *Hemitheconyx caudicinctus*. Sample sizes are given in Appendix A.

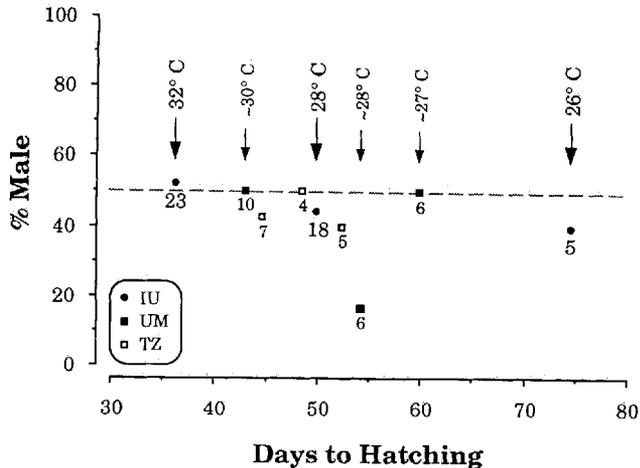


Fig. 2. Hatching sex ratio (expressed as percent male) as a function of days to hatching in *Coleonyx variegatus*. Sample sizes are indicated by each point. Incubation temperatures in the larger font varied no more than $\pm 0.2^\circ\text{C}$. Incubation temperatures preceded by \sim varied up to $\pm 0.5^\circ\text{C}$. Incubations at the Tulsa Zoo varied $\pm 1.1^\circ\text{C}$ and are therefore not given a reference temperature. However, for all incubations, total incubation times were accurately recorded. Days to hatching with a sample size of three or less have been omitted. Incubations at the University of Michigan (R. Burke, unpublished data) used animals collected in the same counties of Arizona as our colony stock; incubations at the Tulsa Zoo (R. Grimpe, unpublished data) used animals of unknown origin. IU = Indiana University; TZ = Tulsa Zoo; UM = University of Michigan.

lents are provided for reference (Fig. 2). The combined data strongly support GSD. *Coleonyx brevis* probably has GSD as well (Appendix A).

Gekkonidae

In *Gekko japonicus*, Pattern II (= FMF) TSD has been firmly established (Tokunaga, '85, '89). An earlier report described well-differentiated sex chromosomes for this species (Yoshida and Itoh, '74). In fact, the sex chromosomes of *G. japonicus* are the most strongly differentiated in all of the Gekkota, differing in both heterochromatin content and size (Moritz, '90). Intraspecific variation in sex-determining mode is not totally unprecedented in vertebrates, as GSD and TSD co-occur in two fishes (*Menidia menidia* [Conover and Heins, '87]; *Poeciliopsis lucida* [Sullivan and Schultz, '86]). However, the occurrence of both HSCs and TSD within the same population would be quite surprising. This thus raises some question about the taxonomic status of the populations studied. In any case, these reports suggest considerable evolutionary plasticity in sex-determining mode.

In *Tarentola mauritanica*, only females (N = 33) were produced at 27, 29, and 30°C , and in *T. boettgeri hierrensis*, only females (N = 23) were produced at 28 and 30°C (Nettmann and Rykena, '85). The authors suggested TSD in these two species and in four other species of *Tarentola* as well. Patterns of TSD cannot yet be determined for *T. mauritanica* and *T. boettgeri hierrensis*, and no sex ratio data are provided for the other *Tarentola* species. All species of *Tarentola* require males for reproduction in captivity; thus, these results cannot be interpreted as parthenogenesis.

Although there are few published data concerning sex determination in the genus *Phelsuma*, temperature is frequently mentioned as having an effect (Howard, '80; McKeown, '84; Tyle, in press). Our work with *Phelsuma madagascariensis grandis* indicates that it has Pattern II TSD (Appendix A). Additional support for this claim comes from a private breeder (T. Tyle, unpublished data) and the National Zoological Park (B. Demeter, unpublished data). *P. dubia* probably has TSD as well (0% male at 28°C ; N = 9).

