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Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia, Anura, Hyperoliidae) to its arid environment

I. The skin of *Hyperolius viridiflavus nitidulus* in wet and dry season conditions

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Summary. *Hyperolius viridiflavus nitidulus* inhabits parts of the seasonally very hot and dry West African savanna. During the long lasting dry season, the small frog is sitting unhidden on mostly dry plants and has to deal with high solar radiation load (SRL), evaporative water loss (EWL) and small energy reserves. It seems to be very badly equipped to survive such harsh climatic conditions (unfavorable surface to volume ratio, very limited capacity to store energy and water). Therefore, it must have developed extraordinary efficient mechanisms to solve the mentioned problems. Some of these mechanisms are to be looked for within the skin of the animal (e.g. protection against fast desiccation, deleterious effects of UV radiation and overheating). The morphology of the wet season skin is, in most aspects, that of a "normal" anuran skin. It differs in the organization of the processes of the melanophores and in the arrangement of the chromatophores in the stratum spongiosum, forming no "Dermal Chromatophore Unit". During the adaptation to dry season conditions the number of iridophores in dorsal and ventral skin is increased 4–6 times compared to wet season skin. This increase is accompanied by a very conspicuous change of the wet season color pattern. Now, at air temperatures below 35° C the color becomes brownish white or grey and changes to a brilliant white at air temperatures near and over 40° C. Thus, in dry season state the frog retains its ability for rapid color change. In wet season state the platelets of the iridophores are irregularly distributed. In dry season state many platelets become arranged almost parallel to the surface. These purine crystals probably act as quarter-wavelength interference reflectors, reducing SRL by reflecting a considerable amount of the radiated energy input.

EWL is as low as that of much larger xeric reptilians. The impermeability of the skin seems to be the result of several mechanisms (ground substance, iridophores, lipids, mucus) supplementing each other.

The light red skin at the pelvic region and inner sides of the limbs is specialized for rapid uptake of water allowing the frog to replenish the unavoidable EWL by using single drops of dew or rain, available for only very short periods.

Usually amphibians are restricted to moist habitats with open water accessible most of the year. Until now some species of Australian, American and African anurans are known to live in arid biotopes. Nearly all species avoid the extreme conditions prevailing during the dry season by choosing favorable microclimates (e.g. by burrowing into the ground) (Bentley 1966, McClanahan 1967, Mayhew 1968, Ruibal et al. 1969, Warburg 1972). These anurans developed physiological and morphological adaptations, helping them to survive long lasting dry periods (McClanahan 1967, Lee and Mercer 1967, Loveridge and Crayé 1979). Such adaptations are e.g. tolerance to high concentration of urea in the blood allowing them to tolerate strong desiccation, or formation of cocoons by multiple molting of the stratum corneum reducing their cutaneous water losses. A few years ago, some species of the Rhacophoridae and Phyllomedusinae became known not to avoid, but to withstand the harsh climatic conditions of their seasonally hot and dry habitats rather unprotected. Until now, the eco-physiology of these very peculiar species has not been thoroughly investigated, except for some aspects of excretion, osmoregulation and water budget (Blaylock et al. 1976, Drewes et al. 1977, Loveridge 1970, 1976, Shoemaker et al. 1972, Withers et al. 1977, Loveridge 1970, 1976, Shoemaker et al. 1972, Withers et al. 1982).

One of these unusual species is the hyperoliide frog *Hyperolius viridiflavus nitidulus.* This species inhabits different areas of the West African savanna. During the 5–6 months long (in the most extreme of the Sahelian habitats even longer) dry season *H. v. nitidulus* is sitting motionless in a typical "hunched" posture on dry leaves or blades without feeding. Water (as dew) is more or less regularly available only in the first two months of the dry season. At the beginning of the dry season, the aestivating frogs are 2–4 months old (after metamorphosis) have an average snout-vent length of 1,5 cm (±0,5 cm) and a weight of 300–600 mg. Aestivating adult frogs (snout-vent length over 2 cm and weight 1.5–2.5 g) have been found in the field only occasionally and seem not to survive the prolonged dry season in the Sahalian habitats. The frogs are not in the physiological state of a dryness- and/or heat-induced torpor. They are awake and able to jump at any time if necessary. Often they are completely unshaded and have to stand the direct solar heat load during the whole day. They therefore must have developed very efficient mechanisms preventing them from fast desiccation and protecting them from being injured by UV radiation and from being dangerously overheated. Some of these mechanisms are certainly to be looked for within the integument of the animal. One corresponding hint is a very conspicuous color change

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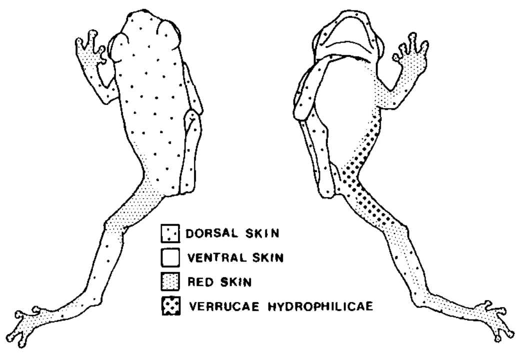


Fig. 1. Distribution of the three types of skin
This schematic shows the distribution of the three types of skin and the verrucae hydrophilicae on the body of *H. v. nitidulus.* The limbs of the right side of the frog are drawn in the typical dry season "hunched" posture

during the adaptation to dry season conditions. The color of the wet season skin changes from light-brown with a dark brown bar at the flanks to a uniformly brilliant white in the dry season state. The processes involved in this color change could be part of the adaptation to the dry season. Therefore, comparing the differences between the wet and dry season skin could give valuable hints on the nature of these protecting mechanisms.

