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Heat tolerance in desert rodents is correlated with microclimate at inter- and intraspecific levels

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Abstract

Physiological diversity in thermoregulatory traits has been extensively investigated in both endo- and ectothermic vertebrates, with many studies revealing that thermal physiology has evolved in response to selection arising from climate. The majority of studies have investigated how adaptative variation in thermal physiology is correlated with broad-scale climate, but the role of fine-scale microclimate remains less clear . We hypothesised that the heat tolerance limits and evaporative cooling capacity of desert rodents are correlated with microclimates within species-specific diurnal refugia. We tested predictions arising from this hypothesis by comparing thermoregulation in the heat among arboreal black-tailed tree rats (*Thallomys nigricauda*), Namaqua rock rats (*Micaelamys namaquensis*) and hairy-footed gerbils (*Gerbillurus paeba*). Species and populations that occupy hotter diurnal microsites tolerated air temperatures (T_a) ~ 2–4 ℃ higher compared to those species occupying cooler, more thermally buffered microsites. Inter- and intraspecific variation in heat tolerance was attributable to ~ 30% greater evaporative water loss and ~ 44 % lower resting metabolic rates at high T_a, respectively. Our results suggest that microclimates within rodent diurnal refugia are an important correlate of intra- and interspecific physiological variation and reiterate the need to incorporate fine-scale microclimatic conditions when investigating adaptative variation in thermal physiology.

Keywords Thermoregulation · Microclimate · Refugia · Variation

Introduction

Numerous studies have revealed that the thermal physiology of ecto- and endothermic animals has been shaped by selective pressures imposed by historical and current environmental conditions (e.g., McNab 1970; Angilletta et al.

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2010; Boyles et al. 2012; Pettersen et al. 2018). Thermal adaptation is evident in taxa ranging from insects to mammals and understanding how climate drives the evolution of thermal physiology is vital for elucidating the processes determining the geographical distribution and range limits of species (Bozinovic and Rosenmann 1989; Bozinovic et al. 2011; Keller and Seehausen 2012).

Patterns such as positive correlations between latitude and cold tolerance and thermal tolerance breadth among insects and ectothermic vertebrates (Addo-Bediako et al. 2000; Sunday et al. 2011; Araújo et al. 2013) provide evidence for the evolution of ectotherm thermal physiology with global gradients of climate. Among endotherms, evidence for similiar global variation associated with environmental temperature has been reported for avian and mammalian basal metabolic rate, evaporative water loss, core body temperature, and minimal conductance (Withers et al. 2006; White et al. 2007; Naya et al. 2013a; Naya et al. 2013b; Clarke and O'Connor 2014; but see also Rezende et al. 2004), whereas summit metabolism is positively correlated with air temperature for birds (Swanson and Garland 2009). In addition, lower and

upper critical limits of thermoneutrality are positively correlated with air temperature, while the breadth of the thermoneutral zone is negatively correlated with air temperature in birds and mammals (Araújo et al. 2013; Bozinovic et al. 2014).

The majority of studies investigating climate-correlated adaptive variation in thermal physiology have done so using broad-scale measures of climatic conditions (Addo-Bediako et al. 2001; Sunday et al. 2011; Araújo et al. 2013). However, there frequently exists a mismatch between broad-scale climate and the microclimates experienced by organisms (Potter et al. 2013). Indeed, microclimate, *in lieu* of broad-scale climate variation, has been found to shape behavioural, physiological and ecological processes (Bell et al. 1986; Sears et al. 2016). For example, California leaf-nosed bats (*Macrotus californicus*) are able to persist in the southwestern Sonoran desert throughout the year provided that they can rely on geothermally heated winter roost sites (Bell et al. 1986). Among sympatric bats in a hot, semi-arid landscape, heat tolerance is greater among species that occupy relatively exposed roosts compared to those that roost in the cooler microsites of tree cavities (Cory Toussaint and McKechnie 2012). Additionally, models predicting changes in species distribution in response to expected global change show considerable differences when incorporating fine-scale microclimatic conditions compared to those only including broad-scale indices of climate (Suggitt et al. 2011; Duffy et al. 2015). These and similar studies raise the possibility that microclimatic conditions, in addition to broad-scale climate variation, comprise an important factor driving thermal adaptation.

Rodents are a particularly well-studied taxon in terms of thermoregulatory adaptation, with several studies examining correlations between broad-scale environmental variables and the thermoregulatory physiology of this group. Interspecific comparisons reveal that thermoregulatory traits are influenced by primary productivity, aridity, and temperature (Haim 1987; McNab and Morrison 1963; McNab 1979; Lovegrove 2003; Bozinovic et al. 2009, 2014; Luna et al. 2017). Similarly, at the intraspecific level, rodent thermoregulatory patterns also vary with primary productivity, aridity, and temperature (Schmidt-Nielsen and Schmidt-Nielsen 1952; McNab and Morrison 1963; Lovegrove 1986; Haim 1987; Weissenberg and Shkolnik 1994; Tracy and Walsberg 2000; Novoa et al. 2005; Bozinovic et al. 2009). Studies focusing on the importance of microclimates in rodent thermoregulation have revealed that the use of fine-scale variation in environmental temperatures is important in their ability to maintain a positive heat balance and can play defining roles in distribution limits (Schmidt-Nielsen and Schmidt-Nielsen 1950; Dawson 1955; Chappell and Bartholomew 1981; French 1993; Degen 1997; Tracy and

Walsberg 2002). For example, white-tailed antelope ground squirrels (*Ammospermophilus leucurus*) exploit substantial variation in operative temperatures between soil surface and subterranean burrows; they tolerate transient hyperthermia while foraging on the soil surface and retreat to subterranean burrows to dissipate heat loads (Chappell and Bartholomew 1981). Similarly, thermally buffered microsites are thought to play a major role in rodents' occupation of desert habitats (Schmidt-Nielsen and Schmidt-Nielsen 1950; French 1993; Degen 1997; Tracy and Walsberg 2002). However, the role of microclimate as a driver of inter- and intraspecific variation in the thermal physiology of rodents has received less attention. Jackson et al. (2002), for instance, reported that interspecific variation in resting metabolic rates in *Otomys* is correlated with microclimate rather than broad-scale climate.