Phelsuma g. guimbeaui produced only females at a wide variety of temperatures (S. McKeown, unpublished data; T. Tyle, unpublished data) (Appendix A). This species is not parthenogenetic, as wild breeding groups consist of one or more males with several females (McKeown, '89). Although several temperatures have been employed, possibilities still exist for the production of males at warmer temperatures or from fluctuating temperatures (as in *Crocodylus johnstoni* [Webb et al., '87]). Although TSD might be questionable, some type of environmental sex determination (ESD) is obvious. Eggs of *Phelsuma guentheri* incubated in situ in breeding cages (day, 30°C ; night, 25°C) produced only one male in 1978 (N = 22) and only two males in 1979 (N = 46) (Bloxam and Tonge, '80). Here again, some type of ESD is obvious. The same probably holds true for *P. laticauda*, as only females were produced from 26 – 30°C (Howard, '80), but sample sizes were not given.

Tyle (unpublished data) has also investigated *P. lineata prusila* (0% male at 28°C ; N = 30), *P. m. madagascariensis* (0% male at 28°C ; N = 12), *P. m. kochi* (39% male at 28°C ; N = 18), and *P. abbotti* (14% male at 28°C ; N = 7). For *P. lineata prusila*, *P. m. madagascariensis*, and *P. abbotti*, TSD is likely, although the data for the latter are not significant. The data for *P. m. kochi* are consistent with either TSD or GSD.

Although precise data are not available, incu-

bation of *Phelsuma cepediana*, *P. ornata*, and *P. sundbergi*, under the same conditions as those used for *P. g. guimbeaui*, produced both males and females (McKeown, unpublished data). Tytle's (unpublished data) results for *P. sundbergi* at 28°C are similar (67% male; N = 6). These results suggest the possibility of GSD in the genus but would be equally compatible with TSD or with some other form of ESD.

Iguanidae

Anolinae

Anolis carolinensis has GSD. Multiple temperature incubations (from 21.5–32°C) had no significant effect on sex ratio, which was close to 1:1 at all temperatures (N = 609) (Appendix A). *A. carolinensis* has been karyotyped, but HSCs have not been reported (Gorman, '73).

Basiliscinae

Basiliscus plumifrons may have GSD (Appendix A). The sample sizes are small, however, so final declaration will require additional data. However, the responses shown fit no known pattern of TSD and probably represent scatter around a 1:1 sex ratio response.

Crotaphytinae

Crotaphytus collaris may have GSD (Appendix A). Clearly, more data are required. No HSCs were reported in the karyotype of *C. collaris* (Gorman, '73).

Iguaninae

The effects of incubation temperature on sex ratio in *Dipsosaurus dorsalis* were studied using eight constant incubation temperatures (Muth and Bull, '81) (Fig. 3). As the results were not consistent with any TSD pattern known at the time, a simple assumption of temperature independence was tested (i.e., GSD). The correlation between temperature and sex ratio was not significantly different from 0, and total sex ratio was not significantly different from 0.5, but the sex ratios at different incubation temperatures were heterogeneous. Although the authors did not so claim, GSD has been cited for *D. dorsalis* in several subsequent articles (e.g., Bull, '83; Raynaud and Pieau, '85; etc.).

Re-examination of these data in light of the subsequent discovery of Pattern II (= FMF) TSD and its occurrence in some lizards (see above) suggest a different conclusion. As demonstrated, the sex ratios at different incubation temperatures are

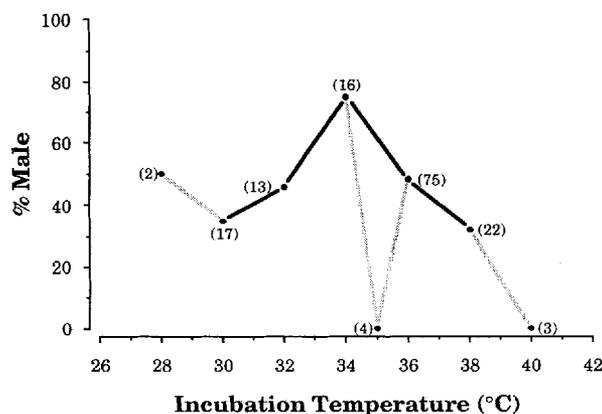


Fig. 3. Hatching sex ratio (expressed as percent male) as a function of incubation temperature in *Dipsosaurus dorsalis*. Data are from Muth and Bull ('81). Numbers in parentheses indicate sample sizes. Solid black lines connect the main samples. Shaded lines connect sample sizes less than five.