Material and methods

Live specimens of *H. v. nitidulus* (Laurent, 1951) were collected in southern Mali, south-eastern Upper Volta, northern Benin and northern Ivory Coast. They were kept and bred in the laboratory at 27°–30° C/23° C (day/night) and about 50–100%/100% R.H. The dry season frogs used here were kept at 27°/24° C and about 30–50%/70 R.H. for 1–2 months before investigation. The ages of the investigated frogs were 1–6 months (young frogs) and 12–15 months (adult frogs) after metamorphosis. Pieces of dorsal, ventral and red skin of the flanks of freshly killed wet season frogs (WSF) and dry season frogs (DSF) were fixed in 6.25% glutaraldehyde in 0.1 M monophosphate buffer (pH 7.2) for 30 min, then washed briefly in phosphate buffer and postfixed for one hour in 1% osmium tetroxide/kaliumdichromate (pH 7.3) (Wohlfarth-Bottermann 1957). The tissue was dehydrated in a graded series of acetone and flat-embedded in Durcupan (Fluka) plastic resin. For light microscopy, sections were cut with a Reichert-Jung Ultracut ultramicrotome at 2 µm and stained with methylene blue/azure II (Richardson et al. 1960). Paraffin techniques have been used, too. These sections were fixed in Bouin's fixative (Romeis 1968) and stained with either Azan B (Romeis 1968) or Alzian-blue (Elkan 1976).

For electron microscopy, thin sections were cut at 60–100 nm using a Diatome diamond knife, stained with uranyl acetate and lead citrate, examined and photographed with a Zeiss EM 10A electron microscope.

The angle between the surface of the skin and the platelets in the iridophores were measured on electron micrographes.

For comparison, species of *Rana esculenta* (age: 4–15 months) were investigated in the same way as *H. v. nitidulus.*

Results

Wet season skin

The epidermis is about 20–25 µm thick and consists of 5 to 7 layers of cells. It is covered with a thin layer of mucus (Geise and Linsenmair 1986) which is often lost during the preparation.

The stratum corneum is 1 to 3 layers thick. The other epidermal and dermal strata conform in principle with the descriptions given elsewhere for other anuran species (Voute 1963, Parakkal and Matoltsy 1964, Farquar and Palade 1965, Carasso et al. 1971, Elkan 1976).

A layer of ground substance which Elkan (1976) did not find in other species of *Hyperolius* is interspersed between stratum spongiosum and stratum compactum. This Alzian-blue stainable layer is about 5 µm thick in young and about 10 µm in adult frogs. It contains irregularly shaped granules of moderate electron-density. Many radial fibers connect the tela subcutanea with the epidermis.

Types of skin. Three types of skin can be distinguished (Fig. 1):

The dorsal skin of young WSF is about 80 µm thick. In adult WSF the thickness is increased to about 120 µm mainly by an enlargement of the stratum compactum. The

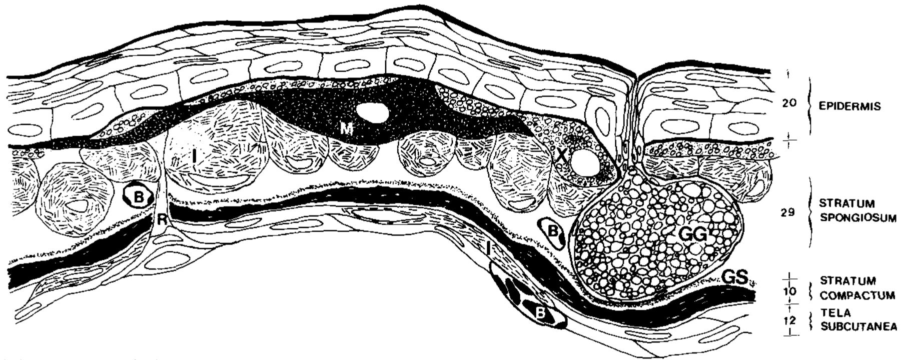


Fig. 2. Dorsal skin, wet season state
The dorsal stratum spongiosum contains one complete layer of xanthophores and globular iridophores. The melanophores are distributed between the iridophores and not below them. Their long processes extend between xanthophores and iridophores. The small stratum compactum is superimposed by a thin layer of ground substance. Squamous iridophores are found in the tela subcutanea.
Symbols: B blood-capillary, *C* ring capillary, *E* erythrophore, *EP* Epidermis, *GS* ground substance, *GG* granular gland, *I* irodophore, *M* melanophore, *MG* mucus gland, *P* platelet, *R* radial fiber, *S* sulcus, *SC* stratum compactum, *SS* stratum spongiosum, *X* xanthopore
(In all Fig. the thickness of the different strata is given in µm)

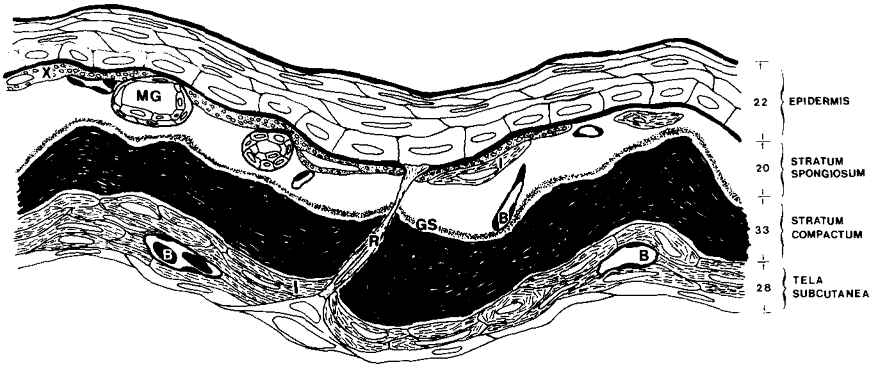


Fig. 3. Ventral skin, wet season state
The ventral stratum spongiosum contains only xanthophores and some squamous iridophores. The stratum compactum is about 5 times as thick as dorsally. No melanophores are present. Squamous iridophores are rather abundant in the tela subcutanea, forming two or three layers. (Symbols see Fig. 2)

