Histochemical and Ultrastructural Analyses of Adhesive Setae of Lizards Indicate that They Contain Lipids in Addition to Keratins

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ABSTRACT We studied the distribution of lipid material and organelles in the epidermal layers of toe pads from two species of lizards representing the two main lizard families in which adhesive scanners are found (gekkonids and polychrotids), the dull day gecko, Phelsuma dubia and the green anole, Anolis carolinensis. Although lipids are a conspicuous component of the mesos layer of squamate reptiles and function in reducing cutaneous water loss, their distribution has not been specifically studied in the highly elaborated epidermal surface of adhesive toe pads. We found that, in addition to the typical cutaneous water loss-resistant mesos and alpha-layer lipids, the Oberha¨utchen (including the setae) on the most exterior layers of the epidermis in P. dubia and A. carolinensis also contain lipid material. We also present detailed histochemical and ultrastructural analyses of the toe pads of P. dubia, which indicate that lipid material is closely associated spatially with maturing setae as they branch during the renewal phase of epidermal regeneration. This lipid material appears associated with the packing of keratin within setae, possibly affecting permeability to water loss in the pad lamella, where the surface area is from 4–60-fold greater compared with normal scales. J. Morphol. 272:758–768, 2011. © 2011 Wiley-Liss, Inc.

KEY WORDS: setae; keratin; Phelsuma; Anolis; gecko; toe pads

INTRODUCTION

The epidermis of amniote vertebrates performs various functions in addition to the ancestral role of protection from mechanical abrasion and as a barrier to excessive water loss (Menon et al., 1996; Alibardi, 2003). For example, geckos and anoles have independently evolved toe pads that bear analogous epidermal structures called setae that allow both to adhere during locomotion to vertically oriented or overhanging substrates (Irschick et al., 2006).

The setae of squamates originate from modifications of the epidermal layers that involve the scales covering the body (Maderon, 1964; Ruibal and Ernst, 1965; Peterson, 1983; Maderon et al., 1998; Alibardi et al., 2007; Alibardi, 2009). Setae appear to be derived from micro-ornamentations (spinulae) commonly produced from the Oberhautchen layer of body scales, but are larger (ranging from ~50 to 100 μm in length and 10 μm in diameter in some species; Maderon, 1970) and usually of more complex form. Setae are shed as part of the normal squamate epidermal regeneration cycle, but both an inner (immature) setal generation and an exposed outer setal generation coexist before shedding.

The morphology of setae (Ruibal and Ernst, 1965; Maderon, 1970; Autumn, 2006) and the anatomical and chemical–physical mechanics of attachment and release have been studied in great detail (Russell, 1981; Autumn and Peattie, 2002; Russell, 2002). Similarly, the molecular biology of setae, particularly the comparative proteomics of keratin and their evolutionary relationship to other keratin-based integumentary structures, such as feathers, has been explored (Alibardi and Toni, 2006; Rizzo et al., 2006; Alibardi et al., 2007; Toni et al., 2007). Perhaps not surprisingly, studies of the derived mechanical function role of setae have focused on keratins more than on other important integumentary components such as lipids.

