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Variation in quantity and composition of cuticular hydrocarbons in the scorpion *Buthus occitanus* (Buthidae) in response to acute exposure to desiccation stress

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ABSTRACT

Scorpions exhibit some of the lowest recorded water loss rates among terrestrial arthropods. Evaporative water loss to the surrounding environment occurs mainly through the integument, and thus its resistance to water loss has paramount significance for the ability of scorpions to tolerate extremely dry habitats. Cuticular hydrocarbons (HCs) deposited on the outer epicuticle play an important role in determining cuticular waterproofing, and seasonal variation in both cuticular HC quantity and composition has been shown to correlate with water loss rates. Precursor incorporation rates into cuticle HCs have been observed to be extremely low in scorpions compared with insects. We therefore used adult male *Buthus occitanus* (Buthidae) in order to test HC profile plasticity during acute exposure to 14 d and 28 d of experimental desiccation. Cuticular HC profile of hydrated scorpions was similar to that reported for several other scorpion species, consisting of similar fractions of *n*-alkanes and branched alkanes, with no evidence for unsaturation. Most abundant of the *n*-alkanes were *n*-heptacosane (C_{27}; 19 ± 2% of total HCs), *n*-nonacosane (C_{29}; 16 ± 1%) and *n*-hentriacontane (C_{31}; 11 ± 1%). Exposure to desiccation stress resulted in a significant increase in the total amount of extracted HCs, and in the relative abundance of branched alkanes at the expense of *n*-alkanes. Together with an increase in HC chain lengths, these changes mimic previously-reported seasonal variation among freshly-collected specimens. This indicates that scorpions respond to water shortage by regulating the properties of their passive integumental barrier to water loss.

1. Introduction

Scorpions are among the most successful terrestrial arthropods inhabiting deserts worldwide (Polis and Yamashita, 1991). A range of behavioral, anatomical and physiological mechanisms underlie the ability of scorpions to withstand the harsh abiotic conditions typical of xeric habitats. Behaviorally, they avoid environmental extremes by retreating to shelters (e.g., burrows, rock crevices) and by a reduced and largely nocturnal surface activity. Still, as small organisms scorpions are faced with a considerable challenge to manage their body water stores in dry environments as a result of their relatively large surface area to volume ratio (reviewed by Hadley, 1990).

Scorpions rarely drink, and gain water from body fluids of their captured prey (Hadley, 1990). Employing mostly a "sit and wait" foraging strategy, scorpions have to manage their body water budget when water gains are often scarce and unpredictable. This is helped by a range of adaptations which limit losses to the environment. Terrestrial arthropods lose water to their environment with their excretions, and to a greater extent through respiratory and cuticular transpiration. Scorpions eliminate nitrogenous wastes primarily as insoluble guanine and uric acid, thus minimizing excretory water losses (Hadley, 1990). A recent study showed that lower water vapor to CO_2 emission ratios account for lower respiratory water losses in xeric compared with closely-related mesic scorpion species (Gefen, 2011). However, it is well established that scorpions, like other terrestrial arthropods, lose water to the environment mainly through cuticular transpiration (Hadley, 1974; Withers and Smith, 1993; Gefen et al., 2009; Gefen, 2011). It is therefore expected that cuticular resistance to diffusion of water vapor would constitute a major target for adaptive responses to desiccating conditions.

The general structure of the scorpion integument is similar to that of insects, and includes a monolayered epidermis and an outer, multilayered, non-cellular cuticle (Hadley, 1990). Experimental evidence suggests that all layers of the scorpion cuticle, and potentially the cellular epidermis, contribute to its exceptionally high resistance to water loss (e.g. Hadley, 1970; Riddle, 1981; Gefen et al., 2009). Nevertheless, lipids on the outer epicuticle provide the principal barrier for water

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vapor diffusion across the integument, and early experiments showed that removal of this layer results in rapid dehydration of the organism (reviewed by Hadley, 1994).

The cuticular wax layer of scorpions typically includes high quantities of hydrocarbons (HCs) and sterols, followed by free fatty acids, aliphatic alcohols and triacylglycerols (Hadley and Jackson, 1977; Toolson and Hadley, 1977, 1979). The best studied among these groups of lipids are cuticular HCs, both for the ease of their extraction and identification, and because they are the most hydrophobic and therefore potentially provide a good barrier for minimizing water loss (Gibbs and Rajpurohit, 2010). The total cuticular extractable lipids and the HC fraction found in scorpions were lower in comparison with the more permeable cuticle of insects (Toolson and Hadley, 1977). Still, similar characteristics of cuticular HC composition are correlated with interspecific variation, and with intraspecific seasonal fluctuations in scorpion desiccation resistance. For example, longer carbon chains and higher proportion of branched alkanes were found in the xeric-adapted *Hadrurus arizonensis* (Iuridae) compared with the more mesic *Pseudouroctonus* (formerly *Uroctonus*) *apacheanus* (Vaejovidae) (Toolson and Hadley, 1977). Likewise, *n*-alkanes accounted for a smaller fraction of total HCs in summer compared with winter samples of *Centruroides exilicauda* (formerly *Centruroides sculpturatus*; Buthidae), with the former also characterized by longer mean HC chain lengths (Toolson and Hadley, 1979).

Scorpion HCs are synthesized mainly in the hepatopancreas, from where they are transferred to the cuticle (Hall and Hadley, 1982). Incorporation of labeled precursors into cuticular HCs in both *C. exilicauda* (Ross and Monroe, 1970) and *Smeringurus* (formerly *Paruroctonus*) *mesaensis* (Vaejovidae) (Hadley and Hall, 1980; Hall and Hadley, 1982) was found to be minimal, suggesting that intermoult biosynthesis of HCs is low. Therefore, the aim of this study was to find out whether scorpions can respond to acute desiccation stress by modifying their cuticular HC composition in order to improve the waterproofing properties of their integument.

