

First record of mating plugs in lizards

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During ongoing research into the courtship behaviour of European lizards in the family Lacertidae (In den Bosch, 1986, 1990) I noticed that more or less recently mated females sometimes produced a faecal pellet consisting not only of the usual brown faecal matter and white nitrogenous waste, but also an additional transparent element. The latter was occasionally also the sole discharge.

In its simplest form this element was Y-shaped, with the lower part shortened and pointing posteriorly (assuming it usually lies in the female genital sinus). The upper bifurcated part was thickened distally, each component pointing somewhat lateroanteriorly (fig. 1). More complex configurations were sometimes found, with flanges and small appendages. It was typically a few millimetres in length and is considered to be a mating plug.

Mating plugs of many sorts have long been known in insects (Parker, 1970), and are also found in mammals (Martan and Sheperd, 1976), but they have not been reported in amphibians or birds. Removal of sperm in birds, however, has been documented (Davies, 1983). Copulatory plugs in reptiles seem to be extremely rare (Whittier and Tokarz, 1992); they have been mentioned only in a few snake species, either as a gelatinous deposit (Devine, 1975) or as an oviducal constriction, as in the adder, *Vipera berus* (Nilson and Andrén, 1982), although the latter may simply serve to decrease sperm loss (Stille et al., 1986). Judging from my observations it appears that the existence of such a plug is more rule than exception in lizards of the West Palearctic Lacertidae; they have been found in the following 36 species: *Algyroides marchi*, *A. moreoticus*, *A. nigropunctatus*; *Eremias arguta*; *Gallotia atlantica*; *Lacerta agilis*, *L. anatolica*, *L. andreanszkyi*, *L. cappadocica*, *L. caucasica*, *L. derjugini*, *L. graeca*, *L. horvathi*, *L. laevis*, *L. monticola*, *L. mosorensis*, *L. nairensis*, *L. oertzeni*, *L. parvula*, *L. praticola*, *L. rudis*, *L. schreiberi*, *L. strigata*, *L. valentini*, *L. viridis*; *Ophisops elegans*; *Podarcis bocagei*, *P. erhardii*, *P. filfolensis*, *P. hispanica*, *P. lilfordi*, *P. muralis*, *P. perspicillata*, *P. tiliguerta*, *P. wagleriana* and *Psammodromus blanci*.

The plugs obviously form a barrier to sperm, either blocking the progress of incoming sperm from a new copulation, or preventing sperm from a previous copulation travelling in the wrong direction. Other effects could include decreased receptivity, by chemical or physical means. The copulatory plug is just one of a series of phenomena I have found in these lizards indicative of female guarding; the majority of such tactics in European lizards seems to be of an ethological nature. All are being dealt with in a review (In den Bosch, in preparation).