very heterogeneous (Fig. 3), and, at 34°C, the sex ratio is significantly different from 1:1, assuming Pattern II as the expected form (one-tailed binomial test, $P < 0.038$). Further, the sex ratios at 30 and 38°C are significantly different from that at 34°C, and the sample at 36°C approaches significance (chi-square; $P = 0.022$, $P = 0.009$, $P = 0.062$, respectively). The data at 28, 35, and 40°C, due to their very small sample sizes (N = 2, 4, and 3, respectively), yield a biased chi-square value (Zar, '74) and thus should be eliminated from the analysis (Cochran, '54). When the data are plotted without these small samples, a curve suggestive of Pattern II TSD is generated (Fig. 3). *Dipsosaurus* has been karyotyped (Paull et al., '76), and no HSCs were found. Thus these criteria are compatible with viewing this as Pattern II TSD. However, the sample at 35°C (four females) seems improbable under such a conclusion.

Brachylophus fasciatus eggs incubated at 25°C ($\pm 1.5^\circ\text{C}$) produced three male hatchlings (Arnett, '79), consistent with either mode of sex determination. "Poor" sex ratios at high incubation temperatures were reported for several *Cyclura* species (Noegel, '89). These results may be due to TSD or differential mortality, as mortality is reported to increase at higher temperatures.

Sceloporinae

Pregnant females of a viviparous lizard, *Sceloporus jarrovi*, were incubated at temperatures ranging from 26–38°C (Beuchat, '88). Although a significant relationship between offspring sex ratio and temperature was found, incubation tem-

perature did not seem to affect sex ratio in any systematic manner.

Sceloporus undulatus hyacinthinus has GSD, as does *S. u. consobrinus* (Appendix A). Incubations (from 24–34°C) in *S. u. hyacinthinus* (N = 66) and incubations at three temperatures (24, 28, and 32°C) in *S. u. consobrinus* (N = 143) had no significant effect on offspring sex ratio. Single temperature (28°C) incubations of *S. u. garmani* and *S. occidentalis* eggs also suggest GSD, although TSD cannot be excluded (50% male, N = 94; 52% male, N = 79). Both *S. undulatus* and *S. occidentalis* have been karyotyped, but no sex chromosomes were reported (reviewed in Gorman, '73).

Lacertidae

GSD has been established for two lacertids. Incubation temperature did not significantly affect the sex ratio of *Lacerta viridis* (Raynaud and Chandola, '69; Raynaud and Pieau, '72), although some modification of the genital system was observed at both low and high temperatures (Raynaud and Pieau, '72, '85). Van Damme et al. ('92) found that incubation temperature differentially affected hatching time, egg survival, and hatchling performance, but not sex ratio, in *Podarcis muralis*.

Janzen and Paukstis ('91b) list *Podarcis pityusensis* and *Podarcis hispanica* as having TSD (although *P. hispanica* is listed as questionable), citing Eichenberger ('81). Eichenberger provided no sex ratio data for *P. hispanica*. His data for *P. pityusensis* show a significant sex ratio skew (1 male:10–15 females) but at only one temperature (29°C). This species thus may well have TSD. If so, the Lacertidae and the genus *Podarcis* will join the list of taxa exhibiting both modes of sex determination (GSD and TSD). Clearly more work involving several temperatures is needed to resolve existing uncertainties.

Scincidae

Both *Eumeces fasciatus* and *E. obsoletus* probably have GSD (Appendix A). Egg incubations from 25–32°C indicated no significant sex ratio skews. However, due to the male bias at low temperatures and the female bias at warmer temperatures in *E. obsoletus*, the unequivocal claim of GSD in this species must await additional data. *E. obsoletus* has been karyotyped, but HSCs have not been reported (reviewed in Gorman, '73).

Teiidae

In *Cnemidophorus inornatus*, incubations at 25°C resulted in 55% males (N = 22), and those

at 30°C resulted in 50% males (N = 20) (Crews, '89). Albeit unlikely, because only two temperatures were employed, the possibility still exists that these data may simply illustrate the two pivotal temperatures in a Pattern II (= FMF) TSD species, but this species probably has GSD. In *C. uniparens*, exclusively females developed at the five temperatures employed, which suggests that, as expected, sex differentiation is independent of temperature in this parthenogenetic lizard (Crews, '89).

A 1:1 hatchling sex ratio was reported for a clutch of *Kentropyx striatus* eggs incubated at 28°C (N = 8) (Dixon et al., '75). These data are consistent with either GSD or TSD.

