Male lizards change their genital skin almost every day: squamate pan-epidermal synchrony refuted

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Abstract: Lacertid lizards frequently shed the outer layers of the epidermis of the paired male intromittent organs, the hemipenes. The hemipenial sloughs so formed are saclike and reproduce all the external features of the hemipenes in great detail. Sloughs are deposited immediately after the first defecation of the day and are produced almost every morning during the breeding season. I have observed these in 48 lacertid species, including members of the genera *Acanthodactylus*, *Adolfus*, *Algyroides*, *Gallotia*, *Holaspis*, *Lacerta* s.l., *Latastia*, *Podarcis*, *Psammodromus*, and *Takydromus*. As these forms represent all three subfamilies of the Lacertidae, the phenomenon is likely to have been present in the common ancestor of the family. It may even be a general squamate feature, as it has also been observed in the distantly related varanid *Varanus acanthurus*. Frequent shedding of hemipenial sloughs violates pan-epidermal synchrony, the belief that the epidermis of squamates is shed simultaneously over all parts of the body, and indicates that the hemipenial epidermis is under different hormonal control from the rest of the skin. The function of frequent shedding of the hemipenial epidermis is not known for certain, but there is a wide range of possible hypotheses. Some of these are involved in increasing the efficiency of copulation and ameliorating its costs, while others emphasise the possible signalling function of the hemipenial sloughs.

Résumé : Les lézards lacertidés rejettent fréquemment les couches externes de l'épiderme de leurs organes d'intromission, les hémipénis. La peau rejetée d'un hémipénis forme une poche sur laquelle se dessinent en détails toutes les structures externes de l'hémipénis. Cette peau est rejetée immédiatement après la première défécation de la journée, presque chaque matin pendant la saison de la reproduction. J'ai observé ce phénomène chez 48 espèces de lacertidés appartenant, entre autres, aux genres *Acanthodactylus*, *Adolfus*, *Algyroides*, *Gallotia*, *Holaspis*, *Lacerta* s.l., *Latastia*, *Podarcis*, *Psammodromus* et *Takydromus*. Ces formes représentent les trois sous-familles de Lacertidae et on peut supposer que le phénomène existait déjà chez l'ancêtre commun de la famille. Il est même possible qu'il s'agisse d'un caractère général des squamates puisqu'il a déjà été observé chez un lézard de parenté éloignée, le varan *Varanus acanthurus*. Le rejet fréquent de la peau des hémipénis viole le concept du synchronisme pan-épidermique qui veut que l'épiderme des squamates soit rejeté simultanément sur toutes les parties du corps et indique que l'épiderme des hémipénis est sous un contrôle hormonal indépendant du reste de la peau. Le rôle du rejet de l'épiderme des hémipénis n'est pas certain, mais donne lieu à plusieurs hypothèses; certaines supposent qu'il améliore l'efficacité de la copulation et conduit à une diminution de ses coûts, d'autres y reconnaissent une fonction de signalisation.

[Traduit par la Rédaction]

Introduction

In squamates (lizards and snakes), the translucent external layer of the epidermis that covers the body externally is shed in its entirety. Such shedding occurs at quite long intervals varying from several days to several months. Between these events the stratum germinativum, the tissue layer that generates the cells which eventually metamorphose into the outer epidermis, first remains fairly static (the resting phase) and later produces the new epithelium that eventually replaces the old one lying above it (the generative phase). Shedding, resting, and generation all occur at the same times right across the external skin and this has been termed pan-epidermal synchrony (Maderson 1984). A possible exception to this synchrony are mite pockets, invaginations of lizard skin that house mites. These often shed epidermis more frequently than the rest of the body surface, although this might be regarded as a wound reaction (Arnold 1986*b*).

Here I report a much more striking exception involving the skin covering the male intromittent organs of lizards, the hemipenes. Although these are housed within the body when not in use, their external tissues are formed from ectoderm and have an epidermis which is continuous with that of the body surface. Unlike that on the rest of the body, the outer layer of the epidermis of the hemipenis is shed extremely frequently during the breeding season, often every day. I first observed this phenomenon during extensive studies of courtship in lacertid lizards (for instance, see in den Bosch and Zandee 2001).

