

Assessment of patterns of temperature-dependent sex determination using maximum likelihood model selection¹

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Abstract: Sex determination in some reptiles is independent of egg incubation temperature and is called genotypic sex determination (GSD). In many other reptiles, sexual phenotype is dependent on incubation temperature. This phenomenon is called temperature-dependent sex determination (TSD). TSD is categorized by three patterns, based on the majority sex produced at lower and higher incubation temperatures, named MF for Male-Female, FM for Female-Male, or FMF for Female-Male-Female. When large numbers of eggs are incubated at many different incubation temperatures, the assessment of TSD pattern is unambiguous, but when few eggs or few incubation temperatures are used, the categorization of TSD pattern is less straightforward. We propose a new methodology based on maximum likelihood model selection that evaluates and ranks the performance of four descriptive models of sex determination for discrete datasets. This method has the added benefit of giving standardized definitions of two commonly reported parameters of TSD: the pivotal temperature and the transitional range of temperature. Standardization of analyses will help facilitate cross-species meta-analyses of TSD in reptiles.
Keywords: crocodile, egg, maximum likelihood, model selection, squamate, temperature-dependent sex determination, turtle.

Résumé : La détermination du sexe chez certains reptiles est indépendante de la température d'incubation des oeufs et est alors appelée détermination génotypique du sexe (DGS). Chez plusieurs autres reptiles, le phénotype sexuel dépend de la température d'incubation et ce phénomène est appelé détermination du sexe dépendante de la température (DSDT). La DSDT regroupe trois patrons qui diffèrent selon le sexe le plus couramment produit en fonction de la température d'incubation. Ces trois patrons sont désignés par MF pour Mâle-Femelle, FM pour Femelle-Mâle et FMF pour Femelle-Mâle-Femelle. Lorsque de grandes quantités d'oeufs sont incubées à plusieurs températures différentes, on peut facilement déterminer le patron de DSDT. Par contre, lorsque seuls quelques oeufs sont incubés, ou encore qu'une faible gamme de températures d'incubation est utilisée, il est plus difficile de déterminer le patron de DSDT. Nous proposons d'utiliser une nouvelle méthode qui sélectionne un modèle de vraisemblance maximale permettant d'évaluer et de classer la performance de quatre modèles descriptifs de détermination du sexe. Cette méthode permet notamment de donner des définitions uniformes pour deux paramètres souvent utilisés dans les études sur la DSDT : la température charnière et l'étendue des températures de transition. L'uniformisation des analyses facilitera les comparaisons de DSDT entre espèces chez les reptiles.
Mots-clés : animaux à écailles, choix du modèle, crocodile, détermination du sexe dépendante de la température, maximum de vraisemblance, oeuf, tortue.

Nomenclature: Bonin, Devaux & Dupré, 1996.

Introduction

Sex determination in many reptiles is sensitive to incubation temperature. This phenomenon is called temperature-dependent sex determination or TSD (Raynaud & Pieau, 1985). It occurs in all crocodylians, many chelonians and sphenodonts, and some squamates. Three distinct patterns of TSD are observed: Pattern TSD IA, or MF, is observed in many turtles and is characterized by the production of males at lower incubation temperatures and females at higher temperatures (Ewert, Jackson & Nelson, 1994). For pattern TSD IB, or FM, females are produced at lower incubation temperatures and males at higher temperatures. This pattern is observed in some lizards (Viets *et al.*, 1994), but was originally described in crocodylians. The recent availability of more complete

data for some crocodile species has revealed that many species actually exhibit TSD II, or FMF, where females are produced at low and high temperatures and males at intermediate ones (Lang & Andrews, 1994). Note that some turtle species also exhibit pattern II, or FMF (Ewert, Jackson & Nelson, 1994).

The relationships among these patterns are subject to debate. It has been proposed that FMF is the general pattern for Reptilia and that FM or MF are simply observed because extreme incubation temperatures have not been studied or because sufficiently low or high incubation temperatures do not result in successful incubation (Pieau *et al.*, 1995). New data are of prime importance in validating this hypothesis, but to date no rigorous method has been available to discriminate among GSD, TSD I, and TSD II patterns. There is some ambiguity in the literature concerning the classification of TSD pattern for species and for different populations of the same species. For instance, Ewert, Jackson and Nelson (1994) proposed MF

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and FMF patterns for two populations of the turtle *Kinosternon flavescens*, although no strict criteria were given for this conclusion.