Adaptive variation in rodent thermoregulation at moderate and cold environmental temperatures is relatively well studied (Brower and Cade 1966; McNab 1979; Downs and Perrin 1990; Haim et al. 1993; Arends and McNab 2001; Jackson et al. 2002; Lovegrove 2003; White 2003; Rezende et al. 2004; Bozinovic et al. 2014; Ruf and Geiser 2015), whereas fewer studies have investigated endothermic thermal adaptation in response to high heat loads (Lovegrove et al. 1991). There are numerous thermoregulatory data sets for rodents at temperatures above the thermoneutral zone, but direct comparisons among studies are often complicated by variation in experimental conditions (Wolf et al. 2017). However, several recent studies of avian and chiropteran thermoregulation under high heat loads have revealed considerable inter- and intraspecific variation in thermoregulatory patterns (Cory Toussaint and McKechnie 2012; Smit et al. 2013; Noakes et al. 2016; Talbot et al. 2017; Czenze et al. 2020a, b). Understanding the evolution of endotherm thermoregulation requires quantifying variation across the full range of environmental temperatures experienced by a taxon. Studies of thermoregulation in the heat are also particularly important in light of rapid anthropogenic global heating.

Here, we aimed to address: (1) the general paucity of studies of microclimate-correlated adaptive variation in mammalian thermal physiology, and (2) the limited data available on adaptive variation in rodent thermoregulation under high heat loads. We hypothesised that heat tolerance and evaporative cooling capacity has evolved in response to microclimate among nocturnal desert-adapted rodents. At the interspecific level, we compared thermoregulation under high heat loads in arboreal black-tailed tree rats (*Thallomys nigricauda*) and Namaqua rock rats (*Micaelamys namaquensis*), sympatric species that occupy diurnal refugia that differ greatly in thermal buffering (Lovegrove and Heldmaier, 1994). During the day, *T. nigricauda* retreat to tree cavities

whose temperatures approach outside air temperature, frequently reaching 40 °C in the southern Kalahari (Coleman and Downs 2010). The subterranean burrows occupied by *M. namaquensis*, on the other hand, are buffered from high daytime air temperature, with burrow temperatures 10–15 °C lower than outside daytime maxima (Jackson et al. 2002). At the intraspecific level, we compared thermoregulation under high heat loads between two populations of hairy-footed gerbils (*Gerbillurus paeba*) and *M. namaquensis* at two sites differing in aridity. Contrasts in the direction of differences regarding macro- and microclimatic conditions allowed us to differentiate between broad-scale climatic effects and fine-scale microclimatic effects (Figs. 1, 2). The diurnal refugia of *G. paeba* at both sites consisted of sandy subterranean tunnels, but one population of *M. namaquensis* used sandy subterranean burrows, whereas the other used rock crevices.

We predicted that more thermally challenging microclimates within diurnal refugia are associated with greater heat tolerance, arising from maximum evaporative heat loss (EHL) / metabolic heat production (MHP) ratios [i.e. overall capacity for evaporative cooling and a significant predictor of heat tolerance limits (Czenze et al. 2020b)]. More specifically, we predicted that the arboreal *T. nigricauda* is more heat tolerant than the co-occurring subterranean *M. namaquensis*. Similarly, we predicted that the population of *M. namaquensis* occupying rock crevice diurnal refugia is more heat tolerant than the population using subterranean refugia, but the heat tolerance of *G. paeba* is indistinguishable between populations. To test these predictions, we measured resting metabolic rate (RMR), evaporative water loss (EWL) and core body temperature (T_b) during exposure to a stepped profile of increasing air temperatures (T_a), ranging from below thermoneutrality to $T_a > T_b$. Our

Fig. 2 Modelled daily maximum air temperature (**a**) and temperature experienced within diurnal refugia occupied by Namaqua rock rats (*Micaelamys namaquensis*) (**b**) and hairy-footed gerbils (*Gerbillurus paeba*) (**c**) at Aggeneys and Radnor, two sites in southern African. Data modelled using the NicheMapR package in R (Kearney 2020). The diurnal refugia of *G. paeba* at both populations consisted of sandy subterranean tunnels. In contrast, the Radnor *M. namaquensis* population occupied sandy subterranean burrows and the Aggeneys population used rock crevices located on a rocky outcrop. Asterisks represent significance at $P < 0.05$

approach is identical to those in a recent series of studies of heat tolerance in birds and bats (Cory Toussaint and McKechnie 2012; Smit et al. 2013; Noakes et al. 2016; Talbot et al. 2017; Czenze et al. 2020a, b).

