Remarks on keeping European lacertids, egg incubation, and sex determination

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INTRODUCTION

Some time ago VAN WESTBROEK (2001) propagated in a Dutch vivarium journal a method for breeding European lizards that made it possible to have hatchlings around New Year. After this batch of "Christmas children", a second reproductive bout could follow in the Spring, yielding another bunch of new-borns. This procedure and recommendations of incubating lizards' eggs in VAN WESTBROEK (2002) (in the present case of Lacertid Lizards, Lacertidae), beside remarks on vitamin dosage, were peculiar. Surprisingly, no commentary was generated in response to VAN WESTBROEK's (2001, 2002) publications. This lack of response is odd because his procedure for soliciting two reproductive bouts is very unnatural as these lizards should be hibernating around Christmas, and his recommendations for incubating Lacertid eggs and his comments on vitamin dosage are both peculiar. Of course, everybody is allowed his own beliefs, but keeping animals is not a religious subject. In matters of vivarium keeping we prefer to work with verifiable methods which, when followed systematically, lead to comparable results. As keeping animals is not the same as following a recipe, some 'experiential' data/commentary is fine and can certainly be helpful, but this should be presented to the reader with substantiating proof to convey knowledge. Such is completely lacking in VAN WESTBROEK (2001, 2002).

This is particularly unfortunate because this method of an additional, artificially induced reproductive period is apparently used frequently in the former Eastern Germany (pers. com. Piet Mantel). Breeding is one of the most important aspects of the terrarium hobby, and evidently many misconceptions still circulate about the most basic conditions that should be complied with, especially during the incubation of the eggs. Variation in a number of important factors will have far reaching consequences. Therefore, this paper presents a brief overview of several of these parameters based on a combination of personal experience and the scientific literature. This will hopefully keep the serious amateur from unnecessary experimenting, and may at the same time increase breeding results without exhausting the adult animals or disrupting their annual rhythm, and thus – lastly – diminish the need for wild-caught terrarium animals.

VAN WESTBROEK (2001, 2002) bred *Algyroides nigropunctatus*, *Lacerta oxycephala* and *Podarcis melisellensis*, but extrapolated his experiences to "European *Lacerta*- and *Podarcis* species". My personal experience with over seventy *Lacerta* and *Podarcis* species, including the aforementioned forms, may allow me some founded annotations.

A two-day old *Lacerta oxycephala*.

Photo: H.A.J. in den Bosch

RESEARCH

During the last decennia, countless scientific publications have appeared that deal definitively with prejudices and half-truths that circulate in the hobby arena. Regrettably, the true vivarium enthusiast rarely takes the time to obtain information from this side of the spectrum. Considerable research has been generated on sex-determination in reptiles, more than the average reptile lover seems to realise (e.g. SPOTILA, SPOTILA & KAUFER, 1994; VIETS et al., 1994).

As in most vertebrates, in many reptiles the gender is determined at conception and the sex ratio is 1:1. This Genotypic Sex Determination (GSD) is sometimes called Chromosomal Sex Determination (CSD). This is seen in all mammals (including man), wherein the females are homogametic and have XX chromosomes while males are heterogametic and have XY. In reptiles (Lacertidae included) heterogamety is frequently found in the females (ZW), while the males are ZZ. However, in several lizard families, e.g. Pygopodidae, Iguanidae and Teiidae, the males constitute the heterogametic sex (GORMAN, 1973). The sex chromosomes of lizards are often microchromosomes and previously might have been overlooked in many of the species. Genotypic sex determination is expected in animals where sex chromosomes (as identified by heterogamety in one of the sexes) are present.

Not all reptiles have GSD. In quite a number of species – including all crocodilians, most turtles and some lizards, but not in snakes – the sexual differentiation of the gonads is sensitive to the incubation temperature during a critical period of embryonic development (literature citations in e.g. PIEAU, 1996). This phenomenon is called Temperature-dependent Sex Determination (TSD). The Tuatara, *Sphenodon* – the only surviving representative of the old order Sphenodontida – also has temperature-dependent sex determination (CREE, THOMPSON & DAUGHERTY, 1995; NELSON et al., 2002; NELSON, 2004; THOMPSON, 1990).