Development of a standardized method for clearly assessing TSD patterns is not simply an esoteric matter. A set method of calculating basic parameters of TSD such as the pivotal temperature, P (the constant incubation temperature that produces a sex ratio of 1:1), and the TRT (the transitional range of temperatures that produce a mixed sex ratio) would facilitate statistical comparisons across studies. For example, a reanalysis of published TSD IA data showed a significant negative relationship between P and TRT for turtle species (Girondot, 1999). Although the ecological meaning of this relationship has yet to be discovered, it does illustrate the potential value of meta-analyses of TSD.

Here we propose a method to stringently distinguish between GSD and TSD and, in the latter case, to distinguish among TSD IA, IB, and II patterns, for discrete datasets. In addition, we provide a standardized method to describe several parameters of the relationship between sex ratio and incubation temperature. We analyze several examples from the literature to show the utility of our method to unambiguously find the TSD pattern that best describes the previously published data.

Methods

INTERPRETING A RESULT OF INCUBATION

The incubation of eggs results generally in male or female embryos for gynodioecious species. However, in some cases the sexual phenotype of embryos is ambiguous. These embryos are generally classified as intersexes. A previous study has shown that juvenile intersex turtles later matured into functional adult males (Pieau *et al.*, 1998). Therefore, we treat "intersexes" as males, for the calculation of overall sex ratios. Another difficulty is determining the sex of embryos that do not survive to the end of development. Late stage dead embryos can sometimes be accurately sexed, but earlier preterm dead embryos, in particular those that died at stages near or before the stages of sexual differentiation, cannot be accurately sexed. For the present analysis, we used data only from sexed embryos or hatchlings, and all were classified as males (including intersexes) or females. Sex ratios are expressed in terms of proportion of males (*i.e.*, 0.0=all females and 1.0=all males).

LIKELIHOOD ESTIMATES

The observed data (*d*) consist of the number of male (m_i) and female (f_i) offspring produced at each incubation temperature (t_i) for a total of u incubation temperatures. We use the observed data (*d*) to estimate the set of parameters $\{p\}$ for some model (*m*) where *m* and $\{p\}$ reflect the hypothesized pattern of sex-determination (GSD, TSD IA, TSD IB, TSD II). These parameter estimates determine the expected sex ratio (sr_i) at each of the studied incubation temperatures (t_i). Let $P(d|m)$ represent the binomial probability of the observed data given the model. Over all u incubation temperatures, the total probability of the experiment is:

$$P(d | m) = \prod_{i=1}^u \left(C_{m_i}^{m_i + f_i} sr_i^{m_i} (1 - sr_i)^{f_i} \right) \quad [1]$$

Let $L(m; d)$ represent the likelihood of the observed data. It is a property of likelihood that $L(m; d) \neq P(d|m)$ where the constant of proportionality is arbitrary (Edwards, 1972). The difference between the probability and likelihood approaches is that whereas the probability approach describes the data for a given and fixed hypothesis, the likelihood approach seeks the hypothesis that best describes the data.

If there is some uncertainty (or variation) associated with a particular mean incubation temperature, all possible temperatures around the particular temperature are checked and the best likelihood is used.

MODELING THE RELATION BETWEEN INCUBATION TEMPERATURE AND SEX RATIO

GSD MODELS

The first model is simply that sex determination is insensitive to incubation temperature and the expected sex ratio is 0.5 ($sr_i=0.5$). This model is called GSD 0.5 (GSD for genotypic sex determination). No parameter is estimated from the data for this model. The second model also assumes that sex determination is insensitive to incubation temperature but the expected sex ratio is different from 0.5. This model is called GSD *x*, with *x* being the mean value of the resultant sex ($sr_i=x$). One parameter is estimated from the data for this model.

TSD I MODELS

For TSD IA, several mathematical equations from the literature can be used to describe the relationship between sex ratio and constant incubation temperatures (note that the equations have been modified here to simplify the description of the parameters' influence). From these equations, we calculated two parameters that have been classically used to describe TSD patterns: the pivotal temperature (P), which is the temperature that produces equal numbers of each sex, and the transitional range of temperatures (TRT), which is the range of constant temperatures that results in both sexes (Mrosovsky & Pieau, 1991). The boundaries of TRT cannot be determined with accuracy based simply on incubation data because their precision depends on the number of different incubation temperatures and the number of eggs per temperature used in the experiment (Girondot, 1999). As an alternative, we follow Girondot (1999) and define TRT_x as the range of temperatures that produce sex ratio between *x* and $1 - x$, with $x=0.05$ being used unless otherwise stated.

