Elucidating the Chemical Crypsis Mechanism in South African Snakes by Determining Microscale and Nanoscale Structure-Function Relationships in Snake Skin Sheds and Replicas

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Principal Investigator(s): Robert Shepherd

User(s): Rachel Miller

Affiliation(s): Materials Science and Engineering, Cornell University

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Contact(s): rfs247@cornell.edu, rm973@cornell.edu

Website: orl.mae.cornell.edu

Primary CNF Tools Used: NanoScribe GT2

MATERIALS

Abstract:

Humans have been studying and mimicking animal's visual crypsis using wearable camouflage since the 18th century. Yet, only recently we have documented and attempted to understand the origins of chemical crypsis, the ability to become imperceptible to olfaction. Specifically this work aims to elucidate the mechanism of chemical crypsis in the first vertebrate shown to exhibit this trait, a snake known as the Puff adder (*Bitis arietans*).

The major feature distinguishing the Puff adder from its non-cryptic brethren such as the Night adder (*Causus rhombeatus*) is the skin surface structure. The skin's micron-scale, high aspect ratio, curved features known as 'fingers' create an array of wells for odorants to pool, significantly reducing odorant volatility and therefore rendering the Puff adder imperceptible to smell. To study and quantify this phenomena independent of snakeskin material, snakeskin surface biomes, and environmental contaminants, we employ various imaging and 3D printing processes to create a variety of detailed and accurate scaled models of both the puff adder skin and night adder skins. Nano-focused computed tomography (nano-CT) is used to create three-dimensional renderings of skins. To maximize accuracy of replicas, nano-CT image segmentation is informed by focused ion beam (FIB) and scanning electron microscopy (SEM) images. These digitally rendered surfaces are printed in epoxy using 3D printing, requiring the use of two photon polymerization (2PP) on the NanoScribe GT2 for micron-scale feature resolution.

We show the use of 2D Fourier transform analysis sequentially along the long axis as a means to quantify periodicity and investigate the degree of quasi-ordered orientation as a predictor for surface thermodynamic phenomena. Physical experiments on printed models and topographical analysis of digital renderings are used together to analyze and quantify the effects of structure periodicity and surface-area-to volume ratio on adhesion, wetting, and evaporation.

Summary of Research:

It has been shown that many snakes may actually hide not in plain sight using visual camouflage, but instead in plain "smell". Instead of being visually cryptic, these South African snakes are chemically cryptic [2]. Specifically, work done by A.K. Miller et al demonstrates that the Puff adder relies on chemical crypsis in order to effectively ambush its prey [1]. Extensive behavioral studies by A.K. Miller et al have suggested that additionally, current unpublished work by A.K. Miller is showing that many snakes in South Africa and the surrounding region also are chemically cryptic.

Previously, Puff adder skins have also been noticed for their microornamentation. Their skins have high aspect ratio "fingers" that protrude out from the skin base. It has been proposed that these types of ornamentation on a similar species were used to create optical effects in the skin. Snakeskin is home to large amounts of bacteria and other odorant producing biota. We hypothesize that these skins are not chemically cryptic because of their lack of odorants, but rather because there is a structure to their skin that keeps odorants from vaporizing and being detected by predators,

