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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

E. Gefen [a,*], S. Talal [a], O. Brendzel [a], A. Dror [b], A. Fishman [b]

[a] Department of Biology and Environment, University of Haifa — Oranim, Israel
[b] Department of Biotechnology and Food Engineering, Technion, Haifa, Israel

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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

* Corresponding author. Tel.: +972 4 9838837.
*E-mail address:* gefene@research.haifa.ac.il (E. Gefen).

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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.

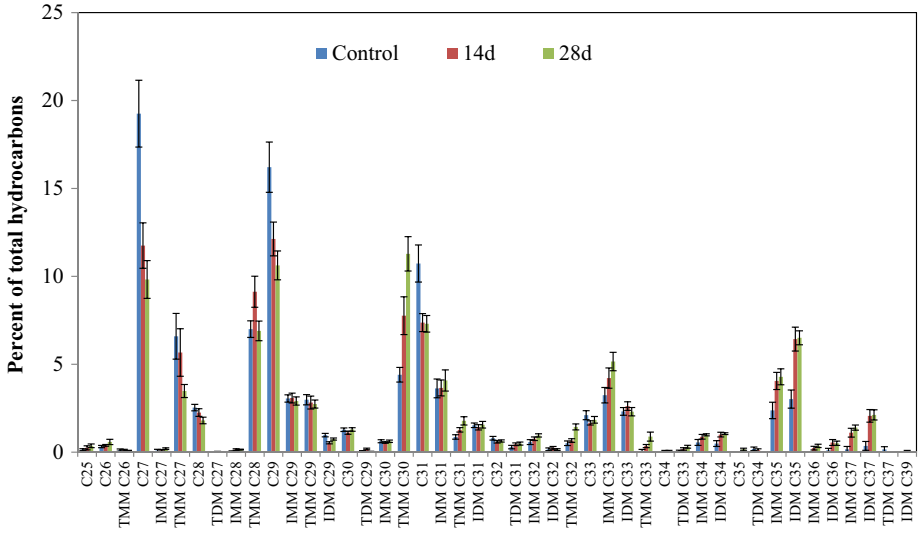


**Fig. 1.** The effect of desiccation at 30 °C on the relative prevalence (means ± s.e.m.) of cuticular hydrocarbons in adult male *B. occitanus*. See Table 1 for sample sizes. Abbreviations: IMM, internal monomethyl; TMM, terminal monomethyl; IDM, internal dimethyl; TDM, terminal dimethyl. Numbers indicate the carbon chain length.

## 3. Results

Cuticular HCs in *B. occitanus* were all saturated, and ranged in carbon-atom chain length from 25 to 39 (Fig. 1). The largest group of cuticular HCs in hydrated scorpions was *n*-alkanes (53 ± 3%) (Fig. 2). This included chain lengths of 25–33 carbon atoms, largely dominated (91%) by odd-number chain lengths. Most abundant were *n*-heptacosane ($C_{27}$; 19 ± 2% of total HCs), *n*-nonacosane ($C_{29}$; 16 ± 1%) and *n*-hentriacontane ($C_{31}$; 11 ± 1%). Internally-branched monomethylalkanes (7-, 9-, 11-, 13-methylalkanes; IMMs; 14 ± 2% of HCs) were dominated by IMM $C_{29}$, IMM $C_{31}$, IMM $C_{33}$ and IMM $C_{35}$ (Fig. 1). Terminally-branched monomethylalkanes (5-, 2- or 4-, 3-methylalkanes; TMMs; 23 ± 2% of HCs) were similarly distributed among odd- and even-numbered carbon chains ($C_{26}$ to $C_{33}$). More than 85% of the internally-branched dimethylalkanes (IDMs; 9 ± 1% of HCs) had odd-number carbon chains, with increasing prevalence as chain length increased ($C_{29} < C_{31} < C_{33} < C_{35}$). Terminally-branched dimethylalkanes (TDMs; 3,7- and 3,9- dimethylalkanes) comprised <1% of total cuticular HC content. No significant relationship was found between total HCs or arcsine-transformed percentages of any HC group and surface area or body condition index of hydrated scorpions ($p > 0.05$).