The integument of tetrapod vertebrates has been the subject of extensive comparative study, particularly with regard to structural and material variation and consequences for resistance to water loss (review Lillywhite, 2006) and protection against mechanical abrasion (Chuong et al., 2002). Cutaneous water impermeability derives largely from extracellular lipids isolated within one or more
regions of a highly stratified epidermis. In squa-
mate reptiles, waterproofing lipids are produced
and secreted by lamellar (mesos) granules in the
mesos layer of a multilayered outer generation
that is periodically sloughed and replaced by an
underlying inner generation. The secreted lipids
surround meso-layer corneocytes and fill intercel-
lar spaces, composing relatively water-imperme-
able sheets within the stratum corneum (Land-
mann et al., 1981; Landmann, 1988). Corneocytes
and lipids are arranged like “bricks” and “mortar”
(Menon et al., 1996; Lillywhite, 2006). The water-
impermeable mesos layer is protected from physi-
(Man et al., 1981; Landmann, 1988). Corneocytes
and lamellar (mesos) granules in lepidosaurs) seem not to be present in the
β-keratogenic cells (Menon et al., 1996; Mader-
southeast of the stratum corneum. Details of the structural organization of the
squamate integument have been described for
many species and many different regions of the
body. However, to our knowledge, the highly spe-
ialized epidermis of the adhesive toe pads of lizards
such as geckos and anoles has not been described
apart from morphological descriptions of their
main features, the setae. Moreover, analyses of
setae have largely focused on their structure rela-
tive to their function in adhesion. However, setae,
as modifications of the outer layers of epidermis,
are likely to impact other functions of the epider-
mis. For example, setae are likely to substantially
increase the effective surface area of toe pad epi-
dermis relative to the spinulate surface of scales
covering other regions of the body, begging the
question of whether the basic structural organiza-
tion of and distribution of lipids in the epidermis
of toe pads is different from the typical squamate
pattern described for areas exclusive of adhesive
toe pads. In addition to their important role in waterproof-
ing, the distribution of epidermal lipids in adhe-
sive toe pads is also of interest because lipids may
be involved in the organization and development of
β-keratin filaments that compose setae. In fact,
other corneous and elongated microstructures, the
barbules of feathers, seem to contain lipids and
keratin material (Bell and Thathachari, 1963; Ali-
bardi, 2005; Alibardi and Toni, 2008). Observations of the spatial proximity and stage of appearance
during the shedding cycle may indicate a potential
chemical-molecular role for nonkeratin molecules
in the condensation and polymerization of β-kerja-
tin filaments into setae. Similar observations have
been made for birds (Alibardi et al., 2009). Usually,
lipids are deposited in the mesos layer of the epi-
dermis of lizards (Lillywhite and Maderson,
1988; Menon et al., 1996; Lillywhite, 2006), but
the presence or absence of lipids in other layers in
regions of the epidermis that are highly modified
(e.g., toe pads) has not been specifically studied. In
numerous studies of β-keratogenic cells (Ober-
häutchen and β-cells) lipids appear to be produced
and to be sparsely accumulated in small droplets
that eventually mix with the prevalent keratin-
material in mature cornified cells (Maderson et al.,
1998; Alibardi, 2003, Rizzo et al., 2006; Toni et al.,
2007). However, typical lamellar granules (mesos
granules in lizards) seem not to be present in the
β-keratogenic cells (Menon et al., 1996; Mader-
southeast, PA). Given the specialized function of adhesive toe
pads, we aim at a morphological characterization of morphologically the adhesive setae of Phelsuma
dubia and Anolis carolinensis in relation to their
lipid content. P. dubia and A. carolinensis are two
representative species from squamate lineages that
independently derived adhesive toe pads bearing
setae (Irschick et al., 1996). Two aspects of the spe-
cialization of the toe pad epidermis for adhesion
were of particular interest to us. First, we wanted to
describe the spatial distribution of lipids in the
toe pads of P. dubia and A. carolinensis and see if
it is different from the typical “bricks and mortar”
organization of body scales of squamates
(Maderson, 1970; Menon et al., 1996). Second,
using ultrastructural analysis, we wanted to char-
terize the general configuration of the epidermis
and the distribution of lipid material in both
mature and immature generations of setae from P.
dubia. In particular, the thick protective layer of β-
Keratin and Oberhäutchen of squamate scales that
protect the deeper lipid-based barrier from cutane-
ous water loss (CWL) would seem to be at odds
with the ability of toe pads to maximize contact
with the substrate through flexibility and compli-
ance. Our overarching goal for this study was to
determine if the structural composition of toe pad
epidermis in two representative species fits the
general archetype for squamate epidermis reported
for scales sampled from other areas of the body.

