TEMPERATURE AND SEX DETERMINATION IN REPTILES WITH REFERENCE TO CHELONIANS

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Introduction

Keeping tortoises as pets can be fun and breeding them also exciting. But there is a serious side to this pastime. Nowadays tortoises in the wild are endangered animals and threatened with extinction. So, their conservation is a really worthwhile activity. A successful breeding conservation strategy will not only ensure their survival but also increase their population. The main purpose of this article is to show that temperature has a profound effect in determining the development of certain artificially incubated reptile eggs into male or female individuals. In particular, it concentrates on recent laboratory and field investigations on sex determination in chelonians. The article concludes that erroneous incubation temperature recommendations are likely to have serious effects on chelonian sex determination and ultimately their conservation.

Sex determination

In many organisms the sex of an offspring will be irreversibly determined by its sex chromosomes, or rather, a set of genes on the chromosomes, regardless of any environmental variation. This condition is known as 'genotypic sex determination'. However, in some organisms the immediate environment determines whether the offspring will become a male or a female, a condition referred to as 'environmental sex determination'. Ultimately, however, environmental sex determination is also controlled by genes. Genotypic Sex determination

Many animals have a pair of sex chromosomes which determines whether their offspring will be male or female. In all mammals and most insects the male gamete has either an X or a Y chromosome, half the sperms carrying the X chromosome and half the Y chromosome. The male is thus heterogametic. The female gamete has only X chromosomes, the unfertilised eggs each carrying an X chromosome. The female is thus homogametic. A male (XY) offspring is produced when a Y sperm fertilises an (X) egg and a female (XX) offspring is produced when an X sperm fertilises an (X) egg. Hence, the male determines the sex of the offspring.

Birds, some amphibia and a few insects also have XX and XY sex chromosomes but here the sexes are reversed, the male being homogametic (XX) and the female heterogametic (XY). Hence, the female gamete determines the offspring's sex in these examples. In reptiles, sex chromosomes have been found in many snakes, several lizards and a few terrapins (Kinosternidae). Only males are heterogametic in the terrapins, only females are heterogametic in the snakes, but both are known in the lizards. Most fishes and some amphibia have chromosomes which appear undifferentiated and the development of either male or female offspring depends on small differences between a pair of like chromosomes.

Environmental (temperature) Sex determination

Temperature profoundly affects the biology and behaviour of all cold-blooded animals, including chelonians. Temperature influences their sex ratio, length of incubation, emergence from the nest, growth, activity, survival, nesting intervals, hibernation, and distribution. Temperature-dependent sex determination in embryos is probably the most important component of environmental sex determination.

Temperature sex determination and sex chromosomes are apparently mutually exclusive sex determining mechanisms. Sex chromosomes are unknown in any animals in which temperature sex determination occurs, but they occur in most animals which do not show any sex determination response to temperature. Among reptiles, sex chromosomes are missing in the tuatara, crocodiles, most tortoises, turtles and terrapins, and a few snakes and lizards.

Laboratory investigations
Laboratory studies over the past fifteen years have shown that in some reptiles sexual differentiation depends on the temperature prevailing during incubation of the eggs. This appears to be a widespread phenomenon in chelonians and has been found in at least a dozen species studied so far. In all experiments, the sex of the hatchlings was diagnosed by inspection of the structure of the reproductive system under a dissecting microscope.

In these reptiles, the effects of egg incubation temperature on sex determination are striking. Low temperatures (20-27°C) produces one sex, higher temperatures (30°C and above) produces the other. Within a narrow range of temperature, known as the threshold (critical/pivotal) temperature, both males and females, and sometimes intersexes, are produced. Generally, this threshold lies between 28°C and 31°C.

In tortoises, terrapins and turtles males will develop at cool temperatures and females at warm temperatures. In marked contrast, lizards and alligators produce males at warm temperatures and females at cool temperatures. Crocodiles and snapping turtles develop into females at both cool and warm temperatures and males at intermediate ones. Finally, some terrapins and turtles, lizards and snakes are apparently uninfluenced by incubation temperatures.

Generally, among most chelonians incubation of eggs at cool temperatures will produce males and incubation of eggs at warm temperatures will produce females, although there are a few exceptions. Some examples will illustrate this point.

Eggs of the Spur-thighed Tortoise (Testudo graeca) produced males at 29.5°C and females at 31.50°C; both sexes were produced at the threshold temperature of 30-31°C. Eggs of the European Pond Terrapin (Emys orbicularis) produced males at 27.5°C and females at 29.5°C; both sexes and intersexes were produced at 28-29°C. Eggs of the Loggerhead Turtle (Caretta caretta) kept at 28°C or below all developed into males, those kept at 300C or above all developed into females, and at 290C both males and females were produced. Map Terrapins (Graptemys spp.), the Slider Terrapin (Pseudemys scripta) and the Painted Terrapin (Chrysemys picta) all produced mostly males at 28°C and mostly females at 30°C; both sexes were produced at 29°C. In contrast, the Snapping Turtle (Chelydra serpentina) both extremes of warm (above 30°C) and cool (20°C) temperatures produced mainly females while intermediate (22-28°C) temperatures produced mainly males. Finally, sexual differentiation in Soft-shelled Turtles (Trionyx spiniferus) was independent of incubation temperature. There are a few exceptions to the patterns given above. Thus, Mud Terrapins (Kinosternon sp.) and the Alligator Snapping Turtle (Macrochelys temminckii) produced females at a temperature 25°C and above while cooler temperatures produced both males and females.

