

Statistical description of temperature-dependent sex determination using maximum likelihood

Marc Girondot*

*URA Evolution et Adaptations des Systèmes Ostéomusculaires, Université Paris 7 and CNRS
(case 7077), 2 place Jussieu, 75251 Paris Cedex 05, France*

ABSTRACT

Temperature-dependent sex determination has been described in turtles, crocodilians, lizards and sphenodonts. In such amniotes, sexual phenotype is determined based on the incubation temperature during embryonic development. The description of temperature-dependent sex determination has often been purely qualitative, without any statistical inference. A new statistical method describing temperature-dependent sex determination with two parameters, the pivotal temperature and the transitional range of temperature producing both sexes, has been developed. The estimates and their standard deviation are produced using maximum likelihood. This method is applied using data from the literature.

Keywords: environmental sex determination, reptile, sex determination, temperature-dependent sex determination, turtle.

INTRODUCTION

In many reptiles, as well as in some actinopterygians and amphibians, sexual phenotype can be determined at least in part by the incubation temperature of eggs (Ewert *et al.*, 1994; Pieau, 1996). This phenomenon has been termed ‘temperature-dependent sex determination’ (TSD). Its occurrence in reptiles (many turtles, all crocodilians, both sphenodonts and some lizards) is puzzling from an evolutionary point of view, because its adaptive significance remains largely unknown (Burke, 1993). Several aspects of TSD require further study, including the primary sex ratio under natural conditions (Bull and Charnov, 1989) and the quantitative evaluation of TSD within and between populations (Ewert *et al.*, 1994). One way of addressing this last point is to study the results of incubation at several constant incubation temperatures (Mrosovsky and Pieau, 1991).

Originally, TSD was described simply based on the pivotal temperature (also called the critical or threshold temperature) – the incubation temperature producing an equal number of both sexes (Pieau, 1976; Bull, 1980; Mrosovsky and Yntema, 1980). There is controversy, however, as to how this temperature should be evaluated. Some authors propose drawing a straight line between the boundary temperatures above and below 50% (Mrosovsky, 1988; Mrosovsky and Pieau, 1991), whereas others adopt total incubation temperatures (Limpus

* e-mail: mgi@ccr.jussieu.fr

et al., 1985; Godfrey, 1997). From a statistical point of view, the first method is inappropriate because it takes account of little information. Moreover, the percentage of males may cross the 50% line several times owing to fluctuations in sample size, making the straight line method unsuitable (for example, the frequency of *Chelonia mydas* males from Surinam is 0.66, 0.41, 0.53 and 0.47 for incubation temperatures of 28.5, 29.25, 29.4 and 30°C respectively; see Mrosovsky *et al.*, 1984; Godfrey, 1997). The method of Limpus *et al.* (1985) provides an estimate of the pivotal temperature (or $SDT_{50\%}$) and its standard deviation. However, this estimate is performed using a crude, manually performed graphical method which could be subjective (Litchfield and Wilcoxon, 1949). Godfrey (1997) performed a logistic regression with the percentage of males at different temperatures. This method is not suitable when incubation temperatures produce 0 and 100% males in the same experiment because the logarithm of 0 is not defined. Moreover, the logistic transformation is in fact incorrect because the function describing sex ratio based on incubation temperature should exhibit two asymptotes.

More recently, a new parameter has been proposed to describe TSD: the transitional range of temperature (TRT) (Mrosovsky and Pieau, 1991). The TRT is the range of constant temperatures for which the incubation produces both sexes, and is presumed to describe more adequately the consequence of TSD in natural conditions: where the TRT is narrower, more nests should be unisexual (Mrosovsky and Pieau, 1991). However, TRT is fully described by three values (the low and high incubation temperatures, or TRT_L and TRT_H respectively, and the range of temperatures, $TRT_H - TRT_L$) which cannot be statistically independent according to the method used for their estimates. Moreover, the boundaries of TRT cannot be determined with accuracy because their precision will simply depend on the number of different incubation temperatures and the number of eggs per temperature used in the experiment (Mrosovsky and Pieau, 1991).

