



Exceptional thermal tolerance and water resistance in the mite *Paratarsotomus macropalpis* (Erythracaridae) challenge prevailing explanations of physiological limits



Grace C. Wu¹, Jonathan C. Wright^{*}

Department of Biology, Pomona College, Claremont, CA 91711, United States

ARTICLE INFO

Article history:

Received 19 June 2015

Received in revised form 3 August 2015

Accepted 5 August 2015

Available online 6 August 2015

Keywords:

Mite
Heat tolerance
Water loss

ABSTRACT

Physiological performance and tolerance limits in metazoans have been widely studied and have informed our understanding of processes such as extreme heat and cold tolerance, and resistance to water loss. Because of scaling considerations, very small arthropods with extreme microclimatic niches provide promising extremophiles for testing predictive physiological models. Corollaries of small size include rapid heating and cooling (small thermal time constants) and high mass-specific metabolic and water exchange rates. This study examined thermal tolerance and water loss in the erythracarid mite *Paratarsotomus macropalpis* (Banks, 1916), a species that forages on the ground surface of the coastal sage scrub habitat of Southern California, USA. Unlike most surface-active diurnal arthropods, *P. macropalpis* remains active during the hottest parts of the day in midsummer. We measured water-loss gravimetrically and estimated the critical thermal maximum (CT_{max}) by exposing animals to a given temperature for 1 h and then increasing temperature sequentially. The standardized water flux of $4.4 \text{ ng h}^{-1} \text{ cm}^{-2} \text{ Pa}^{-1}$, averaged for temperatures between 22 and 40 °C, is among the lowest values reported in the literature. The CT_{max} of 59.4 °C is, to our knowledge, the highest metazoan value reported for chronic (1-h) exposure, and closely matches maximum field substrate temperatures during animal activity. The extraordinary physiological performance seen in *P. macropalpis* likely reflects extreme selection resulting from its small size and resultant high mass-specific water loss rate and low thermal time-constant. Nevertheless, the high water resistance attained with a very thin lipid barrier, and the mite's exceptional thermal tolerance, challenge existing theories seeking to explain physiological limits.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Comparative studies have made important advances in understanding limits to physiological performance (Huey and Bennett, 1987; Somero, 2002). Variation in tolerance limits across different habitats and environmental gradients (Hoffmann, 2010; Spellerberg, 1972; Stillman and Somero, 2000; Tomanek and Somero, 1999) and results of laboratory selection studies (Gilchrist and Huey, 1999; Telonis-Scott et al., 2014) both demonstrate the evolutionary response of thermal tolerance to selection. Laboratory selection trials frequently result in enhanced physiological performance, for example, thermal tolerance (Condon et al., 2015; Gilchrist and Huey, 1999; Williams et al., 2012), and desiccation resistance (Gibbs et al., 1997; Telonis-Scott et al.,

2014), indicating that selection on these traits in natural populations is subject to complex constraints.

Upper and lower tolerance limits tend to be broadly comparable across a given taxon within certain geographic limits (e.g. thermal tolerance limits between 32 and 42 °C for European trochid topshells for a 5-h exposure; Newell, 1979) and across developmental stages within a taxon, but show considerable variation among higher taxa (e.g. across insect families; Dixon et al., 2009). Wide variation is seen similarly in optimal temperatures for physiological performance, for example among lizard families (Huey and Bennett, 1987; Pianka, 1986). Such taxonomic (and phylogenetic) variation indicates different adaptive capacities (Klok et al., 2004; Pörtner, 2001), including differing degrees of heat-shock response (Tomanek, 2002), stemming from biophysical, genetic or multifunctional constraints (Hochachka and Somero, 2002; Somero, 2002). Nevertheless, comparisons of performance limits across taxa indicate ultimate thresholds applying across major phylogenetic groups (Klok et al., 2004; Pörtner, 2001; Somero, 2002).

^{*} Corresponding author.

E-mail address: jcwright@pomona.edu (J.C. Wright).

¹ Energy and Resources Group, University of California, Berkeley, United States.

Examples include the apparent limits to mass-specific metabolism seen in the smallest (1–2 g) homeotherms (Ochościńska and Taylor, 2005), the rapidly diminishing numbers of animal species able to osmoregulate in extreme salinities (Gajardo and Bearmore, 2012; Herbst et al., 1988), limits to upper thermal tolerance in eukaryotes (Chevaldonné et al., 2000; Cooney and Emerson, 1964), and the maximum resistance to water loss attainable by the lipid barriers of arthropod cuticles (Hadley, 1981, 1994). These indicate the presence of ultimate constraints in underlying molecular, cellular or metabolic processes (Guderley and St. Pierre, 1996; Somero, 2002).

Thermal tolerance limits among eukaryotes lie well below those of hyperthermophilic Archaea, which can reproduce in temperatures as high as 122 °C (Kurr et al., 1991; Stetter, 2006; Takai et al., 2008). Generally, upper thermal limits for chronic exposure in thermophilic metazoans range from 45 to 47 °C (Pörtner, 2001). Among the few species that exceed this range are the thermophilic ostracod (*Potamocypris* sp.), with lethal temperature at 50% mortality (LT_{50}) values of 55.75 °C and 50.44 °C for 1-min and 60-min exposures respectively (Wickstrom and Castenholz, 1973), and the Saharan silver ants *Cataglyphis bombycina* and *Cataglyphis bicolor* with respective CT_{max} values of 53.6 °C and 55.1 °C for a short (<10 min) though unspecified duration (Gehring and Wehner, 1995; Wehner et al., 1992). Other species showing exceptional thermal tolerance include alvinellid polychaetes inhabiting hydrothermal vents (Desbruyères et al., 1998; Girguis and Lee, 2006), and the pseudoscorpions *Eremogarypus perfectus* and *Garypus levantinus* (Heurtault and Vannier, 1990), although in both cases lab measurements of long-term thermal tolerance are lacking.

Thermophilic terrestrial species face physiological challenges not only in terms of thermotolerance but also for water retention. Water retention is typically the primary contributor to, and indicator of, an organism's ability to thrive in arid environments (Hadley, 1994; Gibbs et al., 2003). This depends on effective desiccation resistance, both respiratory and transpiratory, and efficient mechanisms of water gain. Desiccation tolerance, or the maximum fractional water loss an organism can withstand, can also promote survival under conditions where avenues of water gain are spatially or temporally unpredictable. High desiccation resistance requires an epicuticular lipid barrier possessing not only high water resistance but also high thermal stability (Gibbs, 1998; Hadley, 1994). Potential means of water gain include imbibing nocturnal dew (Zheng et al., 2010), fog-basking and dew condensation (Guadarrama-Cetina et al., 2014; Parker and Lawrence, 2001), and active water vapor absorption (O'Donnell and Machin, 1988), in addition to dietary and metabolic water production.

