STATISTICAL DESCRIPTION OF TEMPERATURE-DEPENDENT SEX DETERMINATION IN MARINE TURTLES

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INTRODUCTION

In many reptiles, as well as in some actinopterygians and amphibians, the sexual phenotype is determined at least in part by the incubation temperature of eggs (Pieau et al., 1995). This phenomenon has been called temperature-dependent sex determination (TSD). Its occurrence in reptiles (many turtles, all crocodilians, both sphenodons, and some lizards) is puzzling from an evolutionary point of view because its adaptive significance remains largely unknown. TSD occurs in all marine turtles and is generally first characterized in a species by the results of incubation at several constant incubation temperatures. Originally, TSD was described simply using the pivotal temperature (also called the critical or threshold temperature), which is the constant incubation temperature producing an equal number of both sexes (Mrosovsky and Pieau, 1991). More recently, a new parameter has been proposed to describe TSD: the transitional range of temperature (TRT) (Mrosovsky and Pieau, 1991). TRT is the range of constant temperatures in which the incubation produces both sexes. TRT is presumed to describe more adequately the consequence of TSD in natural conditions: when the TRT is narrower, more nests should be unisexual.

A new procedure has been recently introduced to calculate conjointly the pivotal temperature, the transitional range of temperature, and their variance (Girondot, 1999). The estimation is performed using maximum likelihood. We present rapidly here the method, its use to characterize TSD in marine turtles, and a test to compare populations.

Material and method

The statistical methodology used is fully described in Girondot (1999) and is summarized here briefly. It is based on the best-fit curve of the sex ratio on the incubation temperatures by the function:

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

equation 1

This equation has all the prerequisites for describing TSD: sr goes from 0 to 1 for S being positive or from 1 to 0 for S being negative 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 range of temperature producing males and females (TRT) is then simply $|S.k_l|$ with k_l being a constant dependent on l, the sex ratio limit chosen to define TRT (for example $TRT_{5\%}$ indicates that TRT is defined as the range of temperatures producing between 5% and 95% of males): $k_l = 2 \ln(l/(1-l))$

The probability (p_i) of the observed distribution of males and females for each u incubation temperature is obtained using a binomial distribution with m_i, f_i, and n_i ($n_i = m_i + f_i$) being the observed number of males, females, and total of sexed embryos, respectively, for a particular temperature t_i and the theoretical sex ratio at this temperature, $sr(t_i)$ based on equation 1:

$$p_i = C_{n_i}^{m_i} sr(t_i)^{m_i} (1 - sr(t_i))^{f_i}$$

equation 2

The likelihood of the experiment (L) is then simply: $L = \displaystyle\prod_{i=1}^{u} p_i$

equation 3

The adjustment of the *P* and *S* parameters for a particular set of incubation temperatures and sex ratio is performed using maximum-likelihood analysis and the variance of the parameters are obtained by the values of the second order derivative at the maximum-likelihood *S* and *P* values (Girondot, 1999). The test for significant difference between populations is based on the likelihood ratio test. The null hypothesis (H$_0$) being that no difference exists between populations; the likelihood of the combined data of all experiments (*L$_c$*) is calculated as well as the likelihood of each of the experiments (*L$_j$*). The test is then performed using a χ^2 with 2λ-2 DF:

$$\chi^2 = -2 \ln \left. L_c \middle/ \prod_{j=1}^{\lambda} L_j \right.$$

equation 4

Computer program for MS-DOS and MacOS is freely available on the web page:
http://www.biop7.jussieu.fr/perso/girondot.html

Results

The pivotal temperature (*P*) and TRT$_{5\%}$ are shown in **Table 1** for the available data in literature about marine turtles. Three species need to be discussed in further detail.

