Temperature dependence of water loss rates in scorpions and its effect on the distribution of *Buthotus judaicus* (Buthidae) in Israel

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Abstract

Scorpions of the family Buthidae have been shown to be more desiccation resistant in comparison with sympatric Scorpionidae species. This has been attributed to the surface-dwelling existence of the former, which unlike most other scorpion species do not avoid environmental extremes by burrowing. Still, within Buthidae, the mesic *Buthotus judaicus* showed better osmoregulatory capacities than the xeric *Leiurus quinquestriatus*, largely as a result of its high resistance to water loss. However, *B. judaicus* exhibited poor ability to regulate its haemolymph osmolarity at 37 °C. In this study we report a sharp increase in water loss rates of *B. judaicus* at the 30–35 °C temperature range compared to that measured for *L. quinquestriatus*, which could explain the poor osmoregulatory performance of the former at higher ambient temperatures. The increase in water loss rates of *B. judaicus* at high temperatures is not coupled with a similar increase in respiratory rate, suggesting an increase in cuticular permeability. We suggest that this increase in cuticular permeability, which may result from a relatively low critical transition temperature, contributes to limiting the distribution of *B. judaicus* to habitats of moderate environmental conditions.
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1. Introduction

The almost impermeable cuticle of many insects and arachnids provides an effective barrier to water loss, which allows them to exploit a wide range of terrestrial habitats (Cloudsley-Thompson, 1975). In particular, scorpions show some of the lowest transpiration rates of all terrestrial arthropods (Edney, 1977; Hadley, 1990, 1994). For example, the nocturnal *Hadrurus arizonensis* (Iuridae) loses water at a rate ten times lower than that of the desert tenebrionid beetle *Eleodes armata*, which is active during the hot daytime hours (Hadley, 1970).

The distribution of arthropods is largely dependent on water availability and temperature (Chown and Nicolson, 2004). Available data for a variety of terrestrial arthropods, including scorpions, showed good relationships between transpiration rates and environmental conditions in their respective habitats (Edney, 1977; Hadley, 1990). However, the mesic *Buthotus judaicus* (Buthidae) exhibited the lowest body water loss rate among four studied scorpion species, two of which were predominantly xeric in distribution (Gefen and Ar, 2004). The authors concluded that low water loss rates were characteristic of the Buthidae, and may have evolved as a result of the surface-dwelling existence of scorpions of this family.

Within the Family Buthidae, *B. judaicus* (Simon, 1872) and *Leiurus quinquestriatus* (Hemprich and Ehrenberg, 1829), a predominantly xeric species, showed similar haemolymph osmoregulatory capacities when subjected to prolonged desiccation at 30 °C (Gefen and Ar, 2004). However, despite its high resistance to water loss and the resulting enhanced ability to regulate haemolymph osmolarity, the geographical distribution of *B. judaicus* in Israel is limited to the mesic Mediterranean climatic region (Warburg and Ben Horin, 1978; Levy and Amitai, 1980; Fig. 1). Interestingly, the haemolymph osmotic concentration of *B. judaicus* increased by 5% when desiccated to 10% loss of initial body mass at 30 °C and 50% relative humidity (RH) (Gefen and Ar, 2004), whereas at 37 °C and 0% RH, it

and *L. quinquestriatus* at temperatures of 25, 30 and 35 °C, at both 0 and 50% RH conditions. We hypothesized that the lower water loss rates exhibited by *B. judaicus* are limited to relatively moderate environmental conditions, thus limiting its geographical distribution to mesic habitats. In an attempt to attribute increases in water loss rates to either the cuticular or the respiratory channel, we also measured oxygen consumption rates at the same experimental temperatures.

2. Materials and methods

2.1. Scorpion collection

B. judaicus were collected from an area of pine woodlands near Migdal Ha'emek, in the mesic lower Galilee (mean annual precipitation, ~525 mm), whereas *L. quinquestriatus* were collected from the stony areas near Yerucham in the Negev desert (mean annual precipitation, ~100 mm) (Fig. 1). Scorpions of both species were found under small size rocks.