On a physiological level, sex hormones play a role as do enzymatic processes (the enzyme aromatase is frequently mentioned) in which androgens can be converted into estrogens; a thermosensitive factor intervenes, directly or indirectly, in the transcriptional regulation of the aromatase gene (PIEAU, 1996).

In *Sphenodon*, an ancient taxon, temperature-dependent sex determination is found.

Of course the distinction between environmental and genotypic sex determination is not absolute, because individuals living in a heterogeneous environment may have a genotypic mechanism that operates under some conditions but is subject to environmental control under others. For instance, in *Gekko japonicus* – a species that apparently has differentiated sex chromosomes (YOSHIDA & MSAHIRO, 1974) – TSD has also been determined (TOKUNGA, 1985).

However, in many species it has been established which sex-determining mechanism operates so that a well-founded choice of incubation method is possible; in those species playing around with several incubation techniques while ignoring the wealth of available information, will lead to suboptimal results and may waste time unnecessarily.

GENDER

It is regrettable that in hobby circles incorrect notions on sex determination in lizards, especially in the lacertids, which are bred widely in the Netherlands and Germany at this time, keep on circulating and are repeatedly falsely supported by papers with uncorroborated hypotheses like those of VAN WESTBROEK (2002). The reptile-lover that accepts these as truth is unnecessarily deceived into various experiments with different incubation temperatures and substrate types. These experiments are completely gratuitous as the basic data on temperature and/or genotypic dependent sex determination have been available for over a quarter century in most cases, and the rudiments of a proper technique to be used for egg incubation are well-known (summaries e.g. in IN DEN BOSCH, 1996; KÖHLER, 1997).

It is therefore lamentable that VAN WESTBROEK (2002) once again suggested that the gender of the embryo in the incubating lacertid egg can be influenced by the temperature: "…it seems that around 24.5°C … more female hatchlings appear". The critical reader would like to see this 'seeming' confirmed with data and a proper statistical test. It turns out that this is simply an incorrect assumption: in Lacertidae the sex is determined by genotype. These reptiles possess sex chromosomes; more or less similar to mammals and birds, which renders the temperature during incubation irrelevant.

Historically GORMAN & ATKINS (1966) and COLE, LOWE & WRIGHT (1967) were the first to determine sex chromosomes in a lizard (*Anolis* and *Sceloporus* respectively). The first discovery of sex chromosomes in a lacertid was in *Lacerta bilineata* by RAYNAUD & PIEAU (1972). More recently JI & BRAÑA (1999) again (previously demonstrated in VAN DAMME et al., 1992) confirmed clearly that in the lacertid *Podarcis muralis* incubation temperatures did not affect the sex ratio.

Naturally, Temperature-dependent Sex Determination is a system proven to be active in other reptiles including turtles (BULL & VOGT, 1979). In an older overview of sex determination in reptiles, BULL (1980) already mentioned that sex chromosomes – indicative of GSD – are commonly found in snakes and lizards (the group Squamata), but are apparently rare in turtles and are absent in crocodilians and the Tuatara, in which TSD is customary.

In reptiles that depend on TSD, the temperature to which the embryo is exposed

Emys orbicularis is an example of a species of turtle where sex determination is temperature-dependent.

Photo: H.A.J. in den Bosch

Copulation of *Podarcis pityusensis.*

Photo: H.A.J. in den Bosch

during a certain portion of the incubation period is essential. For most reptiles with TSD, the thermosensitive period is in the middle third to half of embryonic development (e.g. BULL & VOGT, 1981). At the so-called critical temperature, but more properly referred to as the pivotal range, an equal sex ratio is observed. High and low temperatures are relative notions; in the Tuatara these are in the range of 18-22°C, whereas in many other reptiles it falls between 25-33°C. Three types of TSD have been recognised (summary in DEEMING & FERGUSON, 1991). In type I females develop at higher temperatures than those which induce development of males (this can be seen in many turtle species). For type II, which is evident in some crocodilians – e.g. *Alligator mississippiensis* (see FERGUSON & JOANEN, 1983) – and a few lizards, e.g. *Eublepharis macularius*, male determining temperatures are higher than those are for females. For type III, females are produced at low and high temperatures, with males resulting at intermediate temperatures (e.g. *Gekko japonicus*, *Crocrodylus johnstoni*, *Chelydra serpentina*).