With increasing temperature, the vapor pressure of water in an animal's body fluids increases, elevating the vapor pressure gradient for water loss in any given relative humidity (RH). This problem is compounded for small animals inhabiting the ground surface. Firstly, the steep thermal gradients near the ground surface will generate an inverse gradient in microclimate RH, increasing the vapor pressure deficit between the animal and surrounding air ($VP_{air} - VP_{animal}$). The saturation vapor pressure of water increases approximately 1.7-fold for each 10 °C increase in temperature between 30 and 50 °C, meaning that the RH of a given air mass will decrease by the same proportion: an ambient RH of 30% at 30 °C would decrease to 10.3% at a ground surface temperature of 50 °C. Secondly, the high surface area to volume ratio of small animals means that a given water loss rate translates into a large proportional (mass-specific) water loss rate (Schmidt-Nielsen, 1984). And thirdly, high metabolic rates of small thermoconforming animals at high temperatures will depend on high respiratory gas fluxes, contributing an additional problem for water retention.

The erythracarid mite *Paratarsotomus macropalpis* (Banks, 1916) (Acarina: Anystidae: Erythracarinae) is a diurnally active arachnid, inhabiting the ground surface of the coastal sage scrub (CSS) ecosystem in Southern California, USA (Banks, 1916; Otto, 1999). It exploits a similar microhabitat to several other thermophilic species in the CSS, among them the California harvester ant *Pogonomyrmex californicus* and thief ant *Solenopsis molesta*. *P. macropalpis* is peculiar in its exceptional running speed and timing of activity (Wu et al., 2010). Preliminary observations showed that this species remains active on the exposed ground surface throughout the late morning and afternoon hours in June and July when ground surface temperatures apparently preclude surface activity of other arthropods. Species of the closely related genus, *Tarsotomus*, have been reported to show similar behavior and tolerance of extremely hot substrates (Barilo, 1984; Berlese, 1886). Due to its very small size (ca. 1 mm, 300 µg), *P. macropalpis* is condemned to being a thermoconformer, that is, its body temperature will closely match that of its immediate surroundings (Hadley, 1970). Based on field measurements of ground surface temperature, we hypothesized that this species would have a critical thermal maximum (CT_{max}) exceeding 50 °C. Although the regional CSS is a less xeric and thermally stressing habitat than the more easterly Colorado Desert, exposed ground surface temperatures still routinely exceed 60 °C in the summer months, above any documented CT_{max} for metazoans. Diurnally active arthropods on exposed surfaces in the CSS may therefore attain thermal tolerances comparable to those of strict desert specialists.

In this study, we quantify thermal tolerance, operative field temperatures, and water loss rates in *P. macropalpis*. Given the documented capacity for active water vapor absorption (WVA) in some mite genera (Wohltmann, 1998; Yoder, 1998), we also tested whether *P. macropalpis* is able to utilize WVA as a means of water recovery.

2. Materials and methods

Adults, deutonymphs and tritonymphs of *P. macropalpis* were collected at the Robert J. Bernard Field Station of the Claremont Colleges, California. Animals were found on bare sand and gravel substrates in the coastal sage scrub habitat, where they run intermittently between the sparse ground vegetation and small masses of windblown debris. We subsequently found populations of these mites on concrete driveways in Claremont. Mites were only active on warm days, when the ambient temperature exceeded ca. 30 °C and ground temperatures exceeded 40 °C. Individuals were collected using an aspirator and promptly transported to the lab where they were maintained at 22 °C and lab humidity (ca. 15–30%) in covered glass dishes. Thermal tolerance studies were conducted within 48 h. Mites used for measures of water-loss rate were transferred to saturated vials for a pre-treatment period (see below) within 6 h of field collection. Since animals are routinely exposed to temperatures in the 20–25 °C range at night and during overcast days, the lab holding periods were considered unlikely to have a significant acclimatory effect on water loss rates or thermal tolerance.

2.1. Water balance

Determination of water loss was gravimetric and followed standard methods (Benoit et al., 2005; Wharton, 1985). All experimental trials were conducted at lab temperature (22 ± 1 °C) unless otherwise indicated. Individual mites were weighed to obtain the initial mass (M_i) using an Orion Cahn C-34 microbalance (Thermo Electron Co., Beverly, MA), with a resolution and reproducibility of 1.0 µg. They were then maintained in 100% relative humidity

(RH) without food for a pretreatment period of 1–2 days to allow for voiding of fecal matter. Following this, animals were reweighed and transferred to separate 20 × 60 mm vials and dried in a desiccator over silica gel. The desiccator humidity was measured using a RH probe (VWR digital hygrometer) inserted through a substitute Plexiglas cover and equilibrated to <1% RH. Different experimental groups of 5–15 mites were weighed periodically to record the mass after *n*-days (M_n). Following lethal desiccation, which was determined by complete lack of mobility after disturbance, mites were dried for a further 24 h at 70 °C and reweighed to obtain dry mass (M_d). Unless otherwise stated, mass-losses were only used for permeability calculations when the mites were alive and ran actively prior to the second weighing.

To study the effects of temperature on water loss, gravimetric losses were measured for mites exposed to dry air in an incubator set to temperatures ranging from 20 to 80 °C. For 22 °C weighing trials, mites were weighed either daily or over intervals of 3–6 days. At higher temperatures, the drying duration was limited to 29 h or less. Following lethal desiccation, some animals were dried for additional periods (up to approximately 50% mass loss) to determine dead water loss rates at the respective temperature. All water losses ($M_i - M_n$) are expressed as either whole-animal mass loss rates (ng h^{-1}) or as standardized fluxes ($\text{ng h}^{-1} \text{cm}^{-2} \text{Pa}^{-1}$) corrected for surface area and vapor pressure deficit. Surface area was estimated using Edney's formula (Edney, 1977):

$$SA (\text{cm}^2) = 12 \cdot M^{0.67} \quad \text{where } M \text{ is the mass in grams}$$

Vapor pressure deficit was determined using standard vapor pressure tables, assuming the animal's tissues to be at the saturation vapor pressure for the experimental temperature, and assuming an ambient relative humidity of 0%.

