The Mechanism of Temperature Dependent Sex Determination in Crocodilians: A Hypothesis

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SYNOPSIS. Incubation of alligator eggs at 30°C produces 100% females, at 33°C 100% males; temperatures in between produce varying sex ratios. Wild nests of Alligator mississippiensis show similar effects and the populations are biased towards females. The incidence and patterns of temperature dependent sex determination (TSD) in other crocodilians are reviewed. Temperature also affects hatching size and pigmentation patterns, post-hatching growth rates and thermoregulation by juvenile crocodilians. The significance of temperature sensitive periods defined by temperature shift experiments is questioned in relation to a hypothesis to explain the mechanism of TSD in crocodilians. It is postulated that there is an initial sex differentiation mechanism which involves a quantum period of time and a threshold for a dose of a male determining factor. The conditions for induction of males are precise but exhibit variation between individuals within the population. Females develop by default. The hypothalamus may have an important role in a later sex differentiation mechanism. The hypothesis is used to explain the late temperature sensitive periods defined by high to low temperature shift experiments, why cooler temperatures are more effective at determining sex, how intermediate temperatures can produce both sexes, the differences in the pattern between turtles and crocodilians and geographical similarities in the pattern of TSD within crocodilians despite differing climates. The phylogenetic advantages of TSD in crocodilians are concerned with the overall reproductive strategy of the animals. Those crocodilians which are incubated and grow to maturity under optimal environmental conditions will be both large and male. Larger males are more likely to produce more offspring. A review of the effects of the environment on sex determination in amphibians and fish suggests that there is a general relationship between size and sex in vertebrates.

INTRODUCTION

The factors that determine vertebrate sex are genetic and/or environmental. In Alligator mississippiensis the temperature of egg incubation determines the sex of the hatchlings (Ferguson and Joanen, 1982, 1983) and temperature dependent sex determination (TSD) is prevalent in all species of crocodilians examined (Table 1). No hermaphroditic or intersex hatchlings have been observed and sex is irreversibly determined at hatching (Ferguson and Joanen, 1983; Webb and Smith, 1984). Heteromorphic sex chromosomes are absent or not recognisable in all crocodilian species examined (Cohen and Gans, 1970).

TSD has been observed in many cheloni-ans and some squamates (see Bull, 1980, 1983; Gutzke, 1987; Packard and Packard, 1988; Deeming and Ferguson, 1988). In many cheloni-ans low temperatures induce males and higher temperatures females, whereas in other cheloni-ans low temperature females are also produced. Lizards exhibit a pattern similar to crocodilians. In all cases, studies of TSD have involved examining the effects of differing temperatures on the sex ratio. Shifts from a male producing to a female producing temperature (and vice versa) have defined temperature sensitive periods (TSPs) when temperature is effective at altering the sex of the embryos. The TSP in all types of reptile appears to occur around the same approximate morphological stages despite the differences in stage of development at oviposition (Bull, 1987).

This paper first summarises the patterns of TSD in crocodilians. Second, it suggests a hypothesis for a mechanism of temperature dependent sex determination in reptiles. Finally, the phylogenetic advantages of TSD in crocodilians, other reptiles and
TABLE 1. The incidence of temperature dependent sex determination in the Crocodylia.*

<table>
<thead>
<tr>
<th>Species</th>
<th>Pattern</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alligator mississippiensis</td>
<td>F-M</td>
<td>Ferguson and Joanen (1982, 1983)</td>
</tr>
<tr>
<td>Caiman crocodilus</td>
<td>F-M</td>
<td>Lang et al. (1989)</td>
</tr>
<tr>
<td>Crocodylus niloticus</td>
<td>F-M</td>
<td>Hutton (1987)</td>
</tr>
<tr>
<td>Crocodylus siamensis</td>
<td>F-M</td>
<td>Lang (1987)</td>
</tr>
<tr>
<td>Crocodylus palustris</td>
<td>F-M-F</td>
<td>Lang et al. (1989)</td>
</tr>
</tbody>
</table>

* The pattern of the sexes from low to high temperatures is included. F = female, M = male.

Vertebrates are described in relation to the new hypothesis.