2.2. Scorpion maintenance in the laboratory

Following collection the scorpions were kept in the laboratory in round (9 cm diameter) transparent plastic boxes, with soil from their respective collection sites as substrate. The scorpions were kept at room temperature (~25 °C), and fed ad libitum with adult crickets until initiation of measurements, two weeks following their capture.

2.3. Water loss rate

Water loss rates (WLR) were measured at 25, 30 and 35 °C, and relative humidities (RH) of 0 and 50% under conditions of 12D:12L photoperiod. The scorpions were fed ad libitum until 48 h before initiation of the measurements. Scorpions were placed in individual plastic boxes with perforated bottoms, which were then put in sealed tanks. Dry air was used for 0% RH measurements, and 50% RH atmosphere was achieved by mixing dry air and air saturated with water vapour at the experimental temperature. Air supply at a 100 mL·min^{-1} was sufficient to limit reduction of oxygen concentration by no more than 0.25–0.5% (at 25 and 35 °C, respectively, see Oxygen consumption rates). Flow rates were controlled with pre-calibrated flow controllers (5800 Series, Brooks, Veenendaal, Holland), and humidity levels were validated using a humidity sensor (±2% RH; Almemo, Holzkirchen, Germany). Animal weighing (±0.1 mg) was performed every second day, and WLR was determined by the mass difference between successive measurements (excluding excretions' dry mass, weighed to the nearest 0.1 mg). Assuming similar metabolic rates and catabolism of the same metabolic fuels by the two species, interspecific differences in calculated mass loss rates reflect differences in WLR (Gefen and Ar, 2004).

In order to avoid overestimation of transpiration rates resulting from excretory water loss, means of days 8–14 transpiration rates were calculated after excluding WLR values of scorpions whenever excretions were found in the
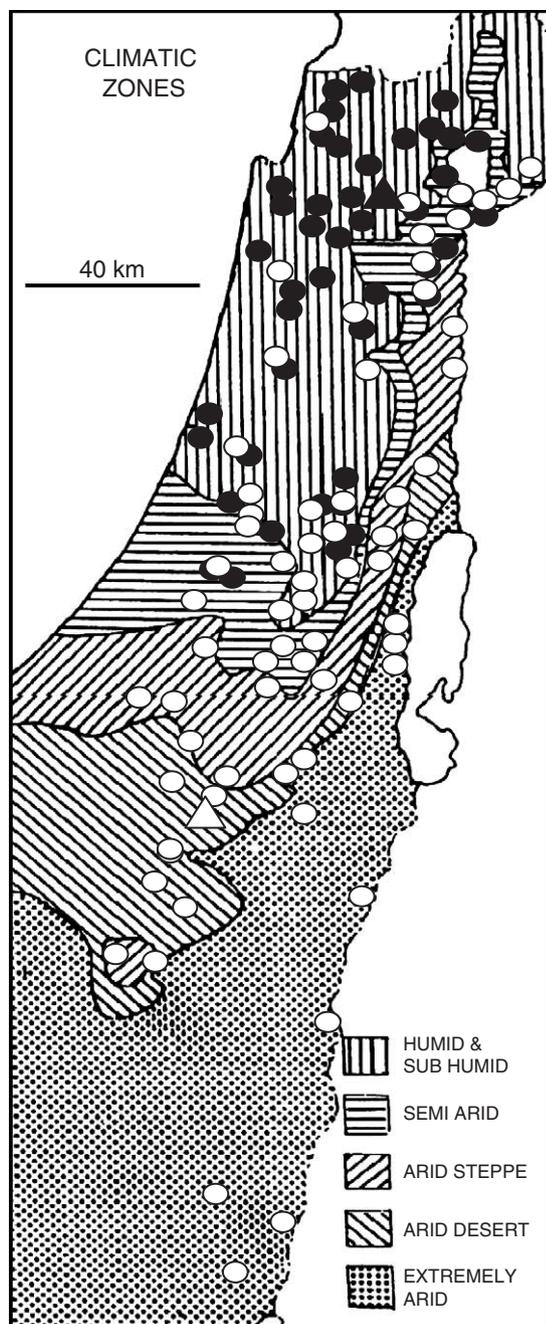

Fig. 1. Records of distribution of *B. judaicus* (●) and *L. quinquestriatus* (○) (redrawn from Levy and Amitai, 1980) superimposed on a map of climatic regions in Israel (after Dan and Raz, 1970). Collection sites of *B. judaicus* and *L. quinquestriatus* for this study are indicated by a filled and open triangle, respectively.

increased by 16% following the loss of only 6.5% of initial body mass (Warburg et al., 1980).