2. Materials and methods

Scorpions

Adult male *Buthus occitanus* (Buthidae) were collected in HaRo'a campsite (30°52′29.6″N 34°47′09.7″E), near Sde Boqer in the Negev Desert in Israel, using ultraviolet light detection in August 2013. The scorpions were placed in round, 9.5 cm diameter plastic containers on a thin layer of soil from the collection site. The scorpions were kept in the lab for two weeks at room temperature (~24 °C), and were provided with three food items during that time: one adult cricket (*Acheta domesticus*) and two cockroaches (*Nauphoeta cinerea*). The scorpions were offered the last prey item 72 h prior to initiation of measurement, when they ($N = 46$; body mass, 1.2366 ± 0.0236 g) were randomly assigned to one of three experimental groups. One group of scorpions were weighed to the nearest 0.1 mg and immediately killed by freezing. In the other two groups the scorpions were weighed, transferred to identical empty containers and placed in a controlled-temperature cabinet (30.0 ± 0.5 °C) for 14 d and 28 d, respectively, and prevented access to food/water. At 14 d and 28 d these scorpions and their dry excretions were weighed for gravimetric estimation of water loss. The scorpions were then killed by freezing.

A dissecting microscope with a micrometer eyepiece was used to measure scorpion carapace length. A body condition index (CI) was calculated as body mass (g) divided by carapace length (mm). This could provide a measure of the scorpion nutritional status as adult scorpions do not molt, and therefore carapace length remains unchanged during adulthood.

Scorpion surface area was estimated using the formula:

$$S = k \cdot W^{2/3}$$

where S is the surface area (cm^2), k is a species-specific value and W is the body mass (g) (Hadley, 1994). A k value of ~12 is typical of many terrestrial arthropods, and a value of 12.07 was reported for *Leiurus quinquestriatus* (Buthidae) (Warburg et al., 1980) and is used here because of the species' morphological resemblance to *B. occitanus*.

Cuticular lipid extraction

Scorpions synthesize *n*-alkanes with predominantly odd-number of carbon atoms, with chain length typically ranging from 19 to 33 (see Trabalon and Bagnères, 2010). Only trace amounts, if any, of *n*-docosane (C_{22}) have been reported from scorpion cuticular lipid extracts (also confirmed in preliminary runs in this study) and therefore *n*-docosane served as an internal standard. Following preliminary assays, in which scorpions were transferred to fresh solvent every 10 min it was concluded that the optimal time for complete cuticular HC extraction at room temperature was 20 min.

We therefore placed individual scorpions in a glass beaker, containing 20 mL HPLC grade *n*-Hexane (Merck) with 2.5 μg *n*-docosane (#43942; Fluka) as an internal standard. Following removal of the scorpion, the solvent with the extracted lipid mixture was passed using a Pasteur pipette through a Florisil® (#220736; Sigma-Aldrich) column. The beaker was washed through the column with additional 1–2 mL of hexane, and the HC-containing eluent was transferred to a glass test tube and dried under a stream of dry nitrogen. The HC mixture was then washed from the test tube (2×1 mL) and transferred to 2 mL glass vials (C4000-1W; National Scientific, Rockwood, TN, USA) where it was dried again under nitrogen. Finally, the mixture was transferred again (2×100 μL) to 0.3 mL polyspring® inserts (C4010-630; National Scientific) placed in empty 2 mL glass vials, where the mixture was dried under nitrogen. The vials were then capped and stored dry under nitrogen at −20 °C.

Hydrocarbon analysis

The dried hydrocarbon extracts were resuspended with 100 μL ethyl acetate prior to gas chromatography analysis. Samples were analyzed using a GC 6890N (Agilent Technologies, CA, USA) instrument equipped with a capillary HP-5MS column (30 m, 0.25 mm, 0.25 μm, Agilent Technologies) filled with phenyl methyl siloxane, and an HP-5975 mass spectra detector (Agilent Technologies, CA, USA). 2 μL of each sample were injected with split ratio of 1:1. Helium was used as carrier gas and the flow rate was maintained at 1 $mL \cdot min^{-1}$. The initial oven temperature was kept at 70 °C for 5 min, raised to 150 °C at 20 $°C \cdot min^{-1}$, held for 1 min, raised to 300 °C at 3 $°C \cdot min^{-1}$ and maintained at this temperature for 25 min. Additional temperature settings were as follows: Front inlet 250 °C, Thermal AUX 280 °C, MS Quad 150 °C and MS source 230 °C.

Identification of hydrocarbons (with minimum peak area >0.5% of the largest peak) was based on mass spectral fragmentation patterns and retention index (KI, Kovats index) (Carlson et al., 1998; Blomquist, 2010). Alkane calibration curves were used for hydrocarbon quantification based on integrated peak areas. A C_7–C_{40} saturated alkane mixture in hexane (#49452-U; Supelco, Bellefonte, PA, USA) stock solution (1000 $\mu g \cdot mL^{-1}$ each) was diluted with hexane to 100 $\mu g \cdot mL^{-1}$ working solution. Using five concentrations (10, 30, 50, 80 and 100 $\mu g \cdot mL^{-1}$) by diluting the working solution with ethyl acetate, we constructed linear calibration curves ($R^2 > 0.98$) for all *n*-alkanes across the biologically-relevant retention time range. Linear equation parameters were assumed to change linearly as a function of retention time between values for two adjacent *n*-alkanes.

Statistics

Statistical analyses were carried out using Statistica for Windows (ver 8.0) (StatSoft, Tulsa, OK, USA). Values throughout the text represent means ± s.e.m.