TEMPERATURE DEPENDENT SEX DETERMINATION IN CROCODYLIANS

Two patterns of TSD are observed in crocodilians (Table 1). The first is exemplified by Alligator mississippiensis and is characterised by the production of female hatchlings at low incubation temperatures (≤30°C) with 100% male clutches occurring at 33°C. The incidence of males at intermediate temperatures increases as incubation temperature increases (Ferguson and Joanen, 1982, 1983; Joanen et al., 1987; Deeming and Ferguson, 1988). TSD is reproducible under artificial and natural conditions. Three types of nest mounds were observed for A. mississippiensis in Louisiana, USA (Ferguson and Joanen, 1982, 1983). The warmest nest temperatures, recorded in dry levees, produced only male hatchlings. Wet marsh nests, the coolest and wettest, produced only females. Dry marsh nests, with intermediate temperatures, produced both sexes with the warmest parts of the nest producing males.

Temperature shift experiments on eggs of A. mississippiensis defined temperature sensitive periods (TSP) for sex determination (Ferguson and Joanen, 1983). Shifts from 30 to 34°C revealed a TSP between 14 and 21 days of incubation (Table 2). By contrast, shifts from 34 to 30°C delayed the TSP which occurred between days 28 and 35 (Table 2). The conventional view that these TSPs are the time of sex determination was not supported by a shift twice, pulsed temperature experiment (Deeming and Ferguson, 1988). A week long pulse of one temperature on the background of another was insufficient to dramatically alter the predicted sex ratio (Table 3; Deeming and Ferguson, 1988). TSPs are now considered to represent the beginning and end of a quantum period of incubation during which sex is being determined (Deeming and Ferguson, 1988; see later).

The second pattern of TSD is exemplified by Crocodylus johnstoni (Table 1; Webb et al., 1983a, 1987; Webb and Smith, 1984). Both low and high temperatures induce female hatchlings, with males occurring at intermediate temperatures. There is no single temperature that produces 100% male hatchlings. By contrast, Crocodylus porosus and Crocodylus palustris do show temperatures (32°C, 33°C; 32.5°C, respectively) that produce 100% males, though high temperature females are present (Webb et al., 1987; Webb and Cooper-Preston, 1989; Lang et al., 1989). In C. johnstoni nests that produce 100% males have been observed (Webb and Smith, 1984; Smith, 1987). The length of the incubation period appeared to influence sex of the hatchling more than temperature per se; male hatchlings occurred after 72 to 82 days of incubation (Smith, 1987). Similarly, in C. palustris the mean length of incubation for 100% male nests was between 64 and 66 days with some male hatchlings emerging from other nests after incubation periods of 60 to 71 days (Lang et al., 1989).

Temperature shift experiments carried out for C. porosus and C. palustris have revealed similarities with A. mississippiensis (Webb et al., 1987; Lang et al., 1989). Temperature shift experiments in C. johnstoni have been hard to interpret owing to the lack of an exclusively male producing temperature (Webb et al., 1987). Shifts from 29°C, 30°C and 31°C to 32°C were effective in inducing development of males as early
TABLE 2. Details of a single shift experiment, originally described in Ferguson and Joanen (1983), carried out on eggs of Alligator mississippiensis.*

<table>
<thead>
<tr>
<th>Day of incubation eggs were shifted</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
<th>49</th>
<th>56</th>
</tr>
</thead>
<tbody>
<tr>
<td>30°C to 34°C</td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number of males</td>
<td>20</td>
<td>20</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number of females</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>18</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>34°C to 30°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of males</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>12</td>
<td>19</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Number of females</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>19</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Eggs were incubated at either 30°C (100% females) or 34°C (100% males) for varying lengths of time and then switched to the other incubation temperature. On day 60 of incubation the sex of the hatchlings was determined (Ferguson and Joanen, 1983). There were 20 eggs in each group.

as 3–5 days of incubation, despite conditions that would normally produce females (Webb et al., 1987). Nearly 66% of the incubation period was temperature sensitive and could alter the sex ratio (Smith, 1987).

Other effects of temperature in crocodilians

The temperature of egg incubation affects other aspects of the development of crocodilians, both before and after hatching. High incubation temperatures induce faster growth and development of the embryo compared to lower temperatures (Webb et al., 1987; Deeming and Ferguson, 1988; Lang et al., 1989). The time of hatching is brought forward (by 8 days in A. mississippiensis [Joanen et al., 1987] and by 15 days in C. porosus [Webb and Cooper-Preston, 1989]). Hatchling mass is smaller at the higher temperature compared to lower temperatures and more abdominal yolk is present. The pigmentation pattern of A. mississippiensis hatchlings is also affected by the incubation temperature. Hatchlings from eggs incubated at 33°C have darker black stripes compared to hatchlings incubated at 30°C incubation (Fig. 1). The number of white stripes on the body of male hatchlings is increased compared to females (Table 4).