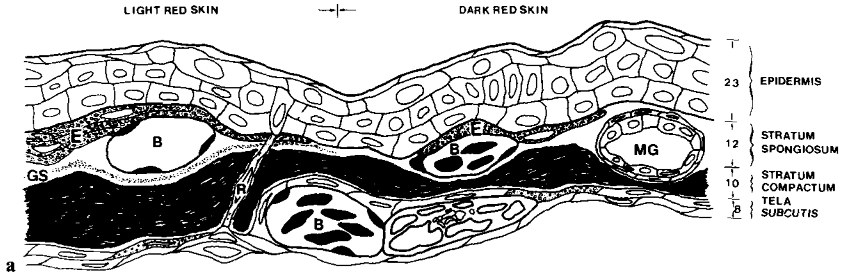
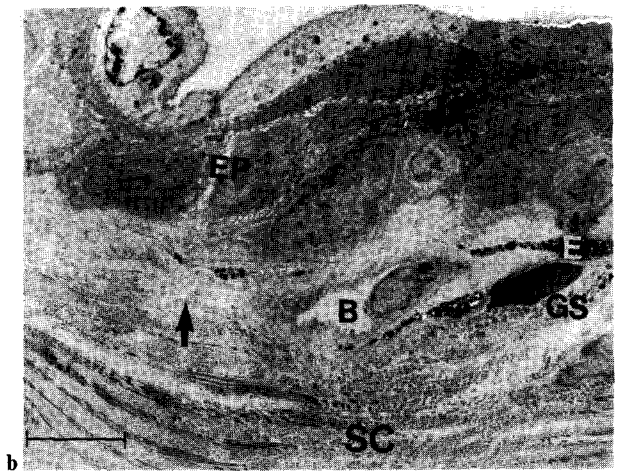


Fig. 4a, Red skin
The red skin is about half as thick as ventral skin. A dark red part (left side of the schematic) adjacent to the dorsal or ventral skin or white skin of the limbs can be distinguished from a light red central portion. The epidermal cells are not as degenerated or ceratinized as in the other two types of skin. The only chromatophores in red skin are erythrophores. They form 2 or 3 layers in the dark red stratum spongiosum and 1 incomplete layer in the light red part. They can be found in the tela subcutanea as well. Red skin contains big conspicuous blood capillaries. (Symbols see Fig. 2)



b Transition from dark red to light red skin
Dark red skin contains 2–3 layers of erythrophores and a layer of ground substance which is about half as thick as dorsally. In light red skin ground substance is absent (arrow) and the erythrophore layer becomes incomplete. The stratum corneum of the epidermis is lost by preparation.
(Scale: 5 μm, magnification: ×2,600, symbols see Fig. 2)

skin contains xanthophores, melanophores and iridophores. The iridophores of the stratum spongiosum are often shaped like globes or inverted cones and contain a round nucleus which is often found near the base of the cell. They form one or two layers. There are only a few squamous iridophores in the tela subcutanea. In the stratum spongiosum a network of blood capillaries (101 ± 11 meshes/mm^2) is placed below the iridophore layer (Fig. 2).

The ventral skin of young and adult WSF is about 100 μm thick. The increased thickness of the skin of young WSF is caused by an enlarged stratum compactum, which is about 5 times thicker than dorsally. No melanophores are present. Iridophores of the squamous type are rather abundant in the tela subcutanea, forming two or three layers. The stratum spongiosum contains only a few squamous iridophores. The network of blood capillaries in the stratum spongiosum is reduced to 63 ± 5 meshes/mm^2. The capillaries in the tela subcutanea are often surrounded by irodophores (Fig. 3).

The skin at the pelvic region and inner sides of the limbs is about 50 μm thick. In young frogs this skin is nearly uncolored and translucent. While growing up the color of this skin turns more and more to a brilliant carmine red. Because of this conspicuous color we call this type of skin "red skin". A dark red part adjacent to the dorsal or ventral skin or skin of the limbs can be distinguished from a light red (or translucent) central portion. The epidermal cells in the red skin are not as degenerated or ceratinized as in the other two types of skin. The only chromatophores in the red skin are erythrophores. They form 2 or 3 layers in the dark red stratum spongiosum and 1 incomplete layer in the light red part. They can be found in the tela subcutanea as well (Fig. 4a). In dark red skin the layer of ground substance has about half the thickness of that in dorsal skin and is absent in light red skin (Fig. 4b). Red skin is found on those parts of the body where the big arterial and venous cutaneous vessels (A.+V. cutanea, A.+V. iliaca and A.+V. subclavia) are in close contact to the