Possible water vapor absorption (WVA) was examined using saturated salt solutions to generate specific relative humidity (RH) values. Freshly collected mites were pre-desiccated to about 20% mass loss and placed in individual 5 mL vials within capped 25 mL glass vials containing 1 mL of a saturated solution of either potassium nitrate (KNO_3) for 93% RH or potassium sulfate (K_2SO_4) for 97% RH (Winston and Bates, 1960). Animal masses were monitored every 24 h. Since hemolymph water activity (a_w) will be approximately 0.99 (Hadley, 1994) any mass-gain measured below 99% RH can be attributed to WVA (O'Donnell and Machin, 1988). In the absence of food and liquid water, the only way mass can be maintained in sub-saturated air would be through absorption of water vapor.

2.2. Thermal tolerance

For thermal tolerance trials, individual mites were placed in Plexiglas fly vials with a foam plug and immersed in a water bath. Each vial was lined with a wick of moist filter paper to maintain saturated humidity and preclude evaporative cooling. Bath temperatures were referenced against simultaneous measurements of the interior vial surface using an inserted copper-constantan thermocouple with a digital thermometer (Digi-Sense Type K, Cole Parmer, Vernon, IL). To provide a preliminary estimate of the upper lethal temperature, we used the dynamic method introduced by Cowles and Bogert (1944), incrementally increasing the exposure temperature. Mites were transferred from ambient lab temperature (22 °C) to an initial bath temperature of 50 °C and held for 10 min. If they remained active or assumed normal locomotion within 10 min of removal, they were deemed fully recovered and exposed to a temperature 2 °C greater than used in the previous trial. Animals not moving within 10 min were scored as dead and held for a further 24 h for confirmation. After

determining the approximate lethal temperature from pilot studies, we subsequently tested animals for a 60-min exposure, beginning at temperatures from 50 to 56 °C and increasing in steps of 1–2 °C. For all treatments above 56 °C, we used a constant ramping rate of 1 °C h^{-1} . Between trials, the bath temperature stabilized at each new set point in 5–10 min.

The CT_{max} is defined as the temperature at which coordinated locomotory activity ceases (Cowles and Bogert, 1944; Lutterschmidt and Hutchinson, 1997) and is often estimated using “knockdown” temperatures (e.g. Klok et al., 2004), at which an animal loses the ability to show a normal climbing or righting response. For *P. macropalpis*, thermal tolerance determinations were straightforward because no distinction between an upper lethal temperature and CT_{max} could be made. We therefore quantified the upper thermal tolerance limit as the temperature resulting in 50% mortality (LT_{50}). The LT_{50} was calculated by transforming the sigmoidal mortality curve using Probits (Armitage and Berry, 1994; Finney, 1971) and then extrapolating the LT_{50} from a linear regression of the data between 54 and 62 °C.

Because of the very small size of these mites (idiosomae of deutonymphs, tritonymphs and adults range in length from 0.5 to 1.2 mm), their operative field temperatures are assumed to be identical to ground surface temperatures (Hadley, 1970). Ground temperatures were measured on a concrete driveway using a copper-constantan thermocouple during periods of observed mite activity to assess the congruence between mite operative temperatures and the estimated CT_{max} . Accompanying ambient temperature measurements were taken in the shade at chest height (1.4 m) using a digital thermometer.

3. Results

3.1. Desiccation resistance

Hydrated masses of juvenile and adult mites ranged from 58 to 365 μg . When mites were weighed daily, water loss rates were higher than with less regular weighing, a result that may be attributable to elevated activity and metabolism following disturbance. Accordingly, we used a longer weighing interval where practical; at 22 °C, mites were weighed after 3 days, but this interval was reduced to 6 h at 50 and 60 °C.

Standardized loss fluxes ($\text{ng h}^{-1} \text{cm}^{-2} \text{Pa}^{-1}$) for the different desiccation temperatures are shown in Fig. 1. Water fluxes in the different treatment groups were compared against those measured at 22 °C using 2 sample *t*-tests. Between 22 and 40 °C, mites showed similar mean fluxes (3.65–4.70 $\text{ng h}^{-1} \text{cm}^{-2} \text{Pa}^{-1}$; mean = 4.4 $\text{ng h}^{-1} \text{cm}^{-2} \text{Pa}^{-1}$). At 50 °C, this rate increased sharply and significantly to 12.90 $\text{ng h}^{-1} \text{cm}^{-2} \text{Pa}^{-1}$. Although these mites remained viable, all animals exposed to 60 °C were dead after 6 h and showed mass-losses from 23% to 48%. Loss rates were also measured in animals following lethal desiccation at 22 and 40 °C to compare rates against those of living animals. In each case, these showed similarly elevated standardized loss fluxes with no significant difference between the two temperature treatment groups or when compared against rates for living animals at 50 and 60 °C (Fig. 1).

When mites were exposed to 70 or 80 °C for 1 h, standardized fluxes again increased abruptly (Fig. 1) to mean values of 66.7 and 53.0 $\text{ng h}^{-1} \text{cm}^{-2} \text{Pa}^{-1}$ respectively.

3.2. Water vapor absorption

Pre-desiccated mites kept in 93% and 97% RH consistently showed small mass-losses, and no indication of any capacity for WVA.

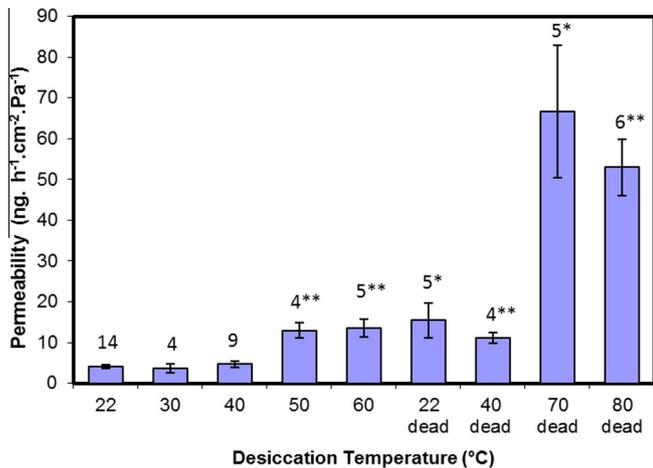


Fig. 1. Standardized water loss fluxes in different temperatures. Numbers above each bar give the sample size (the number of individual mite desiccation trials) and error bars show \pm SEM. Treatment categories marked 'dead' comprise water loss fluxes measured in animals following lethal desiccation (see text) or exposure to lethal temperatures (70 and 80 °C). Animals dried at 60 °C were alive initially but had all died by the end of the drying period. Asterisks denote a statistically significant increase relative to fluxes measured for live animals at 22 °C (data for 30–60 °C), and relative to fluxes measured at 60 °C (data for 70–80 °C); * $p < 0.05$, ** $p < 0.01$.