Materials and methods

Study site and study species

This study was conducted at two sites in South Africa, Radnor farm (26°6′23″S, 22°52′54″E), near Vorstershoop, Northwest Province, and Black Mountain Mine Conservation Area (29°12′14″S, 18°50′31″E), just south of the town of Aggeneys, Northern Cape. Data were collected between 6 October and 28 December 2018. Black Mountain Mine Conservation Area is dominated by grasses, including various species of *Stipagrostis*, and scattered shrubs (typically *R. trichotomum*) (Kemp and McKechnie 2019). Vegetation at Radnor is characterised as Molopo Bushveld, consisting of a mix of open woodland and closed shrubland dominated with *Vachellia erioloba* and *Boscia albitrunca* trees and *Lycium cinereum*, *L. hirsutum* and *Rhigozum trichotomum*
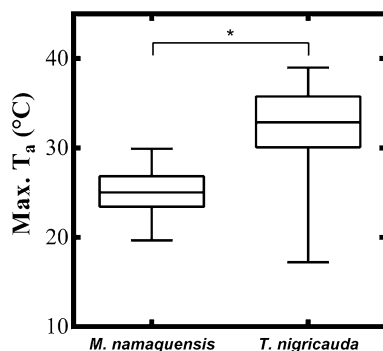

Fig. 1 Modelled daily maximum air temperature (T_a) experienced within diurnal refugia of sympatrically occurring Namaqua rock rats (*Micaelamys namaquensis*) and black-tailed tree rats (*Thallomys nigricauda*). Data modelled using the NicheMapR package in R (Kearney 2020). During the diurnal period, *M. namaquensis* at this site occupies sandy subterranean burrows and *T. nigricauda* occupies tree cavities. Asterisks represent significance at $P < 0.05$
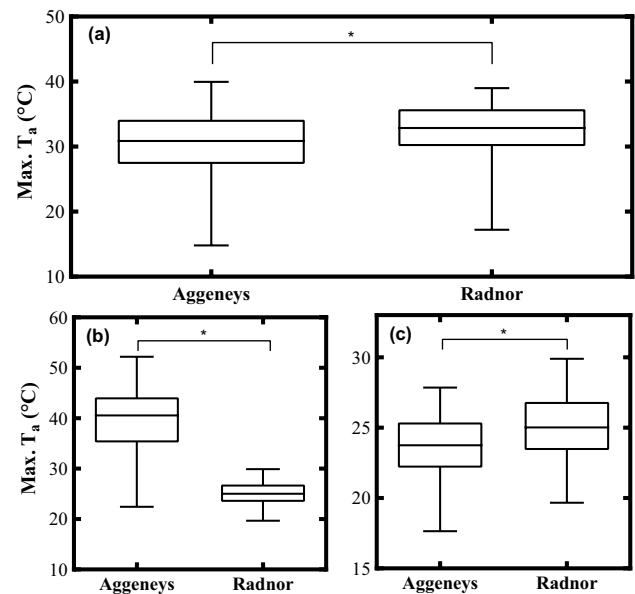
shrubs (Rutherford et al. 2006). During the summer months, October–February, maximum air temperature ranges from 32.6 to 36.6 °C and 27.8 to 33.3 °C, for Radnor and Black Mountain Mine Conservation Area, respectively (Fick and Hijmans 2017) and precipitation received over the summer months is ~ 221 mm and ~ 57 mm, respectively (Fick and Hijmans 2017).

Twenty *G. paeba*, 20 M. *namaquensis* and eight *T. nigricauda* were caught using Sherman traps baited with oats and peanut butter. Mean ± s.d. body mass (M_b) for *G. paeba*, *M. namaquensis* and *T. nigricauda* was 26.6 ± 6.3 g, 61.9 ± 23.5 g and 79.5 ± 9.2 g, respectively. The rodents were held in standard laboratory rodent cages and supplied an ad libitum supply of rodent pellets, super worms (*Zophobas morio*) and water. Rodents were kept for no longer than 24 h prior to experimentation.

Macro- and microclimate analysis

Macro- and microclimate conditions, for 1 October 2017–31 March 2018, were modelled using the NicheMapR package (Kearney 2020) in R 3.5.2 (R Development Core Team 2018). Daily maximum temperatures experienced during the 6-month sampling period were compared using *t* tests. For the interspecific comparison, maximum daily soil temperatures at a depth of 20 cm were compared to air temperature (2 m above ground surface), to quantify the different thermal regimes experienced by the arboreal *T. nigricauda* and subterranean *M. namaquensis*. Tunnel depths of *M. namaquensis* were approximately 20 cm below ground (B. van Jaarsveld, *pers. obs.*) and *T. nigricauda* tree-cavity temperatures approach outside air temperature (Coleman and Downs 2010). *Gerbillurus paeba* used sandy burrows as diurnal refugia at both sites. In contrast, *M. namaquensis* used sandy burrows at Radnor and rock crevices located on rocky outcrops at Aggeneys. Again, soil temperatures were modelled at a depth of 20 cm (B. van Jaarsveld, *pers. obs.*) to represent microclimate experienced by *M. namaquensis* and *G. paeba* within sandy subterranean tunnels at Aggeneys and Radnor. To create a comparable indicator of temperature regimes experienced by *M. namaquensis*, temperatures were modelled assuming daily refugia were located 5 cm (B. van Jaarsveld, *pers. obs.*) below a rock substrate.

