

SEX DETERMINATION IN THE CRITICAL RANGE OF TEMPERATURE FOR MARINE TURTLES

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Sex determination is often classified in two distinct types: Genotypic Sex Determination and Environmental Sex Determination (Bull, 1983). Marine turtles are in the second group because the influence of temperature in sex determination has been demonstrated in all marine turtles. This communication proposes to consider that the genetic basis of sex determination in marine turtles and, by extension, in reptiles with environmental sex determination, is more important than is usually thought. The data used to demonstrate this phenomenon are those obtained in marine turtles as well as in other species of reptiles, particularly in the laboratory of developmental biochemistry, in Claude Pieau's group.

Studies of temperature influencing the sex-ratio in marine turtles often stopped at the determination of "threshold" temperature, "pivotal" temperature or "critical" temperature according to the authors, one same temperature giving theoretically both sexes in equal proportion. Only two studies on marine turtles have looked for a possible genetic basis for this threshold temperature : Nicholas Mrosovsky (1988) and Colin Limpus et al. (1985) in *Caretta caretta*. In both cases, a significant difference of threshold temperatures has been determined for different clutches. These data have been interpreted as different individual responses to temperature with a possible geographic difference among the populations.

After the work of Patrick Zaborski, Mireille Dorizzi and Claude Pieau published in 1979, 1982 and 1988 with *Emys orbicularis*, a European fresh water turtle, a new description of temperature sensitivity has been proposed. In this model, the temperature acts on top of the genotypic sex determination. Sex determination around what is called threshold temperature is under genetic control by a unique gene, as in genotypic sex determination. Classical systems of genotypic sex determination are XX/XY and ZZ/ZW as a function of the heterogametic sex. This denomination does not imply cytologic difference of the sex chromosome.

Let us consider two alleles, a and b. Below a temperature t1, embryos differentiate as males, and above a temperature t2, embryos differentiate as females. Between t1 and t2, embryos differentiate within their genotype. The definition of a t3 temperature is necessary, because temperature sensitivity of heterozygote ab is certainly different than that of homozygotes aa or bb of the same sex (Fig. 1). By analogy with a genotypic sex determination, a and b could be renamed XY or ZW. At t1, t2 and t3 incubation temperatures, sex determination will result from the interaction of temperature with sexual genotype.

Differences of threshold temperature found in different clutches for the same species could be differently interpreted with this hypothesis. Consider two theoretical cases, a clutch with only aa genotypic embryos (from aa female crossed with aa male) and a clutch with only bb genotypic embryos (bb crossed bb). In the first case, the threshold temperature will seem to be t1, and t2 in the second. All the intermediates are possible. Moreover, Harry et al. have demonstrated the fertilization of a female by several males in *Caretta caretta*.

For example let us consider a female of ab genotype mated with two males, one of ab genotype and one of bb genotype. If each male contributes 50% in the fertilization, the offspring genotypic frequencies for this clutch will be: 12.5% of aa genotype, 50% of ab genotype and 37.5% of bb genotype. If we consider, that t3 is in the middle of t2 and t1, the threshold temperature (temperature which gives 50% of each sex) is shown on Figure 2.

At the molecular level it appears now clearly that estrogens are implicated in the sex differentiation. Hormonal thresholds have been forwarded by several authors as the cause of difference of sex differentiation among both sexes. If the enzymatic systems which produced estrogens are sensitive to temperature in reptiles with ESD, the

classical response of sex ratio to temperature could be obtained (Fig. 3). Data obtained in our laboratory confirm this hypothesis. The genetic difference described here could be due to different levels of estrogens among the genotype or different sensitivity within the genotype for the same hormonal level.

The data of restriction fragment length polymorphism described at the previous workshop on sea turtles at Jekyll Island by Wachtel and Demas (1989) in *Chelonia mydas* and *Lepidochelys kempii* could also be explained by the same mechanism. They have described a restriction fragment of male specific DNA. But we don't have any information about the incubation temperature of eggs and probable parental relation between the individuals. However, this experiment is very interesting because it confirms the results of Wellins obtained in 1987 with H-Y antigen in *Chelonia mydas*. This species was classified as XX/XY sex determination. In this case, the restriction fragment of male specific DNA could be a marker of the Y chromosome.