A new procedure is introduced to calculate simultaneously the pivotal temperature, the transitional range of temperature, and their variances. The estimates are made using maximum likelihood, although other statistical parameter-fitting packages (SysStat or SAS) could be used. Some examples are taken from the literature to illustrate its use.

MATERIALS AND METHODS

A mathematical equation will be used to describe the sex ratio, sr (expressed as the frequency of males among hatchlings), as a function of constant incubation temperature:

$$sr = \frac{1}{1 + e^{(1/S \cdot (P - t))}} \quad (1)$$

where t is the incubation temperature, P is the pivotal temperature, and S defines the shape of transition from masculinizing to feminizing temperature.

This equation has all the prerequisites for describing TSD: sr goes from 1 to 0 when S is negative and from 0 to 1 when S is positive, with a more or less sharp transition according to the S parameter and with an sr value equal to 0.5 for $t = P$. For species with two pivotal temperatures, this function should be used twice.

A set of u values $\{t_i; sr_i; n_i\}$ – where n_i is the number of eggs incubated at temperature t_i , among which a fraction sr_i develops as males – describes an experiment. The numbers of males and females incubated at temperature t_i are denoted m_i and f_i respectively. The

probability of obtaining m_i males and f_i females for incubation at temperature t_i can be found by applying a binominal distribution to equation (1):

$$p(sr = sr_i) = C_{n_i}^{m_i} \left(\frac{1}{1 + e^{(1/S) \cdot (P - t_i)}} \right)^{m_i} \left(\frac{e^{(1/S) \cdot (P - t_i)}}{1 + e^{(1/S) \cdot (P - t_i)}} \right)^{f_i} \quad (2)$$

The likelihood of the experiment is the combination of the u probabilities from equation (2):

$$V(sr_1 \dots sr_u) = \prod_{i=1}^u \left(C_{n_i}^{m_i} \left(\frac{1}{1 + e^{(1/S) \cdot (P - t_i)}} \right)^{m_i} \left(\frac{e^{(1/S) \cdot (P - t_i)}}{1 + e^{(1/S) \cdot (P - t_i)}} \right)^{f_i} \right) \quad (3)$$

and

$$\ln(V(sr_1 \dots sr_u)) = \sum_{i=1}^u \left(\ln(C_{n_i}^{m_i}) + \frac{f_i}{S} (P - t_i) - n_i \cdot \ln(1 + e^{(1/S) \cdot (P - t_i)}) \right) \quad (4)$$

The P and S values maximizing the likelihood are found by resolving:

$$\frac{\partial \ln(V(sr_1 \dots sr_u))}{\partial S} = \frac{1}{S^2} \sum_{i=1}^u \left(\frac{(P - t_i)(-f_i + m_i \cdot e^{(1/S) \cdot (P - t_i)})}{1 + e^{(1/S) \cdot (P - t_i)}} \right) = 0 \quad (5)$$

$$\frac{\partial \ln(V(sr_1 \dots sr_u))}{\partial P} = -\frac{1}{S} \sum_{i=1}^u \left(f_i - n_i \cdot \frac{e^{(1/S) \cdot (P - t_i)}}{1 + e^{(1/S) \cdot (P - t_i)}} \right) = 0 \quad (6)$$

The resolution of these equations is done using a procedure derived from a simplex method based on $\{P = 25, S = -2\}$, $\{P = 25.02, S = -2\}$ and $\{P = 25.01, S = -1.99\}$ (Nelder and Mead, 1965), but a screening of the complete domain of variation of S and P is possible as the computing time required is not important. The estimates of the variances of the parameters are obtained using the second derivative of the likelihood at the solution points $\hat{P}$ and $\hat{S}$. Parameters estimated using maximum likelihood are normally distributed.

$$S_P^2 = -1 / \left[\frac{\partial^2 \ln(V(sr_1 \dots sr_u))}{\partial^2 P} \right]_{P=\hat{P}, S=\hat{S}} \quad (7)$$

$$S_S^2 = -1 / \left[\frac{\partial^2 \ln(V(sr_1 \dots sr_u))}{\partial^2 S} \right]_{P=\hat{P}, S=\hat{S}} \quad (8)$$