Air and body temperature measurements

To measure air temperature (T_a) in the metabolic chambers, a hole was drilled in the side of each chamber through which a 3-mm-diameter thermistor probe (Sable Systems, Las Vegas, NV, USA) was inserted and sealed with a rubber grommet. Core body temperature (T_b) was continuously measured using a temperature-sensitive passive integrated transponder

(PIT) tag (BioTherm, Biomark, Biose ID, USA) injected intraperitoneally into each bird (Whitfield et al. 2015) was used to obtain continuous measurements of body temperature (T_b), with the antenna of a portable transceiver system (HPR+, Biomark, Biose, ID, USA) placed adjacent to the chamber. A circulating water bath (F34, Julabo, Seelbach BW, DE) was used to calibrate PIT tags against a digital thermocouple reader (RDXL12SD, Omega, Stamford, CT, USA). The PIT tag readings deviated from actual temperatures by 0.06 ± 0.05 °C ($n = 20$) and T_b all measurements were corrected.

Gas exchange measurements

Rates of carbon dioxide production (mL min^{-1}) and evaporative water loss (EWL; mg min^{-1}) were measured by flow-through respirometry. Each metabolic chamber was outfitted with a metal mesh platform positioned ~ 5 cm above a 1-cm layer of mineral oil at the bottom of the chamber to prevent excreta from affecting water vapour levels. Mixing of air within each chamber was maximised by positioning an air inlet near the top and an outlet near the bottom. A 3-L container was used for *T. nigricauda* and *M. namaquensis*; whereas for *G. paeba*, a 1-L container was used. A modified 75-L ice chest, within which T_a was regulated using a thermoelectric air cooler (TC 720 OEM, T.E. Technology, Traverse City MI, USA) controlled by a digital controller (TC-720 Temperature Controller, T.E. Technology, Traverse City MI, USA), served as a temperature-controlled environment within which the chamber was placed. An oil-free compressor supplied atmospheric air that was scrubbed of water vapour using a membrane dryer (Champion®CMD3, Champion Pneumatic, Quincy IL, USA). The airstream was then split into the baseline and experimental channels supplying the metabolic chambers. In the experimental channel, flow rate was regulated by a mass flow controller (0–30 SLPM, Alicat Scientific Inc., Tuscon, AZ, USA), calibrated against a Gilibrator 2 (Sensidyne, St Petersburg, FL, USA). Flow rate in the baseline channel was regulated at ~ 1 L min^{-1} by a needle valve (Swagelok, Solon, OH, USA). Flow rates were adjusted during measurements to maintain low humidity (< 1 kPa) within the chamber to keep animals calm and avoid impedance of evaporative heat dissipation (Gerson et al. 2014; Whitfield et al. 2015; van Dyk et al. 2019). The difference in [CO_2] between incurrent and excurrent airstreams was typically ~ 200 ppm, whereas the corresponding value for [H_2O] was ~ 1 ppt. The flow rates we used varied from 2 to 14 L min^{-1}, with adjustments made on the basis of T_a and the activity of rodents. A respirometry multiplexer (MUX3-1101-18 M, Sable Systems, Las Vegas, NV, USA) in manual mode sequentially subsampled

excurrent air from the experimental and baseline channels. Subsampled air was pulled through a CO_2/H_2O analyser (LI- 840A, LI-COR, Lincoln, NE, USA). The CO_2 sensor of the analyser was regularly calibrated using pure N_2 and an analytically certified 2000-ppm CO_2 span gas (AFROX, Johannesburg, South Africa). The H_2O sensor was zeroed with N_2 and spanned using humidified air produced by a dewpoint generator (DG4, Sable Systems, Las Vegas, NV, USA). All tubing in the system was Bev-A-Line IV tubing (Thermoplastic Processes Inc., Warren, NJ, USA). An analogue–digital converter (UI-3, Sable Systems, Las Vegas, NV, USA) digitised analogue voltage outputs from the analysers and thermistor probes, with data recorded every 5 s using Expedata software (Sable Systems, Las Vegas, NV, USA).

Experimental protocol

Before measurements, individuals were weighed on an electronic balance (EJ-160, A&D, Tokyo, J.P.) and held in the metabolic chamber without food or water for at least 90 min at $T_a = 20$ ℃. Rodents were exposed to a stepped profile of increasing T_a, with T_a increased in 5-℃ increments at $T_a = 20–30$ ℃ and in 2-℃ increments at $T_a = 32–44$ ℃. Measurements started with a baseline air subsample for ~ 5 min, whereafter excurrent chamber air was subsampled once T_a had stabilised at the target value, followed by another 5-min baseline. The majority of individuals spent ~ 15–20 min at each T_a value, before measurements were taken. The average 99% equilibrium time (Lasiewski et al. 1966) was 3.17 ± 0.41 min.

Due to ethical considerations, individuals that exceeded a T_b value of 41 °C were considered to have reached their thermal endpoints, approximately 2 °C lower than previously reported critical thermal maxima for rodents (Erksine and Hutchinson 1982). The activity and behaviour of the individuals were continuously monitored using a CCTV video camera and an infrared light source. Upon completion of experimental run, a cotton pad soaked in ethanol was rubbed on the rodent's body to increase heat loss. Rodents were placed back in their cages to rest, once T_b stabilised at normothermic levels (34–36 °C). An ad libitum supply of water and food was provided until their release at the site of capture. *Gerbillurus paeba* and *M. namaquensis* lost less than 5% body mass as faeces and water during measurements; whereas *T. nigricauda* lost ~ 10%.