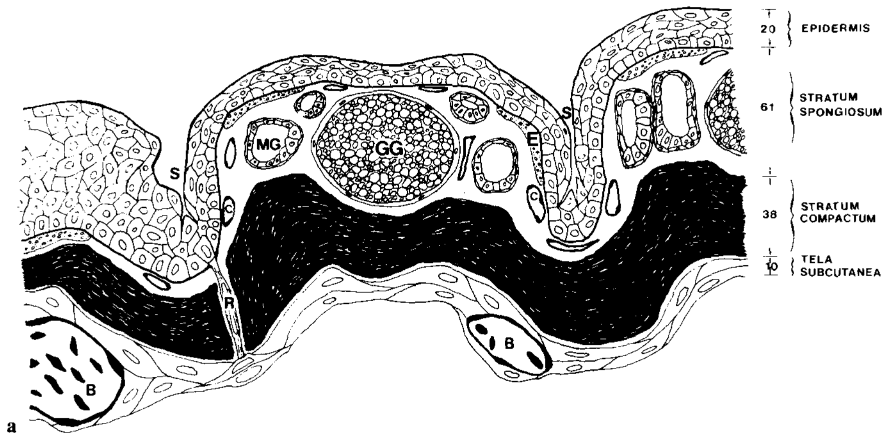
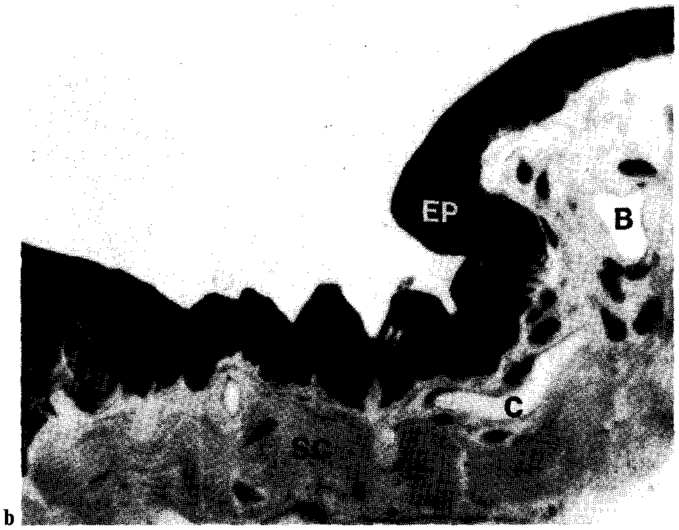


Fig. 5a. Verruca hydrophilica
Every verruca contains one centrally placed granular gland and 4–6 mucus glands surrounding the granular gland more or less regularly. A ring-shaped blood capillary is placed in the stratum spongiosum close to the basal lamina near the bottom of the sulcus. Smaller capillaries form a dense network (206 meshes/mm^2) in the verruca. These capillaries are never embedded in the basal lamina and compared with the ring capillary they are placed about twice the distance away from the surface (20–23 µm). (Symbols see Fig. 2)



b Bottom of a sulcus
Irregularly shaped hollows at the bottom of the sulcus are often near the ring capillary reducing the distance from the capillary to the surface to 8–12 µm.
(Semi-thin section of a Durcupan embedding, stained with Methylenblue/Azur II, magnification: about ×450, symbols see Fig. 2)

skin. The verrucae hydrophilicae differ in some aspects from those described by Drewes et al. (1977). The diameter of the polygonal verrucae is between 0.20–0.35 mm, which is about 1/2–1/3 the diameter of the verrucae of *Chiromantis petersi* (Drewes et al. 1977). They are restricted to those portions of the red skin of the pelvic region and of the thighs which are hidden, while *H. v. nitidulus* is sitting in its typical "hunched" posture. They are completely absent in the other types of skin. Every polygonal verruca contains one centrally placed granular gland and 4–6 mucus glands which surround the central gland in a regular pattern. This glandular pattern is not restricted to red skin, but is found in ventral skin as well. Every verruca contains a ring-shaped blood capillary, which is placed in the stratum spongiosum close to the basal lamina near the bottom of the sulcus. The bottom of the sulcus has irregularly shaped hollows reducing the distance to the ring capillary to 8–12 µm (Fig. 5b). This distance is in accordance with the data for *Chiromantis petersi* (Drewes et al. 1977). Smaller blood capillaries branching from this capillary form a dense network (206 ± 22 meshes/mm^2) in the verruca. These capillaries are always below the basal lamina and not embedded in it. They are about 20–23 µm away from the surface of the skin (Fig. 5a).

Glands. Mucus and granular glands are present in all types of skin. There is no obvious difference in morphology between these glands and the glands described for *Rana pipiens* (Noble and Noble 1966). Lipid glands which play an important role in protecting phylomedusine frogs from desiccation (Blaylock et al. 1976) are absent in *H. v. nitidulus*.

Chromatophores. Consistent with other species that exhibit rapid color change, epidermal pigment cells are absent (Bagnara 1976). The xanthophores which form a single layer just beneath the basal lamina, conform with the description given by Bagnara (1976). The iridophores form a complete layer beneath the xanthophores. The ultrastructure of the iridophores is similar to that described in Bagnara (1976). The almost squared platelets have sides 2–4 µm long. Their thickness is 120–180 nm. The platelets consist of guanine and hypoxanthine in a relation of 10:1 (dry weight, Kobelt unpublished). Adenine present in the skin of *Rana pipiens* (Bagnara and Stackhouse 1961) could not be detected. Many platelets are arranged in stacks with variable numbers of platelets (3–20). Besides their differences in shape, the ultrastructural morphology of the globular, dorsal and the squamous, ventral type of iridophore is the same. The ultrastructure of the melanophores does not differ from the description given elsewhere (Bagnara 1976). But the morphology of the processes is different from those of *R. pipiens*. 3–7 big processes extend from the cell