The sex ratio versus incubation temperature equation used by Girondot (1999) is based on a logistic equation:

$$sr(t) = \left(\frac{1}{1 + e^{\left(\frac{1}{S}(P-t) \right)}} \right)^{-1} \quad [2]$$

with *t* being the incubation temperature, *P* being the pivotal temperature, and *S* defining the shape of transition from masculinizing to feminizing temperature: *sr* goes from 1 to 0 for *S* being negative or from 0 to 1 for *S* being positive, with a transition more or less sharp

according to the S parameter and with an sr value equal to 0.5 for $t=P$. The TSD IA pattern is thus modeled for $S < 0$ and the TSD IB pattern is modeled for $S > 0$. Note that the shape is symmetrical around the P value, but there is no *a priori* justification for this. Therefore, we also used a modified form of the logistic equation to introduce the possibility of asymmetry around P :

$$sr(t) = \left[1 + \left(2^{e^K} - 1 \right) e^{\left(\frac{1}{S} (P-t) \right)} \right]^{-1/e^K} \quad [3]$$

with K being a parameter defining the asymmetrical shape of the equation: if K is equal to 0, the equation reduces to logistic equation [2]; otherwise, the equation shape is asymmetrical, with K , negative or positive, describing the shape. This equation hereafter will be referred to as the A-logistic equation (A referring to asymmetrical). The sex ratio sr value is equal to 0.5 for $t=P$. When K is large ($K > 3$) and $\text{SIGN}(P - t) = \text{SIGN}(S)$, an approximation of equation [3] must be used to avoid a computing overflow:

$$sr(t) = 0.5 e^{S e^K \frac{t-P}{S}} \quad [4]$$

The transitional range of temperatures that produces a sex ratio between x and $1 - x$, TRT_x is

$$\text{TRT}_x = \left| S \cdot \text{Ln} \left(\frac{x^{-e^K} - 1}{(1-x)^{-e^K} - 1} \right) \right| \quad [5]$$

For K large ($K > 3$), the following approximation must be used to avoid a computing overflow:

$$\text{TRT}_x = \left| S e^K \text{Ln} \frac{1-x}{x} \right| \quad [6]$$

When a logistic equation [2] is used, the inflexion point (P_I , point at which the maximum rate of change of sex ratio versus temperature is observed) is simply P . When the A-logistic equation [3] is used, the equation for the inflexion point (P_I) is

$$P_I = P + S \text{Ln} \left(\frac{2^{e^K} - 1}{e^K} \right) \text{ or} \quad [7]$$

$$P_I = P + S e^K \text{Ln} 2 - KS \text{ when } K > 3$$

Comparisons between P and P_I values help to interpret the relative effect of changing the incubation temperature. Three parameters (P , S and K) are estimated from the data for this general model (two if K is set to 0 to produce a symmetric shape). Note that several other equations can also be used to describe a sigmoidal curve (*e.g.*, the Hill or Weibull equations, review in Haefner, 1996), but they will not be considered here because they performed less well than equations [2] or [3] (not shown). However, they are included in the computer package that is freely available (see below).

TSD II MODELS

A more complicated function describing the relationship between incubation temperature and sex ratio is

needed for species with TSD II (FMF pattern). This new function should produce a sex ratio between 0 and 1 describing the sex ratio versus incubation data. For this reason, we did not consider the third-order polynomial fitting used by Ewert, Jackson and Nelson (1994), because it cannot take into account sex ratios from 0 to 1. Berec, Boukal and Berec (2001) used equation [8] to fit the sex ratio versus incubation data for a TSD II pattern turtle species:

$$sr(t) = e^{\left(-\frac{1}{k_2} (t-k_1)^{2k_3} \right)} \quad [8]$$

In this equation, the parameter k_2 is the centre of the male portion of the FMF pattern, and the k_1 parameter governs its width. The k_3 parameter is an integer value that influences the shape and pattern of the transition for both the FM and MF portions of the curve. However, a limitation of this function is that it is strictly symmetric around the k_2 value. Three parameters are estimated from the data for this model, and the equation will be called double-Berec equation hereafter.

Alternatively, the equation [3] previously used to describe TSD I can be altered to describe TSD II by simply multiplying the function $sr(t)$ with parameters $\{p\}$ by its complement $[1 - sr(t)]$ using another set of parameters $\{p'\}$. We define this equation as the double-A-logistic function. Six parameters are estimated from the data for this model, unless a symmetrical shape is assumed and K is set to 0, in which case only four parameters are estimated (double-logistic function). The estimates of the variances of the parameters are obtained using the second derivative of the likelihood at the solution points. Parameters estimated using maximum likelihood are normally distributed (Edwards, 1972).