Post-hatching growth rates are affected by incubation temperature. Hatchlings of A. mississippiensis from eggs incubated at intermediate temperatures (30.6°C and 31.7°C) had faster growth rates under controlled conditions (30°C) than those from extreme temperatures despite being smaller at hatching. Male hatchlings grew faster than females from eggs incubated at the same temperature (Joanen et al., 1987).

TABLE 3. Details of a shift twice, pulsed temperature experiment carried out on eggs of Alligator mississippiensis.*

<table>
<thead>
<tr>
<th>Week</th>
<th>Days</th>
<th>Temperature</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 = 30°C, 2 = 33°C</td>
<td>1 = 33°C, 2 = 30°C</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>Total</td>
<td>Males</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>7-14</td>
<td>5</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>14-21</td>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>21-28</td>
<td>7</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>28-35</td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>35-42</td>
<td>11</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>42-49</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td>8</td>
<td>49-56</td>
<td>15</td>
<td>29</td>
</tr>
</tbody>
</table>

* Eggs from each group were incubated either at 30°C (exclusively female producing) or 33°C (exclusively male) and exposed to 7 day periods of the other temperature during different weeks of the incubation period (pulse week). Macroscopic and histological sexing (Ferguson and Joanen, 1983) was carried out on day 60 of incubation. Groups 1 and 2 are controls. (After Deeming and Ferguson, 1988.)
Crocodylus niloticus male hatchlings were smaller in length than females, but by three months of age they were significantly larger (Hutton, 1987). The thermal preferences of juveniles of Crocodylus siamensis were affected by the temperature of egg incubation. Males (from eggs at 32.5 to 33.5°C) consistently selected higher temperatures (Lang, 1987).

**The Significance of Temperature Shift Experiments**

In all reptiles studied the temperature sensitive period associated with an increase in incubation temperature occurs well before the TSP associated with a decrease in temperature, both in terms of morphological age and incubation period (Yntema, 1979; Bull and Vogt, 1981; Pieu and Dorizzi, 1981; Ferguson and Joanen, 1983; Bull, 1987). This relationship is opposite to what one might expect, i.e., higher initial temperature, which accelerates external development (Webb et al., 1987; Deeming and Ferguson, 1988), delays the TSP and lengthens the period of embryonic sexual lability, measured as absolute incubation time or by stage of development.

Shift twice, pulsed temperature experiments did not significantly override sex ratios associated with background incubation temperatures (Table 3; Deeming and Ferguson, 1988). The sex determining mechanism responded minimally to 7 day pulses of a single temperature at specific developmental stages attributed to the TSPs defined by single shift experiments. Similar dual shift experiments were carried out with eggs of the turtle Graptemys ouachitensis. The length of the pulse of 25°C,
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TABLE 4. The pigmentation patterns in hatchlings of Alligator mississippiensis associated with temperature of incubation. *

<table>
<thead>
<tr>
<th>Tip to tail—White</th>
<th>30°C</th>
<th>33°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(x)</td>
<td>SD</td>
</tr>
<tr>
<td>Total number of stripes</td>
<td>18.78</td>
<td>1.05</td>
</tr>
<tr>
<td>Number of stripes on the tail</td>
<td>11.21</td>
<td>0.97</td>
</tr>
<tr>
<td>Number of stripes on the trunk</td>
<td>7.64</td>
<td>0.63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tip to tail—Black</th>
<th>30°C</th>
<th>33°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(x)</td>
<td>SD</td>
</tr>
<tr>
<td>Total number of stripes</td>
<td>18.35</td>
<td>0.79</td>
</tr>
<tr>
<td>Number of stripes on the tail</td>
<td>11.06</td>
<td>0.56</td>
</tr>
<tr>
<td>Number of stripes on the trunk</td>
<td>7.29</td>
<td>0.47</td>
</tr>
</tbody>
</table>

* The pattern is described as the number of white stripes on the dorsal surface of the animal starting from the crown of the head to the tip of the tail. The patterns of embryos incubated at two temperatures are described: 30°C (100% females) and 33°C (100% males). Those embryos with a white tipped tail are distinguished from those with a black tip. Mean (\(x\)), standard deviation (SD) and sample size (n) are included.

upon a background of 31°C (female inducing) required to produce males was shorter than the period at 31°C to induce females upon a background temperature of 25°C (Bull and Vogt, 1981).