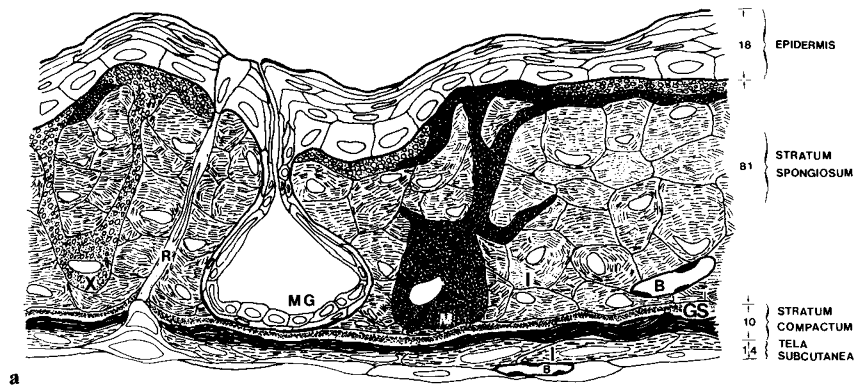
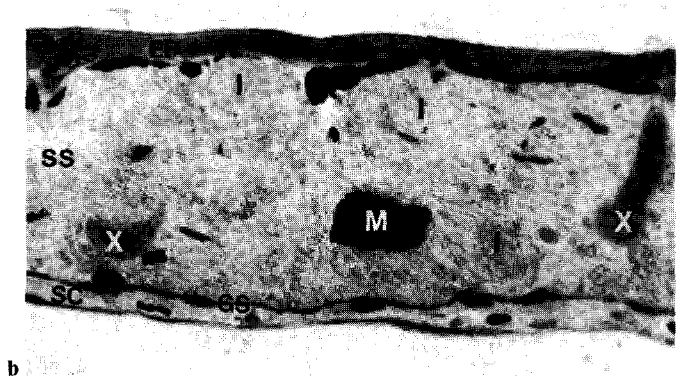
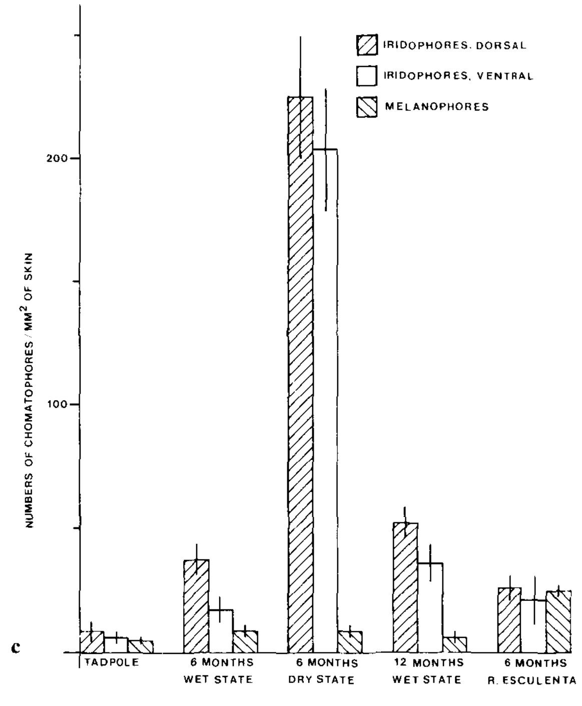


Fig. 6a. Dorsal skin, dry season state
In dorsal and ventral skin, the number of iridophores is increased 4–6 times, compared to that of wet season skin. Now the iridophores are placed in the stratum spongiosum and the tela subcutanea of dorsal and ventral skin. The numbers of melanophores and xanthophores do not change. (Symbols see Fig. 2)



b Melanophore and xanthorphore, dry season state
Both chromatophores are shifted to the bottom of the stratum spongiosum under 3–4 layers of iridophores. The processes of these chromatophores remain at the places they had occupied already in the wet season skin. (Semi-thin section of a Durcupan embedding, stained with Methylenblue/Azur II, magnification: about × 315, symbols see Fig. 2)



c Densities of chromatophores in wet and dry state skin
In the dorsal and ventral dry state skin, the number of iridophores increases 4–6 times, compared to that of wet season skin. In frogs, which are kept under wet conditions only, for 12 months or longer the numbers of iridophores do not increase as much as in dry season skin. 6 months old wet state *H. v. nitidulus* and *R. esculenta* have about the same number of iridophores in their skin. Compared to *R. esculenta*, the skin of *H. v. nitidulus* contains a reduced number (about 30%) of melanophores. The numbers of melanophores (and xanthophores) are not affected by adaptation to dry season conditions. (Bares indicate standard deviation)

body between the iridophores and the xanthophores, where they split up into smaller processes of variable diameter and length. These processes do not surround the iridophores.

Although the ultrastructure of each of the three types of chromatophores is just like that of other species, their arrangement in the wet season stratum spongiosum differs from the dermal chromatophore unit described by Bagnara (1966). The proportion in numbers of xantohophores: iridophores: melanophores is 2:5:1. The processes of one melanophore cover about 4–7 iridophores and the xanthophores cover 2–4 iridophores. The number of melanophores is reduced to about 30% of that of *R. pipiens*. These pigment cells are placed between the iridophores rather than beneath them. Xanthophores are occasionally found between and under the iridophores, too.