3.3. Desiccation tolerance

The mean dry mass of mites was $33.2 \pm 2.8\%$ of the hydrated mass ($n = 17$). The calculated dry mass was subtracted from the measured mass of each mite, giving an estimate of total water content. This value was divided by the initial (assumed hydrated) water content to calculate the fractional water losses under the given desiccation regimes described above. Several mites tolerated water losses of 37–45% during desiccation treatments, but the largest loss tolerated was only slightly greater than this (49.3%). Four animals were dead after desiccating to 54–57% total water loss. While data do not permit a more precise determination, the tolerance limit for desiccation can thus be assumed to fall between 45% and 55% (equivalent to a total mass loss of 30–38%).

3.4. Thermal tolerance

Thermal tolerance trials were conducted using summer-acclimatized animals collected between May and August. Mites tolerated 1-h exposures to remarkably high temperatures with little mortality seen below 58 °C and a few animals making a full recovery after exposure to 60 and 61 °C (Fig. 2). No animals survived a 1-h exposure to 62 °C. The estimated LT_{50} following linear regression of the Probit-transformed survivorship data was 59.4 °C ($r^2 = 0.78$; 95% confidence intervals = 59.0–60.8 °C). Control vials in which the temperature of the lining filter paper ($n = 6$) and inner vial surface ($n = 6$) were measured using a thermocouple, showed that vial temperatures were consistently slightly higher than the bath set temperature, but in all cases within 0.8 °C. The bath temperature was therefore taken as an acceptable measure of the exposure temperature for all test trials.

Frequently, following exposure to temperatures of 56 °C and above, mites would display an apparent heat coma, clinging motionless to the filter paper and remaining immobile when touched with a brush. In some cases, animals would fall to the base of the vial with the legs folded ventrally, appearing dead. However, within a few minutes following transfer to lab temperature (22 °C), living animals extended their legs and either resumed activity or would crawl if touched with a brush. The resumption of activity

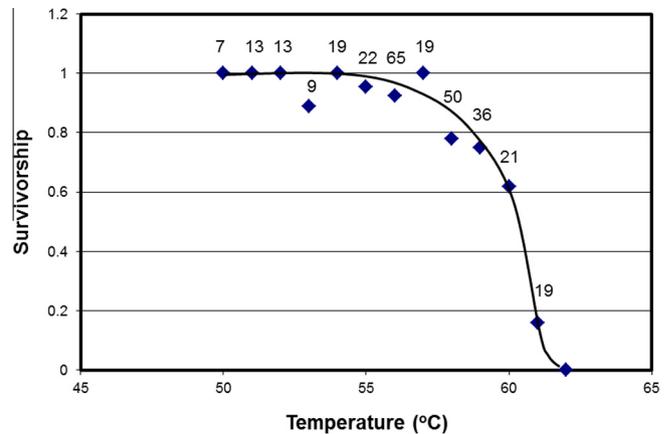


Fig. 2. Thermal tolerance curve for *Paratarsotomus macropalpis* based on temperature exposures of 1 h and using the dynamic method of Cowles and Bogert (1944). The plot is compiled from 16 trials, using variable numbers of mites. Numbers above survivorship values show the total number of animals from which each value is derived. Regression analysis of the Probit-transformed survivorship data between 54 and 62 °C yields an estimated LT_{50} of 59.4 °C.

was unambiguous and occurred within 10 min if at all; dead mites remained contracted with little or no movement. Mites reviving following heat coma remained viable 24 h after heat exposure.

3.5. Ground surface temperatures

In situ measurements of the ground surface temperature of a concrete driveway during periods of mite activity in July and August are shown in Fig. 3. Even with ambient temperatures exceeding 38 °C in the early afternoon, substrate temperatures did not increase high enough to prevent mite activity. The highest measured temperature range of 60.1–61.8 °C indicates that mites remain active very close to their CT_{max} . Several animals were tracked for over 5 min in the early afternoon of August 11, 2012, when ground temperatures fluctuated around 60 °C, remaining motionless on the ground surface between periods of extremely rapid running. Activity at the highest temperatures did not appear to be dependent on size or developmental stage, with adults and tritonymphs (0.8–1.2 mm) and deutonymphs (ca. 0.5 mm) all present.

4. Discussion

4.1. Thermal tolerance

Despite the publication of hundreds of studies examining thermotolerance in metazoans, there are few attempts to synthesize these into a causative framework (Hochachka and Somero, 2002; O'Brien et al., 1991; Pörtner, 2001). Although comparative studies of ecotonal specialists tend to show a consistent picture of higher CT_{max} in more thermally stressed habitats (Newell, 1979; Stillman and Somero, 2000), the inherent trade-offs in such specialization and the ultimate limits for upper thermal tolerance are poorly understood (Guderley and St. Pierre, 1996). The most thermotolerant animals reported come, not surprisingly, from deserts, hot springs and hydrothermal vent ecosystems. Several desert insects have critical thermal maxima above 45 °C including the lepismatid firebrat *Thermobia domestica* (Sweetman, 1938) and the desert tenebrionid beetles *Onymacris plana* and *Physadesmia globus*, both with reported CT_{max} values of 51 °C (Roberts et al., 1991; Ward and Seeley, 1996). The highest CT_{max} values documented for land arthropods are for the Saharan ants *C. bombycina*

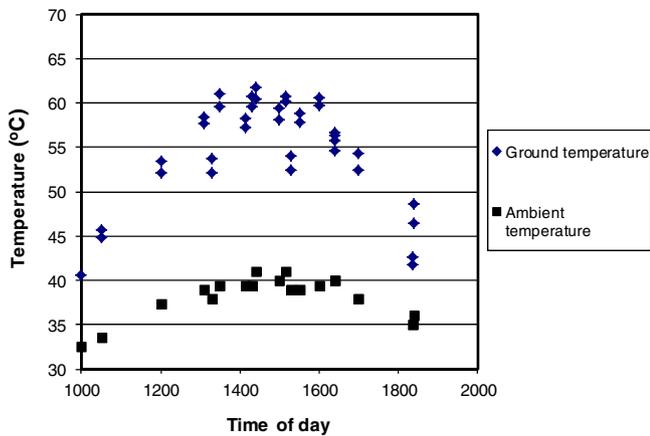


Fig. 3. Ground surface temperatures (°C) measured on a concrete driveway in Claremont, CA, during observed mite activity. Each range shows the upper and lower extremes measured over a 5-min period. Simultaneous ambient temperatures (square symbols) measured at 1.4 m above ground are shown for comparison. Mite activity was not observed earlier and later in the day when substrate temperatures fell below 40.6 °C.

