From air temperature to nest temperature to sex ratio in *Emys orbicularis*: a long chain with uncertainties

Marc Girondot
Laboratoire Ecologie, Systématique et Evolution
Paris, France

**Discovery of temperature-dependent sex determination in Reptile**

**TSD in Chelonians**

**Compilation of artificial incubation for 1293 eggs at 16 different incubation temperatures**
For a long time, the main challenge was to get temperature data from field.

With the development of meteorological satellites, climatic models and interconnection of meteorological stations, more and more data are available.

The new challenge is no more to get temperature data, but to use them correctly in the particular context of TSD analysis.
When sexual phenotype is dependent on temperature during development?

The concept of Thermo-Sensitive Period

The Thermo-Sensitive Period (TSP) must be defined based on embryonic stages and not on incubation duration.

The TSP is located at the middle-third of embryonic development but not at the middle-third of incubation.

The middle-third of incubation is the middle-third of development only for constant incubation data.
How to define the position of TSP?

To define the position of TSP, we need to have a model of embryonic growth dependent on temperature.

Reaction norm of embryonic growth dependent on temperature

TSP at non-constant temperature

TSP at non-constant temperature
How to integrate temperature changes during development?

Constant-Temperature Equivalent

Georges et al. (1989, 1994) have demonstrated this idea in an experiment with constant mean temperature but varying amplitude:

- The highest the amplitude of temperatures, the more feminized are the embryos: in a cyclic pattern, higher temperatures have more influence than lower ones because embryo develop faster at these temperatures


Constant-temperature equivalent

- Using an embryonic growth rate model, we can estimate when is the TSP;
- But how to estimate sex ratio based on temperatures within TSP?
- Based on observations, Pieau (1974) has proposed that the more embryo develops at one temperature, the more this temperature will influence the sex
- But rate of development is higher at higher temperatures...

CTE: Estimate

\[ CTE = \sum Ti \cdot Wi \]

\( Ti \): Temperature

\( Wi \): Weight for ponderation
In short

- Arithmetic average of any-temperature measured in nest is a biased estimate of CTE that best described TSD
- The weights to correct for growth rate dependent on temperature necessitate a model for embryonic growth

How to integrate genetic difference among individuals?

Are there genetic variation than can express in natural condition?
Search for difference in the mean genetic profile of males and females obtained after hybridization with several microsatellite probes.

**Bam HI**
Only one band of very large molecular weight proving the GGCT locus is shared by only one locus.

**Mbo I and Sau 3AI**
Four alleles are detected with a statistical significant difference between male and female genotypic frequencies (p<0.05). No difference for Mbo I and Sau 3AI digestions is obtained proving the male/female difference is not due to difference in DNA methylation.

**Alu I**
Seven alleles are detected with a statistical significant difference of between male and female genotypic frequencies (p=0.027).

Sex determination in laboratory experiments ➔ Constant incubation temperature
Sex determination in natural conditions ➔ Incubation temperatures fluctuate

Therefore, sex determination in *Emys orbicularis* in natural conditions is much more genetically-determined than environmentally-determined.

Is it possible?
How to integrate seasonality in nesting?
Nesting season can vary from year to year.

How turtles decide to begin to nest?

- Do the turtles use a calendar:
  
  An increase in the daily amount of illumination induces in the testes of *Pseudemys elegans* a new spermatogenetic cycle.

- Do the turtles use climatic proxies:
  
  The results suggest that nesting behaviour is related to warm air and water temperatures in *C. picta* and to rainfall in *E. macquarii* and *C. expansa*.

How to integrate thermal heterogeneity in nesting site?
Scale of difference is important.

Scaling the thermal heterogeneity

- It is recognized since very long time that temperature is not homogeneous in a ecosystem, mainly due to cooling due to shade.
Scaling the thermal heterogeneity

- Based on temperature recording, thermal heterogeneity in soil at very small scale (10m) can be very large (up to 3°C) even in the absence of shading difference.
- Physics of thermal properties of soil is very complex. Energy transfer depends on soil structure and composition. Particularly moisture in soil have a very complex interaction with energy loss and will change over time.

Scaling the thermal heterogeneity

- Heterogeneity in soil surface temperature is also observed, but its relationship with soil temperature is not known.

Scaling the thermal heterogeneity

- Climatic models use a 25km x 25km grid to give estimate of past and future climate.
- This scale is not relevant at all to take into account thermal micro-habitat.
Conclusions

- In few years, we shift from crude mechanical thermometer to numeric and satellite data acquisition.

- The main gap now is on the interpretation of the thermal data in the context of TSD.