Material and methods

Lacertid species observed are listed in Table 1. Primary observations were made on lacertids from the West Palearctic region, in-

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Table 1. Lacertid species in which frequent shedding of the hemipenial epidermis has been observed.

Galliotiinae
Gallotia atlantica, G. galloti
Psammodromus algirus, P. blanci, P. hispanicus
Lacertinae
Adolfus jacksonii
Algyroides fitzingeri, A. marchi, A. moreoticus
Holaspis guentheri laevis
Lacerta s.l.: *Lacerta anatolica anatolica, L. aurelioi, Lacerta bedriagae ferrerae, L. bonnali, L. brandtii, L. chlorogaster, L. clarkorum, L. cyreni, Lacerta danfordi danfordi, L. d. bileki, L. derjugini, L. fraasii, L. horvathi,* all (seven known) *L.* cf. *kulzeri* forms, *Lacerta laevis laevis, L. l. troodica, L. mosorensis, L. nairensis, Lacerta oertzeni ibrahimi, L. o. pelasgiana, L. parva, L. parvula, L. praticola, Lacerta saxicola* cf. *brauneri, L. schreiberi, Lacerta valentini lantzicyreni*
Latastia longicaudata
Omanosaura cyanura
Ophisops elegans
Podarcis bocagei, P. erhardii, P. filfolensis, P. gaigeae, P. lilfordi, P. milensis, P. muralis, P. peloponnesiaca, P. pityusensis, P. wagleriana
Takydromus septentrionalis
Teira andreanszkyi, T. perspicillata
Timon princeps princeps
Eremiainae
Acanthodactylus erythrurus, A. tristrami

cluding Europe and the Mediterranean area. These are essentially temperate places, where lizards usually hibernate in the winter. The forms concerned were members of the genera *Algyroides*, *Gallotia*, *Lacerta* s.l., *Podarcis*, and *Psammodromus*, which were observed methodically every day during the spring and summer and their behaviour was frequently recorded using video. A wide range of other lacertids, including members of the genera *Acanthodactylus*, *Adolfus*, *Holaspis*, *Latastia*, and *Takydromus*, were also examined, although less intensively. Commonly, 5–10 males of each species were included in the study; in rare cases with uncommon species (e.g., *Timon princeps*) only 1 male was available.

Secreted blobs were carefully lifted from the substratum immediately after deposition or soaked off the substratum with water when they had dried. They were then placed in water (or later in Ringer's fluid) and gently spread with dissecting needles under a low-power binocular microscope.

Results

Initial observations

When lacertids emerge from their retreats, both in the field and in the terrarium, they typically bask for a time before beginning to their usual activities of hunting and interacting with other lizards. At the end of the initial basking period or soon after it, most individuals defecate. In both sexes the white uric acid from the kidneys is discharged first, immediately followed by the usually brown faecal matter from the gut. Although this was apparently not previously noticed, a further evacuation occurs very frequently in males, consisting of a seemingly slimy blob of matter that is opalescent whitish to transparent. Blobs vary from about 1 mm in diameter in smaller lacertid species (with snout to vent lengths of 50–80 mm) to 2–3 mm in bigger ones (up to 145 mm from snout to vent). As the blob is being deposited, males usually press the cloaca against the substratum and take a few steps forward. This movement stretches the blob into a more elongate shape and it rapidly adheres to the substratum, where it quickly dries. Typically this transformed blob is flat and V-shaped with the two arms of the "V" directed more or less posteriorly and the base of the V pointing towards the head of the lizard.

Composition of the blob

Under a low-power binocular microscope it was clear that the main component of the blob is the shed outer epidermis of both hemipenes, joined together at their bases, in a slightly viscous fluid. When this hemipenial slough is pressed onto the substratum by the lizard, it is the two hemipenes that form the arms of the V. The slough is several cells thick and shows all the external features of the hemipenes themselves, including the gutter-like sulcus spermaticus of each, the regular plicae that are usually present on their lobes, and their micro-ornamentation of minute spines or crown-shaped tubercles (for an explanation of these terms see Wöpke 1930; Böhme 1971; Arnold 1973, 1986*a*). The proximal border of the hemipenial slough, where it was detached from the rest of the epidermis, is smooth and more or less straight-edged, indicating that there is probably a preformed zone of weakness that permits the slough to separate easily (Fig. 1).