Data analyses

Rates of $\dot{V}_{CO2}$ and EWL were estimated from the lowest 5-min stable traces, using Eqs. 9.5 and 9.6 from Lighton (2008) and assuming 0.803 mg H_2O ml vapour^{-1}. We calculated resting metabolic rate RMR (W) from $\dot{V}_{CO2}$ using 20.9 J ml^{-1} CO_2 (i.e., RER = 1.0) and evaporative heat loss (W) was calculated assuming a latent heat of vaporisation of water of 2.406 J mg H_2O^{-1} (Walsberg and Wolf 1995; Tracy et al. 2010). Given the high flow rates, it was not possible to measure oxygen consumption and thus the respiratory exchange ratio (RER) could not be calculated; since rodents were kept without food or water for at least 90 min before measurements, it was thus assumed that RER = 1.00. The efficiency of thermoregulatory heat dissipation by evaporation was calculated as the ratio of evaporative heat loss/ metabolic heat production (i.e., EHL/MHP).

All statistical analyses were performed in R. Inflection points in EWL, RMR, T_b, EHL/MHP as a function of T_a (Table 1) were identified by fitting piece-wise linear regression models fitted separately to data above and below each inflection. Functions described by Muggeo et al. (2014) were used to account for pseudoreplication when calculating the inflection points. A two-part analysis, using linear mixed-effects models using the *lme4* package (Bates et al. 2015), was conducted to test the hypothesis that inter- and intraspecific differences in thermoregulation at high temperatures are correlated with microclimatic conditions. Interspecific differences in thermoregulation related to microclimate were assessed by combining data for *T. nigricauda* and *M. namaquensis* individuals caught at Radnor. Separate linear mixed-effects models were fitted to data above and below each

Table 1 Air temperature (℃) inflections, ± 95% CI, for evaporative water loss (EWL), resting metabolic rate (RMR), ratio of evaporative heat loss to metabolic heat production (EHL/MHP), and core body temperature (T_b) of black-tailed tree rat (*Thallomys nigricauda*), hairy-footed gerbil (*Gerbillurus paeba*) and Namaqua rock rat (*Micaelamys namaquensis*)

| | Interspecific | | Intraspecific | | | |
| | Black-tailed tree rat | Namaqua rock rat | Hairy-footed gerbil | | Namaqua rock rat | |
			Aggeneys	Radnor	Aggeneys	Radnor
EWL	35.8 (34.9–36.8)	34.8 (32.8–36.9)	35.4 (34.8–35.2)	36.6 (36.0–37.3)	37.3 (36.7–37.9)	35.1 (33.7 -36.5)
RMR	33.3 (30.3–36.4)	28.2 (25.4–31.0)	30.8 (29.9–33.4)	31.6 (29.0–32.5)	35.1 (33.6–36.5)	33.8 (30.8–36.8)
EHL/MHP	36.1 (35.7–36.7)	32.1 (31.1–33.2)	37.8 (37.4–38.3)	35.7 (35.0–36.4)	37.2 (36.1–38.1)	35.6 (33.7–37.5)
T_b	31.3 (29.9–32.9)	31.5 (30.5–32.4)	32.4 (31.6–33.3)	33.0 (31.9–34.1)	33.5 (32.8–34.2)	33.0 (32.0–34.0)

inflection point (Table 1). The global model contained T_a (or $T_a - T_b$), species, and M_b and all two and three-way interactions as potential predictor variables. Given the potential for bias when M_b is correlated with other variables of interest, *Gerbillurus paeba* was excluded from the interspecific comparison because of substantial differences in M_b (Freckleton 2009). To assess whether microclimate conditions are correlated with intraspecific differences in thermoregulation at high temperature, the data from *G. paeba* and *M. namaquensis* individuals caught at Radnor and Aggeneys were pooled for each species. Separate linear mixed-effects models were fitted to the data above and below the inflection points. The global model contained T_a (or $T_a - T_b$), site, and M_b and all two- and three-way interactions as potential predictor variables. If inflection points differed between populations/species, the highest inflection point was used as the lower limit when sub-setting data above the inflection point and vice versa. Model selection was conducted using the *dredge* function from the *MuMIn* package (Barton 2019), *dredge* was constrained so that all possible models contained site and species as an explanatory variable for the inter- and intraspecific comparison, respectively. Body mass did not emerge as a significant predictor for any of the response variables (Table S1, supplementary material). Since multiple measurements were taken from the same individuals, pseudoreplication was accounted for by including rodent identity as a random effect. Maximum EHL/MHP of each individual and T_a values at which animals reached thermal endpoints were compared with *t* tests. Values are presented as means ± SD.

Results

Climate and microclimate

Modelled temperatures of microclimates revealed *T. nigricauda* experience higher maximum temperatures ($\bar{x} = 32.38 \pm 0.31$, $n = 182$ days) within tree-cavities compared to those of *M. namaquensis* in subterranean tunnels ($\bar{x} = 25.24 \pm 0.18$, $n = 182$ days), (*t* test: $t = 19.610$, $df = 294$, $P < 0.001$; Fig. 1). Modelled maximum air temperatures varied significantly (t-test: $t = 4.185$, $df = 356$, $P < 0.001$; Fig. 2) between Aggeneys ($\bar{x} = 30.36 \pm 0.36$, $n = 182$ days) and Radnor ($\bar{x} = 32.38 \pm 0.31$, $n = 182$ days). Maximum air temperatures experienced within microclimates by rodents also varied between Aggeneys and Radnor, but in opposite directions. Maximum air temperatures within the subterranean burrows of *G. paeba* were significantly lower (*t* test: $t = 4.185$, $df = 356$, $P < 0.001$; Fig. 2) in Aggeneys ($\bar{x} = 23.61 \pm 0.16$, $n = 182$ days) compared to those at Radnor ($\bar{x} = 25.24 \pm 0.18$, $n = 182$ days). In contrast, maximum air temperatures within the rocky crevices of *M. namaquensis* at Aggeneys ($\bar{x} = 39.43 \pm 0.48$, $n = 182$ days) were

significantly higher (*t* test: $t = 27.73$, $df = 352$, $P < 0.001$; Fig. 2) compared to those in the subterranean burrows ($\bar{x} = 25.24 \pm 0.18$, $n = 182$ days) used by this species at Radnor.