Theoretical intermediate states between GSD and TSD are feasible (PIEAU, 1996; SARRE, GEORGES & QUINN, 2004). In Lacertidae this intermediate state of sex determination is quite unlikely. This is corroborated by ODIERNA et al. (1993), who reported that sex chromosomes are present in all Lacertidae, and there has been no proper data series published to lead to any other hypothesis. Unfounded conclusions like those of VAN WESTBROEK (2002) lead to incorrect rumours in hobby circles that temperature is a gender-determining factor. There is, by the way, a report in Dutch hobby literature that seems to support the finding of VAN WESTBROEK (2002) mentioning that temperature is involved in sex determination in some lacertids: EICHENBERGER (1981) stated that when incubating eggs of *Podarcis pityusensis* at 29°C he obtained "1 male to 10-15 females". This piece of information has been cited in the international scientific literature (JANZEN & PAUKSTIS, 1991; VIETS et al., 1994) and as such has taken on a life of its own as a fact about Lacertidae. A more recent consultation with Paul Eichenberger revealed that at the time of original publication he believed there were only two males among the 25-30 offspring bred, as the remainder were brown in colour similar to the adult female of the breeding pair. Later he learned that males can look like females, notably when housed with really stocky males in the same enclosure. Many

Larva of *Pleurodeles waltl*; a species in which both temperature and genetics can play a role in sex determination.

Photo: H.A.J. in den Bosch

experienced lacertid keepers are familiar with this phenomenon. Such apparent females can obscure the issue of temperature-dependent sex determination.

My own experiences with the species using the same temperature (although with only 12 eggs) do not point to a skewed sex ratio. Nevertheless, Piet Mantel (pers. com.) suspects differently as "there were always just a few males in that species [*P. pityusensis*] (but these can be just purely accidental observations)". Michael Kroniger has been breeding the species for years and reports (in litt.) that while some clutches show a biased sex ratio, on average the sex ratio is 1:1. Also Nico Ross (pers. com.) is quite familiar with breeding *P. pityusensis pityusensis* and *P. pityusensis formenterae* and he states that in over 200 offspring, incubated at 29-30°C, the sex ratio was on average equal. In his experience, young animals are indeed difficult to sex purely morphologically, but the number of transversal rows of ventrals was significantly higher in females (≥27-29) than in males (≤25-27).

Temperature-dependent sex determination certainly exists in lizards. Actually, the first account of the phenomenon was by CHARNIER (1966) in *Agama agama*. To complicate matters, in an amphibian species the overriding of TSD in an animal that typically displays GSD has been demonstrated. Under the effect of heat treatment (30-32°C) ZW [=female] larvae of the amphibian *Pleurodeles waltl* (a species with ZZ/ZW genotypic sex determination) become phenotypic males (discussed in PIEAU, 1987). When heat-induced ZW neomales of *P. waltl* are crossed with ZW standard females, the sex ratio of the progeny is three females to one male. Some females of this offspring have a WW genotype. If they are bred with ZZ standard males, the offspring are 100% phenotypic males. It shows that GSD and TSD may co-exist in the same species, with the latter overriding the former. In reptiles under special circumstances, an environmental overriding of a weak ZW/ZZ sex determining mechanism was suspected (e.g. ZABORSKI, DORIZZI & PIEAU, 1988; for amphibians see e.g. WALLACE, BADAWY & WALLACE, 1999). What these facts illustrate, is that there is a need to keep an open mind, as new possibilities continually present themselves, but also that only carefully documented findings can really help increase our understanding of any new data that arises.