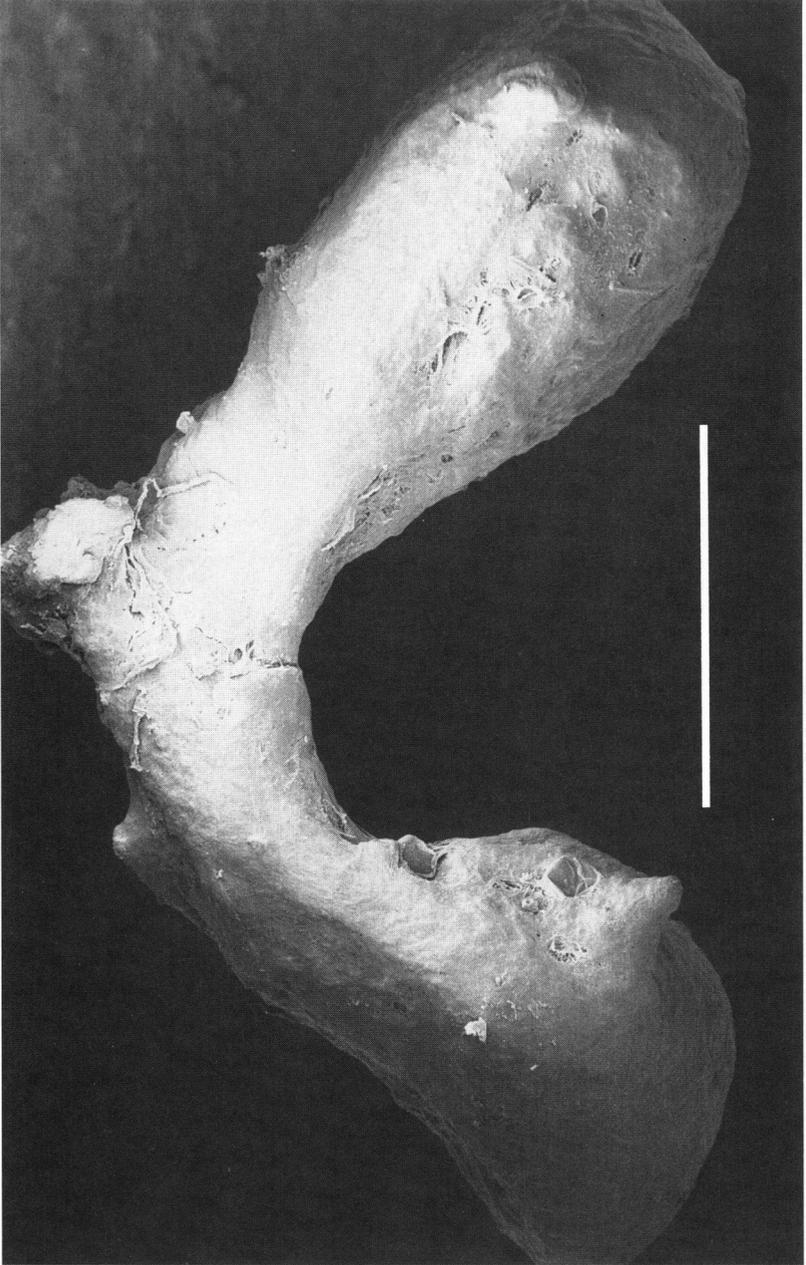


Figure 1. Copulatory plug of *Lacerta horvathi*. The white bar represents one millimetre.

Initially I presumed that the element contained sperm, and so serial sections were cut, and stained with haematoxylin-eosin. No clear sperm aggregations were found on the surface of expelled plugs or within them. A few plugs examined under the scanning electron microscope (fig. 1) had an exterior that looked almost polished. No special surface structures were noticed. The few cracks were probably caused by handling and fixation. The plug appears to be a perfect mould of the female genital sinus, including the exits of the uteri, represented by the small nipple on each of the horns.

Some males were seen to deposit these gelatinous elements on the substrate, sometimes while defaecating. No especial behaviour was associated with this and deposition could occur either in the presence or absence of females. Deposition did not seem to have any influence on females or on their later performance. Thus the plugs may be prefabricated, at least in some species, by the male, and the observation would indicate that the plugs are regularly formed anew. Such a preformation in the male would be remarkably unlike the procedure in rodents (Matthews and Adler, 1977) where the plug is a coagulation of seminal fluids in the vaginal-cervical junction. It remains to be determined if these are really the same in detail as plugs ejected by females, or just an overflow from active glands.