The relationship between the transitional range of temperature and S is dependent on the sensitivity of detection of the less common sex in the experiments (Mrosovsky and Pieau, 1991). When n_i eggs are incubated at temperature t_i , the expected mean number of embryos of each sex is $n_i \cdot sr_i$ and $n_i \cdot (1 - sr_i)$. According to sr_i and n_i , these values can be lower than 1, and this temperature will be considered as producing unisexual nests. Therefore, in any experiment, the observed TRT is obtained from the difference between the two boundary temperatures giving sex ratios of l and $1 - l$, respectively:

$$TRT_l = |S \cdot K_l| \quad (9)$$

where K_l is a constant equal to $[2 \cdot \ln(l/1-l)]$. Therefore, S is a measure of the TRT, independent of the incubation temperature and of the number of eggs in the experiment.

To detect a bias in the estimates of P and S , 1000 sets of data were produced using P and S values chosen at random from the intervals [26, 34] and $[-0.8, 0.8]$ respectively. For each incubation temperature from 25 to 35°C (in steps of 0.5°C), the sex of 10 embryos was assigned using the sex-ratio probability from equation (1). The estimated values of P and S were determined using the procedure previously described.

Sets of incubation data previously published were used to re-evaluate the estimates of the pivotal temperature of several species and the transitional range of temperature. When available, comparisons between the previous estimates were also performed. The computations were performed at the 'Centre de Calcul Recherche' of University Paris 6 and 7 using FORTRAN 90 and Maple Vr4.

RESULTS AND DISCUSSION

Determination of the pivotal temperature and the transitional range of temperature was assessed using the artificial incubation data for the species with the most published data from the same geographic origin, *Emys orbicularis*. The curve shown in Fig. 1 is generally consistent with the observed number of males and females for each incubation temperature.

The relationship between actual and estimated values of the pivotal temperature (P) and the transitional range of temperature (using S) for the 1000 sets of incubation data is shown in Fig. 2. Both parameters were accurately estimated ($r^2 = 0.963$ for S and $r^2 = 0.996$ for P).

The estimates of P and S and their standard deviations for different species are presented in Table 1, together with the previously proposed pivotal temperatures. Previous estimates were within the range of $P \pm 2$ standard deviations. The only standard deviation of pivotal temperature previously calculated was for *Caretta caretta* from Australia. The present standard deviation estimates were slightly lower, making discrimination between populations more likely. The *Dermochelys* and *Gopherus* data require some explanation owing to the null standard deviation and the small sample size. In these cases, only one incubation temperature produced both sexes. With only this information, an infinity of curves can be obtained, all of them fitting the data perfectly and resulting in an artefactual null standard deviation. Whereas for *Gopherus* the problem is a lack of incubation temperatures in the

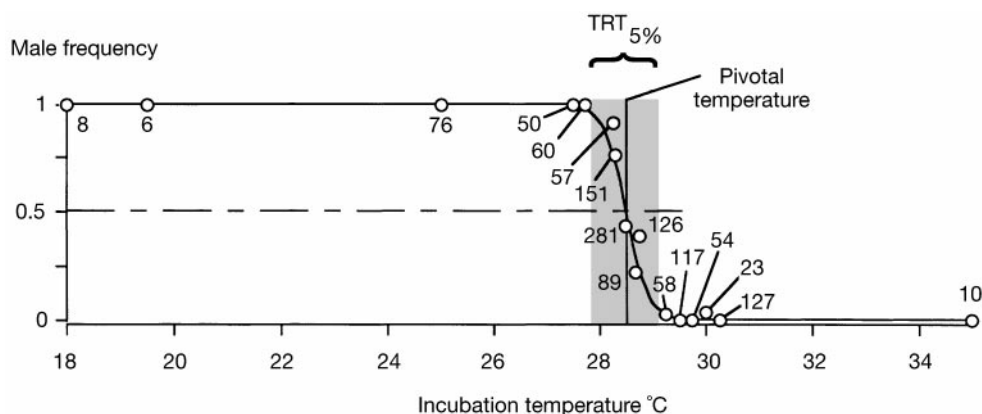


Fig. 1. Fit of the male percentage of *Emys orbicularis* according to the incubation temperature. The number of sexed embryos is shown for each incubation temperature. Data from Pieau (1971, 1972, 1973, 1975a,b, 1976, 1978), Zaborski *et al.* (1982, 1988) and Girondot *et al.* (1994).