Varanidae

Male-biased hatchling sex ratios in *Varanus salvator* were reported by Hairston and Burchfield ('92). At 30.6°C ($\pm 0.6^\circ\text{C}$), 67% males were produced (N = 6), at 31.2°C, 100% males were produced (N = 11), and at 31.4°C ($\pm 0.8^\circ\text{C}$), 75% males were produced (N = 4). Although the sizes of the first and third samples are small and offer little information, the second sample is significantly different from a 1:1 sex ratio (one-tailed binomial test, $P < 0.001$). However, in a different breeding group of *V. salvator*, H. Andrews (personal communication) found only females among 16 individuals that had hatched from eggs incubated at 31–32°C or cooler but for which gender diagnosis had been delayed for over a year. Thus, much uncertainty remains in our understanding of the sex-determining response for this species.

Our data for *V. niloticus* are consistent with either GSD or TSD (Appendix A). A probable ZZ/ZW chromosome system was described in *V. niloticus*, but no sex chromosomes were found in *V. salvator* (King and King, '75). Presently, there are no data inconsistent with TSD in *V. salvator*.

DISCUSSION

Despite the small number of snake species examined, incubation temperature has been shown to cause differential mortality and to affect post-hatching physiology. These effects, most often sought in TSD species, show the importance of investigating the effects of incubation temperature in GSD species as well. Indeed, such studies may provide the information needed for a more satisfactory understanding of the evolution of sex-determining modes and mechanisms in reptiles.

In comparison to the snakes, many more lizard species have been studied for the response of sex

determination to different incubation temperatures. The most salient overall finding is the large degree of diversity in these responses. The families of lizards thus far studied for temperature response all have GSD in at least some species either demonstrated, probable (Chamaeleontidae), or inferred from HSCs (Gekkonidae). TSD co-occurs with GSD in the Agamidae, Eublepharidae, Gekkonidae, and, possibly, the Lacertidae and Varanidae. The pattern of response for *Dipsosaurus dorsalis* (Fig. 3) suggests that TSD may also co-occur with GSD in the Iguanidae.

Considerable evolutionary plasticity in sex determination is also evident from karyotypic studies. Sex chromosomes occur in 85 of 607 karyotyped species of lizards in the last summary available (Olmo, '86). These are widely dispersed across families, suggesting that sex chromosomes have evolved multiple times. In fact, sex chromosomes apparently evolved several times in single genera (e.g., *Anolis*: Gorman and Atkins, '68; *Sceloporus*: Cole, '71). The wide variety of chromosomal sex-determining systems found also suggests multiple origins. Male heterogamety, female heterogamety, multiple sex chromosome systems, and homomorphic sex chromosome systems are all found in lizards (Gorman, '73; Olmo, '86). Several different mechanisms may occur within a given family, genus, species, or population. For example, in the Gekkota (geckos), both male and female heterogamety have been described in the same subfamily (Moritz, '90). In *Phyllodactylus marmoratus*, three distinct chromosome races have been observed, only one having HSCs (King and Rofe, '76). In *Gehyra purpurascens*, all females had HSCs, but these were of six different types (Moritz, '84). In *Heteronotia binoei*, HSCs occur in three of six karyomorphs but only in some populations (Moritz, '90).

Recent studies have increased considerably the diversity of responses to temperature known in turtles (Etchberger, '91; Ewert et al., this issue) and decreased those known in crocodylians (Lang and Andrews, this issue). A comparatively much smaller proportion of the squamates have been examined. Nonetheless, the evolutionary plasticity already evident suggests that much more variation in patterns of TSD, and possibly in sex-determining modes, will be discovered as the number of species studied increases.

The considerable diversity of chromosomal mechanisms demonstrated within some small clades, taken with the occurrence of both HSCs and TSD within populations at least nominally

belonging to *Gekko japonicus*, is particularly interesting. Considerable caution should be exercised in concluding that the presence of HSCs in some taxa of a clade suggests that the other members of the clade will also have temperature-independent sex determination.

Pattern II (= FMF) is the only pattern of TSD common to the three major reptilian lineages that have TSD (Ewert and Nelson, '91), and it may be the primitive state in reptiles (Webb and Smith, '84; Deeming and Ferguson, '89). However, there may be a difference in Pattern II expression between lizards and turtles. In Pattern II turtles, a few males are produced at cool incubation temperatures in many species, but no males are produced at warm incubation temperatures (Ewert and Nelson, '91). In *Eublepharis macularius* and *Hemitheconyx caudicinctus*, two of the best-studied Pattern II lizards, a few males are produced at the warmest viable constant incubation temperatures, but none are produced at the coolest incubation temperatures (Viets, '93; Viets et al., '93; this study).

