Sex in turtle pets

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With the growing market of turtle pet, veterinarians are confronted to new demands. Owners or breeders of turtles generally know that temperature-dependent sex determination occurs in these species, but they lack specific knowledge on how such sex determination works. Furthermore, they need advices on the way to control incubation conditions to take into account this phenomenon. A guide to answer the most common questions is proposed: (i) In which species sex is determined by temperature, sex chromosomes or both? (ii) How to choose incubation temperature for eggs of turtles to optimize hatchling success, performance of juveniles or sex ratio? (iii) How to decipher sexual phenotype of juveniles?

Key words: Turtles, pet, sex determination, sex identification, eggs incubation.

INTRODUCTION

Turtle pet market is growing in all the studied countries (Honegger, 1974; Williams, 1999; Wise et al., 2002) and veterinarians are confronted with recurrent demands about information on take care of eggs and young turtles. A regular concern is about control or knowledge of sexual phenotype of juveniles. Indeed, turtles pet owner generally knows the concept of thermosensitivity of sex determination but specific details necessary to answer some specific questions are lacking. The importance of sexual phenotype of turtles for owners becomes evident when they want to perform some breeding. The objectives of this review is to bring the up-to-date information about thermosensitivity of sex determination in turtles and to share this information to owner of pet turtles and to answer some specific questions: (i) In which species sex is determined by temperature, sex chromosomes or both? (ii) How to choose incubation temperature for eggs of turtles to optimize hatchling success, performance of juveniles or sex ratio? (iii) How to know sexual phenotype of juveniles?

Sex determination in turtles

Many species of oviparous reptiles, including crocodilians, a majority of turtles, some lizards and the two closely related species of Sphenodon have been shown to display temperature-dependent sex determination (TSD). In these species, the differentiation of gonads into ovaries or testes depends on the incubation temperature of the embryo during a critical period of embryonic development designated by the thermosensitive period (TSP) (Mrosovsky and Pieau, 1991; Yntema and Mrosovsky, 1982). This period begins with the appearance of gonad during embryogenesis and encompasses the middle third of embryo development. It approximately underlies the same embryonic stages.
Table 1. Sex determination among families or subfamilies of turtles. When GSD is indicated, it can be male heterogametic (XY), female heterogametic (ZW) or unknown status (modified from Janzen and Krenz, 2004). Average pivotal temperature and its standard deviation has been calculated for MF pattern of sex determination using data from Hulin et al. (2009). “na” indicates unavailable data and species with FMF pattern are indicated.

<table>
<thead>
<tr>
<th>Family or Subfamily</th>
<th>Sex determination</th>
<th>Average pivotal temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelomedusidae</td>
<td>TSD</td>
<td>31.79°C (SD 0.50°C)</td>
</tr>
<tr>
<td>Chelidae</td>
<td>GSD</td>
<td>-</td>
</tr>
<tr>
<td>Carettochelyidae</td>
<td>TSD</td>
<td>Na</td>
</tr>
<tr>
<td>Trionychidae</td>
<td>GSD</td>
<td>-</td>
</tr>
<tr>
<td>Staurotypidae</td>
<td>GSD</td>
<td>-</td>
</tr>
<tr>
<td>Kinosternidae</td>
<td>TSD</td>
<td>Na</td>
</tr>
<tr>
<td>Dermatemysidae</td>
<td>TSD</td>
<td>Na</td>
</tr>
<tr>
<td>Chelydrinae</td>
<td>TSD</td>
<td>FMF</td>
</tr>
<tr>
<td>Dermochelyidae</td>
<td>TSD</td>
<td>29.47°C (SD 0.06°C)</td>
</tr>
<tr>
<td>Cheloniidae</td>
<td>TSD</td>
<td>29.35°C (SD 0.62°C)</td>
</tr>
<tr>
<td>Emydidae</td>
<td>GSD or TSD</td>
<td>28.56°C (SD 0.87°C)</td>
</tr>
<tr>
<td>Emydinae</td>
<td>GSD or TSD</td>
<td>27.82°C (SD 0.70°C)</td>
</tr>
<tr>
<td>Deirochelyinae</td>
<td>TSD</td>
<td>28.87°C (SD 0.76°C)</td>
</tr>
<tr>
<td>Bataguridae</td>
<td>GSD or TSD</td>
<td>Na</td>
</tr>
<tr>
<td>Testudinidae</td>
<td>TSD</td>
<td>30.09°C (SD 0.82°C)</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td>29.33°C (SD 1.07°C)</td>
</tr>
</tbody>
</table>

TSD is widely distributed among family of turtles (Table 1). Eleven families are currently monomorphic for the pattern of sex determination and two (Emydidae and Bataguridae) include species with both TSD and GSD. TSD could be the ancestral state in turtles. During evolution, 5 groups of turtles have acquired, independently, sex chromosomes (Janzen and Krenz, 2004). Then, it is possible to tentatively infer a pattern of sex determination (GSD or TSD) knowing the family or sub-family belongs to a species, except for sub-family Emydinae and the family Bataguridae where the analysis should be done at the genus level.