Threshold temperature must be redefined. Classical definition was "--temperature which produces 50% of each sex," and it must be computed in the system described here by: $(aa)' \cdot t_1 + (ab)' \cdot t_3 + (bb)' \cdot t_2$ where $(aa)'$, $(ab)'$ and $(bb)'$ are the offspring genotypic frequencies of a population. As the genotypic frequencies of the adult are not strictly constant in time, even if this population is at equilibrium, this threshold temperature could not be defined for a species nor for a population. The only non-fluctuant parameters as a function of the population structure are t_1 , t_2 and t_3 . A possible geographic differentiation on these parameters can be evaluated, but it is very difficult to determine experimentally temperatures t_1 , t_2 and t_3 . These temperatures must be considered as the real pivotal temperature. The temperature conditions to obtain both sexes differentiation must not be defined by a threshold temperature but by an interval of temperature. This interval is limited by two temperatures, t_m and t_f which are the lower and higher limits of the temperature range within which both sexes can be produced. t_m temperature is not necessarily equal to t_1 temperature, and t_f is not necessarily equal to t_2 , because t_1 and t_2 are temperature means and t_m and t_f are the lower and higher limits for these temperatures (Fig. 4). t_1 , t_2 and t_3 will be named the pivotal temperature for sex determination for a specific genotype, and the interval between t_m and t_f could be named the critical range of temperature for sex determination.

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Figure 1

Different responses to temperature as a function of genotype

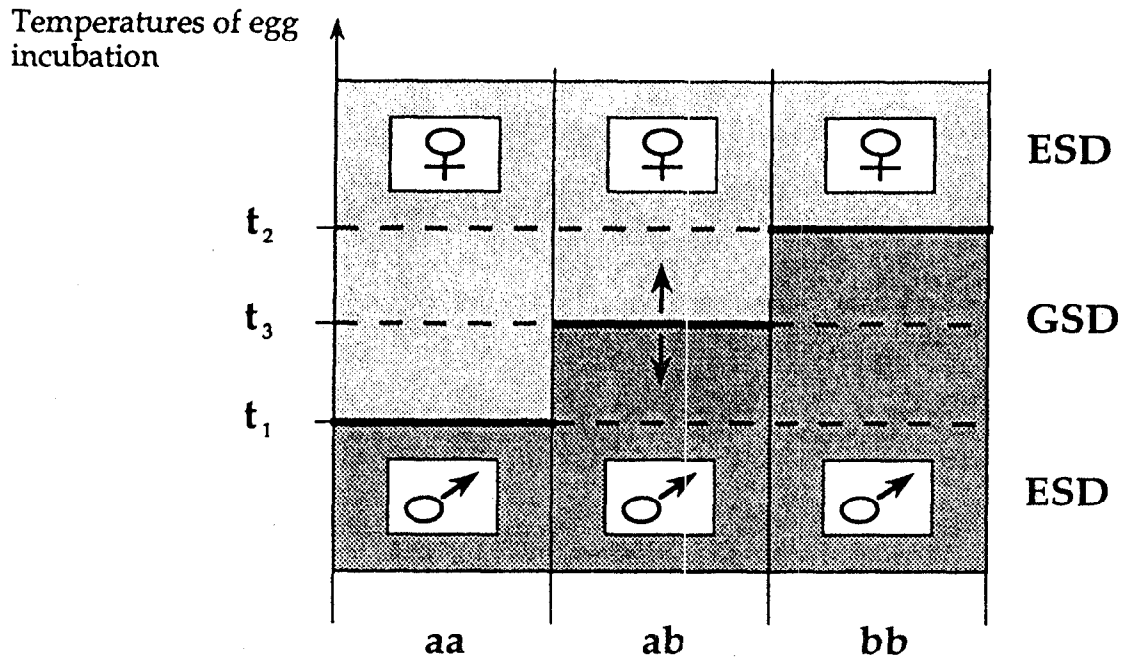


Figure 2

Theoretical threshold temperature for a clutch

		aa	ab	bb	Genotype frequencies of offspring with equal paternal contribution
Female ab	First male ab	0.25	0.5	0.25	
	Second male bb	0	0.5	0.5	
Total in the clutch		0.125	0.5	0.375	