and *C. bicolor* with CT_{max} estimates of 53.4 and 55.1 °C (Gehring and Wehner, 1995; Wehner et al., 1992). The garypid pseudoscorpion *E. perfectus* from the Namib Desert, and the related species *G. levantinus* from Italy, showed respective heat stupor points of 65 °C and 60.6 °C (Heurtault and Vannier, 1990), and are sometimes cited as the most thermotolerant metazoans. In that study, however, animals were warmed rapidly (0.5 °C min⁻¹) and in dry air, so chronic thermal tolerances for these species remain unknown. Among aquatic species, the freshwater ostracod *Potamocypis* sp. has a reported LT_{50} of 50.44 °C for a 1-h exposure (Wickstrom and Castenholz, 1973), and the hydrothermal vent polychaete *Paralvinella sulfincola*, tolerates chronic (7-h) exposure to 50 °C and acute (15-min) exposure to 55 °C (Girguis and Lee, 2006). Other thermophilic vent alvinellids such as the Pompeii worm *Alvinella pompejana* may show comparable upper thermal tolerance, at least for brief periods of exposure (Chevaldonné et al., 1992), but controlled lab studies permitting accurate determination of CT_{max} values are needed (Chevaldonné et al., 2000; Desbruyères et al., 1998).

For many of these species, the CT_{max} determinations were derived using only brief exposure times. For chronic exposure periods (≥ 1 h), *P. macropalpis* has a CT_{max} that apparently exceeds the published value for any metazoan. This mite is also a clear contender for the most thermophilic metazoan based on *in situ* measurements of field substrate temperature. Hydrothermal vent species clearly tolerate chronic exposure to very high temperatures, and *P. sulfincola* actively seeks out temperatures between 40 and 50 °C when placed in a thermal gradient (Girguis and Lee, 2006). However, active *P. macropalpis* routinely forage at ground surface temperatures exceeding 55 °C and summer-acclimatized animals remained active for extended periods when surface temperatures fluctuated between 60.1 and 61.8 °C. Extrapolating allometric data published for lizards (Bell, 1980), the heating and cooling thermal time constants (τ_h , τ_c) for *P. macropalpis* ($M = 58$ – 365 μ g) will be extremely small – in the order of 30–100 ms. Given that individual mites were observed to remain exposed in full sun on the ground surface for several minutes, their body temperatures will conform very closely (<1 °C) to the measured substrate temperatures.

The paucity of species that survive temperatures exceeding 50 °C suggests that metazoan thermotolerance may be functionally constrained by one or more ultimate limiting factors (Somero, 2002). Comparing data for a range of fish and invertebrates,

Hochachka and Somero (2002) show that Arrhenius break temperatures (ABT) for mitochondrial respiration increase as a function of ambient or acclimation temperature (T_a), and the regression of ABT against T_a intersects a hypothetical 'line of identity' (ABT = T_a) between 50 and 60 °C. This represents a probable upper limit for mitochondrial adaptation and is a plausible limiting factor for eukaryote thermotolerance generally. ABT values for mitochondrial oxygen consumption closely match ABT values for cytochrome c oxidase, and mitochondrial respiratory enzymes may lose activity as a result of denaturation and limited homeoviscous adaptation by the membrane lipids (Hochachka and Somero, 2002; O'Brien et al., 1991). Thermotolerance limits for metazoans and other extreme thermophilic eukaryotes, such as the fungus *Chaetomium thermophile* which grows in temperatures as high as 55 °C (Cooney and Emerson, 1964), broadly concur with the estimated limits for mitochondrial respiration. The estimated CT_{max} of 59.4 °C for summer-acclimatized *P. macropalpis* may lie close to the limit attainable for mitochondrial pathways. It is notable that metabolically dormant eukaryotes, such as cryptobiotics and desiccated plant shoots, tolerate chronic exposure to much higher temperatures (Kappen, 1981), the records being 120 °C for a 30-min exposure for the moss *Syntrichia caninervis* (Stark et al., 2009) and 130 °C for a 10-min exposure for the cryptobiotic rotifer *Philodina roseola* (Mertens et al., 2008).

4.2. Desiccation resistance

The estimated permeability of *P. macropalpis* shows a similarly extreme adaptive specialization. The mean standardized flux in temperatures from 22 to 40 °C is 4.4 ng h⁻¹ cm⁻² Pa⁻¹, a value comparable to or below measurements for the most desiccation-resistant arthropods described to date (Hadley, 1994). Among the few arthropods that show comparably low permeability are the diapausing pupae of *Tenebrio molitor* (Holdgate and Seal, 1956) and *Glossina morsitans* (Bursell, 1958). Excluding these, the only arthropods showing superior desiccation resistance appear to be the spider beetles (*Mezium affine*) described by Benoit et al. (2005). *P. macropalpis* did not show any capacity for WVA in 93% and 97% RH. Given that mites remain active for successive days when overnight humidity remains well below saturation, metabolic and dietary (preformed) water must provide the primary sources from which transpired water losses are replenished.

Two abrupt increases in the permeability of *P. macropalpis* are seen as temperature is increased. The increase in permeability at 50 °C and the similar values measured for dead animals at 22, 40 and 60 °C are consistent with the permanent opening of the respiratory stigmata, similar to the widely documented effect of spiracle opening in dead insects (reviewed in Hadley, 1994). This interpretation would require that respiratory losses are strongly constrained by intermittent opening of the stigmata at lower temperatures. Between 40 and 50 °C, the increase in standardized water flux is approximately 3-fold. At 70 and 80 °C, water losses increase again, this time approximately 5-fold beyond the fluxes measured at 50 and 60 °C. This may be attributable to the surpassing of a critical or transition temperature (T_c ; Beament, 1959; Wigglesworth, 1945) at which the structural and functional integrity of the lipid barrier is lost.