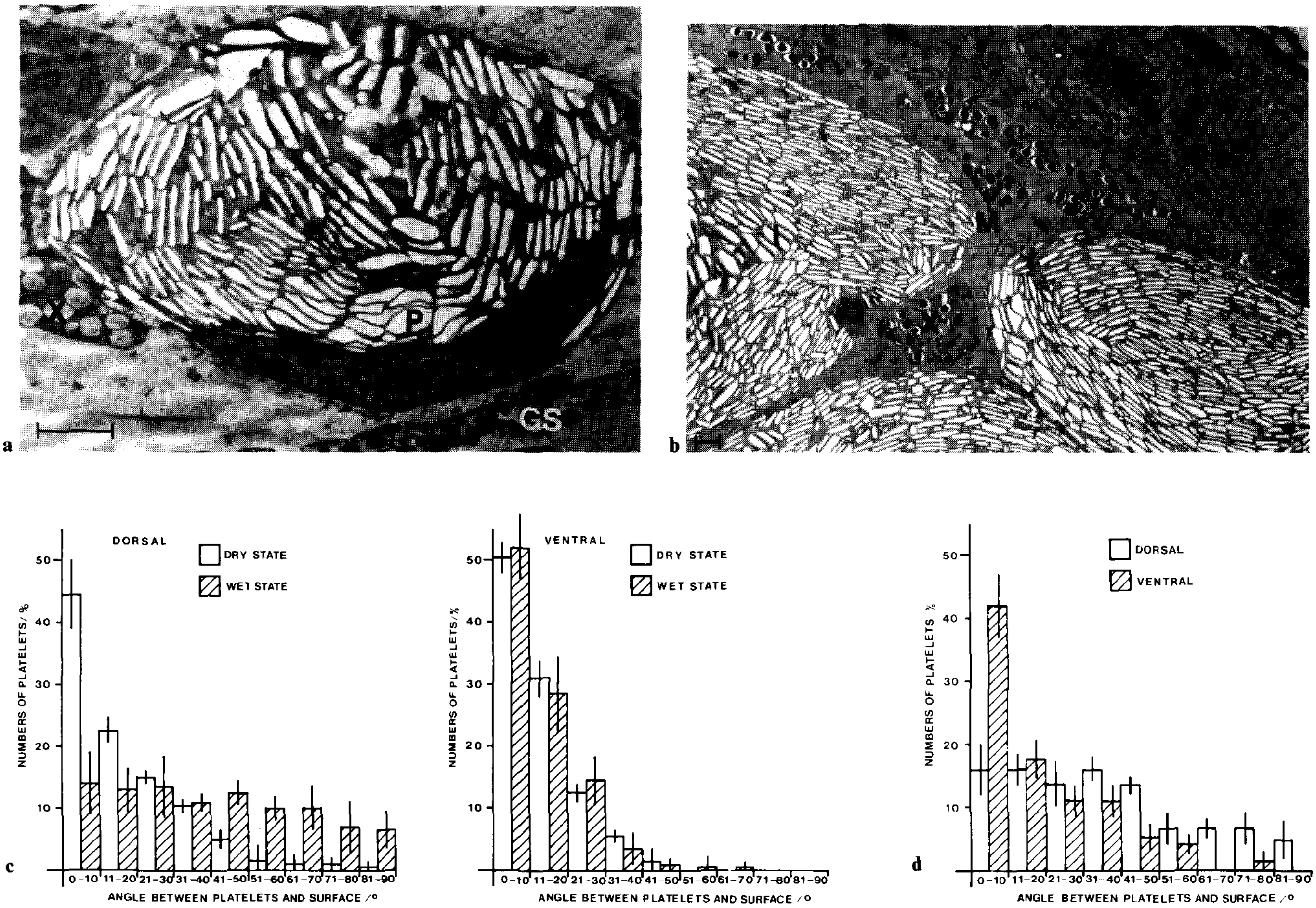


Fig. 7a–d. Orientation of the platelets in WSF (**a, c**), DSF (**b, c**) and in *Rana esculenta* (**d**)
In wet season dorsal skin the stacks of platelets are irregularly distributed (**a, c**). In the dry season skin, many stacks in the globular, dorsal iridophores are oriented in a plane almost parallel to the surface of the skin (**b, c**). The squamous ventral iridophores have stacks with parallel arrangement in wet and dry season skin (**c**).
In *R. esculenta* (same age as frogs in (**a, b**) in platelets of the dorsal iridophores are nearly randomly distributed. The ventral iridophores have platelets with a parallel arrangement comparable to that of the ventral skin of *H. v. nitidulus*.
(**a** Scale: 1 µm, magnification: × 10,000; **b** Scale: 1 µm, magnification: × 3,135, **c, d** bares indicate standard deviation)
CHI^{-2}-test: **c** dorsal/wet – random: $P>0,05$, df = 16 **d** dorsal – random: $P>0.05$, df = 16
dorsal/wet – dorsal/dry: $P<0.001$, df = 14 dorsal – ventral: $P<0.001$, df = 10
ventral/wet – ventral/dry: $P>0.05$, df = 8
dorsal/dry – ventral/dry: $P>0.05$, df = 8
dorsal/wet – ventral/wet: $P<0.001$, df = 8

Dry season skin

In the wet season state, during the first weeks of their postmetamorphosis life the dorsal skin of young frogs shows a light-brown color with a dark brown bar at the flanks. Dry season coloration differs greatly. The skin looses its pattern completely and gets a more uniform color. Above 37° C the skin turns to a brilliant white with tiny parts of it showing a red, yellow or green iridescence. At air temperatures between 20°–35° C, the color of the whole skin often becomes uniformly brown or grey. Thus, during the dry season *H. v. nitidulus* retains its ability for rapid color change. The light after transmission through the isolated skin is unsaturated blue. This color is seen in the dorsal and ventral skin in its dry state and may also be found in the ventral skin in its wet state. In dorsal and ventral skin, the number of iridophores increases 4–6 times, compared to that of wet season skin, filling the whole stratum spongiosum (Fig. 6c). Now the iridophores are placed in the stratum spongiosum and the tela subcutanea of dorsal and ventral skin. As a result the thickness of dorsal and ventral skin increases to about 125 µm. The numbers of melanophores and xanthophores do not change during the transition from wet to dry season state (Fig. 6c). These chromatophores are shifted to the bottom of the stratum spongiosum under 3–4 layers of iridophores. Such an unusual shifting has never been described before. The processes of these chromatophores remain at the places they had occupied already in the wet season skin. Now both chromatophores look much like an opened umbrella with 3–7 handles and a big knob (Fig. 6b).