Embryos of *C. johnstoni* remain sexually labile for a long period of incubation (Webb et al., 1987) but due to the absence of an exclusively male producing temperature TSPs have not been accurately defined. The bimodal pattern of females at high and low temperatures, with males at intermediate temperatures, is also seen in the turtle *Chelydra serpentina* (Yntema, 1976, 1979). The initial incubation temperature affects the time of the TSP but in this species less incubation at 30°C (to Yntema [1968] stage 16) was required to determine femaleness than was required to determine maleness at 26°C (stage 19) (Yntema, 1979).

Data on temperature sensitive periods in crocodilians and chelonians have remained puzzling with no unifying explanation of temperature mediated sex determination. We suggest that previous authors tended to assume that the TSP equated with time of embryonic sex determination (Bull, 1987; Gutzke, 1987; Lang et al., 1989), which would, therefore, differ by at least 2 to 3 stages of development depending on initial temperature. This erroneous assumption (Webb et al., 1987) was compounded by misleading concepts and nomenclature of TSPs and TSD. A brief summary of these contradictory or unexplained aspects of TSD is in order before elaborating our own mechanistic hypothesis of TSD in crocodilians and other reptiles.

First, why does the TSP vary according to the initial incubation temperature: intuitively higher temperatures would be expected to have an earlier TSP (Yntema, 1979; Bull and Vogt, 1981; Pieau and Dorizzi, 1981; Ferguson and Joannen, 1983; Bull, 1987)? Second, with the exception of *C. serpentina*, how are cooler temperatures more effective at determining sex in reptiles (Yntema, 1979; Ferguson and Joannen, 1983)? Third, how can intermediate temperatures, between exclusively male and exclusively female temperatures, produce both sexes (Ferguson and Joannen, 1982, 1983; Bull, 1980)? Fourth, the two patterns of TSD exhibited by crocodilians are also observed in the turtles and lizards (Yntema, 1976, 1979; Vogt et al., 1982; Tokunaga, 1985; reviewed by Deeming and Ferguson, 1988). It is not clear why two patterns should occur. Are these species differences significant and what is the evolutionary relationship between them? Fifth, how is it that the same (and different) species with TSD living in different geographical locations with hot or cool climates (e.g., a comparison of Florida and North Carolina for *A. mississippiensis* or a comparison of this species with *C. porosus*) usually have remarkably similar threshold temperatures for TSD (Ferguson and
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Joanen, 1983; Webb and Cooper-Preston, 1989)?

The determination of the sex of an individual is considered to occur when the cells that express a certain sex cease to be totipotent and are set on a course of development that leads to only one tissue type. The developmental processes by which this occurs are described as sexual differentiation (Austin et al., 1981). Mammalian and avian sex determination occurs at conception and differentiation of the gonad is under genetic control. We are proposing that crocodilian embryos are undetermined and sexually labile and that two phases of sex differentiation occur, during different parts of the incubation period, which determine sex in crocodilians.

Initial sex differentiation mechanism

It is our suggestion (initially postulated in Deeming and Ferguson [1988] and amplified here) that the temperature sensitive periods as defined by single shift experiments do not define precise points at which sex is being determined but rather indicate the initial sex differentiation period. TSPs as defined from the lowest to the highest viable incubation temperature shifts and vice versa define the earliest and latest times, respectively, during incubation that the sex of the embryo can be altered. In effect the TSP between days 14 and 21 (defined from a shift from 30 to 34°C in A. mississippiensis [Ferguson and Joanen, 1983]) is the start of a quantum of time during incubation that temperature is effective. The TSP defined as 28 to 35 days from a shift from 34 to 30°C in A. mississippiensis indicates the end of this quantum period. To define the complete quantum period for A. mississippiensis, however, temperature shifts between the highest (34°C) and lowest (29°C) viable incubation temperatures need to be carried out. It is important to note that the precise length of the quantum period can be determined by this method for a population of embryos but not for individual embryos.

Maleness is conferred upon an embryo by a series of events during the quantum period. The embryo has to be exposed to a specific “dose” of a male determining factor (the nature of which is described later). If embryos are not exposed to an adequate dose of the factor or for an adequate time, then females develop, essentially by default.