Frequency and timing of hemipenial-skin shedding

During intensive observation of captive lizards that had previously hibernated, hemipenial sloughs were produced by most males almost daily from March to June or even July, covering the usual courtship and breeding season of the species concerned. Similar frequent hemipenial skin shedding was also observed in wild animals during this period, specifically in *Lacerta laevis* and members of the *Lacerta kulzeri* complex, but also in, for example, *Lacerta fraasii* and *Ophisops elegans*, which were observed in Lebanon in the early summer of 1993–1999. Over 50 such events were seen in the field, involving almost every male encountered defecating in the early morning. Literally hundreds of such acts were witnessed in the laboratory.

General shedding commonly occurs once in the weeks after hibernation, then roughly 1–3 times during the rest of the year. Hemipenial ecdysis independent of the general shedding

Fig. 1. Hemipenial slough from a hemipenis of *Omanosaura cyanura* (Arnold, 1972). The paired structure of the organ is clearly visible. Details of the plicate surface of the two lobes of each hemipenis, the sulcus spermaticus, and the outer lip of the sulcal branch are easily discerned. The straight edge of the exuvium near the basal stalk indicates a preformed tear zone (photograph by H.A.J. in den Bosch).

of the body skin occurs, albeit infrequently, also in all months outside the breeding season that are not spent in hibernation, from February to October. The phenomenon does not occur during hibernation itself. The first defecation after emergence in the spring is usually aberrant, no or very little white uric acid being produced and the faecal matter often being greenish and not followed by a hemipenial slough. The first slough is observed 8 days after emergence from hibernation (in *Lacerta brandtii*).

No details are available as to when the production of hemipenial sloughs begins during ontogeny, but males of at least some European species of *Algyroides*, *Podarcis*, and *Lacerta* s.l. (*A. marchi*, *P. filfolensis*, *P. gaigeae*, *P. milensis*, *L. brandtii*, and several *L.* cf. *kulzeri* forms) produce them in their first spring. At this time the lizards are less than 1 year old and not full-grown, and some are not yet sexually active.

Other aspects of the production of hemipenial sloughs

The whole procedure of depositing a hemipenial slough usually takes only a few seconds and rarely occurs outside the context of defecation. It also nearly always takes place without the hemipenes being everted, although hemipenial protrusion can occur outside the context of copulation. However, a male may exceptionally raise the hindquarters by extending the hind legs, and then evert one or both hemipenes for a few seconds. During retraction, these may be rubbed on the substratum so that a hemipenial slough is deposited. Usually such atypical deposition is not preceded by defecation, although this has been seen in a single *L. brandtii*.

Although female lizards may excrete mating plugs (in den Bosch 1994), there is no evidence that they regularly deposit the epidermal lining of their genital sinus in a manner analogous to the production of hemipenial sloughs in males.

Taxonomic distribution of frequent hemipenial-skin shedding

The frequent production of hemipenial sloughs is extremely widespread in the Lacertidae (Table 1). It occurs in members of all three subfamilies, indicating that it was likely to have been present in the common ancestor of all modern lacertids. The phenomenon is found in species from all climates and most habitats inhabited by the family.

No systematic search for frequent hemipenial-skin shedding has been made in other squamates, but production of a hemipenial slough after defecation was observed in the varanid lizard *Varanus acanthurus* (a captive specimen from offspring originally from Mount Isa, Queensland, Australia, living in Rotterdam Zoo).