Interspecific comparison

Evaporative water loss

Below inflection points (Table 1), rates of EWL were similar for *T. nigricauda* and *M. namaquensis* ($t = 0.450$, $df = 16$, $P = 0.659$; Fig. 3). Minimal rates of EWL, 0.15 ± 0.06 g h^{-1} ($n = 18$), occurred at $T_a = \sim 20$ °C in both species. At T_a values above the inflection point, there was a significant species $\times T_a$ interaction ($t = 4.461$, $df = 38$, $P = 0.001$; Fig. 3). Evaporative water loss of *T. nigricauda* increased to a maximum of 2.09 ± 0.42 g h^{-1} ($n = 4$) at $T_a = \sim 44$ °C, and 0.65 ± 0.40 g h^{-1} ($n = 6$) at $T_a = \sim 40$ °C for *M. namaquensis*.

Resting metabolic rate

Only one inflection point in RMR was present for both *M. namaquensis* and *T. nigricauda* across the range of temperatures (Table 1.). Above the inflection points, RMR was similar between the two species ($t = 0.561$, $df = 15$, $P = 0.583$; Fig. 3) at 0.52 ± 0.13 W ($n = 18$). T_a was not a significant explanatory variable for RMR (Electronic supplementary file) above the inflection points. At T_as below inflection points, RMR were higher in *M. namaquensis* compared to *T. nigricauda* ($t = 3.462$, $df = 16$, $P = 0.003$; Fig. 3) and decreased with increasing T_a ($t = 4.292$, $df = 18$, $P < 0.001$; Fig. 3). At $T_a = \sim 25$ °C, RMR for *M. namaquensis* was 0.64 ± 0.12 W ($n = 10$), and *T. nigricauda*, 0.47 ± 0.13 W ($n = 8$) approximately 27% lower than in *M. namaquensis*.

Evaporative heat loss / metabolic heat production

When EHL/MHP was plotted against $T_a - T_b$ an inflection point was identified at $T_a - T_b = -2.7$ °C for *T. nigricauda* and $T_a - T_b = -3.5$ °C for *M. namaquensis* (Fig. 3). Above these inflection points, EHL/MHP increased linearly and significantly with $T_a - T_b$ ($t = 14.610$, $df = 40$, $P < 0.001$; Fig. 3) and was similar between species ($t = 0.935$, $df = 15$, $P = 0.365$; Fig. 3). The linear regression for $T_a - T_b$ vs. EHL/MHP intercepts the y-axis at ~1, meaning that 100% of MHP is dissipated evaporatively when $T_b = T_a$. Below the inflection point, EHL/MHP was similar between species ($t = 0.467$, $df = 15$, $P = 0.647$; Fig. 3) and increased significantly with $T_a - T_b$ ($t = 7.272$, $df = 50$, $P < 0.001$; Fig. 3). Maximum EHL/MHP values were significantly greater (*t* test: $t = 19.610$, $df = 294$, $P < 0.001$; Fig. 4) in *T. nigricauda* ($\bar{x} = 2.12 \pm 0.20$, $n = 6$) than *M. namaquensis* ($\bar{x} = 0.64 \pm 0.13$, $n = 9$).
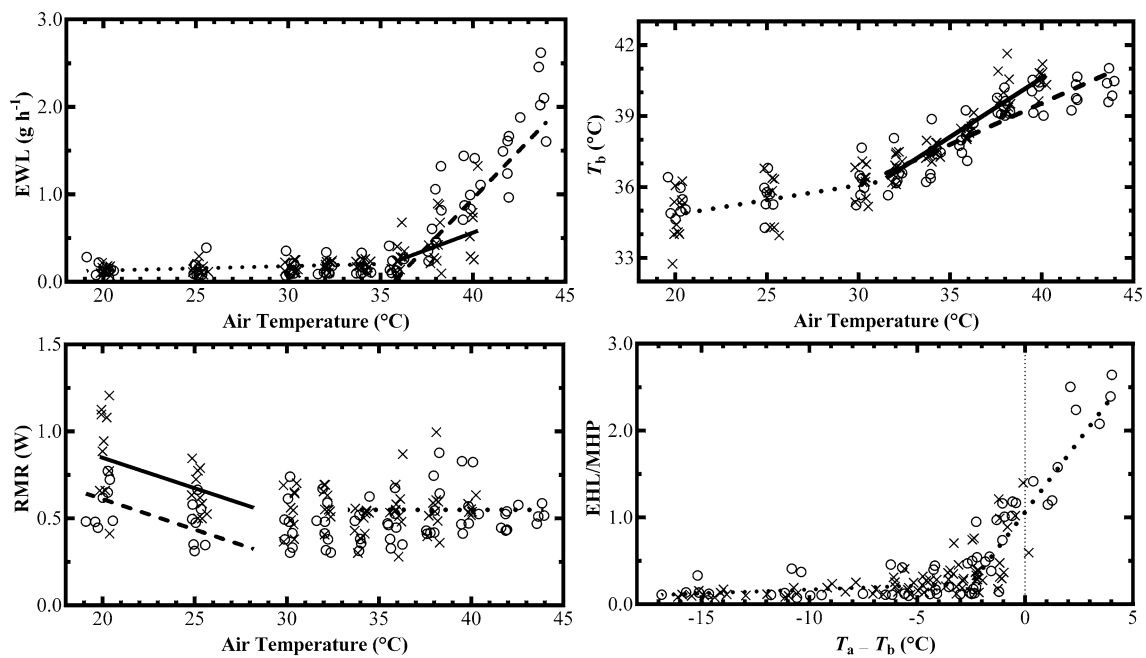
Fig. 3 Relationships between evaporative water loss (EWL), resting metabolic rate (RMR), evaporative heat loss/metabolic heat production (EHL/MHP), core body temperature (T_b) and air temperature (T_a) in sympatric Namaqua rock rats (*Micaelamys namaquensis*) and black-tailed tree rats (*Thallomys nigricauda*). Data were obtained from 10 *M. namaquensis* (crosses) and eight *T. nigricauda* (circles) individuals using open flow-through respirometry. Lines indicate linear mixed-effects regression models fitted at T_a above and below inflection points; regression lines are fitted where there are significant differences between *M. namaquensis* (solid) and *T. nigricauda* (dashed). At $T_a >$ inflections, *M. namaquensis*: EWL $= 0.07T_a - 2.58$ and *T. nigricauda*: EWL $= 0.22T_a - 7.87$ and, RMR $= 0.55$, EHL/MHP $= 0.33(T_a - T_b) + 1.06$, *M. namaquensis*: $T_b = 0.50T_a + 20$ and *T. nigricauda*: $T_b = 0.35T_a + 25.59$. At $T_a <$ inflections, EWL $= 0.005T_a + 0.03$, *M. namaquensis*: RMR $= -0.04T_a + 1.55$ and *T. nigricauda*: RMR $= -0.04T_a + 1.31$, EHL/MHP $= 0.26(T_a - T_b) + 0.009$, $T_b = 0.13T_a + 32.32$