However, the exact reaction norm of sex ratio versus incubation temperatures is more difficult to anticipate except if the eggs come from an already studied species and population (review in Hulin et al., 2009).

Now let us discuss an unresolved question: is it possible to have both GSD and TSD at the same time in a single species? When first discovered in turtles (Pieau, 1971, 1972, 1973), thermosensitivity of sex determination was thought to superimpose to putative chromosomal sex determination (C. Pieau, pers. comm. see also Zaborski et al., 1979). But rapidly a strong dichotomy between GSD and TSD species has been drawn by some authors, and GSD and TSD were described as two incompatible states (Bull, 1983). However, genetic component in sex ratio has been demonstrated for eggs incubated in the range of temperatures where both sexes can be produced in the European Freshwater turtle (Emys orbicularis), the common snapping turtle (Chelydra
serpentina), the painted turtle (Chrysemys picta), and the map turtle (Graptomys ouachitensis) (Bull et al., 1982; Girondot, 1993; Janzen, 1992; Rhen and Lang, 1998). However, this genetic component was thought not to be able to express in natural condition (Bull et al., 1982).

In the 80'th, the serological minor histocompatibility antigen H-Y was supposed to be a marker of the heterogametic sex and has been extensively studied in the European Freshwater turtle, Emys orbicularis. When incubated at male or female-producing temperatures, high and low expression levels were observed in serum of juveniles, irrespective of their sexual phenotype. On the other hand, when incubated at both sexes-producing temperatures, males hatchlings had low-level of expression and females had high-level of expression. This polymorphism of expression of H-Y antigen had been proposed to reflect the genetic component influencing sex determination at intermediate temperature (Zaborski et al., 1982, 1988). Low or high temperatures override this genetic component. Then for incubation at constant temperature, most of the embryos have their sexual phenotype that is determined by incubation temperature except for the narrow zone of temperature where both sexes are produced. When H-Y antigen was studied among adults from natural population, 80% of individuals have their H-Y antigen status (that is sexual genotype) conformed to their sexual phenotype (Girondot et al., 1994b). How a species could have TSD when incubated in artificial condition and a sex determination that looks like GSD in 80% of the individuals in natural conditions? It was proposed that when incubation temperatures fluctuate from male to female temperature, the genetic variability in sex determination could influence sex of the gonad and that only when temperature fluctuates around very high or very low temperature, it overpasses the genetic component. The question was debated until a similar system was deciphered in an Australian lizard with both sex chromosomes and TSD (Radder et al., 2008). However, we still lack a molecular marker for this genetic component to validate definitively this model for turtles.

Both in Green turtle, Chelonia mydas (Demas et al., 1990), and European Freshwater turtle, Emys orbicularis (Girondot, 1993; Girondot et al., 1994a) sex specific DNA RFLP bands have been found but, in both cases, validation with eggs incubated in known conditions were lacking. Further researches should be done in this direction.

As a conclusion for this section, a veterinarian should look at the Table 1 to identify the family or sub-family of the species. The main pet species are reported in Table 2 with their pattern of sex determination. For uncommon

### Table 2. Twenty most common turtle pet species exported from US between 2002 to 2005 (Senneke, 2010) with their pattern of sex determination (Ewert et al., 2004; Paukstis and Janzen, 1990).