Under mild desiccation conditions scorpions regulate their haemolymph volume and osmotic concentration by mobilising water from hepatopancreatic stores (Gefen and Ar, 2005). However, increasing rates of water loss to the environment under more severe desiccation conditions could outweigh the contribution of this compensatory mechanism, which may be limited by body water availability and rates of mobilisation (Gefen and Ar, 2005). We therefore measured water loss rates of *B. judaicus*

boxes. Calculations of transpiration rates and Q_{10} values of WLR and metabolic rates are based on the respective sample means.

2.4. Oxygen consumption rate ($\dot{M}_{O_2}$)

Measurements of $\dot{M}_{O_2}$ and WLR were performed on different sets of individual scorpions. For $\dot{M}_{O_2}$ determination, scorpions were starved for 48 h and were acclimated to the measuring temperatures for 24 h prior to measurement. Scorpions were then weighed, and their $\dot{M}_{O_2}$ was determined in a modified close-system method (Withers and Smith,1993). The scorpions were put individually in 100-mL plastic syringes previously washed with dry air with the plunger withdrawn to the 100-mL mark, together with a pre-weighed mesh bag containing silica gel (4–8 g). The syringes were sealed and placed in a controlled temperature cabinet set for the measuring temperature (± 0.2 °C) and under constant light conditions. After 2–6 h, depending on temperature and scorpion body size, approximately 60 mL of the gas contents of the sealed syringes were injected into a model OA 272 Servomex oxygen analyzer (Crowborough, Sussex, UK). Oxygen concentration drop, compared to control a dry-air filled control syringe, was determined to ± 0.01%. Oxygen

Table 1
Water loss rates (mg·g^{-1}·h^{-1}) at temperatures of 25, 30 and 35 °C, and RH of 0 and 50%

T (°C)	RH (%)	Buthotus judaicus	Leiurus quinquestriatus
25	0	0.131* ±0.014 (10)	0.294 ±0.023 (11)
30		0.227* ±0.025 (10)	0.376 ±0.048 (7)
35		0.561 ±0.056 (11)	0.545 ±0.047 (12)
25	50	0.099* ±0.015 (10)	0.158 ±0.016 (11)
30		0.168* ±0.025 (10)	0.272 ±0.035 (9)
35		0.351 ±0.029 (11)	0.376 ±0.036 (11)

Values are mean±S.E. (n) of rates recorded from days 8–14 of measurements.
*Significant difference between species (ANCOVA, $p < 0.01$).

consumption rates were calculated using standard equations, and assuming densities of 1.6 and 1.0 g mL^{-1} for the silica gel and scorpion, respectively.

2.5. Statistics

Statistical analysis was carried out using Statistica for Windows 5.0 software (Statsoft, Tulsa, OK, USA).

3. Results

3.1. Water loss rate

Initial body mass of B. judaicus (2.432±0.244 g, mean±S.E.) was significantly lower in comparison with that of L. quinquestriatus (3.266±0.248 g) (ANOVA, $p < 0.05$; see Table 1 for sample sizes). Water loss rates (WLR) are shown in Fig. 2, and mean days 8–14 WLR values are given in Table 1. Mean WLR of B. judaicus was significantly lower than those of L. quinquestriatus at 25 and 30 °C, at both experimental RH conditions (ANCOVA, $p < 0.01$). However, no significant difference was found between the WLR values of the two species at 35 °C, both at 0% RH ($p = 0.92$) and 50% RH ($p = 0.57$) (Table 1; Fig. 2). Q_{10} values of 1.6–3.0 were calculated for L. quinquestriatus at both 25–30 and 30–35 °C. In contrast, a similar Q_{10} value recorded for B. judaicus at 25–30 °C, was followed by considerably higher values at 30–35 °C (Table 2).