Reports (PACKARD, PACKARD & GUTZKE, 1985; PACKARD et al, 1987) that the water potential of the substrate affects sex determination (in e.g. the turtle *Chrysemys picta*, and the lizard *Podarcis muralis*) have been discredited (see PACKARD, PACKARD & BIRCHARD, 1989; JI & BRAÑA, 1999), as has the idea that the restriction of gas and water vapour conduction in alligator eggs affects the sex ratio (DEEMING & FERGUSON, 1991). On the 'why' of TSD and GSD and the advantages and disadvantages of both systems much has been written (there is a recent summary in SARRE, GEORGES & QUINN, 2004). These types of sex determining mechanisms should not be viewed as fundamentally different, but rather as opposite ends of a continuum. This is not the right place to elaborate on these aspects. The main factors to take into account are life span, age of maturity, social structure, and ecological conditions. As an example, FERGUSON & JOANEN's (1983) analysis of *Alligator mississippiensis* can be consulted.

In nature conservation and reintroduction programs, females are often considered more valuable because of their greater reproductive potential. It may consequently not be surprising that some Americans (Crews & Wibbels) patented (US 5201280 / 19930413) a 'Method for preferential production of female turtles, lizards, and crocodiles'. The inventors summarise their procedure as follows: "A method for sex reversal in reptiles and their derivatives comprising the steps of sterilising the surface of a fertilised egg; injecting the egg with a material that causes sex reversal at least before the first two-thirds of incubation of the egg has passed; and sealing any holes in the egg. In a preferred embodiment, the material that causes sex reversal includes natural estrogens or its synthetic mimics. In an alternative embodiment, the material is applied to the surface of the egg. By practising the aforesaid method, sex reversal in reptiles and their derivatives is accomplished and viable all female sexed hatchlings are capable of being produced thereby."

Water potential (kPa)	Incubation medium (dry) (g)	Water (g)
-150 (wet)	300 g vermiculite	337.5
-150 (wet)	4000 g sand	38.8
-200 (wet)	300 g Perlite	150.0
-550 (average)	300 g vermiculite	84.5
-550 (average)	4000 g sand	28.0
-800 (dry)	300 g vermiculite	56.5
-950 (dry)	300 g vermiculite	51.2
-950 (dry)	4000 g sand	24.0

Table. The table illustrates the quantities of water and incubating substrate needed in combination to attain different humidity levels. The recommendations are based on a temperature of approximately 29°C in a closed box (after PACKARD, PACKARD & GUTZKE (1985) and PACKARD et al. (1987)). The vermiculite used is by Terra Lite and has a grain size of 3; the sand is coarse river sand.

INCUBATION

It is sad that authors like Van Westbroek (2002) did not search for more information and/or were not supplied with adequate background literature by their editorial board. These writers consequently take their own limited experience as a standard, in this case concerning incubation techniques. Neither the hobby literature on incubating lacertid eggs published in Dutch in the same periodical (e.g. In den Bosch, 1996), nor a well-known book (Köhler, 1997) was consulted.

Had this been done, it would have become clear that in essence it is quite irrelevant which incubating medium is used or in what kind of incubator the eggs are deposited. It is all about humidity and temperature within reasonable bounds. The rest is extraneous. Indeed, it is difficult to measure humidity in terms of water potential (in kPa). That is why e.g. In den Bosch (1996) presented a table (reprinted here) that mentioned the combination of specific amounts of substrate and water needed to obtain the correct humidity. The same applies to substrates as sand and vermiculite, both of which Van Westbroek (2002) rejected. There is at first sight nothing inherently wrong with that author's final choice of cactus potting soil as his incubating medium, but his reasoning for discarding other possibilities is less than well-founded. None of the problems encountered by Van Westbroek can be traced back to the material used, as it is more likely personal errors are responsible. In addition, the large-scale aspect, which seems to fascinate the author, turned up again: one can stack the eggs in the cactus potting soil so that more eggs can be incubated in the same incubator. There is no overview, which means that a rotten egg could contaminate the rest, and possible problems during hatching can be missed. The reader is interested in knowing more about this cactus soil. What fractions is it composed of? Is fertiliser added? What is the pH? Is the eggshell affected in any manner? How much water is added to maintain the proper humidity levels?

Alligator mississippiensis hatching.