<table>
<thead>
<tr>
<th>Rank</th>
<th>Scientific name</th>
<th>Common name</th>
<th>Family or Sub-Family</th>
<th>Pattern of sex determination</th>
<th>Exported from the US (2002-2005)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trachemys scripta elegans</td>
<td>Red-eared Slider Turtle</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>15186735</td>
</tr>
<tr>
<td>2</td>
<td>Pseudemys nelsoni</td>
<td>Florida Redbelly Turtle</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>7019251</td>
</tr>
<tr>
<td>3</td>
<td>Pseudemys concinna</td>
<td>River Cooter Turtle</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>2566312</td>
</tr>
<tr>
<td>4</td>
<td>Trachemys scripta scripta</td>
<td>Yellowbelly Slider</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>1255398</td>
</tr>
<tr>
<td>5</td>
<td>Pseudemys alabamensis</td>
<td>Alabama Redbelly Turtle</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>961772</td>
</tr>
<tr>
<td>6</td>
<td>Pseudemys floridana</td>
<td>Florida Cooter</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>939583</td>
</tr>
<tr>
<td>7</td>
<td>Graptemys pseudogeographica kohni</td>
<td>Mississippi Map Turtle</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>680795</td>
</tr>
<tr>
<td>8</td>
<td>Chelydra serpentina</td>
<td>Common Snapping Turtle</td>
<td>Chelyridae</td>
<td>TSD FMR</td>
<td>634838</td>
</tr>
<tr>
<td>9</td>
<td>Trachemys scripta troostii</td>
<td>Cumberland Slider</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>366648</td>
</tr>
<tr>
<td>10</td>
<td>Trachemys scripta gageae</td>
<td>Big Bend Slider</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>338396</td>
</tr>
<tr>
<td>11</td>
<td>Aplone ferox</td>
<td>Florida soft-shell Turtle</td>
<td>Trionychidae</td>
<td>GSD</td>
<td>256650</td>
</tr>
<tr>
<td>12</td>
<td>Pseudemys peninsularis</td>
<td>Peninsula Cooter</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>191606</td>
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<tr>
<td>13</td>
<td>Chrysemys picta</td>
<td>Western Painted Turtle</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>173315</td>
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<tr>
<td>14</td>
<td>Pseudemys rubriventris</td>
<td>American Redbelly Turtle</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>104711</td>
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<tr>
<td>15</td>
<td>Macrochelys temminckii</td>
<td>Alligator Snapping Turtle</td>
<td>Chelyridae</td>
<td>TSD FMR</td>
<td>90466</td>
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<tr>
<td>16</td>
<td>Apalone spinifera</td>
<td>Spiny soft-shell Turtle</td>
<td>Trionychidae</td>
<td>GSD</td>
<td>85072</td>
</tr>
<tr>
<td>17</td>
<td>Sternomochus odoratus</td>
<td>Common Musk Turtle</td>
<td>Kinosternidae</td>
<td>TSD FMR</td>
<td>65734</td>
</tr>
<tr>
<td>18</td>
<td>Graptemys pseudogeographica</td>
<td>False Map Turtle</td>
<td>Deirochelyneae</td>
<td>TSD MF</td>
<td>40979</td>
</tr>
<tr>
<td>19</td>
<td>Apalone mutica</td>
<td>Smooth soft-shell Turtle</td>
<td>Trionychidae</td>
<td>GSD</td>
<td>20892</td>
</tr>
<tr>
<td>20</td>
<td>Sternomochus carinatus</td>
<td>Keelbacked Musk Turtle</td>
<td>Kinosternidae</td>
<td>TSD FMR</td>
<td>14901</td>
</tr>
</tbody>
</table>
species, a search in specific literature must be done (Ewert et al., 2004). If the species is not listed anywhere, the only solution is to refer to the pattern in the most related species based on a composite phylogeny of turtles build from the most recent sources (Bowen et al., 1993; Fujita et al., 2004; Krenz and Janzen, 2000; Noonan, 2000; Sasaki et al., 2004; Serb et al., 2001; Shaffer et al., 1997; Spinks et al., 2009) and to infer pattern of sex determination based on parsimony principles for reconstructing evolution (Maddison and Maddison, 1992). However, a doubt will subsist.

How to choose incubation temperature for eggs of turtles?

When an owner of turtle pet gets some eggs, he will need information on the best incubation procedure. The three main factors that should be taken into account are cleanness, humidity and temperature (Gunther, 2005). Cleanness is important to prevent eggs rotten (Girondot et al., 1990) and pathogen transmission to the eggs (Feeley and Treger, 1969). If humidity is lower than required, eggs will dehydrate and die and if higher than required, eggs will break out or rot. The use of moist vermiculite is recommended to maintain viable hygrometry (Delmas et al., 2008). Similarly, too high and low incubation temperatures could have detrimental effect on survival of embryos and intermediate ones must be preferred. However, by chosen a specific temperature, the owner can influence the sex of offspring as seen previously but also their performance (for example see Bobyn and Brooks, 1994; Delmas et al., 2007; O’Steen, 1998).

There is no general conclusion on the best-required temperature of incubation for turtle eggs. For example, incubation at feminizing has been advocated as a conservation measure for threaten species in the case of reintroduction purpose (Vogt, 1994) but this recommendation has been criticized from a ecological point of view (Lovich, 1996), lack of knowledge for the consequence to disrupt fluctuation of temperature in natural incubation of eggs (Mrosovsky and Godfrey, 1995) and adverse effects for individual of strongly feminizing conditions (Girondot et al., 1998).