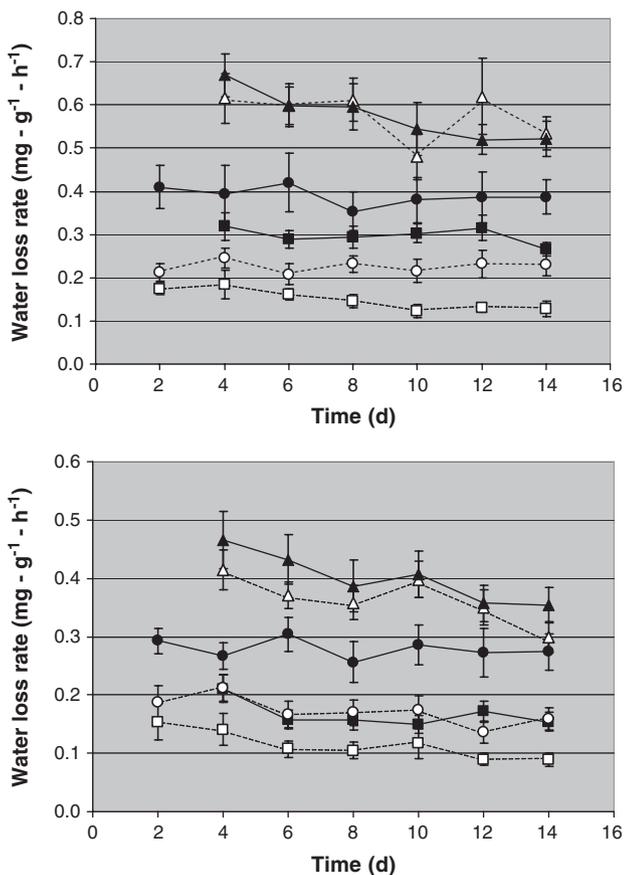Transpiration rates (Table 3) accounted for 90% or more of total WLR in all experimental conditions.

Fig. 2. Mass-specific water loss rates (mean±S.E.) as a function of time from initiation of desiccation at 25 (squares), 30 (circles) and 35 °C (triangles) and RH of 0% (top) and 50% (bottom). Scorpions were weighed in 2-day intervals. B. judaicus, open symbols; L. quinquestriatus, filled symbols.

Table 2
Q_{10} values of water loss rates over temperature ranges of 25–30 °C and 30–35 °C, and RH of 0 and 50%

Temperature range (°C)	25–30	30–35	25–30	30–35
RH (%)	0		50	
B. judaicus	3.0	6.1	2.9	4.4
L. quinquestriatus	1.6	2.1	3.0	1.9

Table 3
Calculated transpiration rates ($\mu g \cdot cm^{-2} \cdot h^{-1} \cdot Torr^{-1}$) of the scorpions at different experimental conditions, and the corresponding water vapour pressure differences between the scorpion interior and its environment

RH (%)	Temperatrue (°C)	ΔP (Torr)	Buthotus judaicus	Leiurus quinquestriatus
0	25	24	0.46	1.52
	30	32	0.63	1.22
	35	42	1.09	1.41
50	25	12	0.84	1.62
	30	16	1.16	1.65
	35	21	1.54	1.85

Surface area is calculated using the equations of Warburg et al. (1980).

3.2. Oxygen consumption rate

Mean body mass ($\pm$ S.E.) of the scorpions used for metabolic rate determinations were 1.616 ± 0.094 g and 1.824 ± 0.159 g for *B. judaicus* and *L. quinquestriatus*, respectively ($n = 10$ for each experimental temperature). $\dot{M}_{O_2}$ measurements showed a significant effect of temperature on metabolic rates (ANOVA, $F_{2,56} = 62$, $p < 0.001$). No significant difference in $\dot{M}_{O_2}$ was found between the two species in any of the experimental temperatures (ANCOVA, $p > 0.2$; Table 4). Calculation of Q_{10} values for measured mass-specific $\dot{M}_{O_2}$ yielded values of 2.1–2.7 for both species (Table 5).