Core body temperature

Below the inflection point, T_b values did not differ between species ($t = 0.717$, $df = 15$, $P = 0.484$; Fig. 3) and increased linearly and significantly with T_a ($t = 7.902$, $df = 30$, $P < 0.001$; Fig. 3). At $T_a = \sim 25\,°C$ mean normothermic T_b values were $35.5 \pm 0.9\,°C$ ($n = 15$). At T_a values above the inflection point, there was a significant species $\times T_a$ interaction ($t = 3.330$, $df = 75$, $P = 0.001$; Fig. 3). *Thallomys nigricauda* reached thermal endpoints at higher T_a values than *M. namaquensis* (t-test: $t = 4.650$, $df = 11$, $P < 0.001$; Fig. 4). *T. nigricauda* reached thermal end points at $T_a = 44.6 \pm 2.1\,°C$, whereas *M. namaquensis* reached thermal endpoints at $T_a = 40.4 \pm 1.3\,°C$ ($n = 10$).

Intraspecific comparison

Evaporative water loss

Above inflection points, rates of EWL increased linearly and significantly with T_a for both species (*G. paeba*: $t = 5.825$, $df = 17$, $P < 0.001$; *M. namaquensis*: $t = 7.490$, $df = 20$, $P < 0.001$; Figs. 5, 6), and did not vary between
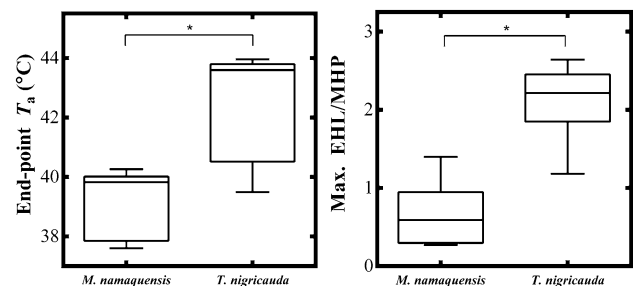

Fig. 4 Maximum air temperature (T_a) at thermal endpoints and corresponding maximum evaporative heat loss/metabolic heat production (EHL/MHP) in sympatric Namaqua rock rats (*Micaelamys namaquensis*) and black-tailed tree rats (*Thallomys nigricauda*). Data were obtained from 10 *M. namaquensis* and eight *T. nigricauda* individuals using open flow-through respirometry. Thermal endpoints are the T_a at which $T_b = 41\,°C$. Asterisks represent significance at $P < 0.05$

sites for either species (*G. paeba*: $t = 1.773$, $df = 17$, $P = 0.094$; *M. namaquensis*: $t = 1.346$, $df = 18$, $P = 0.195$; Figs. 5, 6).

Below the inflection points (Table 1), rates of EWL were lower for individuals at Radnor compared to

Aggeneys ($t = 2.199$, $df = 18$, $P = 0.041$; Fig. 5) for *G. paeba*; 0.08 ± 0.01 g h^{-1} compared to 0.11 ± 0.06 g h^{-1} ($n = 10$) at $T_a = 30$ ℃. Converlsely, rates of EWL below inflection points (Table 1) were minimal, 0.13 ± 0.07 g h^{-1} ($n = 20$) at $T_a = 30$ ℃ for *M. namaquensis* and did not vary between sites ($t = 1.161$, $df = 18$, $P = 0.261$; Fig. 6).