However, another explanation is that the diverse structures (ornamentation, plicae, spines, terminal awns) found on the hemipenis are not without a direct function as is believed (Böhme, 1971, 1988), but that they play a role in the removal of previously inserted, smooth plugs. If this is the case then hemipenes do not have a single function, as is often assumed (Arnold, 1986), and so their use in taxonomy must be re-evaluated. Spines have been implicated in the removal of plugs in the Bank vole (*Clethrionomys glareolus*) in combination with successive intromissions (Milligan, 1979). The eversible structure of the squamate hemipenis may make an ideal suction plunger. If the previous plug is removed and not immediately expelled by the male, (which procedure I have observed in *Lacerta agilis*), it has to be stored somewhere. This is most likely to be in the retracted hemipenis, or in the cloaca. Since the hemipenes are paired, one could carry out the removal, the other the actual insemination. My observations on double copulations which often occur during one session in *Lacerta cappadocica*, *L. danfordi* and *L. oertzeni* groups in which the male first copulates on one side of the female, then on the other side, could support this hypothesis. The first "copulation" would serve to remove the plug, the second would be the proper copulation. Alternatively, the first intromission could be for sperm transfer and if the male actually does prefabricate his plugs, the second could be the plug insertion (Here one has to remember that such a prefabricated plug would initially point the wrong way and would have to be turned around).

Some South African *Pedioplanis* species show hard awns at the tips of the hemipenial lobes (Arnold, 1991). These may allow the lizards to penetrate plugs rather than to extract them.

In the West Palearctic Lacertidae, in which in my experience a female commonly mates more than once and often with more than one male, the removal of a previously deposited plug would certainly be of selective advantage to a male. The alternation of

hemipenis use in *Anolis* in successive copulations with a longer interval (1-24 hours) has been considered (Tokarz and Slowinski, 1990) to be a behavioural means to increase sperm transfer. This is different strategy from the double intromissions in *L. cappadocica*, *L. danfordi* and *L. oertzeni*.

The period of time for which plugs remain in the female is variable. Some females excrete the plug almost immediately (with or without faeces) after copulation, others do so later, after as much as 24 h. This is much shorter than the period in gartersnakes (*Thamnophis*), which may be as long as two weeks (Devine, 1984). Defecation does not necessarily mean that the lizard plug is also discharged; this can happen later. When no plug is found some time afterwards, this of course does not mean that there was none: beside the restriction of the posterior uteri as recorded in *Vipera berus*, it could have been resorbed, though my impression is that in these lizards the plug is commonly excreted. Sometimes male lizards discharge a tiny, shrivelled, opaque, thread-like "plug" with two bulbous ends. These are the exuviae of hemipenes, shed independently from the general moult.

Further investigations will be necessary to show whether the time for which a plug remains inside a female is individually determined or more species dependent (as is my understanding now). Factors such as temperature, insolation and food intake appear to have some influence.

Detailed morphological and physiological studies will be necessary to elucidate the relationships between the plug and the female genital tract, the actual sperm transfer and the time required to achieve fertilization. It also remains to be determined exactly how and where the plug is formed, and of what material it is constructed. It was reported in snakes that the plug is formed from male kidney secretions (Devine, 1975), and seminal fluid is thought to be the main component in rodents (Martan and Sheperd, 1976). As snakes and lizards are closely related, they might be expected to share the same mechanism of plug production, with secretions from the sexual segment of the male kidney running along the sulcus of the hemipenis after the semen has passed and slowly replacing the lobes of the hemipenis as these are drawn back into the hemipenial stem.

Acknowledgement. Discussions with Dr. E.N. Arnold (Natural History Museum, London) were highly appreciated.

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Received: October 28, 1993. Accepted: November 12, 1993.

Diel variation in thermoregulatory set points of the lizard *Podarcis muralis*

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Diel cycles of temperature preference in lizards have been reported by several workers (e.g. Rismiller and Heldmaier, 1987; Sievert and Hutchison, 1989), and may be endogenous (Cowgell and Underwood, 1979; Innocenti et al., 1993). The experiments have usually been performed in the artificial conditions of laboratory gradients. The development of a system using a pyroelectric vidicon infra-red camera as a radiation thermometer to measure body temperatures (T_b s) non-invasively (Jones and Avery, 1989) has made it possible to determine values for both lower and upper set point temperatures (Firth and Turner, 1982) in unrestrained lizards which can move freely about an arena. Under these conditions the animals can utilise a normal range of thermoregulatory