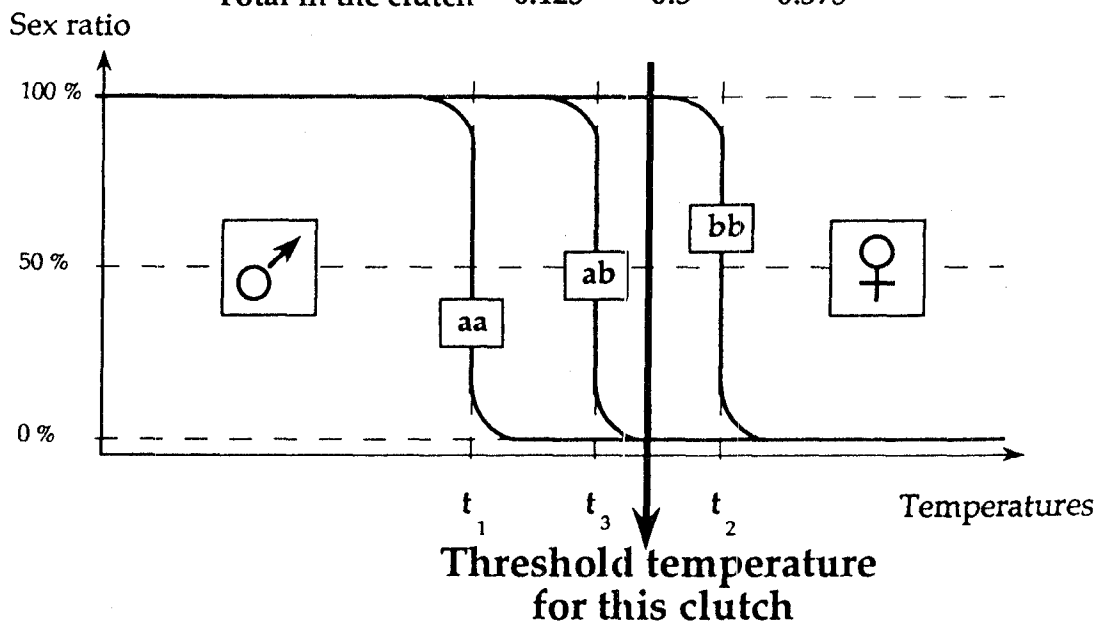


Figure 3

Synthesis of estrogens as a function of the temperature in aa, ab and bb individuals (theoretical curves).

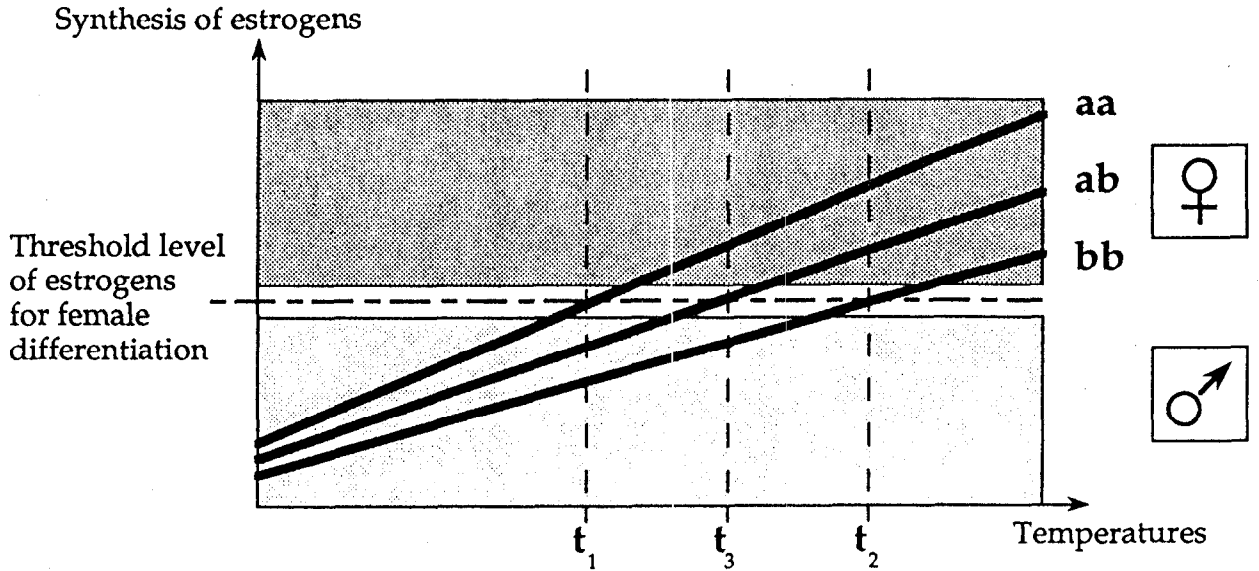


Figure 4

Conclusion : new terminology in interaction of temperature with genotypic sex determination