MATERIAL AND METHODS
Toes from adult gecko (P. dubia, n = 3; pes, digit 4) and
anoles (A. carolinensis, n = 3; pes, digit 4) were fixed in 10%
normal buffered formalin for 4 h, then dehydrated in 80% etha-
nol (three times, 45 min each), 95% ethanol (two times, 30 min
each), 100% ethanol (three times, 45 min each), and immersed
in xylene (two times, 45 min each). Specimens were embedded
in paraffin (56–58°C) (Surgipath Medical Ind., Richmond, IL)
and cut into 5 μm thick sections using a Leica RM2255 auto-
mated rotary microtome (Leica Microsystems; Bannockburn,
IL). Some sections were stained with Hematoxylin and Eosin
(H and E) and others with Masson’s Trichrome stain. Other sec-
tions were processed following standard histochemical protoco-
ls for periodic acid-Schiff reaction for carbohydrates and glyco-
lipids or the Alcian Blue staining protocol for acid mucopolysac-
charides. Sections were then viewed using an Olympus bright
field microscope (Olympus BX51; Olympus America, Center Val-
ley, PA).

For staining of neutral triglycerides and lipids in frozen sec-
tions, toes of adult P. dubia and A. carolinensis (n = 2 each; pes

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digit 4) were embedded in Tissue-Tek® O.C.T.™ Compound (Sakura Tissue-Tek, Zoeterwoude, The Netherlands). Several fresh frozen sections were taken in the sagittal plane to include both the dorsal and ventral toe surface and the setae. The sections were stained with oil red O according to standard protocols (Bancroft et al., 1990) and counter stained with Harris’ Hematoxylin.

Other digits (pes digit 4) were collected for ultrastructural examination from two more geckos (P. dubia), fixed in 3% glutaraldehyde in 0.1 mol phosphate buffer at pH 7.2, and left at 4°C for 15 h. Tissues were rinsed in the buffer and postfixed in 2% OsO4 for 2 h, then rinsed in the buffer for 10 min, transferred to distilled water for 15–20 min (two changes), post-fixed in toto with 2% uranyl acetate, dehydrated in ethanol (70% for 30 min, 90% for 30 min, 100% with two changes over 45 min), then in acetone (100%) for 40 min, then immersed in pure Epon resins for 5–8 h to improve penetration, and finally embedded in Epon resin for 2 days at 60°C. Tissue containing the adhesive setae was sectioned with an ultra-microtome (LKB-Novia) to obtain semithin sections of 1–3 μm in thickness that were stained with 1% toluidine blue. From areas containing the setae, thin sections of 40–80 nm were stained with uranyl acetate and lead citrate according to routine procedures (Alibardi and Toni, 2007). Observations were made on a Philips CM-100 transmission electron microscope operating at 60 or 80 kV.

All protocols complied with the regulations of The University of Akron IACUC, protocol #07-4G. Work was completed during 2009 in laboratories at the University of Akron and Summa Health System (Akron, OH) and The University of Bologna (Bologna, IT). P. dubia were obtained from California Zoological Supply, and A. carolinensis were obtained from a local pet shop.

RESULTS

Light Microscopic Observations

The toe pads of P. dubia and A. carolinensis are covered with a multilayered epidermis comprised of modified outer corneous epidermal strata on a living epidermis 2–4 layers thick, all resting on what appears to be a less compact dermis in our sections (Fig. 1). In sections from toes with epidermis in the renewal phase, the inner and outer setae are clearly visible (Fig. 1A,E,F). Although the size of setae differs between Anolis and Phelsuma, the general features of the layers of the epidermis and the overall distribution of lipids is similar in both species. In particular, lipid staining can be observed in the stalks of the normal spinu-lae (Fig. 1D), in the outer setae (Fig. 1A–D), and in the inner setae (where present, Fig. 1A,E,F), as lipid droplets or vesicles in the dermis, and in apparently high abundance in the hinge regions of the ventral surface of toe pad lamellar scales. In Phelsuma, large lipid cells can be seen in the dermis (Fig. 1E), especially near the hinge regions of pad lamellae, an observation later confirmed using electron microscopy (see later description). High magnification of Phelsuma setae reveal that lipids are present within both the horizontally extensive Oberhäutchen-β-layer that supports the setae and the vertically extensive setae (Fig. 1C,D) in a distribution pattern that suggests association with filamentous material making up the stalk (β-keratin). The lipid-stained material appears to be both associated with the outer surface of the setae, pre-