Significant increase in total cuticular HC content was recorded after two weeks of desiccation, but not between the 14 and 28 d treatments (Table 1) (ANCOVA, with surface area as a covariate, followed by Tukey HSD test for unequal N; $\alpha = 0.05$). The increase in *n*-alkane, TMM and IDM content was significant ($p < 0.001$; ANCOVA, with body surface area as a covariate) following 14 d of desiccation, whereas a significant increase in IMM ($p = 0.004$) compared with controls, was found only in the 28 d treatment. The content of TDM remained unchanged throughout the experimental exposure to desiccation.

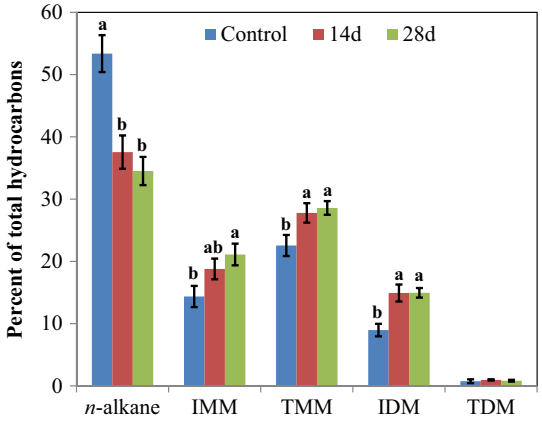


**Fig. 2.** The effect of desiccation at 30 °C on cuticular hydrocarbon branching in adult male *B. occitanus* (means ± s.e.m.). See Table 1 for sample sizes. Abbreviations as in Fig. 1. Different letters indicate significant differences ($\alpha = 0.05$).

Nevertheless, despite the increasing amounts among all cuticular HC groups there was a significant change in their respective relative prevalence following exposure to experimental desiccating conditions (Figs. 1, 2). Despite the increase in absolute amounts the fraction of *n*-alkanes from total cuticular HC decreased significantly ($p < 0.001$; ANCOVA on arcsine-transformed percentages, with surface area as a covariate). Instead, there was a significant increase in the fraction of IMMs ($p < 0.05$), TMMs ($p < 0.01$) and IDMs ($p < 0.001$) (Fig. 2). The increase in IMM ($C_{33}$, $C_{35}$ and $C_{37}$) and IDM ($C_{35}$ and $C_{37}$) quantities was largely associated with long-chain branched alkanes (Fig. 1). In contrast, the increase in TMMs resulted from an increase in shorter, and the generally less abundant even-number HC chains, namely 4-methyl triacontane, and to a lesser extent 4-methyl dotriacontane. The former increased from 0.09 ± 0.01 $\mu g \cdot cm^{-2}$ in the control group to 0.43 ± 0.12 $\mu g \cdot cm^{-2}$ following two weeks under desiccation stress. When exposure to desiccation stress was extended to 28 d, the amount of 4-methyl triacontane increased to 0.75 ± 0.12 $\mu g \cdot cm^{-2}$, totaling ~12% in mass of the entire cuticular HC mixture.

Only three individuals out of sixteen control *B. occitanus* exhibited branched heptatriacontane. In contrast, with the exception of one individual, monomethyl- and dimethyl-heptatriacontane (or both) were present in the HC mixture of all scorpions following a 28 d exposure to desiccation. The effect of desiccation stress on HC chain length is highlighted in Fig. 3a. The increase in the fraction of methyl-branched $C_{30}$, $C_{33}$, $C_{35}$ and $C_{37}$ (Fig. 1) is coupled with a decrease

**Table 1**
Initial body mass and condition index, and the effect of experimental treatments on cuticular hydrocarbon content of male *B. occitanus*.

| Treatment | Control | 14 d | 28 d |
|---|---|---|---|
| N | 16 | 14 | 16 |
| Initial body mass (g) | $1.2657 \pm 0.0386^{ab}$ | $1.3027 \pm 0.0465^{a}$ | $1.1497 \pm 0.0297^{b}$ |
| Initial condition index | $0.2079 \pm 0.004^{ab}$ | $0.2124 \pm 0.006^{a}$ | $0.1944 \pm 0.003^{b}$ |
| Total HC ($\mu g \cdot cm^{-2}$) | $1.80 \pm 0.17^{b}$ | $3.66 \pm 0.64^{a}$ | $6.29 \pm 0.87^{a}$ |

Different superscript letters indicate significant differences ($\alpha = 0.05$).