Photo: www.gatorland.com

It is worth reminding ourselves that in nature lizard eggs are not shielded from outside influences. One can be of the opinion that in common potting soil eggs discolour to "ugly brown" (Van Westbroek, 2002), but whether this is really a bad thing, is a matter of dispute. It is a familiar fact that crocodile eggs fail to hatch, or hatch with much difficulty, when incubated under very hygienic circumstances (Ferguson, 1981c; McCartney, 1990). Why does this occur? In nature, in the nesting mounds constructed by the female, a certain acidity develops when the plant material disintegrates causing the egg shell to thin from the outside (Ferguson, 1981a, b; 1982). Ferguson (l.c.) described this as follows. As incubation progresses, the outer, densely calcified layer of the alligator eggshell shows progressive crystal dissolution with the production of concentrically stepped erosion craters. This dissolution is caused by the acidic metabolic by-products of nest bacteria. Extrinsic degradation serves to gradually increase the porosity and decrease the strength of the eggshell. If the decomposition of the outer layer is prevented, then the full-grown embryo is virtually imprisoned (Ferguson, 1981c) and will die.

In many species of Lacertidae whose eggs I incubated on Perlon aquarium filter material – pH 6.5-7.0 – the problem of a too thick outer eggshell in the last phase of incubation never occurred. This does not mean that in other reptiles such a contingency should be disregarded. A good example would be the Dutch Grass Snake *Natrix natrix* that likes to oviposit in dunghills and compost heaps. In general, one would expect a lower pH under more anaerobic circumstances, thus in species that make nests or use decomposing matter in some form. Most reptiles do not do this, so a neutral pH would be expected where they deposit their eggs.

To prevent tipping the scale to the other side – using an acid incubating environment for arbitrary species – it is good to know that the embryo uses all of the nutrients present in the egg optimally. This is also true for the egg shell, in which the calcium present not only increases the strength of the outer casing, but is also used by the developing embryo in a complex phasing (e.g. FERGUSON, 1982; JI et al. 1997; SAHOO et al., 1998). The calcium left in the eggshell during the last days of incubation, is transported to the remainder of the yolk and can be used by the young reptile soon after hatching (e.g. DEEMING & FERGUSON, 1991; PACKARD & PACKARD, 1989). An artificially weakened eggshell can thus have a disastrous influence on both the embryo and the hatchling.

VITAMINS

For all animals, but certainly for Lacertidae in terraria, a well-balanced supplement of vitamins and minerals in the food is essential. These basics have been known for years and there is little or no need to deviate from proven, perfectly functioning dosages. Nevertheless VAN WESTBROEK (2002) advised "vitamin AD aquosum, 50.000 and 10.000 IU/ml respectively, 1 ml added to 1 litre water". Disregarding the fact that he should have explicitly mentioned vitamin D_3 (the other D vitamins have no effect at all in the calcium metabolism: see for instance MANTEL, 1994), this is an overly large dosage of vitamin A (with a large risk of hypervitaminosis, resulting in – among other problems – an excessive production of skin cells) and relatively little vitamin D_3 since for egg bearing females we prefer 20.000 IU (see: How to keep… European Lacertids http://www.podarcis.nl). The addition of calcium lactate by VAN WESTBROEK (2002) is a good idea, although the dosage could be specified with more clarity and greater detail than "a teaspoon with a head in a beer glass of water", and it should be mentioned that calcium and the other necessary minerals can be provided in other ways (IN DEN BOSCH & KLAASSE, 1998).

NATURE - TERRARIUM

Keeping animals in a vivarium creates an unnatural situation: however much one tries, it is just an approximation of what happens in the wild. Most vivarium enthusiasts nevertheless strive for an approach that most closely resembles the natural rhythm of their animals. Thus I was rather surprised that apparently nobody was disturbed by the by VAN WESTBROEK (2001) propagated double reproductive periods, whereby he deceived his European lizards by inducing a short and very early hibernation that led to an extra oviposition episode in November-December. This is an absurd situation that puts unnecessary stress on the animals. His own casual remarks about "strange behaviour", "pregnant females and brightly coloured males at the end of September", "many eggs are unfertilised" clearly prove this. Besides, the value of this additional breeding effort is extremely dubious, unless one intends to walk the path of intensive ranching where the yield should be as high as possible.