The actual barrier resistance in *P. macropalpis* will be somewhat higher than our calculated values at 22 °C because gravimetrically determined mass-loss includes not only respiratory water loss but also the mass-loss of respired carbon. The extremely small size of these mites does not permit an accurate measurement of resting and active metabolic rates but approximations can be made using published allometric equations. Standard metabolic rates for spiders (J h⁻¹) at 20 °C fit the following allometric equation (Withers, 1992):

$$E_{\text{met}} = 1M^{0.65}$$

where M is the animal mass in grams. For a 0.2 mg *P. macropalpis*, this gives an estimate of 3.9 mJ h^{-1} or $0.92 \text{ } \mu\text{W}$. This, in turn, would require the oxidation of $0.14 \text{ } \mu\text{g h}^{-1}$ protein, assuming a protein energy yield of 23 kJ g^{-1} . The mean mass loss of a 0.2 mg animal in dry air at $22 \text{ } ^\circ\text{C} = 3.7 \text{ } \mu\text{g h}^{-1}$ so the estimated contribution of metabolic substrate catabolism is comparatively small, in the order of 4%. This value will increase sharply during locomotion, and we have previously shown these mites to run at speeds exceeding 10 cm s^{-1} in temperatures between 40 and $50 \text{ } ^\circ\text{C}$ (Wu et al., 2010). However, mites are relatively quiescent at lab temperature, and remain largely inactive during prolonged desiccation. We therefore consider the measured mass losses to provide a reasonable estimate of the combined respiratory and transpiratory water losses for inactive animals.

The epicuticular lipid layer confers the primary barrier resistance of the cuticle in most arthropods (Gibbs, 1998; Hadley, 1994; Machin and Lampert, 1985). There is an extensive literature on this subject, and while our understanding of lipid barriers is still rather poor, resistance is generally positively correlated with lipid melting point, and related to the specific lipid composition (Gibbs, 1998; Gibbs and Rajpurohit, 2010; Hadley, 1981; Rourke and Gibbs, 1999). The importance of lipid thickness, surprisingly, is less clearly demonstrated (Gibbs, 1998), and total cuticle hydrocarbon quantity did not change in *Drosophila melanogaster* stock populations following selection for enhanced desiccation resistance over 100 generations (Gibbs et al., 1997). There is a good correlation between transition temperatures and lipid melting points estimated using chain lengths (Rourke and Gibbs, 1999), with longer-chain, saturated alkanes generally showing the highest melting points and branched and unsaturated lipids having lower melting points (literature reviewed in Gibbs, 1998). Several species have been shown to undergo seasonal adaptations in T_c associated with increases in the mean chain lengths and melting points of the cuticular lipids (Hadley, 1977; Toolson and Hadley, 1979). The extreme thermal tolerance of *P. macropalpis* is associated with a very high T_c between 60 and $70 \text{ } ^\circ\text{C}$, equaling or exceeding the estimated CT_{max} . This is consistent with the pattern seen for many other arthropods, although some species show partial melting of lipids at lower temperatures, perhaps relating to the importance of lipid fluidity in pheromone communication, lipid dispersal or other functions (Gibbs, 1998).

The presumptive lipid barriers of this mite confer exceptionally high barrier resistance with a very thin layer. Estimates of the thickness of the entire cuticle, based on scanning electron microscopy of air-dried specimens, are only $3\text{--}5 \text{ } \mu\text{m}$ so the thickness of the lipid layer is probably less than $1 \text{ } \mu\text{m}$. While epicuticular lipid layers of many arthropods are of comparable thickness (Hadley, 1994), many are thicker. The high barrier resistance in *P. macropalpis* presents a paradox to Fick's Law which predicts that barrier resistance should be positively correlated with lipid thickness (Machin, 1980). This, again, calls into question the importance of lipid thickness and may indicate that the primary water barrier in arthropod cuticles generally is conferred by a very thin layer of high resistance lipids, with the additional lipids of thicker layers serving different roles.

Whole-animal cuticular transpiration is predicted to scale in proportion to surface area ($M^{0.67}$), assuming a uniform barrier resistance, and respiratory losses should scale in proportion to metabolic rate ($M^{0.75}$). Published comparative data for water flux in air-breathing arthropods provide a scaling exponent of 0.697 (Nagy and Peterson, 1988) which broadly supports these predictions. Given that the respective mass-specific exponent is negative (-0.303), smaller animals will tend to lose proportionally more water per unit time, and species exploiting xeric habitats should

experience severe selection for water retention. This is consistent with the extreme water economy demonstrated by *P. macropalpis*, and suggests that even lower transpiratory fluxes may be found in other very small arthropods.

Acknowledgments

We thank Dr. Mark Judson (Museum of Natural History, Paris) for identifying this mite species and providing additional information on the taxonomy and natural history of *Tarsotomus* and *Paratarsotomus* spp., and to two anonymous reviewers for helpful comments. This work was carried out in part at the Robert J. Bernard Field Station of the Claremont Colleges and was supported by funds from Pomona College and the Rose Hills Foundation.