Several authors have suggested a relationship between sex-determining mechanism and sexual dimorphism. The most precise suggestion (Ewert and Nelson, '91; Ewert et al., '94) is that species with female-smaller sexual dimorphism usually have Pattern II TSD (pattern-dimorphism congruence). A major limitation of the evidence has been that many of the species on which it was based fall into two clades, so that the data might alternatively be accounted for in part by phylogenetic inertia (Ewert and Nelson, '91; Janzen and Paukstis, '91a; but see Ewert et al., this issue). This suggests an examination of sex-determining mechanisms in fairly closely related species that have contrasting patterns of sexual size dimorphism. The eublepharid geckos provide such a case.

Two members of the Eublepharidae, *Eublepharis macularius* and *Hemitheconyx caudicinctus*, are male-larger species (for *E. macularius*: Minton, '66; for *H. caudicinctus*: Loveridge, '47), and also have Pattern II TSD. This corresponds with the pattern-dimorphism congruence hypothesis developed for turtles. In contrast, *C. variegatus*, a species in which females are somewhat larger than males (Parker, '72), has GSD. Similarly, on preliminary data, *C. brevis*, also a female-larger species (Dixon, '70), also appears to have GSD. Although the pattern-dimorphism congruence hypothesis makes no predictions relative to the occurrence of GSD, it would predict that female-larger species should not have Pattern II TSD.

These data on sex-determining mechanisms in the Eublepharidae are thus compatible with this hypothesis.

A confounding variable here is overall body size. Adult *C. variegatus* and *C. brevis* are much smaller than adults of any of the other eublepharids. Grismer ('88) considered the small snout-vent length of *C. variegatus* and *C. brevis* to be the derived state. So it is possible that GSD and female-larger dimorphism might also be correlated with overall body size.

Other aspects of the biology of these animals provide partial support for an adaptive interpretation of these differences. In *E. macularius* and *H. caudicinctus*, adult males are distinctly larger than females and are highly territorial and antagonistic toward one another (for *E. macularius*: Thorogood and Whimster, '79; for *H. caudicinctus*: B. Viets, unpublished data). Unlike most of the eublepharids, however, *C. variegatus* is not a male-larger species. Males of this species are "much less belligerent" than other geckos, often rest close together (Greenberg, '43), and move extensively in the field during the breeding season, evidently to increase the likelihood of mating (Parker, '72). This reproductive strategy is much different than that of most male-larger lizard species, where males are typically territorial (reviewed in Stamps, '83).

One possible cause of adult sexual size dimorphism is differential hatchling growth. Several studies in crocodylians have demonstrated that post-hatching growth and survival, important fitness parameters, are affected by incubation temperature (Hutton, '87; Joanen et al., '87; Webb and Cooper-Preston, '89). If hatchling fitness, as well as sex, varies with incubation temperature, average relative fitness could be greater under TSD than under GSD, in accord with the model developed by Charnov and Bull ('77).

Pattern II TSD thus would be adaptive in the male-larger species if it acted to allocate males to temperatures where they would become largest. In *H. caudicinctus*, hatchling mass and length are unaffected by incubation temperature. However, after 350 days (i.e., maturity), hatchlings of both sexes incubated at 31°C, a mostly male-producing temperature, were significantly larger than those from other, more female-producing temperatures (Viets, '93). Because clutch size is fixed in *H. caudicinctus*, and because there is intense, presumably size-dependent, male-male competition, males would benefit more by being large, relatively speaking, than females. TSD allows males

to be produced at temperatures which apparently maximize their fitness.