A Canary Island lacertid, *Gallotia galloti eisentrauti*, hatching.

Photo: H.A.J. in den Bosch

Everyone who is familiar with the reptile scene, including Van Westbroek, is aware that the market for these small lizards is not large and has been close to saturation for years. Thus high numbers of offspring are not necessary, and ethically speaking are even unwanted. This not only goes for The Netherlands, but applies abroad as well. Even in Germany, which has one of the largest herpetological societies in the world (Salamandra, with over 8000 members), the species bred by VAN WESTBROEK (2001) are very difficult to dispose of. Sure, at commercial events wild-caught specimens exchange hands readily, but we are talking here about serious reptile keepers, who

Tail-biting in *Algyroides nigropunctatus*: an introduction to mating.

Photo: H.A.J. in den Bosch

already have what they want and/or have friends who breed when requested so that getting rid of lacertid offspring can be very difficult.

By the way, it would have been correct of VAN WESTBROEK (2001) when he had referred to EICHENBERGER (1981) who twenty years earlier, in a more thoughtful way, employed the same method of breeding in winter. In this article Eichenberger used the same method of breeding in winter as VAN WESTBROEK (2001), but he quickly realised that a large surplus of lizards was created by this approach, and subsequently released the offspring at the locality of their parents and ceased using the method.

CONCLUSION

It continues to be a remarkable fact that experienced lacertid breeders now and then report skewed sex ratios. How to interpret these statements remains a difficult question to answer. As described above, apparent females (when trying to determine the sex of juvenile animals) may thoroughly disturb the picture. Rarely does the amateur keeper do a more thorough morphological or chromosomal examination. Quite often, this is impossible to do at a later date when the offspring is housed elsewhere with fellow-enthusiasts. The repeatability of the incubation circumstances can be another weak point, as not all of the thermometers used are accurate; an aberration of 2-3°C is not uncommon. Sometimes a perfectly good thermometer is available, but then the incubator is placed in an environment that is so warm or cold that the aimed for temperature is only reached during a part of the season. Prudence is called for in interpreting the data offered by hobby herpetologists.

As argued before (IN DEN BOSCH, 1996), and backed up with a considerable amount of data in the scientific literature, it is recommended to incubate flexible, parchment-like reptile eggs like those of the Lacertidae, in fairly humid (-400/-150 kPa) environment at reasonably high (up to 30°C) temperatures, and a pH of 6.5-7. In fact, normal room temperature is alright too – in some species an even lower temperature is no problem – but my experience and that of other researchers (e.g. VAN DAMME et al., 1992) suggest that the temperature range of 25-30°C is most favourable. There are no indications that temperature or humidity influences the sex of the embryo in lacertids. Reproduction in those lizards should be confined to the single yearly natural (albeit quite possibly with multiple ovipositions) breeding period.

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SUMMARY

In response to two previously published accounts, it is pointed out that successful incubation of lizard eggs does not primarily depend on the medium on or in which the eggs rest, nor on the incubator. Successful incubation is solely based on the two physical variables of humidity (measured as water potential in kPa) and temperature. When these variables are within the proper range (-400/-150 kPa and 25-30°C) then the chief criteria are met. The inaccurate suggestion that the sex of lacertids is related to incubation temperature is rejected because the gender of the young in this group of reptiles is determined genetically. The single report cited in the scientific literature that discussed TSD in a lacertid (*Podarcis pityusensis*) is refuted by considering larger samples of incubated eggs. Similarly, reports that water potential of the

substrate affects sex determination have also been discredited in the scientific literature.

The sometimes in hobby literature propagated short, artificially induced additional hibernation soon after the summer to bring on an additional reproductive period with egg production in November/December, is objectionable. The stress on the animals is such that the health of the lizards is compromised. Moreover, among serious reptile enthusiasts there is only a limited demand for lacertids, discounting the need for so many offspring.

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