Especially in dry season skin, many stacks in the globular dorsal iridophores are much better oriented in a plane almost parallel to the surface of the skin than in wet season skin. The squamous ventral iridophores have always stacks with parallel arrangement (Fig. 7).

Cells containing purine platelets can be found in other endothelia like the pericard, too.

In the young WSF portions of the dark red skin cannot be hidden while the frog is sitting in its "hunched" posture. During the adaptation to dry season conditions these red portions become whitened by a thick layer of iridophores which obviously do not immigrate from the adjacent white skin into these parts.

Discussion

In general, the morphology of the wet season skin of *H. v. nitidulus,* is in most aspects, that of a "normal" anuran skin. The two types of skin glands conform to the description given by Noble and Noble (1966). The ultrastructure of the different chromatophores described in this study does not differ greatly from those described by Bagnara (1976). During the dry season the sun radiates a very high energy load upon the motionless animal. This high radiation from near UV to far IR is one of the major problems the frog has to deal with. The frog needs mechanisms a) to avoid being dangerously overheated by the radiative head load and b) to minimize UV-absorption within the body. The frog, weighing only 300–600 mg, cannot regularly use evaporative cooling to keep the body temperature below the lethal limit. The stored water would not last for more than a very small fraction of the dry season and the frog would have to replenish the lost water very frequently. During long lasting periods of the dry season this is impossible. In its Westafrican biotops dew is available dayly only in the first month of the dry season. Then dew fall occurs only occasionally in the next 2–3 months, and is normally absent in the last 2–3 months (or even more). Thus the frog has to use its stored water in most parsimonious ways by minimizing every loss. According to this vital need of water conservation evaporative cooling is used only if air temperature reaches the upper lethal limit (Geise and Linsenmair 1986).

Another way to reduce the solar heat load, which is the main source of energy (Parkhurst and Loucks 1972) at the places the frog is usually sitting during the dry season, is to reflect as much of the incoming radiation as possible, since the reflected part of the light cannot effect body temperature. The iridophores filled with numerous reflecting platelets obviously represent such a reflector. Their increased number and the changes of their inner structure during the adaptation to dry season conditions strongly support the assumption that this is their main biological role. *H. v. nitidulus* is able to perform rapid color change, which might, in addition to other functions, be involved in thermoregulation by changing the absorptivity of the skin. Most effectively this could be achieved by placing all three types of chromatophores in the epidermis. The epidermal layers, however, are frequently lost by molting (Geise and Linsenmair in prep.). To renew all chromatophores every 2 or 3 days would cost the frog most probably too much energy and additionally the formation of new chromatophores could most probably not be achieved in the short time between two moltings. That might be the reason why the reflector is placed in the dermis which is not lost by molting. As a result the epidermis has no or no obvious protection against the mutagenous UV radiation. The dermal reflector has been developed by a multiplication of the light reflecting iridophores which in DSF occupy the whole dermis in dorsal and ventral skin (without the stratum compactum).

But how is a layer, not more than 0.1 mm thick, able to function as a reflector, reducing the head load effectively?

In such a thin layer, light scattering alone cannot achieve a reflectivity (Fischer 1980) which reduces the solar heat load considerably. Usually, the platelets are arranged in stacks with alternating layers of purine crystals and cytoplasm. This arrangement and the observed coloration of the reflected and transmitted light, which is, according to Land (1966), characteristic for multilayer quarter wavelength reflectors (Huxley 1966, Land 1966), supports the assumption that the stacks are multilayers. When the optical path lengthes of light in the platelets and interspersed cytoplasmic films in a stack are equal, only about 30 double-layers of platelets and cytoplasm are necessary to achieve a 100% reflectivity around the quarter wavelength (Land 1966). The better paralleled orientation of stacks in dry season iridophores is most likely a special adaptation to dry season conditions increasing the reflectivity of the skin. The reflectivity of such a multilayer structure is wavelength-dependent. Computing the best wavelength by the method of Land (1966) gives 1100 ± 250 nm (thickness of the platelets: 150 ± 32 nm, index of refraction of cytoplasm: 1,34 (Land 1966), index of refraction of the platelets: 1,81 (Kobelt, unpublished)). Therefore, the "bandwidth" of high reflectivity is 850–1350 nm. Because of this dependency, all the light outside this range which is about 2/3 of the irradiated energy, would pass the dielectric reflector. The best wavelength is a function of the thickness of the platelets and the cytoplasmic films. Varying this factor from one iridophore to another may increase the bandwidth of high reflectivity. The different colors of iridescense of the iridophores are in accordance with this assumption. Those stacks and platelets which are not in parallel orientation to the surface additionally scatter the light wavelength-independent, increasing the reflectivity of that parts of the spectrum, which are poorly reflected by the multilayer. Therefore, the unparallel platelets and stacks may act as diffuse reflectors reducing the wavelength dependency additionally. Thus, a certain amount of disorder in the arrangement of stacks is no disadvantage but on the contrary a presumably specifically selected advantage.