Ultrastructural Observations of P. dubia

Semithin sections of Phelsuma samples showed two different phases of the shedding cycle for the adhesive pad scanners: renewal and postsheeding (Fig. 2A–D). In the renewal phase, the inner generation of setae appears as elongated bristles 30–40 μm in length, which terminate in apical spatular endings containing several keratin bundles that are stained blue (arrows in Fig. 2B). Among the blue-stained filaments, a pale-brown osmiophilic material is clearly visible, indicating lipid content within the setae, as previously seen in sections treated with oil red O (Fig. 1C). In the post-sheeding phase, the setae are completely cornified and form homogeneous structures. In our sections, the formation of a new generation of setae is at an early stage, as indicated by a long intercellular linear boundary (Fig. 2C,D).

Single bundles of keratin are surrounded by the cytoplasm of apparently syncitial clear layer cells near the setal endings (Figs. 2E and 3A,B). Inspection of seta formation during the renewal stage shows that the cytoplasm of the clear layer cells surrounds each seta to its apical tip (Fig. 2E). Separation of keratin bundles begins about 10–15 μm from the tip, leaving behind the intact plasma membranes of the clear cells (Fig. 3C). In more mature setae, the cytoplasm surrounding the keratin bundles appears degraded and no ribosomes are evident. The electron-pale material in these areas is lipidic in content. Both in immature and mature setae, it appears that at the point of setal branching the keratin bundles become increasingly separated to form the terminal spatulae of the setae (Fig. 3B,D).

The cytoplasm of clear layer cells in the renewal stage contains a diffuse meshwork of keratin bundles, and numerous lipid vesicles or lamelliform bodies (Figs. 2E and 4A–C). Circular lamelliform bodies display an onion-like organization of layers of degenerating membranes that sandwich numerous lipid droplets (Fig. 4C). Lipid vesicles are, in part, derived from the poorly developed Golgi apparatus or from the smooth endoplasmic reticulum of these cells. Sparse areas of the apical cytoplasm of clear layer cells contain denser clumps of granules that resemble glycogen (Fig. 4D). At this stage of differentiation, the setae contain compact materials (keratin) and some interkeratin granulated material of unknown nature (Figs. 4A–C and 5A). The plasmamembrane of the setae is often thickened (80–150 nm) by the deposition of darker material.
Moving toward the base of the clear layer cells and approaching the Oberhautchen layer, keratin bundles become more abundant. The apical cytoplasm of clear layer cells surrounding the branching spatulae is degenerating. The deepest cytoplasm of clear layer cells adjacent to the Oberhautchen cells, from which setae have arisen, contains an even distribution of keratin filaments,