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APPENDIX A

TABLE A. Effects of different constant incubation temperatures on sex ratio in lizards—new data¹

Agamidae		
<i>Pogona vitticeps</i> —GSD	(10); 32°—52% (23) [<i>P</i> = 0.68; see text also]	
	<i>Eublepharis macularius</i> ⁴ —TSD, Pattern II (= FMF)	
28°—52% (25); 30°—57% (7); 32°—47% (17) [<i>P</i> = 0.90]	26°—0% (125); 28°—2% (114); 29°—19% (21); 30°—29% (80); 31°—83% (46); 31.5°—88% (17); 32°—90% (169); 32.5°—74% (219); 33°—80% (5); 34°—7% (61); 35°—4% (23) [<i>P</i> < 0.0001]	
Chamaeleontidae		
<i>Chamaeleo pardalis</i> ² —GSD?	<i>Hemitheconyx caudicinctus</i> —TSD, Pattern II (= FMF)	
Room temp (23° ± 5.5°)—45% (22); 28°—59% (27)	28°—0% (15); 29°—3% (33); 30°—0% (12); 31°—80% (66); 32°—50% (44); 34°—7% (14) [<i>P</i> < 0.0001]	
[<i>P</i> = 0.34]	Gekkonidae	
Eublepharidae		
<i>Coleonyx brevis</i> —GSD?	<i>Phelsuma madagascariensis grandis</i> —TSD, Pattern II (= FMF)	
28°—60% (10)	Room temp. (22.2° ± 1.0°)—0% (6); 26°—0% (40);	
28°—50%; 30°—50%; 32°—50%; total N = 25 ³ [NSP]		
<i>Coleonyx variegatus</i> —GSD		
26°—40% (5); 27°—50% (6); 28°—38% (24); 30°—50%		

28–33% (36)⁵; 32°–0% (6); 28° → 32°⁶–100% (1)

[$P < 0.0002$; not including shift]

Days⁷ ≥ 69–0% (4); 52–68–50% (12); 35–51–24% (38);
≤ 34–0% (3)

Phelsuma g. guimbeaui—ESD

22.8°–0%; 23.6°–0%; 26.7° (±1.0°)–0%; 27.2° (±2.0°)

–0%; 27.8°–0%; 28.9 (±2.0°)–0%; 30.3°–0%;

total N = 84⁸ [NSP]

28°–0% (50)⁵

Iguanidae

Anolinae

Anolis carolinensis—GSD

21.5°–57% (30); 22.5°–49% (37); 24°–54%

(37); 25°–50% (431); 28°–47% (15); 30°–62% (45);

32°–64% (14) [$P = 0.68$]

Basiliscinae

Basiliscus plumifrons—GSD?

28°–60% (5); 30°–33% (6)⁹; 32°–67% (3) [$P = 0.55$]¹⁰

Crotaphytinae

Crotaphytus collaris—GSD?

27°–50% (2); 28°–67% (3); 30°–67% (6); 32°–29% (7)

[$P = 0.52$]¹⁰

Sceloporinae

Sceloporus undulatus consobrinus—GSD

24°–45% (53)¹¹; 28°–50% (40); 32°–50% (50)¹¹

[$P = 0.54$]

Sceloporus undulatus hyacinthinus—GSD

24°–64% (11); 25°–67% (9); 27°–50% (8); 28°–67%

(9); 30°–67% (12); 32°–30% (10); 34°–71% (7)

[$P = 0.54$]

Scincidae

Eumeces fasciatus—GSD

25°–50% (8); 27°–67% (6); 30°–20% (5); 32°–67% (6)

[$P = 0.38$]¹⁰

Eumeces obsoletus—GSD?

25°–60% (5); 27°–67% (6); 30°–29% (7); 32°–20% (5)

[$P = 0.31$]¹⁰

Varanidae

Varanus niloticus—?

28°–0% (3); 30°–67% (3); 32°–0% (3)

APPENDIX B: METHODS FOR NEW INCUBATIONS

The methodology described below is generalized and applies to all species included in Appendix A. Specific methodologies are listed in Appendix C or are described elsewhere: Viets et al. ('93) for eublepharid geckos, Viets ('93) for *Anolis carolinensis*.

Lizards were kept in environmental chambers with controlled light cycles (species specific). Smaller species were fed crickets. Larger species were fed crickets and neonatal mice. Food was sprinkled with a nutritional supplement (Vionate[®], Rich Health) and calcium carbonate. Frequency of supplementation depended on species. Water was provided ad libitum.

Eggs were obtained during 1988–1992. Gravid females were provided with nesting areas of moist vermiculite. Clutches were usually split between incubation temperatures.

Most eggs were incubated in 1:1 water:vermiculite by mass, although some eggs were incubated in drier or moister substrata (Appendix C). In *Eublepharis macularius*, at least, these differences did not affect sex ratio (B. Viets and L. Talent, unpublished data).