References

- Armitage, P., Berry, G., 1994. Statistical Methods in Medical Research, 3rd ed. Blackwell, Oxford.
- Banks, N., 1916. New Californian mites. J. Entomol. Zool. 8, 12–16.
- Barilo, A.B., 1984. New mites of the family Anystidae (Acariformes: Prostigmata) of the fauna of Uzbekistan. Zool. Zhurnal 63, 1734–1738.
- Beament, J.W.L., 1959. The effect of temperature on the water-proofing mechanism of an insect. J. Exp. Biol. 35, 494–519.
- Bell, C.J., 1980. The scaling of the thermal inertia of lizards. J. Exp. Biol. 86, 79–85.
- Benoit, J.B., Yoder, J.A., Rellinger, E.J., Ark, J.T., Keeney, G.D., 2005. Prolonged maintenance of water balance by adult females of the American spider beetle, *Mezium affine* Boieldieu, in the absence of food and water resources. J. Insect Physiol. 51, 565–573.
- Berlese, A., 1886. Note relative agli, Acari et Myriapodi e Scorpioni Italiani, 4.
- Bursell, E., 1958. The water balance of tsetse pupae. Philos. Trans. R. Soc. B 241, 179–210.
- Chevaldonné, P., Desbruyères, D., Childress, J.J., 1992. Some like it hot... and some even hotter. Nature 359, 593–594.
- Chevaldonné, P., Fisher, C.R., Childress, J.J., Desbruyères, D., Jollivet, D., Zal, F., Toulmond, A., 2000. Thermotolerance and the 'Pompeii worms'. Mar. Ecol. Prog. Ser. (Comment) 208, 293–295.
- Condon, C., Acharya, A., Adrian, D.J., Hurliman, A.M., Malekooti, D., Nguyen, P., Zelic, M.H., Angilletta, M.J., 2015. Indirect selection of thermal tolerance during experimental evolution of *Drosophila melanogaster*. Ecol. Evol. 5, 1873–1880.
- Cooney, D., Emerson, R., 1964. Thermophilic Fungi. Freeman and Co., San Francisco.
- Cowles, R.B., Bogert, C.M., 1944. A preliminary study of the thermal requirements of desert reptiles. Bull. Am. Mus. Nat. History 83, 265–296.
- Desbruyères, D., Chevaldonné, P., Alayse, A.-M., Jollivet, D., Lallier, F., Jouin-Toulmond, C., Zal, F., Sarradin, P.-M., Cossou, R., Caprais, J.-C., Arndt, C., O'Brien, J., Guezennec, J., Hourdez, S., Riso, R., Gaill, F., Laubier, L., Toulmond, A., 1998. Biology and ecology of the "Pompeii worm" (*Alvinella pompejana* Desbruyères and Laubier), a normal dweller of an extreme deep-sea environment: a synthesis of current knowledge and recent developments. Deep-Sea Res. II 45, 383–422.
- Dixon, A.F.G., Honěk, A., Keil, P., Kotela, M.A.A., Šizling, A.L., Jarošík, V., 2009. Relationship between the minimum and maximum temperature thresholds for development in insects. Funct. Ecol. 23, 257–264 (Blackwell Publishing).
- Edney, E.B., 1977. Water Balance in Land Arthropods. Springer-Verlag, Berlin.
- Finney, D.J., 1971. Probit Analysis. Cambridge University Press.
- Gajardo, G.M., Bearmore, J.A., 2012. The brine shrimp *Artemia*: adapted to critical life conditions. Front. Physiol. 3 (185), 1–8.
- Gehring, W.J., Wehner, R., 1995. Heat-shock protein synthesis and thermotolerance in *Cataglyphis*, an ant from the Sahara Desert. Proc. Natl. Acad. Sci. USA 92, 2994–2998.
- Gibbs, A.G., Chippindale, A.K., Rose, M.R., 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. J. Exp. Biol. 200, 1821–1832.
- Gibbs, A.G., 1998. The role of lipid physical properties in lipid barriers. Am. Zool. 38, 268–279.
- Gibbs, A.G., Fukuzato, F., Matzkin, L.M., 2003. Evolution of water conservation mechanisms in *Drosophila*. J. Exp. Biol. 206, 1183–1192.
- Gibbs, A.G., Rajpurohit, S., 2010. Water-proofing properties of cuticular lipids. In: Blomquist, G.J., Bagnères, A.G. (Eds.), Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology. Cambridge University Press, pp. 100–120.
- Gilchrist, G.W., Huey, R.B., 1999. The direct response of *Drosophila melanogaster* to selection on knockdown temperature. Heredity 83, 15–29.
- Girguis, P.R., Lee, R.W., 2006. Thermal preference and tolerance of alvinellids. Science 312, 231.
- Guadarrama-Cetina, J.M., Mongruel, A., Medici, M.G., Baquero, E., Parker, A.R., Milimouk-Melnitshuk, I., Gonzalez-Vinas, W., Beysens, D., 2014. Dew condensation on desert beetle skin. Eur. Phys. J. E 37 (109), 1–6.
- Guderley, H.E., St. Pierre, J., 1996. Phenotypic plasticity and evolutionary adaptations of mitochondria to temperature. In: Johnston, I.A., Bennett, A.F. (Eds.), Animals and Temperature: Phenotypic Plasticity and Evolutionary Adaptation. Cambridge University Press, Cambridge, pp. 127–152.