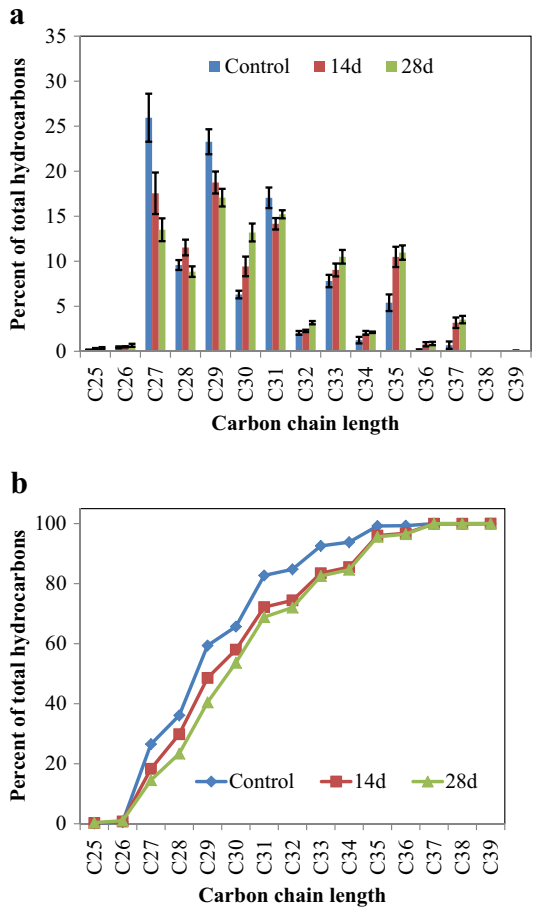


**Fig. 3.** The effect of desiccation at 30 °C on cuticular hydrocarbon chain length in adult male *B. occitanus*. Data presented as mean percentages (±s.e.m.) (a) and cumulatively (b). See Table 1 for sample sizes. Abbreviations as in Fig. 1.

in the relative contribution of $C_{27}$ (*n*-heptacosane and terminally-branched heptacosane) and $C_{29}$ (*n*-nonacosane; Fig. 1). Cumulative frequency distribution of cuticular HCs (following Toolson and Hadley, 1977, 1979) indicates a gradual increase in mean chain length as exposure to desiccation prolongs, and shows that 37-carbon atom chains are only found in trace amounts among hydrated *B. occitanus* (Fig. 3). Principal component analysis (PCA; Fig. 4) confirms that gradual separation of cuticular HC mixtures across a desiccation gradient is largely associated with increased levels of long-chain branched alkanes at the expense of shorter-chain *n*-alkanes. Five specimens from the 28 d treatment were clearly separated from the rest by the second factor, correlating with high levels of long-chain branched alkanes. Interestingly, these five individuals exhibited five of the highest seven mass loss values recorded in this study. Likewise, the one 14 d specimen with the lowest value on the first factor represents a scorpion that had lost 18.0% of its initial body mass, compared with its experimental group mean of 11.1 ± 0.6%.

## 4. Discussion

Cuticular HCs have been found to be the most abundant among lipid components in the scorpion epicuticle (Hadley and Jackson, 1977; Toolson and Hadley, 1977, 1979). As non-polar molecules, they provide scorpions and other terrestrial arthropods with an efficient barrier which limits integumental transpiration. Regulation of cuticular resistance could therefore be achieved by plastic changes in HC quantity and/or composition. However, it is not known whether relatively short-term changes in cuticular wax composition (and resistance to water loss) could contribute to desiccation resistance during acute water shortages, typical of xeric existence entailing unpredictable access to water.