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Fig. 2. Light micrographs (A–D) and transmission electron micrographs (E) of lamellae of *P. dubia*. A: Lamellae with epidermis in renewal stage, showing the inner seta generation deep to the *Oberhäutchen* of the outer generation. Bar, 20 μm. B: Detail of renewal phase epidermis showing the dark keratin bundles in the terminal regions of the setae (arrowheads). Bar, 10 μm. C: Scansor with fully formed outer setae generation located near the tip of this modified scale. Bar, 20 μm. D: Detail of the stratified epidermis of the scansor in which a line (arrowhead) indicates the very beginning of differentiation of the future shedding line. Bar, 10 μm. E: Low magnification transmission electron micrograph of the apical part (corresponding to arrowheads in figure B) of inner generation setae (arrows). Keratin bundles appear separated by the pale cytoplasm of clear layer cells. The second rows of setae (arrowheads) show setae at a deeper level of sectioning (more proximal) with respect to the external row and keratin masses tend to appear merged. This process of compaction of keratin bundles into a single mass is completed in the third row of sectioning (double arrowheads) where setae are sectioned even more proximally. The double arrows indicate fibrous material along the plasma membrane of clear layer cells (not visible here). Bar, 1 μm. a, alpha layer; c, cytoplasm of clear cells; d, dermis; io, inner *Oberhäutchen*; is, inner setae; l, lipid droplets; o, *Oberhäutchen* (outer); os, outer setae. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Fig. 3. Ultrastructural detail of setae in the renewal phase (A and B) and the postshedding (mature) phase (C and D). A: Detail of branching bundles of corneous material (arrowheads) which thin-out forming the apical spatulae (arrows). Bar, 0.5 μm. B: Further enlargement detailing the curved spatular surface (arrows) separated from the cytoplasm of clear cells by the plasma membrane (arrowheads). Bar, 250 nm. C: Cross-sectioned setae at a level below the final branching into spatulae. Most of the plasma membrane is still present (arrows), whereas nearly empty spaces (asterisks), perhaps containing lipid material, are present among the external mass of keratin material. Bar, 250 nm. D: Mature setae showing the external, separated spatulae (arrows), the more compacted bundles of keratin (arrowheads), and the more compacted mass of keratin (double arrowheads). Bar, 1 μm. c, clear cell; dk, dense/compact β-keratin; k, keratin bundles.
Fig. 4. Transmission electron micrographs of clear cells located between the maturing setae. A: Concentric rings of membranes (arrows) among keratin and some lipid droplets. The plasma membrane of clear cells (arrowheads) surrounds setae sections. Bar, 0.5 μm. B: Detail of a large body made of concentric lamellae (arrow) and containing a lipid droplet (arrowhead). Bar, 0.5 μm. C: Detail of free lipid droplets within an arm of clear cells in which cytoplasm "branches" to surround setae (the arrows show the continuity of the plasma membrane). Bar, 0.5 μm. D: Other detail of lipid droplets connected to the vesicle of the Golgi apparatus (arrow). The arrowheads indicate spot desmosomes, whereas the plasma membrane is no longer visible. Bar, 200 nm. c, cytoplasm of clear cells; g, glycogen particles; k, keratin bundles; l, lipid droplet; se, setae.
representing a moderate state of cornification (Fig. 5A,B). The basal pedicels of clear cells surrounding the base of the setae, therefore, appear moderately keratinized, whereas the base of setae show merging bundles of β-keratin packets being assembled into long filaments (Fig. 5B).

Deep to the single Oberhäutchen/β-layer filled with β-keratin packets, the next layer of cells is pale and contains sparse keratin bundles and many vesicles and lipids, resembling cells of the lacunar layer, an immature form of the alpha-layer (Fig. 5C).

Ultrastructural examination of the voluminous fat cells (30–90 μm diameter) present in the connective tissue deep to the scanner epidermis (Fig. 1E) shows that numerous lipid vesicles are continuously formed and merge into central lipid droplets (Fig. 6A). The low electron-density of the droplets indicates they contain mainly saturated lipids. Numerous granules of glycerol are also contained in actively synthesizing lipoblasts (Fig. 6B).

**DISCUSSION**

Studies of the distribution of lipids between the outer layers of the epidermis in squamates have focused mainly on comparisons of the sources, location, and interaction with other epidermal constituents (e.g., keratin) in the context of evaporative water loss (Bell and Thathachari, 1963; Menon et al., 1996; Lillywhite, 2006). Extracellular lipids are typically associated with the mesos layer deep to the Oberhäutchen and β-layer (Lillywhite and Maderson, 1988). In our study, epidermal samples of gecko and anole toe pads reveal abundant

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extracellular lipids in other layers of the epidermis and in the dermis as well.

