GESTATION AND DELAYED FERTILIZATION.

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The complete development of a young reptile from the moment of fertilization of the eggs up to the moment of birth or hatching - the embryonal development - with oviparous species can be divided into:

a. gestation (the period during which the eggs are in the body of the female); and
b. the incubation period (the time the eggs need to develop outside the female's body).

For many reptiles, the gestation period is given approximately in litterature, as is the duration of the incubation. Strikingly, quite a lot of differences can be noticed in the recorded duration of these periods. For example, we read that the incubation period is 55 to 70 days with temperatures ranging from 27 to 30^{0}C. Most readers will know that the incubation period depends on temperature. The duration of gestation is much more difficult to determine, because it is not so easy to determine whether the female is pregnant or not, and it often is not known for sure when fertilization took place.

For those reptiles that are live-bearing (ovoviviparous), gestation synchronizes with the total duration of the complete embryonal development. Yet here too things can be complicated: many ovoviviparous species have been observed under certain conditions to exhibit a short incubation period outside the mother's body after the laying of the not completely developed eggs. In most cases however, the oviposition coincides with hatching.

Not only in books, but also in breeding reports, the gestation duration is being determined rashly.

In many cases the authors start from some assumptions that are often rather dubious:

a. they assume that the last observed copulation actually has been the last fertilizing copulation, whereas this can only be known for sure if the couple has been separated immediately after that copulation. What is more: how could one know exactly which of all those possible copulations has led to fertilization?
b. they assume that there has been an uninterrupted development of the eggs.
c. They assume that fertilization has taken place rather quickly after the copulation, and that the sperm loses its fertilizing potential within a short time.
d. they assume that the incubation period admittedly is influenced by temperature, but that a specific fixed duration can be determined within these boundaries. They consequently do not reckon with the possible shortening of the incubation by the female's withholding the eggs for some reason or another, thus lengthening gestation.

As for the last possibility: eggs of *Elaphe schrenkii* incubated under my care in 37 days (at 30°C), which is very fast, but the eggs were laid several days after the ovipositions of other females which were imported at the same time. It is not impossible that this female withheld her eggs (egg-retention), thus lengthening the gestation period but shortening the incubation period.

In connection with the preceding assumptions, the following remarks must be made:

a. it is not certain that an onserved copulation leads to fertilization. Not all copulations are observed. This is why in breeding reports the following clause is often read: '...days after the last observed copulation'.
b. active sperm can be stored by the female in many species (sperm storage) and be used sometimes

much later, thus inducing fertilization without a new copulation.

c. it is not to be assumed that there is always a continuous development of the eggs. A so called germinative pause can take place, during which the development is being interrupted.

d. egg-retention often occurs.

Some examples of gestation period data found in litterature are here mentioned.

-*Boa constrictor imperator*: From 119 to 196 days, one mention even of 294 days (live bearing)

-*Epicrates inornatus*: 153-206 days (live bearing)

-*Corallus caninus*: 184 days (live bearing)

-*Python molurus*: 80-120 days (oviparous)

-*Python reticulatus*: 81 days (oviparous)

-With the garter snake *Thamnophis sirtalis*, gestation is strongly influenced by temperature: in hot summers 87 days, in cooler summers 116 days (both cases in Michigan). A difference in temperature of 1^0C causes an average lengthening or shortening of gestation of 4 to 5 days (live bearing)

-*Elaphe quatuorlineata*: 54-63 days (oviparous)

-*Crotalus atrox*: 167-253 days (live bearing)

-*Agkistrodon piscivorus*: 166-390 days (live bearing)

In captivity, pregnancy is often indicated, at an early stage by the female's refusal of food. This is the case with most Boids, but often not with other species. About the cause, little is yet known.

The phenomenon of amphigonia retardata (delayed fertilization) was not discovered until 1938 (Kopstein, 1938). It is of the highest importance, as it for instance explains why the gestation periods of reptiles often are so different within one and the same species, measured from the observed copulations onwards. Also, this phenomenon forms a counterevidence against supposed curious