Table 1. Estimates of pivotal temperature and transitional range of temperature for five turtle species using maximum likelihood^a

Species	Geographic location	Pivotal temp. (<i>P</i>) ± s.d. (°C)	Previously published <i>P</i> ± s.d.	<i>S</i> ± s.d.	TRT _{5%} ± s.d. (°C)	Number of incubation temps and (eggs)
<i>Emys orbicularis</i>	Brenne, France	28.51 ± 0.02	28.5	−0.196 ± 0.0008	1.15 ± 0.005	16 (1293)
<i>Gopherus polyphemus</i>		29.10 ± 0.10		−0.09 ± 0.00	0.53 ± 0.00	3 (13)
<i>Dermochelys coriacea</i>	French Guiana	29.52 ± 0.01	29.5	−0.02 ± 0.00	0.12 ± 0.00	13 (282)
<i>Chelonia mydas</i>	Surinam	29.34 ± 0.17	29.4 or 29.1	−1.01 ± 0.24	5.93 ± 1.41	9 (189)
<i>Caretta caretta</i>	North Carolina	29.15 ± 0.11	29 or 29.2	−0.55 ± 0.06	3.23 ± 0.35	8 (155)
<i>C. caretta</i>	Georgia	28.19 ± 0.17	<28.5	−0.79 ± 0.12	4.64 ± 0.71	4 (127)
<i>C. caretta</i>	Florida	29.09 ± 0.11	29.2	−0.54 ± 0.11	3.17 ± 0.65	4 (144)
<i>C. caretta</i>	Australia	27.85 ± 0.22	27.7 ± 0.3	−1.00 ± 0.13	5.88 ± 0.76	10 (140)
(3 nests)	<i>Mon Repos</i>	29.10 ± 0.58		−1.45 ± 0.47	8.53 ± 2.76	4 (34)
		27.05 ± 0.39		−0.96 ± 0.27	5.64 ± 1.58	6 (47)
		27.84 ± 0.24		−0.51 ± 0.07	3.00 ± 0.41	7 (59)
<i>C. caretta</i>	Australia	28.87 ± 0.22	28.7 ± 0.3	−0.78 ± 0.05	4.58 ± 0.29	10 (115)
(3 nests)	<i>Heron Island</i>	28.71 ± 0.30		−0.60 ± 0.03	3.52 ± 0.18	6 (53)
		29.21 ± 0.35		−0.82 ± 0.16	4.82 ± 0.94	5 (34)
		28.12 ± 0.64		−1.00 ± 0.23	5.88 ± 1.35	3 (28)

^a Data are from: (*E. o.*) Pieau (1971, 1972, 1973, 1975a,b, 1976, 1978), Zaborski *et al.* (1982, 1988), Girondot *et al.* (1994); (*G. p.*) Burke *et al.* (1996); (*D. c.*) Rimblot *et al.* (1985), Rimblot-Baly *et al.* (1986); (*C. m.*) Mrosovsky *et al.* (1984), Godfrey (1997); (*C. c.*) Limpus *et al.* (1985), Mrosovsky (1988). Values for each individual nest are shown in italics for the Australian data.