The shift twice, pulsed temperature experiment on eggs of A. mississippiensis (Table 3; Deeming and Ferguson, 1988) indicates some important points. A temperature pulse of 7 days is too short a period to significantly affect the population sex ratio but it can alter the sex of individual embryos. Sex determination in A. mississippiensis is, therefore, not a switch event affecting the whole population at a single developmental time. The small change in the sex ratio associated with a pulse of a high temperature on the background of a lower temperature is only observed early in incubation yet a pulse of a low temperature on a background of a higher temperature is effective over a longer incubation period (Table 3). These data can be explained by variation in the population of embryos. Variation will exist within a clutch of eggs both in the length of the quantum period and the dose of male determining factor required by individual embryos to develop into males. A small proportion of the population will only require a short quantum period and/or a low dose of the male determining factor. The 7 day pulse of the higher temperature is sufficient to induce male differentiation in these embryos. This implies that higher temperatures are more effective at producing male determining factor than lower temperatures (see later) so that some embryos with a low threshold achieve this even with small pulses. The pulse of high temperature must occur early in development; by contrast exposure to a lower temperature during the quantum period affects those embryos that require a long quantum period and/or have a high threshold for the male determining factor. For some pulsed embryos, therefore, the conditions for male differentiation are not maintained and they develop into females by default. Hence,
lower temperatures are more effective in determining the sex of an individual embryo due to an essentially default mode of action. Initial sex differentiation would appear to depend upon the dosage of male determining factor transcribed, the individual threshold for that factor and the individual period during which the factor is required to cause male differentiation.

This hypothesis explains some of the puzzling aspects of the observed patterns of TSD in reptiles outlined previously. TSPs defined by low to high temperature shifts occur early in incubation as the threshold for the male determining factor has to be reached early in development. Any disruption of the quantum period induces females by default. Later TSPs defined by shifts from high to low temperatures indicate the end of the quantum period and are the earliest time that lowering the temperature has no effect on sex determination. In crocodilians, cooler temperatures are disruptive of the male inducing conditions and therefore are more effective at altering sex ratio. In C. serpentina, however, higher temperatures also disrupt the initial sex differentiation mechanism and females develop by default at higher temperatures. At intermediate temperatures both sexes can be produced due to variation within a clutch of eggs in the embryos' requirement for both the quantum period and the threshold dose of the male determining factor. At 31°C in A. mississippiensis, for example, the 13% of the hatchlings which are male (Ferguson and Joanen, 1983) may be those with a low threshold for the male determining factor and/or have a late starting, relatively short quantum period. In A. mississippiensis the optimum temperature for male differentiation and determination is 33°C. Other species may have different optimal incubation temperatures for the production of male embryos. In the turtles, G. ouachitensis and C. serpentina, the optimum incubation temperature for male embryos is 25–26°C (Bull and Vogt, 1981; Yntema, 1976, 1979) whereas in the gekkonid lizard, Eublepharis macularius, it is 32°C (Bull, 1987). Temperatures above and below the optimum result in reduced transcription of the male determining factor and females develop by default. Therefore, the primitive pattern is probably high temperature females, intermediate temperature males and low temperature females. Selection has removed either low temperature (many turtles) or high temperature females (A. mississippiensis) for reasons unrelated to sex determination. Indeed, nest site selection in reptiles is usually correlated to embryo survival rather than predicted sex ratio (Stoneburner and Richardson, 1981; Webb and Smith, 1984; Ferguson, 1985; Schwarzkopf and Brooks, 1987; Lang et al., 1989). Again constraints on the optimal temperatures for embryonic development and survival have led to species in different climatic conditions having similar temperature thresholds for TSD. It is the environment of the nest, and not the climate per se, which is important in sex determination in reptiles.

The nature of the male determining factor

The nature of the male determining factor is currently unclear. There are, however, different patterns in the differentiation of the gonad in embryos of A. mississippiensis at male and female inducing incubation temperatures (Deeming and Ferguson, 1988). At 33°C there is a clear organisation of the medulla of the gonad by day 34 of incubation. At 30°C there is no organisation of this region of the gonad prior to formation of the ovary (Deeming and Ferguson, 1988). Morphometric analysis of growth in alligator embryos has shown that general development is enhanced at 33°C compared to 30°C. It would seem that at 33°C differentiation is rapid at the expense of growth (Deeming and Ferguson, 1988). It is not clear how temperature acts to direct gonadal organisation though it is almost certainly related to transcription and translation of specific gene products.