hybridizations and against supposed discoveries of a case of parthenogenesis (procreation without involving the other sex).
With reptiles, there is no fixed relation between the ovulation period (during which eggs are formed) and copulation. The sperm can be stored in the female and remain fertile a very long time.
In March 1935 a female of *Natrix vittata* was caught, which on 26 March 1935 laid eggs and did so again on 3 May 1935. All eggs were fertilized. Another female of the same species was captured in May 1935 and kept separately. She laid eggs on 31 May 1935, again on 27 September (all fertile), again on 28 January 1936, on 4 March 1936, again on 5 April 1936 and 15 May, then on 19 June 1936, 25 July 1936 and finally on 10 January 1937. Only the last eggs were all infertile. The fertility of the sperm had diminished, though: the eggs from the year after the capture were all fertilized, but not viable (Kopstein, 1938).
The advantage of sperm storage is obvious, especially in areas where males and females are not likely to meet often, sperm storage gives a biological advantage for the survival of the species.
Many snakes from Northern America mate in the autumn as well as in spring. Here also, sperm storage during hibernation is not unlikely, the sperm being available when the female is forming eggs in the spring.
For reptiles, most research has been done on snakes. Proof for sperm storage has been found with a female *Boa constrictor constrictor* which had mated in captivity with a male *Boa constrictor occidentalis* and delivered 50 hybrid young on 29 June 1961. On 3 May 1963 she did so again, without there having been another mating, as the male had died a year and a half before. This happened in the Schönbrunn Zoo.
In 1940 Rahn succeeded in proving the presence of

live sperm in the body of a female *Thamnophis sirtalis*, still able to fertilize after a month, and in any case still motile after three months. Sperm storage has been proved further on in various other snake species.

There are still many questions to be answered about the biological advantages of amphigonia retardata. One would expect that sperm storage would occur with priority in species which live solitarily, but it has conclusively been proven to occur in many Lacertids, which live in groups, whereas the phenomenon has never been proven in, for example, Monitor lizards, which live solitary existences and theoretically would benefit more. Though maybe needless to say, it is important to realize that sperm storage is possible but not necessary. The normal pattern is, that a copulations or a series of copulations causes fertilization, after which eggs are laid or young are born without abnormal delay.
For the practice of breeding in captivity it means, though, that we should not jump to conclusions about gestation periods or about hybridization, especially when gestation seems to last unexpectedly long. As we shall see further on, there are still other problems. Some examples can be given here.
In 1975 the Zoological Museum of Leiden acquired two specimens of *Agkistrodon rhodostoma*. Both animals laid eggs in captivity from 1977 on, which were always infertile. In July 1981 fertilized eggs were laid, though, of which some hatched in September 1981. The authors who described this case, conclude 'that there has been sperm storage with *Agkistrodon rhodostoma* over a period of at least six years, whereafter fertile eggs were laid, from which - be it with some difficulties - viable young were able to hatch'. One could imagine that, if one of these females would have been kept to-

gether with a male of another (sub)species, it would have been very tempting to assume hybridization would have taken place! In the past, such false assumptions have indeed been made.
A second example: In Litteratura Serpentium Vol. 3 I described a hybrid breeding of *Thamnophis (radix) butleri* with *Thamnophis sirtalis parietalis* (Steehouder, 1983). Actually, only the fact that the young indeed had characteristics of both species, proved indeed this male had inseminated the female. Otherwise it would have been possible she had been inseminated before, in the wild, and that the sperm had been stored.
A third example. In Litteratura Serpentium Vol. 4 I described a successful breeding with *Psammophis subtaeniatus sudanensis*. Since there have been three clutches. Between all ovipositions, I observed copulations. For the sake of simplicity, I assumed that each time a new series of copulations led to a new fertilizaton. Yet, the number of fertile and viable eggs diminished (first clutch: 12 eggs, 11 hatched; second clutch: 11 eggs, 10 hatched; third clutch: 10 eggs and one delayed, 5 hatched), a phenomenon normal with amphigonia retardata. On the other hand, the second clutch was of better quality than the first - though this might be caused by the better incubation method I used. It is quite possible that especially the third clutch was a product of amphigonia retardata. You can only be sure if you separate the couple after the first fertilization (or directly after the first oviposition), thus preventing a new copulation.
Last example. In the spring of 1984 I acquired a female *Lampropeltis getulus floridana*, which after some time copulated with a male I already had in my possession. When eggs were laid (an excellent breeding, all hatched) I assumed that the observed copulations had led to them. Strictly taken, I could not really be sure about that.
We need not be too critical, though. The normal

procedure, as mentioned before, is that copulations lead to fertilizations which produce fertile eggs that hatch, in that order. Yet, what I wanted to point out, is that we should be cautious in drawing conclusions about hybridization, paternity and gestation periods.

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