The overall quantities and relative abundance of HCs among other cuticular lipid groups are difficult to compare between studies because of the use of different solvents for lipid extraction (Hadley and Jackson, 1977), dietary effects (Hall and Hadley, 1982) and age and sex-specific variation (Trabalon and Bagnères, 2010). Results in this study suggest that cuticular HC content could also be significantly affected by the hydration state of freshly-collected scorpions. They indicate that epicuticular HC deposition continues, and varies in pattern, when the scorpions have no access to either food or environmental water. The energy investment in HC synthesis and cuticular deposition under stressful conditions, when further environmental resources are limited and unpredictable, suggests a fitness advantage. Cuticular lipid quantification does not allow successive extractions from the same individual, which prevents direct testing of the effect of total HC quantities on desiccation resistance performance. Still, comparative analysis appears to support improved cuticular resistance to water loss in scorpions by means of increased surface density of HCs. Values of 5.8 and 2.9 $\mu g \cdot cm^{-2}$ were measured for the xeric *H. arizonensis* and mesic *P. apacheanus*, respectively, correlating with the lower cuticular permeability of the former (Toolson and Hadley, 1977). Two-fold higher mass-specific cuticular lipid contents were measured in juvenile compared with adult *S. mesaensis* (Trabalon and Bagnères, 2010), perhaps compensating for the high surface area to volume ratio exposing small-sized young scorpions to increased desiccation hazard. The lack of significant relationship between HC content and scorpion condition index reported here supports the observation of Trabalon and Bagnères (2010) that HC quantities are largely independent of body size in adult scorpions. Nevertheless, the increase in cuticular HC content during desiccation suggests a relatively short-term stress-response mechanism. Despite the transfer of scorpions from acclimation to experimental desiccation temperature (24 to 30 °C) the observed increase in HC content in desiccated *B. occitanus* cannot be attributed to temperature-dependent increase in metabolic rates. It was recently shown that exposure of scorpions to identical desiccating conditions resulted in a two-fold decrease in metabolic rates (Kalra and Gefen, 2012).

Variation in total HC content appears to be associated with desiccation stress-response and developmental and ecological constraints in scorpions. However, higher lipid surface-densities have been recorded in insects exhibiting considerably lower cuticular resistance to water loss compared with scorpions (Hadley, 1977, 1994). The typical HC fraction of the cuticular lipid mixture (>70%) in insects is also higher than reported values for scorpions, suggesting that lipid and HC composition may have a significant effect on the waterproofing properties of the cuticle (Hadley and Jackson, 1977; Toolson and Hadley, 1977). In line with all previous reports on scorpions (and unlike HC profile typical of many insects; Lockey, 1988), we did not find evidence for unsaturated cuticular HCs in male *B. occitanus*. Considering the extremely low melting points ($T_m$s) of alkenes compared with similar-size alkanes, this correlates well with the low permeability of the scorpion cuticle (Gibbs and Pomonis, 1995). Similar fractions of normal (*n*-alkanes) and branched alkanes in the control group agree with previously reported values for *S. mesaensis* (Hadley and Jackson, 1977), *U. apacheanus* (Toolson and Hadley, 1977) and winter-collected *C. exilicauda* (Toolson and Hadley, 1979). The significant decrease in the relative abundance of *n*-alkanes during exposure to desiccation (Fig. 2) is also in agreement with previous results. Toolson and Hadley (1979) reported seasonal fluctuations with a two-fold decrease in the fraction of *n*-alkanes from winter to summer collection. That seasonal variation among freshly-collected samples is mimicked during acute exposure in laboratory settings provides support to our hypothesis that stress-response mechanisms in scorpions include compositional plasticity of cuticular HCs.