MODEL SELECTION

The model selection is based on both the likelihood ratio test statistic (LRT) and the minimum theoretical information criteria (AIC) (Akaike, 1974).

The LRT tests the goodness of fit of two models using

$$\delta = -2 \ln \left(\frac{ML_0}{ML_1} \right) \quad [9]$$

where ML_0 is the maximum likelihood under the null hypothesis (simple model) and ML_1 is the maximum likelihood under the alternative hypothesis (parameter-rich model). When the models are nested, the δ statistic is asymptotically distributed as χ^2 with q degrees of freedom, where q is the difference in the number of free parameters between the two models (Kendall & Stuart, 1979).

The AIC is a measure that rewards models for good fit, but imposes a penalty for unnecessary parameters. If L is the maximum likelihood for a specific model using n independently adjusted parameters within the model, then $\text{AIC} = -2 \ln L + 2n$. Smaller values of AIC indicate models that more accurately describe the data. When the models are not nested, only AIC criteria can be used.

TYPE I AND II ERRORS

The quality of the fit is tested using the probability to observe deviance ($-2 \ln L$) for H_0 hypothesis. The

assumption that the deviance is distributed as a χ^2 with $u - p$ degrees of freedom (Burnham & Anderson, 1998) can produce strongly biased results (data not shown). Consequently, the H_0 distribution of the deviance was generated by Monte-Carlo simulations as follows. We built new datasets with the same number of incubation temperatures and number of eggs as the original dataset. For each of these u incubation temperatures, the sex of the n_i eggs was inferred randomly using equation [3] with parameters S , P and K that maximize likelihood. For each dataset, the value of S , P and K parameters was randomly chosen in Gaussian distribution taking into account its standard error. Then the values of S , P and K parameters were fitted again and the deviance calculated. The type I error is the proportion of times the observed deviation is higher than the deviance calculated under H_0 hypothesis.

To test the impact of sample size on model selection, in particular between symmetric and asymmetrical models for A-logistic function (*i.e.*, $K=0$ versus $K \neq 0$), we have developed a measure of the power of the test. To evaluate the power of the test for an experiment of u incubation temperatures with n_i eggs incubated at each temperature, $a - 1$ datasets are built with the same number of incubation temperatures and number of eggs as the original dataset. For each of these u incubation temperatures, sex of the n_i eggs is inferred randomly using equation [4] with parameters S , P and K that maximize likelihood. For each dataset, the value of S , P and K parameters was randomly chosen from a Gaussian distribution taking into account its standard error. Each of these datasets is fitted again independently, using the AIC to test whether setting $K \neq 0$ provides a better fit. The power of the test is the proportion of times the model with $K \neq 0$ is selected. A low value indicates that the A-logistic model ($K \neq 0$) is unlikely to be selected compared to the logistic model ($K=0$) even if the A-logistic model is the true one, sim-

ply because of a lack of data. This test should be used whenever the initial comparison of AIC leads to the conclusion that logistic function is the better choice. The power of the test can detect whether there are sufficient data to fairly choose between logistic and A-logistic functions or the logistic function is imposed simply because of the lack of available incubation temperatures.

Equation manipulations were performed using Mathematica 4.0 (Wolfram Research, Champaign, Illinois, U.S.A.) on a Macintosh PowerPC. Computer software for MacOS and Windows to estimate P , S and K values, as well as to perform LRT, type I, and power tests is freely available at the following URL:

<http://www.ese.u-psud.fr/epc/conservation/TSD/index.html>

INCUBATION DATA

The model was tested using data from three species of turtles in which more than 200 eggs have been incubated at various temperatures. This included *Emys orbicularis* (TSD IA), *Trachemys scripta* (TSD IA), and *Chelydra serpentina* (TSD II) turtles. The model was then used to analyze data from the turtle species *Apalone spinifera* (GSD or TSD II), *Kinosternon flavescens* (TSD IA or TSD II), and the lizard *Ctenophorus decresii* (TSD II or TSD IB). Sources of incubation data are summarized in Table I.