Possible candidates for the male determining factor include: H-Y antigen (Engel et al., 1981; Zaborski et al., 1982; Standora and Spotila, 1985), steroid hormones (Gutzke and Bull, 1986; Bull et al., 1988), the enzyme aromatase (Bogart, 1987), differential gonadal growth (Mittwoch, 1971,
1973, 1983), heat shock proteins (Schlesinger, 1986) and some form of testis determining factor (Page et al., 1987). The expression of H-Y antigen has been of interest in studies of TSD in reptiles (Engel et al., 1981; Zaborski et al., 1982; Standora and Spotila, 1985). At present, however, it is thought unlikely to play any major role in sex determination (Gutzke, 1987). Steroid sex hormones, estrogens and testosterone, applied to reptilian embryos that normally exhibit TSD, can affect the sex ratio (Gutzke and Bull, 1986; Bull et al., 1988) though it is generally considered that these hormones play no role in initial sex determination. Differential growth of the gonad (Mittwoch, 1971, 1973, 1983) cannot explain the data as gonads of alligator embryos incubated at 30°C (female) are retarded in their differentiation compared to their counterparts of equivalent age at 33°C (male): comparison of equivalent stages reveals that at 30°C gonads are longer (Deeming and Ferguson, 1988). A more likely factor is the testis determining factor encoded by a gene on the human Y chromosome (TDY) and its homologue (TDX) on the X chromosome (Page et al., 1987). TDY and TDX genes encode for a zinc finger protein which could bind to both DNA and RNA. It is therefore likely to be a molecule regulating the expression of other genes on autosomes, including those involved in the sex differentiation cascade as well as autosomal genes, e.g., for growth. In mammals it is possible that two doses of TDF occur in males but only one in females due to X chromosome inactivation (Page et al., 1987). Sex determination in Drosophila and a nematode worm are also dose dependent and associated with the number of X chromosomes (Hodgkin, 1987; Nöthiger and Steinmann-Zwicky, 1987; Kimble, 1988). Incubation temperature may be influencing the expression of a TDF-like gene in reptiles (Deeming and Ferguson, 1988). Under this scheme we postulate that at male determining temperatures, gene transcription and translation and enzyme activity are all optimal, therefore a large dose of the male determining factor is produced. Structural gene transcription and translation may therefore be enhanced by this increased amount of regulatory gene product, and, together with the degradation of gene products, may also be directly affected by temperature. At a lower or higher than optimum temperature gene transcription and translation and enzyme activity will be sub-optimal, lower doses of the male determining factor result and females develop by default. Although few data are available it would appear that dose dependent mechanisms may be important in sex determination in animals.

Later sex differentiation mechanism

In adult alligators, and other animals, gonadotrophins are responsible for the physiological control of hormone secretion from both the testis and ovary (Lance, 1987). If it is postulated that gonadotrophins are present in embryonic alligators, they are present in chick embryos (Woods, 1987), then they may be important in controlling later sexual differentiation. Incubation temperature may affect the amount of gonadotrophin secretion (either by direct effects on the pituitary or hypothalamus), the expression of LH and FSH receptors in the medulla and cortex of the gonad, or the affinity of such receptors. All possibilities could affect sex determination and may play a role in the specific conditions for production of males and later default to females. We postulate that later sexual differentiation in alligators is controlled by the embryonic hypothalamus via its secretion of luteinizing hormone releasing hormone (LHRH). The early hypothalamus may detect environmental parameters, such as incubation temperature, pH, pCO₂, pO₂, and thereby set the release of releasing hormones. Alternatively, the regulatory TDY gene may act within the hypothalamus and directly control the release of these same hormones. Either way, it would appear that incubation conditions during development are important in setting the hypothalamus. Optimal incubation conditions during development link the activity of the hypothalamus with the physiological requirements of being male. Incubation temperature also influences thermoregu-
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Population and growth rates of juvenile crocodilians (Joanen et al., 1987; Lang, 1987; Webb and Cooper-Preston, 1989) which are presumably under hypothalamic control (Lance, 1987). The incubation temperature is, therefore, not only establishing the sex of the individual but setting the hypothalamic regulatory system for the rest of the animal’s life.

Size and Sex: The Phyllogenetic Advantages of Environmental Sex Determination?