Discussion

The very frequent shedding of the outer layers of the hemipenial epidermis reported here refutes the widely held assumption that skin shedding is synchronised across all areas of the body, as postulated by Maderson (1984). It also suggests that shedding of most of the epidermis of the body and that of the hemipenes takes place under different mechanisms of hormonal control, and although shedding of the outer layers of the hemipenial epidermis has been reported before, it was in association with shedding of the skin on the rest of the body. For instance, this has been observed in the species of the iguanid genus *Liolaemus*, where it was associated with active protrusion of the hemipenes (W. Böhme, personal observation in Köster and Böhme 1975), something that is characteristically absent in the production of isolated hemipenial sloughs. The shed outer epidermis of the gecko *Oedura monilis*, illustrated by Rösler (1980, 1995), shows an attached hemipenis slough, although the author states that these are usually missing in such body sloughs. Other examples of geckoes and monitor lizards were given by Ziegler and Böhme (1997), who mentioned in passing the (conceivably oldest) record of hemipenial shedding with everted organs (of *Zootoca vivipara*; Leydig 1872).

It is surprising that the regular production of hemipenial sloughs by lizards has not been recognised earlier for what it is, although veterinarians may have inadvertently described sloughs ("accumulations of desquamated cornified epithelial cells … the exact origin is not known"; Zwart 1992*a*, cited in Zwart 1992*b*) in the cavity of retracted hemipenes of snakes and lizards. In captivity such tissue may evidently sometimes accumulate to a pathological extent (for example, see Köhler 1996). Likewise, "smegma" found in *Podarcis sicula* by Furieri (1962) and "seminal plugs" (Ross and Marzec 1990) in giant snakes containing remnants of shed skin may indicate compressed hemipenial sloughs in the lumen of the retracted, inverted organs. Various forms of cloacal rubbing in lizards and snakes have been described, with interpretations diverse as masturbation to achieve more rapid sperm transfer to cleaning the sulcus spermaticus (see examples in Mason 1992; Olsson and Mader 1998), and it could well be that some actually apply to hemipenis shedding. The late discovery of the very frequent production of hemipenial sloughs underlines the continuing importance of detailed observation of living animals.

The production of hemipenial sloughs by animals in the field (see above) confirms that this is not a pathological artefact of captivity. Its frequent occurrence in a wide range of taxa strongly suggests that it has some functional importance, and a wide range of hypotheses can be generated about its utility. Regular shedding may ensure that the surface of the hemipenes is kept pristine, at least during the mating season. This may have the advantages of keeping the hemipenial surface free of debris and producing fresh microornamentation that is not worn or damaged. Possibly this enables the spines or tubercles that constitute the microornamentation to adhere better to the inside of the female genital sinus. Shedding of the hemipenial surface may reduce the chances of infection by bacteria, viruses, and larger parasites during copulation.

Another possibility is that deposited hemipenial sloughs have a communication function. The whitish "V" shape of the dried slough on the substratum is quite conspicuous and may provide a visual and perhaps an olfactory signal to other lizards that a male is present. This may be analogous

to marking parts of the home range with the secretion of the femoral pores, which strongly absorbs ultraviolet light and so is visible to conspecifics (*Dipsosaurus dorsalis*; Alberts 1989). The various hypotheses about the function of frequent shedding of the outer layers of the hemipenial epidermis will be discussed further elsewhere. For now these theories should provide an intriguing starting point for further study by herpetologists as well as physiological and behavioural ecologists.

The presence of the phenomenon in members of two lizard families as distantly related as lacertids and varanids raises the possibility that frequent hemipenial-skin shedding is a phenomenon characteristic of squamates in general, including snakes, all the more so since the paired copulatory organs are considered to be one of the most important synapomorphies of the order Squamata (e.g., Dowling and Duellman 1978).

The hemipenes of squamates are used extensively as a source of characters in systematic research. This is especially true for snakes but applies to lizards as well. Lacertid lizards, for instance, may exhibit substantial differences in hemipenial shape and symmetry between even close relatives, and the pattern of epidermal micro-ornamentation is also significant in their taxonomy (Klemmer 1957; Böhme 1971, 1993; Arnold 1973, 1986*a*). Hemipenial sloughs allow such features to be checked non-invasively in living lizards. Micro-ornamentation, for instance, is not always fully developed when examined by dissecting preserved animals. If this is found to be the case with hemipenial sloughs from living individuals, later sloughs may show the mature condition. Such monitoring can also be important in assessing the sexual state of lizards, both in captivity and in the field.

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