Chelonia mydas: Only data from Godfrey (1997) are used for the estimations of *P* and *S*. Data from Mrosovsky *et al.* (1984) does not permit to reach some maximum likelihood. It can be easily explained because only 4 incubation temperatures were studied and the two intermediate temperatures produced inverse sex ratio tendency compared to the two extreme temperatures. This could be the result of difference in cooling due to change of substrate for incubation (Mrosovsky, pers. comm.). Miller and Limpus (1980) estimate sex ratio for Australian population of this species at 3 incubation temperatures but none produced mixed sex ratio whereas at least two are required to estimate *P* and *S*. Nine intersexes were originally described in this experiment but further studies have shown that their phenotype were in fact identical to the most common sex obtained at this temperature (J.D. Miller and C.J. Limpus, pers. comm.). The probability of the observed number of males and females for each incubation temperature has been established based on the hypothesis that sex determination is the same than for Suriname Green turtles. The probability of the observed number of males and females is >0.99, <0.0001 and >0.99 for incubations at 26, 29 and 33°C respectively. Result at 29°C shows that *C. mydas* from Australia therefore exhibits a significant difference for TSD compared to *C. mydas* from Suriname.

Dermochelys coriacea: Binckley *et al.* (1998) reported sex ratio versus incubation temperatures for Leatherbacks nesting at Playa Grande, Costa Rica (Pacific). Based on a visual comparison with data from Atlantic (French Guiana) TSD in Leatherbacks (Rimblot *et al.*, 1985; Rimblot-Baly *et al.*, 1986), they conclude that: " The two temperature versus percent female curves for Atlantic and Pacific leatherback populations are virtually identical in pivotal temperature and overall shape. ". A reanalysis of these data has concluded that Atlantic and Pacific pivotal temperatures are indeed not significantly different but that TRT is significantly larger for Leatherbacks from Playa Grande (Pacific) compared to French Guiana (Atlantic) (Chevalier *et al.*, 1999).

Caretta caretta: TSD has been studied for 6 different geographic origins of animals and permit to use the likelihood ratio test. A significant global difference for TSD is observed (**Figure 2**). This demonstrates a significant genetic structure of populations for this species as already demonstrated with mtDNA polymorphism (Encalada *et al.*, 1998).

Conclusions

The first method that uses all the information available to describe TSD in reptiles is presented. When applied to available data for marine turtles, this method permits the calculation of pivotal temperature and TRT and also their standard deviations.

This method can be used also to distinguish populations of *Caretta caretta*, *Chelonia mydas* and *Dermochelys coriacea* based on their sensitivity to temperature. This new statistical tool will permit to enhance the ecological and evolutionary work on temperature-dependent sex determination.

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Table 1: Pivotal temperature (P) and $TRT_{5\%}$ for marine turtles (SD is standard deviation).

Species	Locality	P (SD)	$TRT_{5\%}$ (SD)	Ref.
C. mydas	Suriname	29.14 (SD 0.18)	3.23 (SD 0.464)	1
L. olivacea	World	30.06 (SD 0.24)	3.95 (SD 0.047)	2, 3
C. caretta	North-Carolina, USA	29.15 (SD 0.11)	3.24 (SD 0.353)	4
C. caretta	Georgia, USA	28.19 (SD 0.17)	4.65 (SD 0.706)	4
C. caretta	Florida, USA	29.09 (SD 0.11)	3.18 (SD 0.647)	4
C. caretta	Mon Repos, Australia	27.85 (SD 0.22)	5.89 (SD 0.765)	5
C. caretta	Heron Island, Australia	28.87 (SD 0.22)	4.59 (SD 0.294)	5
C. caretta	Brazil	29.15 (SD 0.06)	1.75 (SD 0.070)	6
E. imbricata	Antigua	29.27 (SD 0.04)	0.97 (SD 0.007)	7
D. coriacea	Costa Rica, Pacific	29.43 (SD 0.01)	1.17 (SD 0.040)	8

1: Godfrey (1997); 2: McCoy et al. (1983); 3: Standora and Spotila (1985); 4: Mrosovsky (1988); 5: Limpus et al. (1985); 6: Marcovaldi et al. (1997); 7: Mrosovsky et al. (1992); 8: Binckley et al. (1998).

Figure 2: Significant TSD difference between *Caretta caretta* " populations " (^{2}=107.12, 10 DF, p<0.01). The sex ratios (Y axes) are shown in " male frequency ".