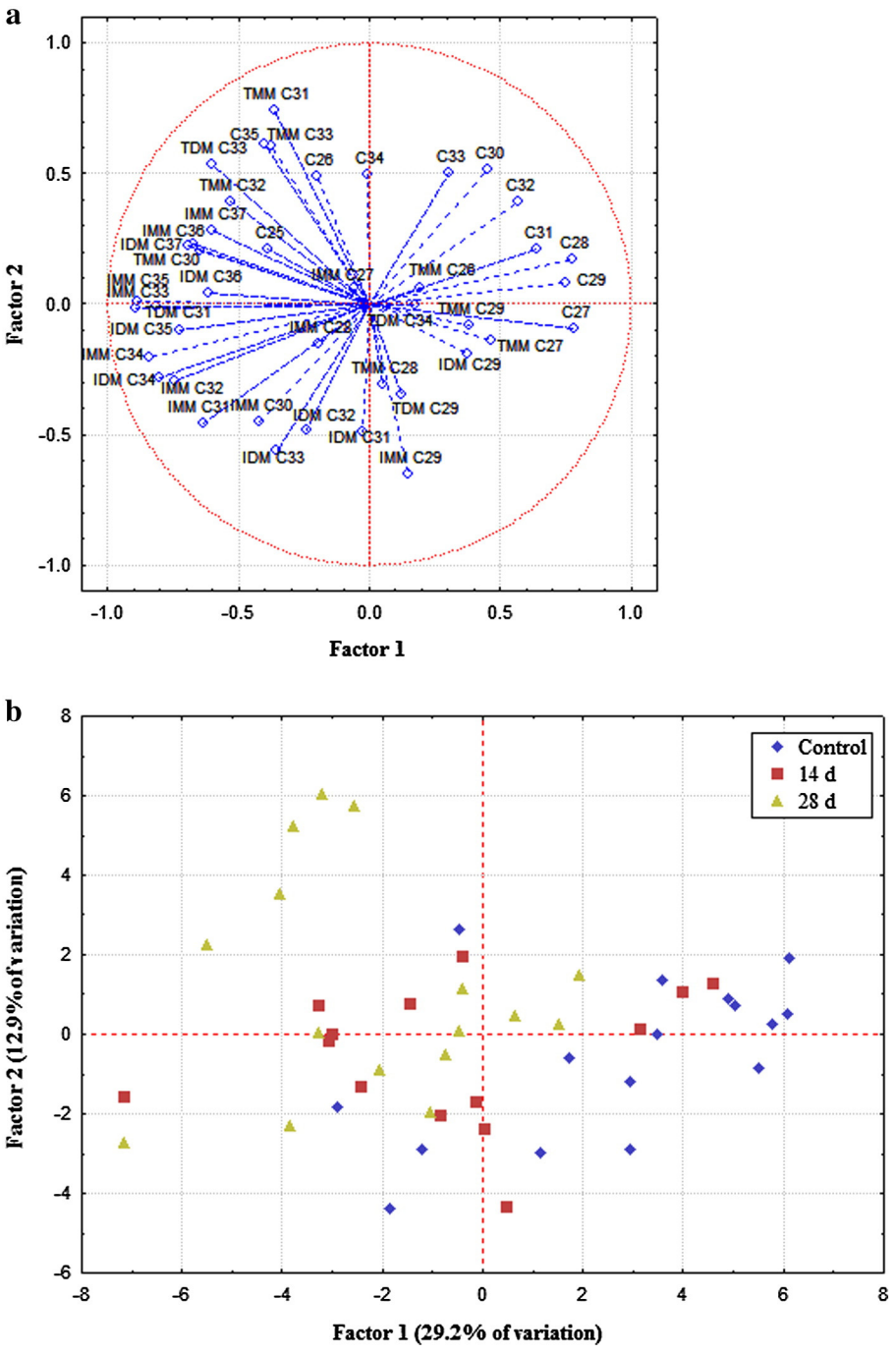


**Fig. 4.** Principal component analysis (PCA), using cuticular hydrocarbons as variables. (a) Factor coordinates, indicating the representation of each of the hydrocarbon percentages on the two main factors. (b) Each point represents an individual scorpion, belonging to one of the experimental groups (fed controls, 14 d and 28 d desiccation). Variables with a single observation (TDM C27 and C34) were omitted from the analysis. Abbreviations as in Fig. 1.

The lipid-melting model is supported by both inter- and intraspecific correlation between cuticular lipid $T_m$s and cuticular lipid "critical" temperatures above which a sharp increase in cuticular permeability is observed (Gibbs, 2002). Among HCs, *n*-alkanes allow closer packing and are thus thought to reduce permeability to water vapor diffusion through the cuticular barrier. The melting points of branched methylalkanes, and internally-branched alkanes in particular, are lower than those of *n*-alkanes of the same carbon-atom chain length (Gibbs and Pomonis, 1995). An increase in the fraction of branched alkanes at the expense of *n*-alkanes under desiccation stress would therefore appear counter-productive to the water budget of scorpions. Still, a decrease in the fractions of *n*-heptacosane ($C_{27}$) and *n*-nonacosane ($C_{29}$) (see Fig. 1) is coupled with a sharp increase in the fraction of a terminally-branched monomethyl, 4-methyl triacontane in *B. occitanus* (Figs. 2, 3 in this study) and a terminally-branched hentriacontane in *H. arizonensis* (Toolson and Hadley, 1979). This suggests a general stress response in scorpions, where an increase in the rate of *n*-alkane synthesis may be complemented by recruitment of alternative pathways for biosynthesis of HCs (Blomquist, 2010), including higher synthesis rates of methyl-branched alkanes. Scorpions are unable to synthesize *n*-alkanes longer than 33–35 carbon atoms (Hadley and Jackson, 1977; Toolson and Hadley, 1977, 1979; This study), and thus rely on alternative pathways for increasing cuticular resistance. Terminally-branched alkanes melt at only slightly lower temperatures compared with *n*-alkanes