- Hadley, N.F., 1970. Micrometeorology and energy exchange in two desert arthropods. *Ecology* 51, 34–444.
- Hadley, N.F., 1977. Epicuticular lipids of the desert tenebrionid beetle, *Eleodes armatus*: seasonal and acclimatory effects on chemical composition. *Insect Biochemistry* 7, 277–283.
- Hadley, N.F., 1981. Cuticular lipids of terrestrial plants and arthropods: a comparison of their structure, composition and waterproofing function. *Biol. Rev.* 56, 23–47.
- Hadley, N.F., 1994. *Water Relations of Terrestrial Arthropods*. Academic Press, San Diego.
- Herbst, D., Conte, F.P., Brookes, V.J., 1988. Osmoregulation in an alkaline salt lake insect, *Ephydra (Hydropyrus) hians* Say (Diptera: Ephydriidae) in relation to water chemistry. *J. Insect Physiol.* 34, 903–909.
- Heurtault, J., Vannier, G., 1990. Thermorésistance chez deux pseudoscorpions (Garypidae), l'un du désert de Namibie, l'autre de la région de Gènes (Italie). *Acta Zool. Fenn.* 190, 165–171.
- Hochachka, P., Somero, G.N., 2002. *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford University Press, New York.
- Hoffmann, A.A., 2010. Physiological climatic limits in *Drosophila*: patterns and implications. *J. Exp. Biol.* 213, 870–880.
- Holdgate, M.W., Seal, M., 1956. The epicuticular wax layers of the pupa of *Tenebrio molitor*. *J. Exp. Biol.* 33, 82–106.
- Huey, R.B., Bennett, A.F., 1987. Phylogenetic studies of coevolution: preferred temperatures versus optimal performance temperatures of lizards. *Evolution* 41, 1098–1115.
- Kappen, L., 1981. Ecological significance of resistance to high temperature. In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (Eds.), *Encyclopedia of Plant Physiology* 12A, *Physiological Plant Ecology*, Vol. 1. Springer, New York, pp. 439–474.
- Klok, C.J., Sinclair, B.J., Chown, S.L., 2004. Upper thermal tolerance and oxygen limitation in terrestrial arthropods. *J. Exp. Biol.* 207, 2361–2370.
- Kurr, M., Huber, R., König, H., Jannasch, H.W., et al., 1991. *Methanopyrus kandleri*, gen. and sp. nov. represents a novel group of hyperthermophilic methanogens, growing at 110 °C. *Arch. Microbiol.* 156, 239–247.
- Lutterschmidt, W.L., Hutchinson, V.H., 1997. The critical thermal maximum: history and critique. *Can. J. Zool.* 75, 1561–1574.
- Machin, J., 1980. Cuticle water relations: towards a new cuticle water proofing model. In: Locke, M., Smith, D.S. (Eds.), *Insect Biology in the Future*. Academic Press, New York, pp. 79–103.
- Machin, J., Lampert, G.J., 1985. A passive two-layer permeability-water content model for *Periplaneta* cuticle. *J. Exp. Biol.* 117, 171–179.
- Mertens, J., Beladjal Alcantara, L., et al., 2008. Survival of dried eukaryotes (anhydrobiotes) after exposure to very high temperatures. *Biol. J. Linn. Soc.* 93, 15–22.
- Nagy, K., Peterson, C.C., 1988. *Scaling of Water Flux Rate in Animals* (University of California Publications in Zoology 120). University of California Press, Berkeley.
- Newell, R.C., 1979. *Biology of Intertidal Animals*, 3rd ed. Marine Ecological Surveys, Ltd., Faversham, Kent.
- O'Brien, J.E., Dahlhoff, E., Somero, G.N., 1991. Thermal resistance of mitochondrial respiration: hydrophobic interactions of membrane proteins may limit mitochondrial thermal resistance. *Physiol. Zool.* 64, 1509–1526.
- O'Donnell, M.J., Machin, J., 1988. Water vapor absorption in terrestrial organisms. In: Gilles, R. (Ed.), *Advances in Comparative and Environmental Physiology*, vol. 2. Springer-Verlag, Berlin, pp. 47–107.
- Ochocińska, D., Taylor, J.R.E., 2005. Living at the physiological limits: field and maximum metabolic rates of the Common Shrew (*Sorex araneus*). *Physiol. Biochem. Zool.* 78, 808–818.
- Otto, J.C., 1999. The taxonomy of *Tarsotomus* Berlese and *Paratarsotomus* Kuznetsov (Acarina: Anystidae: Erythracarinae) with observations on the natural history of *Tarsotomus*. *Invertebrate Taxonomy* 13, 749–803.
- Parker, A.R., Lawrence, C.R., 2001. Water capture by a desert beetle. *Nature* 414, 33–34.
- Pianka, E.R., 1986. *Ecology and Natural History of Desert Lizards*. Princeton University Press, Princeton, NJ.
- Pörtner, H.O., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* 88, 137–146.
- Roberts, C.S., Seeley, M.K., Ward, D., Mitchell, D., Campbell, J.D., 1991. Body temperatures of Namib Desert tenebrionid beetles: their relationship in laboratory and field. *Physiol. Entomol.* 16, 463–475.
- Rourke, B.C., Gibbs, A.G., 1999. Effects of lipid phase transitions on cuticular permeability: model membrane and *in situ* studies. *J. Exp. Biol.* 202, 3255–3262.
- Schmidt-Nielsen, K., 1984. *Scaling: Why is Animal Size SO Important?* Cambridge University Press.
- Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. *Integr. Comp. Biol.* 42, 780–789.
- Spellerberg, I.F., 1972. Thermal ecology of allopatric lizards (*Sphenomorphus*) in southeast Australia. I. The environment and lizard critical temperatures. *Oecologia* 9, 371–383.
- Stark, L.R., McLetchie, D.N., Roberts, S.P., 2009. Gender differences and a new adult eukaryotic record for upper thermal tolerance in the desert moss *Syntrichia caninervis*. *J. Therm. Biol.* 34, 131–137.
- Stetter, K.O., 2006. Hyperthermophiles in the history of life. *Philos. Trans. R. Soc. B* 361, 1837–1843.
- Stillman, J.H., Somero, G.N., 2000. A comparative analysis of the upper thermal tolerance limits of Eastern pacific porcelain crabs (genus *Petrolisthes*): influences of latitude, vertical zonation, acclimation and phylogeny. *Physiol. Biochem. Zool.* 73, 200–208.
- Sweetman, H.L., 1938. Physical ecology of the firebrat *Thermobia domestica* (Packard). *Ecol. Monogr.* 8, 285–311.
- Takai, K., Nakamura, K., Toki, T., Tsunogai, U., et al., 2008. Cell proliferation at 122 °C and isotopically heavy CH₄ production by a hyperthermophilic methanogen under high-pressure cultivation. *Proc. Natl. Acad. Sci. USA* 105, 10949–10954.
- Telonis-Scott, M., Guthridge, K.M., Hoffmann, A.A., 2014. A new set of laboratory-selected *Drosophila melanogaster* lines for the analysis of desiccation resistance: response to selection, physiology and correlated responses. *J. Exp. Biol.* 209, 1837–1847.
- Tomanek, L., 2002. The heat-shock response and patterns of vertical zonation in intertidal *Tegula* congeners. *Integr. Comp. Biol.* 42, 797–807.
- Tomanek, L., Somero, G.N., 1999. Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: implications for limits of thermotolerance and biogeography. *J. Exp. Biol.* 202, 2925–2936.
- Toolson, E.C., Hadley, N.F., 1979. Seasonal effects on cuticular permeability and epicuticular lipid composition in *Centruroides sculpturatus* Ewing 1928 (Scorpiones: Buthidae). *J. Comp. Physiol. B.* 129, 319–325.
- Ward, D., Seeley, M., 1996. Adaptation and constraint in the evolution of the physiology and behavior of the Namib desert tenebrionid beetle genus *Onymacris*. *Evolution* 50, 1231–1240.
- Wehner, R., Marsh, A.C., Wehner, S., 1992. Desert ants on a thermal tightrope. *Nature* 357, 586–587.
- Wharton, G.W., 1985. Water balance of insects. In: Kerkut, G.A., Gilbert, L.I. (Eds.), *Comparative Insect Physiology, Biochemistry and Pharmacology*. Pergamon Press, Oxford, pp. 565–603.
- Wickstrom, C.E., Castenholz, R.W., 1973. Thermophilic Ostracod: aquatic metazoan with the highest known temperature tolerance. *Science* 181, 1063–1064.
- Wigglesworth, V.B., 1945. Transpiration through the cuticle of insects. *J. Exp. Biol.* 21, 97–114.
- Williams, B.R., van Heerwaarden, B., Dowling, D.K., Sgrò, C.M., 2012. A multivariate test of evolutionary constraints for thermal tolerance in *Drosophila melanogaster*. *J. Evol. Biol.* 25, 1415–1426.
- Winston, P.W., Bates, D.H., 1960. Saturated solutions for the control of humidity in biological research. *Ecology* 41, 232–237.
- Withers, P.C., 1992. *Comparative Animal Physiology*. Saunders College Publishing, Fort Worth.
- Wohltmann, A., 1998. Water vapour uptake and drought resistance in immobile instars of *Parasitengona* (Acari: Prostigmata). *Can. J. Zool.* 76, 1741–1754.
- Wu, G.C., Wright, J.C., Whitaker, D.L., Ahn, A., 2010. Kinematic evidence for superfast locomotory muscle in a tenebrionid mite. *J. Exp. Biol.* 213, 2551–2556.
- Yoder, J.A., 1998. A comparison of the water balance characteristics of *Typhlodromus occidentalis* and *Amblyseius finlandicus* mites (Acari: Phytoseiidae), and evidence for the site of water vapour uptake. *Exp. Appl. Acarol.* 22, 279–286.
- Zheng, Y., Bai, H., Huang, Z., Tian, X., Nie, F., Zhao, Y., Zhai, J., Jiang, L., 2010. Directional water collection on wetted spider silk. *Nature* 463, 640–643.