We consider the above mentioned differences from the usual dermal chromatophore unit (Bagnara 1968) another special adaptation to the need of an effective shield against radiation in two aspects: 1) The number of melanophores containing black pigments is reduced, which might be due to the fact that the reflectivity of a white pigment is exponentially reduced when soiled with graded amounts of a black pigment (Kortüm 1969); 2) Because the pigment cells are not tightly bond together as a dermal chromatophore unit, the cell bodies of the melanophores and the xanthophores can more easily be shifted under the reflecting layer (by elongating their 3–7 big processes) during the adaptive modification of the skin to dry season conditions. As the processes of both chromatophores still remain in place, the frog retains its ability for rapid color change. Although there are some hints in the literature that in reptiles color change and thermoregulation are related to each other, it does not play a significant role in those anuran species investigated until now (Waring 1963; Carey 1978, Gates

1980). However, these species either live in humid habitats with open water permanently available and under climatic conditions not at all comparable to those prevailing during the dry season in the habitats of *H. v. nitidulus* or aestivate in favorable microclimates. During the adaptation to dry season conditions, portions of the red skin whiten gradually by iridophores which appear in increasing number in the dermis of this parts. The chemical relationships between pteridines of xanthophores and purines of iridophores are very close and a possible interconversion of these substances has been suggested (Bagnara 1966; Stackhouse 1966). This leads to the conclusion that the change from red to white skin is the result of a transformation of erythrophores (which are xanthophores containing red pigments) to iridophores rather than to a migration of iridophores from adjacent white into the red skin. If this hypothesis is valid the dark red skin bordering the light red skin could be understood as a convertible buffer between light red skin and the iridophore containing skin.

Evaporative water loss (EWL) is another major problem the frog has to deal with. The animal has to reduce water loss as much as possible to survive the dry season. EWL is very low and is fully comparable to that of much larger reptilians living in the same environment (Geise and Linsenmair 1986).

But how can the frog maintain such a low evaporative water loss? Ground substance is discussed to play a role in defense against desiccation by acting as a sponge, absorbing large quantities of water and giving it up only under extreme circumstances (Elkan 1976). Drewes et al. (1977) doubt this. But in the light red skin, highly permeable to water, ground substance is completely absent. This might be a hint that ground substance could be involved in protection against water loss.

Their findings that EWL of some Hylidae and Hyperoliidae is unequal in dorsal and ventral skin and that a corresponding unequal distribution of the iridophores exists, induced some authors to speculate about iridophores as an important morphological barrier against EWL (Drewes et al. 1977, Yorio and Bentley 1977, Withers et al. 1982). As diffusion is linearly correlated to the plane perpendicular to the direction of mass flow, the numerous unsoluble purine crystals in the dry season skin reduce this plane significantly. That may lead to a reduced permeability. This conclusion is in agreement with the results of Kutchai and Steen (1971). They found that the permeability of the swimbladder of the conger eel (Conger conger) is only 10% of that of connective tissue. The silvery layer producing this effect contains 13% guanine (dryed tissue). Most probably a secretion of the numerous mucus glands plays an important role in reducing EWL, as well. This mucus dries very fast and seems to seal the skin, including the ducts of the glands and the small gaps between the body and the limbs (Geise and Linsenmair 1986). EWL increases when this layer is injured. Machin (1974) reports a similar function of mucus in *Otala lactea* (Gastropoda). Reno et al. (1972) found a mucus-cocoon in estivating *Siren intermedia* (Urodela).

The impermeability of the skin of *H. v. nitidulus* seems to be achieved not only by one specialized structure but by the cooperation of several mechanisms, which on their own, cannot reduce water loss as efficiently as in combination. The three different types of skin of the investigated frog may help to answer questions on how the permeability of the skin is controlled and what morphological structures and/or other mechanisms are involved.

Although the frog is very well protected against EWL, water loss due to cutaneous and respiratory transpiration is unavoidable. The frog cannot store enough water to survive during the whole dry season (Geise and Linsenmair, 1986). Therefore it has to replenish the lost water by occasional drops of dew or rain. But this water evaporates very fast. The less permeable dorsal and ventral skin could take up only a very tiny fraction of this small amount of water. Thus, the frog needs a specialized skin structure which a) is able to take up water very fast and which b) is protected against EWL as well as the other types of skin.

When the red skin of only one leg is exposed to the air, the over all water loss of such a frog increases twentyfold (Geise and Linsenmair, unpublished). This skin is much thinner than the other two types of skin and it lacks some morphological structures that could impede diffusion. There are only a few erythrophores in the dermis. Other chromatophores are completely absent as ground substance is, too. This skin is richly supplied with blood vessels and it is the only part of the body that contains verrucae hydrophilicae, which probably are the actual sites of water absorption (Drewes et al. 1977). Most probably *H. v. nitidulus* saves the small drops of water from evaporation by storing it in the sulci until absorption. Thus, the sulci might be water storages rather than surface enlargements. Then, water would be taken up mainly via the sulci and not via the surface of the verrucae. The different ways of diffusion from the hollowed surface of the sulcus to the ring capillary and from the surface of the verruca to the other capillaries might be interpreted as a hint in favor of this conclusion.

In *H. v. nitidulus* the verrucae are restricted to the hidden parts of the red skin. In many other anurans the whole ventral skin is studded with such verrucae (Drewes et al. 1977). This fact and the similar glandular pattern of the ventral skin and of the verrucae leeds to the conclusion that in former times the verrucae were spread over the whole ventral skin. But the ventral skin is often exposed to the air at the aestivating places. As the hidden portions of the red skin are very well protected against EWL (Geise and Linsenmair, 1986) the restriction of the verrucae to this part of the body is another adaptation against high EWL. Thus, the light red skin obviously is specialized for rapid uptake of water and is very well protected against the air by the typical "hunched" posture of the DSF.

The iridophores seem to be one of the most important morphological preadaptations. Their changes in quantity and quality are decisive adaptations to meet the requirements of the frog's seasonally desert-like hot and dry savanna biotope. They probably act not only as reflectors to minimize the harmful effects of radiation, but also as a barrier against desiccation. And as a third function, they presumably store the wast products of the nitrogen metabolism.

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