their length (Gibbs and Pomonis, 1995), and could provide a fitting substitute which could explain their relative high abundance in severely-stressed scorpions (Fig. 4; see Results). It is worth noting that based on data provided by Gibbs and Pomonis (1995), the $T_m$ of 4-methyl triacontane is likely to be ~40 °C, a temperature that the nocturnal, burrow-inhabiting *B. occitanus* is unlikely to encounter in Israel.

Despite their ability to synthesize HCs with $T_m$s higher than ecologically-relevant temperatures, terrestrial arthropods also deposit low-$T_m$ HCs on their cuticles (Gibbs, 2002). A suggested likely explanation is the need to spread deposited HCs across the cuticle in order to increase epicuticular resistance. Available data on regional cuticular permeability in scorpions is contradictory. No difference was found between the permeability of sclerotized and non-sclerotized regions in the integument of *H. arizonensis* (Hadley and Quinlan, 1987), whereas sternite permeability was 2.5-fold lower compared with that of the pleural membrane in *Pandinus imperator* (Scorpionidae) (Hadley, 1994). Considering that the highest water loss rates among scorpions were reported for the tropic *P. imperator* (Hadley, 1994), it is possible that an efficient cuticular barrier to water vapor diffusion depends on low-$T_m$ HCs which may support a more even distribution of high-$T_m$ lipids across the cuticle.

The melting points of dimethylalkanes are considerably lower than those of same-length *n*-alkanes (Gibbs and Pomonis, 1995). However, an intriguing observation is the significant increase in the relative abundance of internally-branched long-chained alkanes, associated with the overall increase in HCs longer than $C_{34}$ following desiccation (6.4 to ~15% of the mixture). From an energy-budget perspective there are better alternatives for low-$T_m$ HCs compared with the energetically-costly long-chain dimethyl pentatriacontane ($C_{35}$) and heptatriacontane ($C_{37}$) (Fig. 1) that would provide the mobility required for spreading the lipid mixture across the cuticle. Importantly, HCs do not appear in isolation on arthropod cuticles, and interaction between two or more HCs and other lipid groups may be at play in determining the overall cuticular resistance. Data is not available for mixtures consisting of dimethyl-alkanes, but the $T_m$ of alkane–methylalkane mixtures was not higher than the weighted average $T_m$ of its components (Gibbs, 1995). An important observation in this regard is the exceptionally high fraction of sterols, mainly cholesterol, in the cuticular lipid mixture of scorpions. Values ranging from 20 to 25% of total lipids in vaejovid scorpions, and 40% in the buthid *C. exilicauda* (Toolson and Hadley, 1977, 1979) are exceptionally high compared with insects for which sterols contribute less than 6% of the total cuticular lipid content (Lockey, 1988). If cuticular sterols serve a role in reducing cuticular lipid fluidity through interactions with HC chains, similar to their effect on cell membrane fluidity, then high sterol levels in the scorpion cuticle could explain its extremely high resistance to water loss. The stabilizing properties of sterols could also compensate for potential biosynthetic constraints resulting in deposition of internally-branched alkanes. Arthropods are not capable of sterol synthesis, and rely on dietary sources. It was therefore suggested that the deposition of this important building block of cell membranes on the cuticular surface supports the functional significance of cuticular sterols (Toolson and Hadley, 1977).

In conclusion, this study shows that acute exposure of scorpions to desiccation stress results in a change in quantity and composition of cuticular HCs. These changes mimic previously reported seasonal fluctuations, and could reflect a direct effect of abiotic conditions and/or response to changes in prey availability.

## Acknowledgments

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