4. Discussion

Observed interspecific differences in water relations traits in scorpions have long been attributed to adaptations of xeric species to their extreme environments (Hadley, 1990). More recent evidence suggests that differences in water and solute management of scorpions are phylogenetically constrained (Gefen and Ar, 2004, 2005). Within Buthidae, *B. judaicus* was shown to be more desiccation resistant than *L. quinquestriatus* when subjected to mild desiccation (Gefen and Ar, 2004). Furthermore, WLR of *B. judaicus* is comparable to those of several other Buthidae species occupying arid habitats (see Hadley, 1990, for a list of studies). Still, the geographical distribution of *B. judaicus* is limited to areas of cooler and more humid climate in comparison with that of *L. quinquestriatus* (Fig. 1; Warburg and Ben Horin, 1978; Levy and Amitai, 1980).

Results in the present study confirm that WLR of *B. judaicus* are lower than those of the xeric *L. quinquestriatus* when subjected to prolonged desiccation at 25–30 °C (Fig. 2, Table 1).

Table 4
Mass specific oxygen consumption rates ($\mu l_{[STPD]} \cdot g^{-1} \cdot h^{-1}$) at ambient temperatures of 25, 30 and 35 °C, values are mean$\pm$S.E. (n)

T (°C)	Buthotus judaicus	Leiurus quinquestriatus
25	78.7	69.8
	± 2.4 (10)	± 6.5 (10)
30	116.2	100.9
	± 8.2 (10)	± 3.7 (10)
35	168.7	165.1
	± 8.6 (10)	± 8.4 (10)

Table 5
Q_{10} values of mass-specific oxygen consumption rates over temperature ranges of 25–30 °C and 30–35 °C

	Temperature range (°C)	
	25–30	30–35
Buthotus judaicus	2.2	2.1
Leiurus quinquestriatus	2.1	2.7

The higher resistance to water loss of the former was maintained at both experimental humidity conditions. However, these interspecific differences in WLR disappeared when scorpions of both species were desiccated at 35 °C (Fig. 2) as a result of the sharp increase in WLR of *B. judaicus* between 30 and 35 °C (Table 1). Again, a similar pattern was observed at both humidity conditions.

A study of scorpion distribution in Northern Israel showed that *B. judaicus* was found in abundance in the Lower Galilee and the Gilboa area, but was absent from the arid area of Mehola, in the Northern part of the Jordan Valley. In contrast, the xeric *L. quinquestriatus* was found in abundance in the latter (Warburg and Ben Horin, 1978). Microclimatic measurements showed differences in both temperature and humidity between the sites, with higher air temperature and lower humidity in Mehola in August and September (Warburg and Ben Horin, 1978). Results in this study suggest that between the two environmental factors, temperature is more likely to be the limiting factor to the distribution of *B. judaicus*. Water loss rates of *B. judaicus* are significantly lower than those of *L. quinquestriatus* at temperatures of 25 and 30 °C, suggesting *B. judaicus* maintains its resistance to water loss even at extremely dry humidity conditions at this temperature range. Moreover, the adverse effect of high temperature conditions on the ability of *B. judaicus* to conserve its body water is evident even in moderate humidity conditions (Table 1, Fig. 2).

The high Q_{10} value for body water loss rate of *B. judaicus* in the 30–35 °C range (Table 2) highlights the abrupt change in its resistance to water loss within this temperature range. This may explain the differences in the reported osmoregulatory capacities of scorpions of this species at 37 °C (Warburg et al., 1980) and 30 °C (Gefen and Ar, 2004). When mildly desiccated, scorpions lose water primarily from hepatopancreatic stores in order to regulate their haemolymph volume (Gefen and Ar, 2005). The compromise in the osmoregulatory capacity of *B. judaicus* between 30 and 37 °C suggests that the sharp increase in water loss to the environment in this temperature range impairs haemolymph volume regulation by water mobilisation from the hepatopancreas. Water loss rates of *B. judaicus* and the xeric *L. quinquestriatus* are similar at 35 °C, but the latter has been shown to compensate better for lost haemolymph water by higher mobilising rate of hepatopancreatic water stores (Gefen and Ar, 2005).