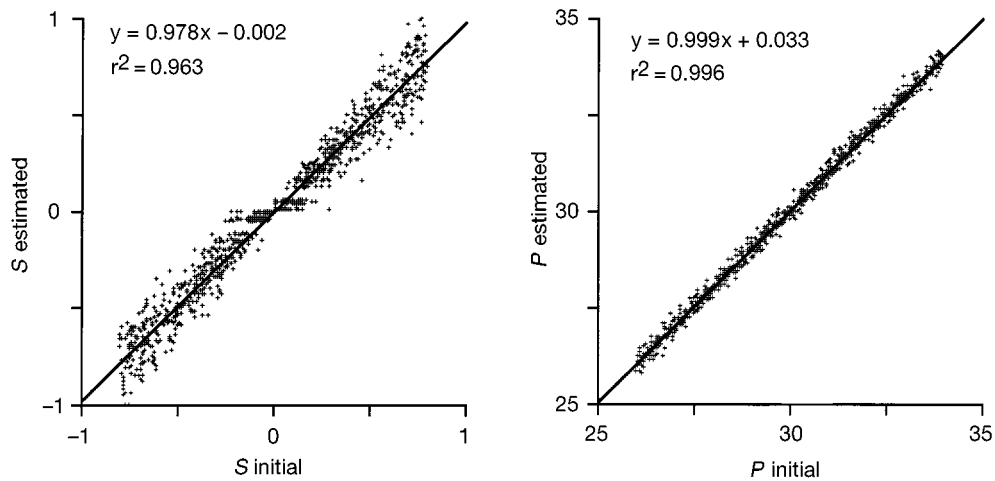


Fig. 2. Actual and estimated values of pivotal temperature (P) and transitional range of temperature (S) for 1000 trials.

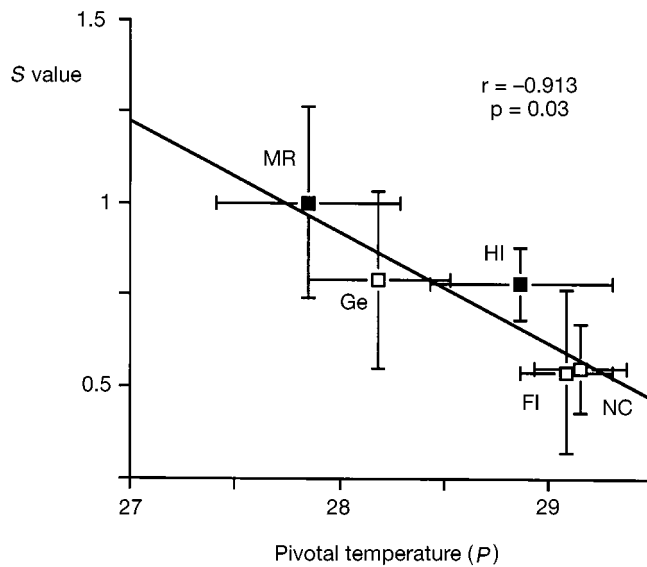


Fig. 3. Pivotal temperature (P) and transitional range of temperature (TRT) measured using the S parameter for *Caretta caretta* nests from five nesting beaches (■, Australia, MR: Mon Repos; HI, Heron Island; □, USA, Ge: Georgia, FI: Florida, NC: North Carolina). Data from Limpus *et al.* (1985) and Mrosovsky (1988). Error bars represent two standard deviations.

experimental design, for *Dermochelys coriacea* the problem is more the straightness of its TRT. Estimates of P or S cannot be performed when less than two incubation temperatures produce a mixed sex ratio. On the other hand, the small standard deviation obtained for *Emys orbicularis* was due to the large number of eggs and incubation temperatures used and the good fit of the theoretical curve to the observed data (Fig. 1).

The data for *Caretta caretta* can be used to demonstrate the use of estimates to compare populations. Three nesting beaches from the Atlantic coast of the USA have been sampled, each with two nests (Mrosovsky 1988), and two nesting beaches from Australia, each with three nests (Limpus *et al.*, 1985). The correlation between the estimates of P and S for the five *Caretta caretta* nesting beaches has been calculated. The two parameters were found to be inversely correlated ($r = -0.913$, $P = 0.03$; Fig. 3). This correlation could correspond to a physiological constraint linking the pivotal temperature to the TRT, or to an adaptive response of populations to particular environmental conditions. The use of statistical estimates of temperature-dependent sex determination could help to choose between these hypotheses.

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