Rates of whole animal CWL are related to the thickness of the epidermis, its composition, and the surface area available for exchange (Lillywhite and Maderson, 1988; Menon et al., 1996). Distinct from any systematic variation in composition of the toe pad epidermis, the epidermal morphology of toe pads creates a highly subdivided surface area when compared with that of the general body surface. For example, in the genus Phelsuma the density of spatulae has been estimated to be as high as $21 \times 10^6$ per mm$^2$ (Peattie and Full, 2007). Assuming that there are 1000 spatulae per setal stalk (Autumn and Peattie, 2002), that setae are simple cylinders of 10 $\mu$m diameter and 100 $\mu$m in length, the setae would result in a ~70-fold surface magnification of the (smooth) epidermis. However, the epidermis of many lizards, including geckos, is ornamented rather than smooth, likely rendering the 70-fold increase in surface area an overestimate (Ruibal, 1968; Stewart and Daniel, 1972; Stewart and Daniel, 1973; Peterson, 1984). Nevertheless, the ornamentation of lizard epidermis (including geckos and anoles) that has been studied is usually described as comparatively small hair-like “spinules,” which vary in size from less than 1 to 2 $\mu$m in thickness in the basal part of the spinulae and from 1 to 3 $\mu$m in height in the outer and inner scale surface. Effects on surface area due to the much larger setae reasonably span a 4–60-fold increase relative to an epidermis covered with spinulae. Because of the surface restriction, we might expect that CWL in toe pads to be significantly higher than in other regions of the body surface. Therefore to limit water loss, lipids may also be increased in keratin-rich tissue, such as the setae and the Oberhäutchen, which normally have limited lipogenic activity. This process might limit water loss in conjunction with the underlying mesos layer. However, it is unclear how the extracellular lipids visible in the outermost layers of the outer generation, including within the setae themselves (Fig. 1), might influence CWL in comparison with epidermis from areas exclusive of the toe pads. Interestingly, the Oberhäutchen–$\beta$-layer and the alternating keratin–lipid multilayered mesos components are not obvious in our sections of toe pad epidermis, as previously observed in other gecko scanners (Alibardi et al., 2007; Alibardi, 2009). Our observations confirm that lipids are also abundant in the mesos and alpha-layers forming the setae. The mesos and the alpha-layers have been associated with the majority of resistance to diffusion of water in CWL (Lillywhite and Maderson, 1988; Menon et al., 1996; Lillywhite, 2006). It is not clear whether a distribution of lipids (as seen in this study) different from the bricks and mortar model would have the same effect in reducing CWL.

The setal epidermis of Phelsuma is very different from that of normal scales in which 4–5 layers of $\beta$-cells are deposited to produce harder and more resistant scales. It is likely that the thinning of the $\beta$-layer in scanners helps maintain the epidermis as a relatively pliable surface, maximizing the number of setae that can come into close contact with the substrate. Such conformation of the toe pad with the substrate would be made difficult if a thick $\beta$-layer formed the foundation of the setae. It is well possible that the thick keratin layers of the $\beta$-section and mesos-section of epidermis found in the scaly covering on the rest of the body is incompatible with the mechanics of adhesion of setae because compliance of setae (Jagota and Bennison, 2002) is essential for substrate contact and for the generation of van der Waals forces (Autumn and Peattie, 2002; Russell et al., 2007). Our observations suggest that most of the $\beta$-keratin in scanners is not used for making a resistant and thick $\beta$-layer, but instead is involved in building long setae. Finally, it is important to note that in Anolis, a significant portion of the lamellar surface extends distally as a free margin beyond the conspicuous cellular region along the proximal portion of the lamella (Ruibal and Ernst, 1965; Ernst and Ruibal, 1966). Whether setae along the free margin are more or less compliant than those in more proximal locations on the lamella is unknown. However, thickened $\beta$-layers are absent in both the proximal and distal portions of the lamellae.