One evolutionary advantage of environmental sex determination (ESD), TSD being a specialised form of ESD, is that incubation conditions determine the sex of a hatchling and its size (Ferguson and Joanen, 1982, 1983; Hutton, 1987; Webb et al., 1987; Deeming and Ferguson, 1989; Webb and Cooper-Preston, 1989). Adult male crocodilians are usually larger than females. Large size within the population of males is relevant to subsequent reproductive success. Large males control larger harems of females and produce more spermatozoa for a longer time than small males thereby passing on their genes to more offspring (Ferguson, 1985). Incubation temperature influences sex, growth rate before and after hatching, metabolic rate, optimal thermoregulatory temperatures plus sexual characteristics such as pigmentation pattern and intensity. The selective advantage is that large, fast growing animals will be males.

Genetic sex determination (GSD) would not guarantee that the eggs containing genetically determined male embryos would be in optimum incubation conditions and therefore grow fast into large males. ESD allows the association of sex and potential post-hatching growth rate as both are determined by incubation conditions. Those hatchlings which were incubated at high temperatures are adapted as adults to grow rapidly to a large size given optimal environmental conditions. Hence the largest animals in a population are males but very large males may not be the oldest animals, rather those individuals which have grown under optimal incubation, and post-hatching environments. Smaller males may be those produced at sub-optimal incubation conditions in optimum post-hatching conditions, sub-optimally incubated males in a sub-optimal environment or optimally incubated males in a sub-optimal environment. In the last case the males from sub-optimal incubation conditions may be at an advantage. The optimal environment is a rare event which may explain the relatively infrequent occurrence of very large male crocodilians and the bottlenecking of males in certain size classes (Webb et al., 1983b). A further advantage of TSD producing 100% male or 100% female clutches in natural nests is in the prevention of inbreeding as all siblings are of a single sex.

The beauty of the mechanism is that not only does TSD associate the potentially largest animals with the male sex but produces a whole range of animals (male and female) each adapted to a different post-hatching environment. Thus a heterogeneous population of males and females, in terms of metabolic rate and potential growth rate, is produced and liberated into a heterogeneous environment. Varying temperature and other conditions of incubation ultimately produce hatchlings that are adapted to differing environments and this improves the capacity of the population to survive changes in the environment and to colonise various ecological niches. A further advantage of ESD is that it may allow populations of crocodilians to recover from catastrophic situations (repeated nest flooding, disease or sustained hunting [Webb and Smith, 1984]). The sex ratios of crocodilian populations are heavily biased towards females (Ferguson and Joanen, 1982, 1983; Webb et al., 1983a; Webb and Smith, 1984; Hutton, 1987; Lang et al., 1989). After a catastrophe, those animals surviving may be wary and preferentially nest close to the water. These nests tend to be cooler (Ferguson and Joanen, 1982, 1983) thereby producing a large number of females and few males. The population is thus able to rapidly expand due to the large numbers of breeding females and the ability of a few males to fertilize these. As competition for nest sites increases, more females are forced to
higher ground. These warmer areas increase the proportion of male hatchlings thereby limiting population expansion.

Size and the sex determining mechanism in other vertebrates

The Charnov and Bull (1977) hypothesis on the evolution of ESD is dependent on three factors. First, there must be selective advantages in being either male or female. Second, the environment must be spatially heterogeneous with some patches improving male fitness whilst other patches favour females. Third, neither the adults nor the offspring can control the environment they enter. TSD in crocodilian populations appears to fit these criteria with large size being associated with maleness. The selective advantage of being large in reptiles has been related to sex and to the species concerned (Head et al., 1987). Large turtles are at a selective advantage if they are female as the size of the body affects female fecundity directly. Male lizards are often territorial and the larger they are the more capable they are in maintaining territories and procuring females (Head et al., 1987).

Exceptions do occur: e.g., the snapping turtle, *C. serpentina*, has large aggressive males and therefore resembles crocodilians (Head et al., 1987). Such a divergence from this normal pattern of ESD and size in turtles may well be explained by further study of the reproductive biology of this turtle.

Temperature dependent sex determination comparable to that observed in reptiles has not been reported in amphibians. Temperature mediated sex change does occur; high temperatures during larval development of a frog induced genetic females to express male phenotypes (Witschi, 1992; Gallien, 1974). Maintenance of female (ZW) larvae of the urodele *Pleurodeles waltlii* at 29.5–30.0°C induced male development. (In this species it is the male which is the larger sex and which fights for females [Shine, 1979].) These males (ZW) were mated to genetic females (ZW) and a ratio of three females (ZW and WW) to one male (ZZ) confirmed that they had a female genotype (Houllion and Dournon, 1978; Zaborski, 1986).