No significant differences ware found in the $\dot{M}_{O_2}$ values recorded for the two species (Table 4), which were in agreement with a range of previously reported values (varying with measuring methods and experimental temperature ranges; Withers and Smith, 1993; Bridges et al., 1997). The effect of

temperature on the $\dot{M}_{O_2}$ of both species was close to the expected Q_{10} value of 2 (Table 5). Hence, the increase in WLR of *B. judaicus* at temperatures higher than 30 °C (Tables 1 and 2) is not matched by a concurrent sharp increase in oxygen consumption rates (and consequently in respiratory water loss) (Tables 4 and 5), suggesting an increase in cuticular permeability.

Transpiration rates of arthropods increase more rapidly with increasing temperature above a critical temperature (Wigglesworth, 1945). Although much of the early work on the transition phenomena did not take into account the increased water vapour pressure gradient between the arthropod and the atmosphere at higher ambient temperatures, this abrupt change in cuticular transpiration rate has since been established in a series of following studies (see Edney, 1977 for a list of studies). Cuticular transpiration is the main route for water loss in scorpions, although respiratory water loss becomes increasingly important at high temperatures (Hadley, 1970). Therefore, the increase in cuticular permeability at temperatures higher than the critical temperature severely compromises the ability of scorpions to conserve body water stores.

The sharp increase in insects' cuticular permeability at a "transition temperature" was attributed to changes in the orientation of epicuticular lipid molecules (Edney, 1977). Hadley (1994) stated that if the area-specific WLR is corrected for water vapour pressure difference between the tissues and the environment, there is little if any changes in the cuticular permeability with temperature at 20–35 °C. Nevertheless, the dramatic change that occurs at the "transition temperature" can not be accounted for by the increasing water vapour difference between the tissues and the environment with increasing temperature (providing relative humidity is kept constant) (Hadley, 1994). The transition temperature phenomenon has already been shown to occur in scorpions (Toolson and Hadley, 1977), although it was later attributed to possible calculation artefacts (Toolson, 1978). Calculated transpiration rates of the two species are presented in Table 3, where rates are expressed as water vapour permeability, namely evaporative water loss rate per unit of the scorpion's surface area, and per unit of water vapour pressure difference between the tissues and ambient air at the various experimental conditions. Table 3 shows that the water vapour permeability of *B. judaicus*, unlike that of *L. quinquestriatus*, increases with ambient temperature. It also confirms that the abrupt increase in the WLR of *B. judaicus* at temperatures of 30–35 °C (Tables 1 and 2) can not be fully explained by the increasing water vapour pressure difference between ambient air and the organism's interior at increasing temperatures.

Despite the lack of a satisfactory explanation to the transition phenomena, comparative studies employing the same methodology on a number of species can highlight interspecific differences and their possible significance (Hadley, 1994). A transition temperature of about 30 °C has been shown for *Blattella germanica* and *Periplaneta*, among other insect species, while the cuticle of insects from dry environments, e.g. the larvae of *Tenebrio molitor*, show similar abrupt changes in transpiration rates at temperatures 20–30 °C higher (Wigglesworth, 1945). Similarly, among the species investigated in the present study, the cuticle of the mesic *B. judaicus* appears to undergo changes at lower temperatures in comparison with that of the xeric *L. quinquestriatus*.

In conclusion, *B. judaicus* exhibits some of the lowest recorded WLR values for scorpions at temperatures of up to 30 °C, coupled with an enhanced ability to osmoregulate its haemolymph for long periods of food and water deprivation. However, results in this study point at a dramatic increase in WLR of *B. judaicus* at temperatures higher than 30 °C, which appears to result mainly from a temperature-dependent increase in cuticular permeability. We suggest that this may contribute to limiting the geographical distribution of this surface-dwelling buthid to areas of moderate climatic conditions.

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