Results

TESTS OF THE MODEL

The European pond turtle, *Emys orbicularis*, is the TSD IA species for which the most data on incubation versus sex ratio are available. Sexual phenotype is known for 1,259 embryos incubated at 16 different temperatures, six of them producing both males and females (review in Girondot, 1999) (Figure 1a). The likelihood of the results

TABLE I. Summary of the incubation data used in the analyses. Refs 1-10: Pieau (1971; 1972; 1973; 1975a,b; 1976; 1978), Zaborski, Dorizzi and Pieau (1982; 1988), Girondot, Servan and Pieau (1994); 11-13: Bull, Vogt and McCoy (1982), Ewert and Nelson (1991), Ewert, Jackson and Nelson (1994); 14-24: Yntema (1976; 1981), Dimond (1983), Packard, Packard and Boardman (1984), Gutzke and Packard (1986), Gutzke and Chymiy (1988), Crews, Wibbels and Gutzke (1989), Brooks *et al.* (1991), Ewert and Nelson (1991), Janzen (1992), O'Steen (1998); 25-27: Bull and Vogt (1979), Vogt and Bull (1982), Bull, Gutzke and Crews (1988); 28: Harlow (1999); 29: Ewert and Nelson (1991).

Species and references	Number of incubation temperatures	Total number of eggs	Possible pattern of sex determination	Selected pattern of sex determination	Selected equation	Parameters*
<i>Emys orbicularis</i> Refs 1-10	16	1,259	TSD IA	TSD IA	Logistic	$P_{MF} = 28.62$; $S_{MF} = -0.1385$
<i>Trachemys scripta</i> Refs 11-13	9	255	TSD IA	TSD IA	A-logistic	$P_{MF} = 28.83$; $S_{MF} = -0.0598$; $K_{MF} = 2.654$
<i>Chelydra serpentina</i> Refs 14-24	18	2,199	TSD II	TSD II	Logistic	$S_{FM} = 0.1932$; $P_{FM} = 21.72$; $S_{MF} = -0.4776$; $P_{MF} = 27.53$
<i>Apalone spinifera</i> Refs 25-27	6	378	TSD II or GSD	GSD	n.a.	n.a.
<i>Ctenophorus decresii</i> Ref 28	6	38	TSD II or TSD IB	TSD IB	Logistic	$P_{FM} = 30.57$; $S_{FM} = 1.8118$
<i>Kinosternon flavescens</i> Ref 29	5	166	TSD IA or TSD II	TSD II	Logistic	See text

*P, S, and K represent the pivotal temperature, the shape of transition from masculinizing to feminizing temperature, and an asymmetrical shape parameter, respectively.

when fitted with the logistic function ($K=0$) was $-\ln L=44.71$ (AIC=93.43), whereas the likelihood and the AIC for the A-logistic function ($K>0$) were significantly better: $-\ln L=41.03$ (AIC=88.07). However, in this case the deviance was very high ($-2 \ln L=82.06$), a value that was never reached in 1,000 replications using the H_0 distribution ($p<0.001$). Half of the deviance was due to a single incubation temperature (28.75°C), at which nine clutches (54 eggs) were incubated. It is unlikely that the discrepancy observed at this temperature resulted from genetic difference among females that, by chance, produced biased sex ratios in the same direction for all nine clutches. It is much more likely that the high deviance was due to uncertainty in the specified mean temperature of one of the original incubators used. If an uncertainty (variation) of 0.15°C is assumed to be correct for the specified mean temperature (*C. Pieau, pers. comm.*), then the deviance falls to 29.7 and the fit cannot be rejected ($p=0.06$ for 1,000 replications). When such an uncertainty around a temperature is used, however, the AIC comparison between logistic and A-logistic functions leads to the choice of logistic function (AIC=33.70 versus 35.69). However, the power of the test for this species was very low, taking into account the uncertainty: <0.001 . This was due to the small $TRT_{5\%}$ (0.83°C) as compared to the uncertainty of incubation temperatures (0.15°C around specified value, so 0.3°C in total).

The common slider turtle, *Trachemys scripta*, also has TSD IA. The best-fit model for this species was also the A-logistic equation, with $K=2.65$ (AIC=27.91 versus 29.78 for the logistic) (Figure 1b). When the uncertainty around a mean incubation temperature was assumed to be 0.15°C for each specified temperature, the fit was not rejected ($p=0.06$). The $TRT_{5\%}$ was much higher for this species (2.52°C) than the value calculated for *Emys orbicularis* (0.83°C), and therefore an uncertainty of 0.15°C for a mean incubation temperature had a reduced impact on the ability of the model to detect a better fit for the A-logistic function. P_I was lower than P , indicating a saturation effect of temperature at higher incubation temperatures.

Other species with TSD IA have a very low power of detection of $K \neq 0$ due to the reduced number of incubation temperatures or eggs used. Indeed the likelihood of the logistic model often was identical to that of the A-logistic model.