Recently, several authors have published data to compare the effect of fluctuating versus constant incubation temperature. For all the tested parameters (growth rate, temperature choice, locomotor performance, immune response), fluctuating temperatures have a differential impact, often beneficial, when compared to constant temperatures (Booth, 2006; Delmas et al., 2007; Les et al., 2009; Paitz et al., 2010).

I will then recommend incubation at diurnal fluctuating temperatures around the pivotal temperature. Average pivotal temperatures for the various turtle families or sub-families are shown in Table 1. In case the owner wants specifically one sex, species specific pattern of sex determination (Hulin et al., 2009) should serve as a reference to choose incubation temperature. An average incubation temperature of 30°C for Testudinidae, 31°C for Podocnemididae and 28.5°C for Emydidae can be chosen with 2°C diurnal amplitude.

Identification of sexual phenotype of juveniles

Many turtle species are highly dimorphic as adults (Gibbons and Lovich, 1990) but external morphology of turtle is generally not sexually dimorphic until subadult stage (Wibbels et al., 2000). Hatchling turtles typically exhibit little or no obvious dimorphism that allows straightforward identification of sex by external observation (Ernst and Barbour, 1989). Significant sexual dimorphism Chrysemys picta and Podocnemis expansa hatchlings were measured using landmark-based geometric morphometric methods. This method had high accuracy in assigning sex when compared with true sex (98 and 90%, respectively), and cross-validation with hatchlings not used in model definition revealed a correct classification rate of 85% (Valenzuela et al., 2004). Multivariate model permits to discriminate males and females in 4-years juveniles Gopherus polyphemus but not in hatchling Gopherus agassizii (Burke et al., 1994). Difference in possibility to differentiate both sexes in Gopherus genus could reveal that shape morphology dimorphism is acquired during early growth. Size difference between one-year old males and females Trachemys scripta elegans were observed when reared in same conditions (Delmas, 2006). However, size assignation cannot be used when rearing conditions were not standardized and alternative techniques must be used.

External gonadal or sexual ducts morphology is generally studied by dissection of dead animals (Ceriani and Wyneken, 2008) which cannot be recommended in the context of this review. But external gonadal morphology has been also observed by laparoscopy on live animals in Green turtles, Chelonia mydas (Wood et al., 1983) and Desert tortoise, Gopherus agassizii (Rostal et al., 1994). Laparoscopy has been successfully used in Desert tortoises as small as 28 g total body mass. Recalling that hatchlings of most turtles weight less than 10 g, example, 6 g in Emys orbicularis (Pleau and Dorizzi, 1981), this method can not be used for very young turtles. Furthermore, this method is very invasive and could be lethal for small turtles (pers. obs.).

The serum testosterone radioimmunoassay procedure has been used conclusively for Lepidochelys olivacea, Chelonia mydas and Gopherus agassizii (Owens et al.,
1978; Rostal et al., 1994; Valverde, 1996). However, species specific sexing criteria must be developed using both plasma testosterone concentrations and laparoscopic examination of the gonads (Coyne and Landry, 2000). The origin of serum testosterone could be extragonadal, perhaps from brain (Withingam et al., 2000), and then a secondary consequence of sexual phenotype as demonstrated in Alligator (Lance et al., 2003). The serum testosterone radioimmunoassay procedure cannot be used for hatching, and estradiol: testosterone ratio has been used conclusively in Caretta caretta egg chorioallantoic/amniotic fluid (Gross et al., 1995) but should be validated for other species as well before to be recommended.

CONCLUSIONS

Whereas the phenomenon of temperature-dependent sex determination in reptiles is generally known, the limit of the current knowledge of this sex determination is often not well understood. Specifically, the exact nature of the relationship between TSD and GSD in a single species is still controversial. The most recent review points out that sex determination should be viewed along a continuum TSD-GSD rather than from a dichotomy (Sarre et al., 2004).

When confronted to the demand for incubation conditions, the best solution will be to mimic natural conditions of incubation and then to incubate eggs with daily fluctuating temperatures within the range of viable incubation temperatures. Only when the owner indicates that he want specifically one sex; for example for breeding purpose, then incubation at low or high nearly constant temperatures could be recommended.

To know the sexual phenotype of hatchlings or juveniles, there is at present for most species no other way than to be patient and to wait that the individual acquires sexually secondary dimorphic characters which can be difference in tail length, eyes colour, claws length, or plastron shape depending on the species (Ernst and Barbour, 1989).

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