Eggs were placed in airtight plastic boxes that were flushed with air daily. The incubation boxes were placed in incubators, and their positions were rotated daily to minimize the effects of any small temperature gradients within the incubators. Temperatures in the upright, cabinet-type incubators seldom varied more than ±0.2°C from the design temperature.

Eggs were incubated at temperatures ranging from 21.5–35°C and, in most cases, were placed at the design temperature on the day of oviposition and remained there until hatching or death. Eggs of *Eublepharis macularius* and *Hemitheconyx caudicinctus* destined for 34°C spent the first day at 32°C, were shifted to 34°C for the bulk of the incubation period, and were shifted back to 32°C for 1–3 days before hatching. These shifts did not affect sex determination but increased the survival of embryos at extreme temperatures (Viets et al., '93).

Gender was diagnosed by the presence of secondary sexual characteristics. Gender was confirmed at maturity and by dissection on all animals that died during the course of the study. See Viets ('93) for more detail on gender determination.

¹ Format is Incubation Temperature (°C) - % Male (N). If incubation temperature varied more than ±0.5°C, the range has been included after the temperature. See Table 1 for more explanation of sex-determining mechanisms. P values listed are for chi-square tests on the effect of incubation temperature on offspring sex ratio. NSP, no statistics possible.

² G. Ferguson, unpublished data.

³ No significant deviations from a 1:1 sex ratio were observed (L. Talent, unpublished data).

⁴ Data are from Viets ('93) and Viets et al. ('93), with the addition of 129 hatchlings from 28, 32, and 34°C.

⁵ T. Tytle, unpublished data.

⁶ Egg started at 32°, shifted to 28°C after 2 weeks.

⁷ Days to hatching were used in lieu of incubation temperatures, which were not available. Groupings represent one and two standard deviations from the mean (B. Demeter, unpublished data).

⁸ S. McKeown, unpublished data.

⁹ S. Tonge, unpublished data.

¹⁰ Chi-square value is biased due to small sample sizes; P value may not be valid.

¹¹ A. Landwer and G. Ferguson, unpublished data.

APPENDIX C

TABLE B. Method of egg obtainment and incubation substratum moisture

Species	Eggs ¹	Substratum ²
<i>Anolis carolinensis</i>	wc	0.75:1, 1:1
<i>Basiliscus plumifrons</i>	cb, wc	1:1
<i>Chamaeleo pardalis</i>	cb	0.7:1
<i>Coleonyx brevis</i>	cb	1:1
<i>Coleonyx variegatus</i>	cb, wc	1.5:1
<i>Crotaphytus collaris</i>	wc	1:1
<i>Eublepharis macularius</i>	cb	Several ³
<i>Eumeces fasciatus</i>	wc	1:1
<i>E. obsoletus</i>	wc	1:1
<i>Hemitheconyx caudicinctus</i>	cb	1.5:1
<i>Phelsuma abbotti</i>	cb	1:1
<i>P. cepediana</i>	cb	0.5:1
<i>P. dubia</i>	cb	0.7:1
<i>P. g. guimbeaui</i>	cb	0.5:1 ⁴
<i>P. lineata prusila</i>	cb	1:1
<i>P. madagascariensis grandis</i>	cb	0.7:1
<i>P. m. madagascariensis</i>	cb	1:1
<i>P. m. kochi</i>	cb	1:1
<i>P. ornata</i>	cb	0.5:1
<i>P. sundbergi</i>	cb	0.5:1, 1:1
<i>Pogona vitticeps</i>	cb	0.75:1, 1:1
<i>Sceloporus occidentalis</i>	cb	0.7:1
<i>S. undulatus consobrinus</i>	cb, wc	0.7:1
<i>S. u. garmani</i>	cb	0.7:1
<i>S. u. hyacinthinus</i>	wc	0.75:1, 1:1
<i>Varanus niloticus</i>	wc	1:1

¹Eggs: cb, eggs obtained from captive-bred females; wc, eggs obtained from wild-caught females.

²Substratum moisture. Listed as parts water:parts vermiculite by mass.

³Ratios 0.5:1, 0.75:1, 1:1, 1.5:1, 2:1. 1:1 and 1.5:1 utilized for most incubations.

⁴Due to adhesive nature of the eggs, some were incubated in situ in ambient humidity.