The most studied species with TSD II is the snapping turtle *Chelydra serpentina* (Figure 1c). Sexual phenotype is known for 2,199 embryos incubated at 18 different incubation temperatures, nine of them producing both sexes. When the data for *C. serpentina* were analyzed with zero uncertainty assumed for each mean incubation temperature, the resultant models ranked by order of merit were the double-A-logistic equation, $-\ln L=42.90$ (AIC=97.81); the double-logistic, $-\ln L=48.64$ (AIC=109.28); and finally the double-Berec equation, $-\ln L=66.3$ (AIC=138.60). However, the overall fit was rejected ($p<0.001$). To avoid having the fit rejected by the Type I Error test, an uncertainty value of 0.45°C was necessary ($p=0.063$). This high value probably reflected more the differences among various experimental procedures than the true uncertainty of the mean incubation temperatures of the incubators. Taking into account this uncertainty of the mean incubation temperatures, the double-logistic model was selected (AIC=60.76 versus 64.64 for the double A-logistic). If only one of the transitions (FM or MF) was modeled using A-logistic function, the best-fit model remained the double-logistic equation (AIC=64.69 for the A-logistic FM transition and 64.68 for the A-logistic MF transition). The power to select the A-logistic function for these data was very low ($p<0.001$).

APPLICATIONS OF THE MODELS

One of the most-studied turtle species with GSD is the spiny softshell turtle, *Apalone spinifera* (= *Trionyx spiniferus*). When plotted together, all available data suggest a possible FMF TSD II pattern (Figure 2a). We calculated the AIC for GSD 0.5, GSD x , and TSD II with A-logistic and logistic models of sex determination. Models ranked by order of AIC merit were GSD 0.50 (AIC=28.28); GSD 0.49 (AIC=30.10); TSD II logistic (AIC=33.88); and TSD II A-logistic (AIC=37.88).

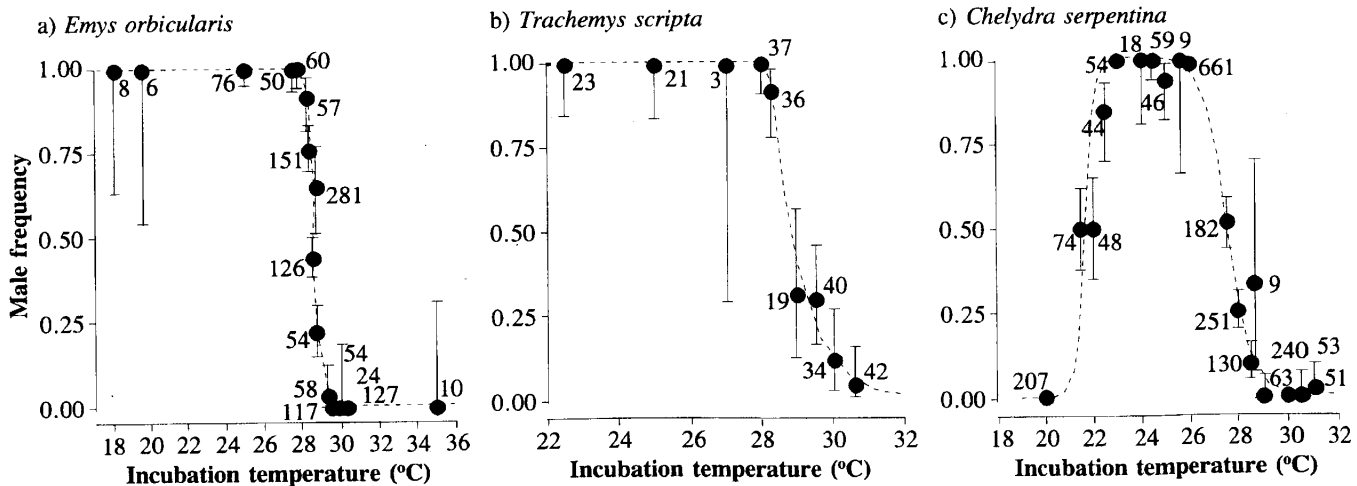


FIGURE 1. Relationship between sex ratio and constant incubation temperature in the turtles *Emys orbicularis* and *Trachemys scripta* with TSD IA (a and b) and *Chelydra serpentina* with TSD II (c). Values next to points indicate the number of sexed embryos, and error bars are ± 2 SE. The curve is the best-fit equation describing sex ratio versus constant incubation temperature.

Therefore, these data are best described by a classical GSD mechanism of sex determination, and there is no need to invoke a possible effect of temperature for this species.