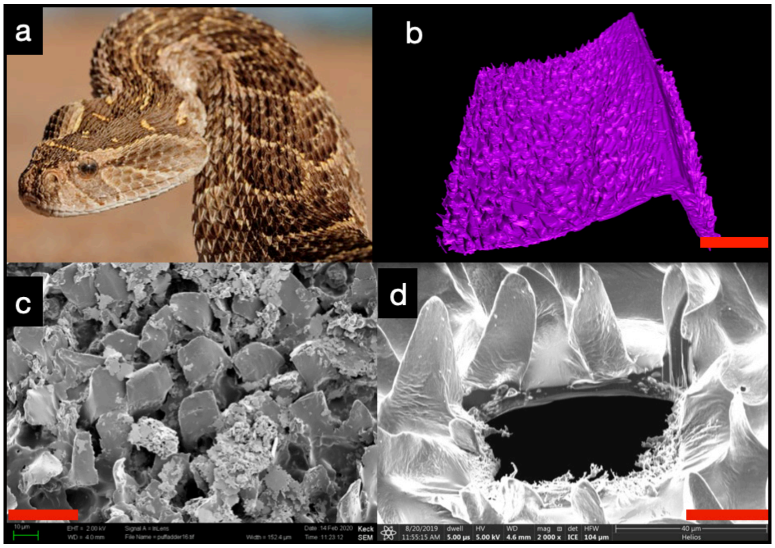

Figure 1: (a) Bitis arietans *(Puff adder), chemically cryptic snake1. (b) Nano CT scan reconstruction of Puff adder skin section with 'fingers,' Scale bar = 100 µm. (c) SEM image of puff adder skin to show pores are more visible than in CT or FIB, Scale bar = 20 µm. (d) FIB image of Puff adder skin. Further reveals structure of 'fingers' on skin, Scale bar = 20 µm.*

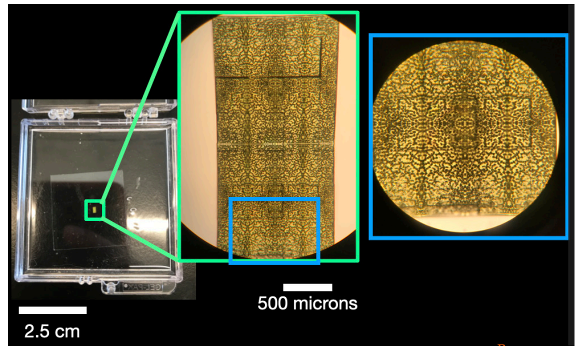

Figure 2: 2PP 3D printed Puff adder snakeskin replicas, photograph and optical microscope images.

prey, or even mates. The high aspect ratio features on Puff adder skin creates an environment on the surface of snake skin which decreases the volatility of odorants, rendering them chemically cryptic.

Using imaging techniques — including but not limited to nano-focused X-ray computed tomography (nano-CT) (Figure 1b), scanning electron microscopy (SEM) (Figure 1c), and focused ion beam microscopy — further reveals structures of 'fingers' on skin (Figure 1d, scale bar = 20 μm (FIB), and we can comprehensively probe Puff adder skin sheds. Together these techniques enable us to render and fabricate accurate digital and physical 3D renderings of skin features via nanoscopic 3D printing on the NanoScribe Photonic Professional GT2 (Figure 2).

Chemical treatment of these nano- and microscale features will be performed using thiolate mediated self-assembled monolayer chemistry, with a final test to be performed using modified substrates that chemically mimic the original skin.

To test these structures' impact on odorant volatility it would be beneficial to be able to fabricate consistent, large area replicas of these features. This could potentially involve reactive ion etching to achieve high aspect ratio features or wet etching methods, depending on the material chosen for these replicas. It is currently being debated as to whether or not it is possible to make these replica structures out of materials that are not silicone, epoxy, or other commercial plastics and instead out of keratin itself or another functionally and/or structurally similar material.

Conclusions and Future Work:

We have successfully created 0.1 cm × 0.1 cm footprint 1:1 Puff adder skin replica prints with submicron scale resolution using the NanoScribe GT2 printing apparatus (Figure 2). Nanoscopic prints and snakeskin sheds will serve as masters for replica molding, the former being non-destructive technique and the latter being a destructive technique.

References:

[1] McKay S, Glaudas X, Alexander GJ, Miller AK, Maritz B. An ambusher's arsenal: chemical crypsis in the puff adder (*Bitis arietans*). Proceedings of the Royal Society B, 282, 2015.

[2] Graeme D Ruxton. Non-visual crypsis: a review of the empirical evidence for camouflage to senses other than vision. Philosophical Transactions of The Royal Society, 364(1516), 2008.