ESD has been described in several species of fish, though the patterns of sex determination in Pisces are more variable. For example, the sex ratio in cichlid, and a poeciliid, fish are influenced by pH of the water; more acidic water produces more male fish (Rubin, 1985). Temperature dependent sex determination occurs in silverside fish (*Menidia* species). Population sex ratios of *Menidia peninsulae* are influenced by water temperature; higher temperatures reduce the predominantly female-biased sex ratio (75–90%) in favour of more males (Middaugh and Hemmer, 1987). In *Menidia menidia* low temperatures and slow growth rates leading to long growing periods produce female fish but warmer water increases growth rates and male fish develop after short growing periods (Conover and Kynard, 1981; Conover, 1984). Temperature shift experiments have shown that the TSP in this species is related not to age but to body size, though (in contrast to reptiles) higher temperatures are more effective at establishing the sex of the fish (Conover and Fleisher, 1986). Populations of *M. menidia* from different latitudes exhibit differing patterns of TSD; northern populations exhibit genetic sex determination over a range of temperatures whereas populations at more southerly latitudes show TSD (Conover and Heins, 1986). Both genetic and environmental sex determination have important roles in silverside fish (Conover and Heins, 1986, 1987) with an association between the environment, larval growth and the pattern of sex determination. When the environment is favourable, with long growing seasons and warm temperatures allowing rapid growth of the fish (southern latitudes) TSD is prevalent. As the environment becomes less favourable, with shorter growing seasons and lower temperatures allowing rapid growth of the fish (northern latitudes) TSD is prevalent. As the environment becomes less favourable, with shorter growing seasons and lower temperatures allowing rapid growth of the fish (northern latitudes) TSD is prevalent.
Many fish are hermaphroditic and exhibit sex change related to various environmental factors. In *Rivulus marmoratus* all fish are initially hermaphroditic but secondary males develop with time in response to annual short-day seasons (Harrington, 1967, 1971). Development of embryonic fish at 25°C or higher induces hermaphroditic fish, but lower temperatures (<20°C) induced the production of primary males which are very rare under natural conditions (Harrington, 1967, 1968). Changes in sex are common amongst fish. In the wrasse *Thalassoma duperrey*, a species which exhibits female to male hermaphroditism, it is the social environment which affects secondary sex determination (Ross et al., 1983). Social interactions affect the growth rate of individuals; large fish grow rapidly in the presence of smaller conspecifics and change from females to males (Ross et al., 1983; Ross, 1984, 1987). This process is mediated through visual contact (Ross, 1987). The advantage of this strategy would appear to be that small individuals put more energy into growth than reproduction to minimise the period of low reproductive fitness (Ross, 1984); larger males are able to defend the preferred spawning sites of the smaller females (Ross, 1984, 1987). The link between relative reproductive success and change in sex is well illustrated by fish of the genus *Symphodus* (Warner and Lejeune, 1985). The four species of fish studied differed in the complexity of the nest the males built. Those species with a lot of male investment in the nest did not show sex change. Less male investment in tending the eggs led to a higher incidence of sex change associated with an ability to spawn more frequently. Larger males are more successful at this strategy. If the reproductive effort associated with being male was relatively low, then female to male sex changes were common (Warner and Lejeune, 1985).

There may well be an association between the size of an individual and its sex and this may be determined by the environment or genetically. Interestingly, in Turner's syndrome in man, affected individuals are generally small and this growth retardation can be corrected by growth hormone treatment (Tanner et al., 1959). One of the few genes (other than TDY) on the human Y chromosome is that which encodes for growth and stature (Goodfellow et al., 1985): a link between size and sex seems to have been preserved even in man. The extent to which genetic sex determination occurs in reptiles is unclear but in crocodilians the dependence on TSD and the apparent advantages that it brings to a population throughout time may well have maintained the strategy of ESD in this group. Other successful groups (birds and mammals) may have evolved genetic sex determination in response to more homogeneous incubation conditions. While it is relatively simple to envisage how TSD evolved into GSD and heteromorphic chromosomes (more uniform incubation conditions may have led to (1) loss of a copy of the gene for sex determination [female heterogamety], as in the case of birds, or (2) inactivation of a chromosome containing the gene [male heterogamety], as in the case of mammals) it is unclear whether TSD is a primitive strategy in terms of sex determination: the data from the fish studies illustrate that TSD can evolve from GSD.

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**References**


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