Recently, Harlow (1999) reported TSD II in the tawny dragon lizard, *Ctenophorus decresii*. We reanalyzed the reported data (Figure 2b) using the different models. The rank order of best-fit models (based on AIC merit) is as follows: TSD IB logistic (AIC=16.77); TSD IB A-logistic (AIC=18.77); TSD II logistic (AIC=20.77); GSD 0.151 (AIC=22.33); TSD II A-logistic (AIC=24.77); and GSD 0.5 (AIC=48.82). TSD IB logistic model is not rejected even when no uncertainty is set up ($p=0.386$ for 1,000 replications). Thus, the published data are actually more consistent with the TSD IB pattern.

The yellow mud turtle, *Kinosternon flavescens*, has been described as having either TSD IA or TSD II patterns (Figure 2c), depending on whether the population under study was from Nebraska, U.S.A. or Sonora, Mexico (Ewert, Jackson & Nelson, 1994). When we analyzed the data for each population separately, the best-fit models for particular TSD patterns ranked by AIC merit for the Sonora, Mexico population were TSD II logistic (AIC=23.38); TSD II A-logistic (AIC=25.14); TSD IA (MF) logistic (AIC=55.88); and TSD IA A-logistic (AIC=57.88). For the Nebraska population, the merit order was TSD II logistic (AIC=22.13); TSD IA (MF) logistic (AIC=23.30); TSD IA (MF) A-logistic (AIC=25.30); and TSD II A-logistic (AIC=26.11). Selected models were not rejected even when no uncertainty was set up ($p=0.05$ and $p=0.12$ for 1,000 replications for Sonora and Nebraska population, respectively). The $-\ln$ likelihood of the combined data analyzed in a single TSD II model was 31.37, whereas they were 7.69 for Sonora and 7.06 for Nebraska when analyzed separately. An LRT test revealed a significant effect ($\delta=33.24$; 4 df, $p < 10^{-6}$) for the origin of the animals. Therefore, data from the two populations of *K. flavescens* were significantly better described by TSD II (FMF) pattern than by TSD IA pattern (MF), but the response to temperature was significantly different between populations.

Discussion

The difference between logistic and A-logistic equations is based on whether or not the curve describing TSD is symmetric around the pivotal temperature (P). Previous statistical descriptions have assumed that the curve is symmetric (Girondot, 1999; Berec, Boukal & Berec, 2001), although there is no *a priori* reason for this. We have shown here that for *T. scripta*, a species for which a great amount of incubation data are available, an asymmetric distribution of sex ratio around the pivotal temperature fit significantly better than a symmetric distribution. In this case, the point of inflexion was located below the pivotal temperature, suggesting that a small change at lower incubation temperatures produces a greater difference in sex ratio than a similar change at higher temperatures. Unfortunately, for no other species are there adequate data to allow a sufficient power for this test. This observation is at odds with reports that embryonic development is more rapid at higher temperatures and therefore higher temperatures count more in the sex determining process than lower temperatures (Georges, 1989). This phenomenon may be related to a saturation effect on the action of temperature, for either the growth of embryos, the growth of the gonads, or the aromatase activity. Aromatase is the enzyme whose product is estradiol, the major component in the sex determination cascade in reptiles (Pieau *et al.*, 1994).

Despite the standardization our method offers in terms of statistical description and analyses, there remains much variation in the actual methods used to incubate reptile eggs. Older studies suffer from imprecise thermal control, and often there were daily fluctuations around the mean incubation temperatures (Mrosovsky, 1988). Although our method can take into account uncertainty (variation) in incubation temperatures, such values are often not included in the original publications. In these cases, the only option is to search for the uncertainty values that do not result in rejection of the model. Note that these data cannot be used to test the validity of the model because of