The presence of extracellular lipids in layers of the epidermis directly adjacent to the setae themselves may be related to functions other than reducing CWL. The macromolecular organization of $\beta$-keratin and the mechanisms responsible for the assembly of long $\beta$-keratin filaments into setae (or feathers) remain unknown (Fraser and Parry, 1996; Rizzo et al., 2006; Fraser and Parry, 2008; Alibardi, 2009). However, the presence of lipids has suggested that they may play an important role in the assembly of the $\beta$-keratin filaments that compose avian feathers (Bell and Thathchari, 1963; Alibardi and Toni, 2008). In our sections of lizard toes, lipids are present in close association with mature setae of the inner and outer generation of P. dubia and the outer generation of A. carolinensis.

Within the inner generation, transmission electron microscopy revealed that during maturation the renewal phase in P. dubia exhibits at least one potential source of lipids to be the lamelliform bodies of the clear layer cells, which are composed of membrane lipids (Figs. 2 and 4). The degeneration of the basal part of the cytoplasm of the clear layer cells that contact the basal part of setae determines the loss of extracellular lipids that may coat the setal membrane. The lipid degeneration in the cells of the clear layer at the base of setae

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may also enable a quick detachment from the underlying Oberhäutchen, allowing shedding. A similar mechanism has been observed in feathers when barbules become separated one from another following the lipid degeneration of the interposed supportive cells (Alibardi and Toni, 2008).

The other lipid localization observed in this study (intracellular in the Oberhäutchen and setae) appears to be linked to the lipid-degeneration of the cytoplasm surrounding the keratin-bundles located in distal regions of the setae. This degeneration may be responsible for the subdividion of the setal tip into numerous branches, terminating in spatulae (Alibardi, 2009). It remains unknown whether lipids also interact more specifically with keratin. Although the mechanisms responsible for keratin filament assembly are of great interest, there is still substantial controversy about the relative roles of intracellular scaffolding (e.g., lipid or membrane-based) versus self-assembly (Norlen and Al-Amoudi, 2004; Meyers et al., 2008; Dufresne et al., 2009; Ghiradella and Butler, 2009; Prum et al., 2009).

Finally, we found large aggregations of fat cells in areas of the dermis of Phelsuma (Fig. 6) underlying scanners, relatively remote from the developing and mature setal generations of the epidermis. It seems unlikely, given their clumped distribution, that these lipids function as a general barrier to water loss. Presumably, fat cells function as a part of a hierarchical compliance system (Russell, 1981; Russell, 2002), which is comprised of muscle, tendons, paraphalangeal elements, and vascular sinuses, critical to maximizing the fraction of setae that come into contact with the substrate. Compliance is an essential feature of the gecko adhesive system and several components operating at considerably different spatial-scales (e.g., branching of the setae and digital vascular system) contribute to compliance in different ways. Over small distances, setae differ in length over the surface of scanners, maximizing spatular contact on substrates with roughness dimensions in tens of microns (Russell and Johnson, 2007). More extreme surface rugosity (on the order of millimeters) can be accommodated by pressure changes within sinuses of the digital vascular system seen in many species. It is possible that fat deposits, like those seen in our sections (Fig. 6), contribute to the compliance provided by the gecko digital vascular system (Russell, 1981; Rosenberg et al., 1992). Relative to our knowledge about the function and mechanics of setae, the complexity of other components of the gecko and anole adhesive system remain to be fully described and analyzed (Russell, 2002; but see Russell and Higham, 2009). However, recent examples of biologically inspired adhesive system prototypes (Rosenberg et al., 1992; Hui et al., 2007; Russell and Johnson, 2007; Sameoto et al., 2008; Lee et al., 2009) have demonstrated improved adhesion on rough surfaces derived from the assembly of compliant systems.

In summary, our study shows that lipids are abundant and widely distributed in layers of the epidermis external to the mesos layer, the part of the epidermis identified as the major barrier to CWL. Moreover, lipid vesicles or lamelliform bodies are present in the clear layer cells of the maturing inner generation setae and the lipids appear to surround the spatular ends of the setae as they progress through branching.

ACKNOWLEDGMENTS

The authors thank Stephanie Lopez, Alyssa Stark, and Brittany Hefin for help with animal husbandry and laboratory protocols. Anthony Cho-myk assisted with histology and light microscopy.

LITERATURE CITED