Experiments to determine the critical temperature-sensitive period for sex differentiation in Map and Painted Terrapin and Snapping Turtle eggs have indicated that sex is determined during the middle of the developmental period, coinciding with the development of the reproductive organs. This period corresponds to a minimal exposure of temperature which results in either all males or all females being produced. This can be as short as twelve days in the European Pond Terrapin for male differentiation at 25°C and female differentiation at 30°C. Recent studies have further shown that in the Snapping Turtle a minimum incubation period of four hours a day at 30°C throughout the critical period was required to produce females; decreasing time produced fewer females.

Most laboratory investigations on egg incubation have been done at constant temperature but it is important to know how sex determination operates when incubation temperature fluctuates, as happens in nature. Eggs of the European Pond Terrapin were incubated at a daily temperature cycle of between 24 and 30°C or 26 and 31°C. Both males and females developed in the second cycle, that is, within its threshold temperature of 28-29°C, whereas only males and intersexes developed in the first cycle. In other experiments, eggs of the Map Terrapin were incubated at a daily fluctuation of 23-33°C or 20-30°C. The first cycle produced only females and the second cycle only males, although the mean temperature of these two cycles differed by only 3°C. Intersexes were not found. Hence, there appears to be no marked differences between incubation under constant or fluctuating temperatures.

Very little is known about environmental factors other than temperature regulating sex differentiation in chelonians. Recent work has suggested that eggs incubated under 'dry' or 'wet' conditions may produce different sex ratios. However, more information is needed before the influence of dryness or wetness on the development of chelonian embryos can be assessed.

Field Investigations

Does temperature determine sex of chelonians in nature? Although more work on temperature-dependent sex determination has been done in the laboratory than in the field, some studies on sex determination from natural nests have recently been made. A large number of nests of Map and Painted Terrapins were located along the shore of the Mississippi river in Wisconsin, USA, hatching was monitored and the hatchlings sexed. Nests with males were located in sand amid shading vegetation, which cooled the temperature, and nests with females were located in the open sand exposed to the sun, with warm temperatures. The sex ratio among emerging hatchlings changed from nearly all females early in the season to all males late in the season. Eggs from Green Turtles (Chelonia mydas) were incubated in artificial nests on a beach in Costa Rica and the temperature monitored daily. Shaded cold and partially shaded cold nests produced mainly males while nests in warm sunny areas produced mainly females. These observations are consistent with the interpretation that temperature affects sex determination in chelonians.

The positions of the eggs within the nest of the Snapping Turtle as well as nest depth is also important in sex determination. Snapping Turtles make deeper nests in which they lay more eggs than many other chelonians. Females were mainly produced from eggs in the top of the nest where the temperature was higher than lower down. In the centre of the clutch mixed sexes were found at a lower temperature. Bottom eggs produced only males at the lowest temperature.

There are discrepancies from field observations that have not yet been fully resolved but there is no reason to reject the view that sex determination in chelonians is largely the same under natural incubation as under artificial incubation. Field observations not only confirmed laboratory experiments but provided additional information. Thus, the sex ratio in chelonians with temperature-dependent determination is influenced partly by the threshold temperature of the embryos and partly by the mother's choice of nest sites and, in large nests, partly also by the position of the eggs within the clutch.
Conclusions

So what does all this add up to? Quite simply, it is this. not only is temperature control important in order to breed chelonians but also it is vital that the eggs must be artificially incubated at a temperature of 29-31°C to obtain both male and female hatchlings. Unfortunately, books and articles on tortoise biology often recommended a temperature for incubating tortoise eggs well below that suggested in this article. I have in my possession nine published works on chelonian biology, ranging from an encyclopedia, several books and diverse booklets. These are all written by well-known and experienced tortoise enthusiasts. On average, the temperature recommended by these authors for artificially incubating tortoise eggs is 25.5°C, ranging from 23.5 to 27.5°C.

Moreover, articles on tortoise breeding published both in Testudo and British Chelonia Group newsletters also frequently advise using incubation temperatures within this range. An average incubation temperature of 25.5°C will most probably produce an all-male tortoise colony; even at the upper temperature range suggested only few, if any, females are likely to be produced. If the main aim of breeding tortoises (and other chelonians) is for their conservation then tortoise owners would be well-advised to incubate eggs at a higher temperature than hitherto recommended. Since little is yet known about the temperature that determines the sex requirements of most European tortoise species, particularly their threshold temperature, ideally one egg batch in a clutch should be incubated at a constant temperature of, say, 27-28°C and a second at, say, 32-33°C to ensure an all male and all female development respectively. Alternatively, keeping a clutch at a constant temperature of 30–31°C will, hopefully, enable the development of individuals of both sexes.

European tortoise eggs will hatch in about 10 weeks (range 8-12 weeks) at an incubation temperature of 30°C, irrespective of humidity, and the middle period is the likely critical time when males and females will be environmentally determined. Among European tortoises temperature sex determination has been studied so far in only the Spur-thighed Tortoise. Although generalisations are sometimes misleading it is probable, however, that these conclusions are also valid for the other species.

REFERENCES

The following list is incomplete and highly selective:

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