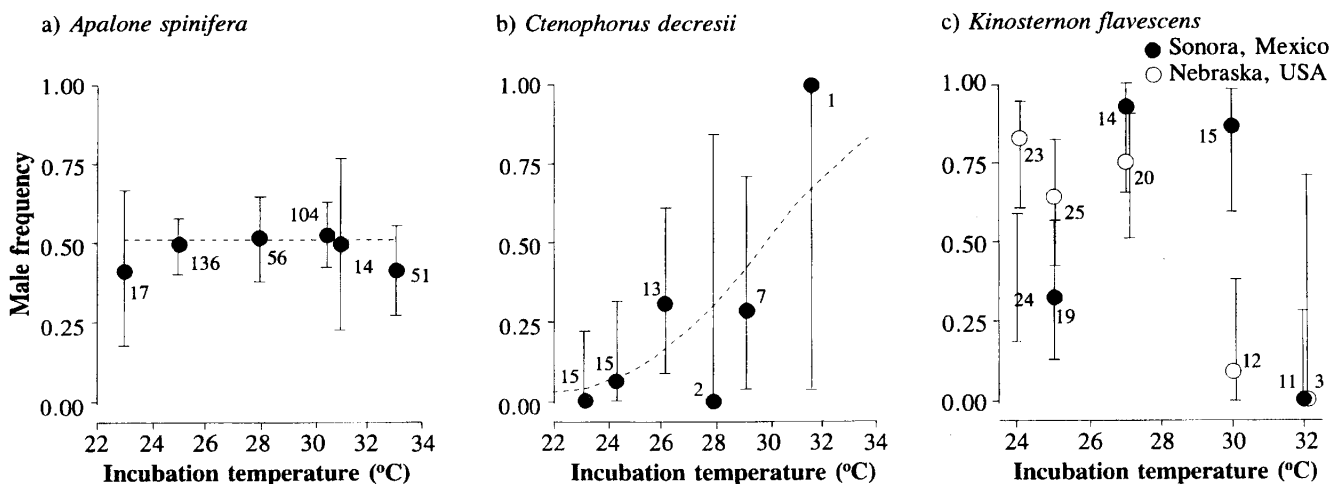


FIGURE 2. Relationship between sex ratio and constant incubation temperature in the turtle *Apalone spinifera* (= *Trionyx spiniferus*) with GSD or TSD II (a), the squamate *Ctenophorus decresii* with TSD IB or TSD II (b), and two populations of the turtle *Kinosternon flavescens* with TSD II or TSD IA (c). Values next to points indicate the number of sexed embryos, and error bars are ± 2 SE. The curve is the best-fit equation describing sex ratio versus constant incubation temperature (not shown in C, see text).

the circular logic. Additionally, fluctuations around the mean incubation temperature tend to have a feminizing effect on turtle eggs (Georges, Limpus & Stoutjesdijk, 1994). Recent technological advances in both measuring devices and incubators have helped increase the thermal precision and accuracy of incubation procedures. Another potential technical problem is the evaporative cooling of eggs during incubation, which means that the actual egg temperature is less than air temperature in the incubator (Hanson, Wibbels & Martin, 1998). Although some authors have measured and reported this effect in their studies (Marcovaldi, Godfrey & Mrosovsky, 1997; Godfrey *et al.*, 1999), most do not. A further complication is that hydric conditions during incubation are not always measured or reported (Steyermark, 1999). Although changes in water potential are less important than changes in temperature for overall sex ratios (Godfrey & Mrosovsky, 2001), humidity should be controlled for in a consistent manner in incubation studies. Thus, in tandem with our statistical method outlined here we recommend that more standardized methods of incubation be undertaken for reptile egg studies in the laboratory.

A different limitation in experiments on TSD is the number of eggs and/or incubation temperatures tested. Sample size is often constrained by a limited availability of eggs, either for biological or conservation reasons. Small sample size may obscure the actual TSD pattern of a species. For instance, in the case of *C. decresii*, the small sample size led Harlow (1999) to assume that this species had TSD II (FMF) pattern. Interestingly, this conclusion was based largely on circumstantial data available from another squamate species and did not take into account the possible variation in the TSD patterns among phylogenetic groups. Our analysis in this paper supports a TSD IB (FM) pattern of TSD in *C. decresii*. We cannot definitively rule out the possibility that *C. decresii* has not the TSD II (FMF) pattern, because future incubation studies may find females produced at higher incubation temperatures (compare Figure 1a,c). Nevertheless, with the data currently available, the conservative strategy is to describe *C. decresii* as a TSD IB pattern species.

Previous attempts to make comparisons across species or even among different populations of the same species have suffered from the lack of a standard description of TSD (Mrosovsky, 1994), and only recently were terms such as "pivotal temperature" and "thermosensitive period" clearly defined (Mrosovsky & Pieau, 1991). The method described in this paper is an extension of previous work developed for TSD in turtles (Girondot, 1999) to all reptile species. In addition to rigorously distinguishing the best-fit TSD pattern for a particular dataset, this method offers clear and precise statistical definitions of pivotal temperature and transitional range of temperatures. In the case of turtles, these rigorous definitions have facilitated cross-population comparisons (Chevalier, Godfrey & Girondot, 1999; Godfrey *et al.*, 1999). In the future, the increased availability of data from different species and taxa, combined with this rigorous method, will facilitate greater cross study comparisons. This may help us understand the evolutionary significance of TSD, which remains a mystery (Shine, 1999) despite having been first described in reptiles over 30 years ago (Charnier, 1966).

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