Maximum rates of EWL for *G. paeba* and *M. namaquensis* were 0.41 ± 0.20 g h^{-1} ($n = 16$) at $T_a = \sim 40$ ℃ and 0.93 ± 0.12 g h^{-1} ($n = 4$) at $T_a = \sim 42$ ℃, respectively.

Resting metabolic rate

Below inflection points (Table 1), RMR decreased with increasing T_a (*G. paeba*: $t = 13.691$, $df = 40$, $P < 0.001$; *M. namaquensis*: $t = 8.298$, $df = 67$, $P < 0.001$; Figs. 5, 6) and did not vary between sites for *G. paeba* ($t = 1.499$, $df = 18$, $P = 0.151$; Fig. 5). However, for *M. namaquensis,* RMR was lower for Aggeneys than Radnor individuals ($t = 7.022$, $df = 18$, $P < 0.001$, Fig. 6). At $T_a = \sim 25$ ℃, RMR for *M. namaquensis* from Radnor was 0.65 ± 0.12 W ($n = 10$), whereas at Aggeneys it was approximately 44%, at 0.36 ± 0.12 W ($n = 10$).

At T_a above the inflection point, RMR increased linearly and significantly with T_a for *G. paeba* ($t = 5.098$, $df = 74$, $P < 0.001$; Fig. 5). However, for *M. namaquensis* T_a was not a significant explanatory variable for RMR (Fig. 6 and Electronic supplementary file). No significant difference was observed in RMR above inflection points between the sites for *G. paeba* ($t = 1.934$, $df = 18$, $P = 0.069$, Fig. 5), reaching a maximum of 0.23 ± 0.04 W ($n = 16$) at $T_{a=} \sim 40$ ℃. In contrast, the RMR of *M. namaquensis* was lower for Aggeneys than Radnor individuals ($t = 5.393$, $df = 5$, $P < 0.001$, Fig. 6). At $T_a = \sim 40$ ℃, RMR for *M. namaquensis* from Radnor was 0.56 ± 0.04 W ($n = 6$), and at Aggeneys, 0.45 ± 0.10 W ($n = 9$) approximately 29% lower than for individuals from Radnor. We recorded minimum RMR $= 0.19 \pm 0.04$ W ($n = 20$) at $T_a = \sim 34$ ℃ for *G. paeba* and 0.49 ± 0.09 W ($n = 6$) at $T_a = \sim 34$ ℃ for *M. namaquensis*.

Evaporative heat loss/metabolic heat production

When EHL/MHP was plotted against $T_a - T_b$, inflection points for *G. paeba* were identified at $T_a - T_b = -2.6$ ℃ at Aggeneys and $T_a - T_b = -2.1$ ℃ at Radnor (Fig. 5). For *M. namaquensis*, inflection points were identified at
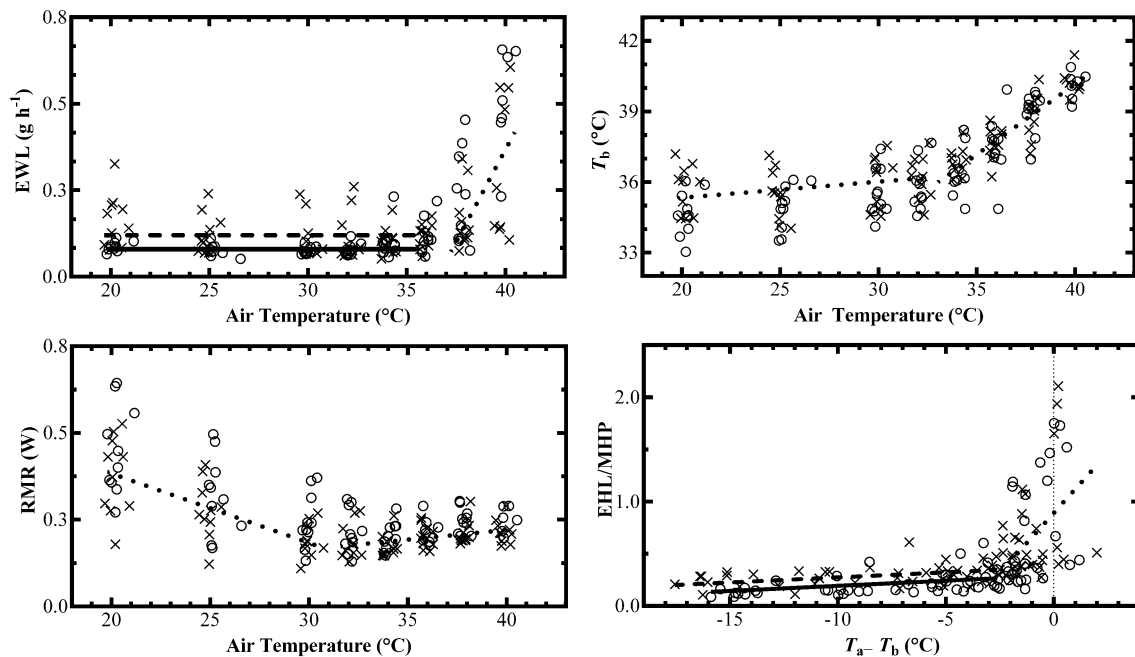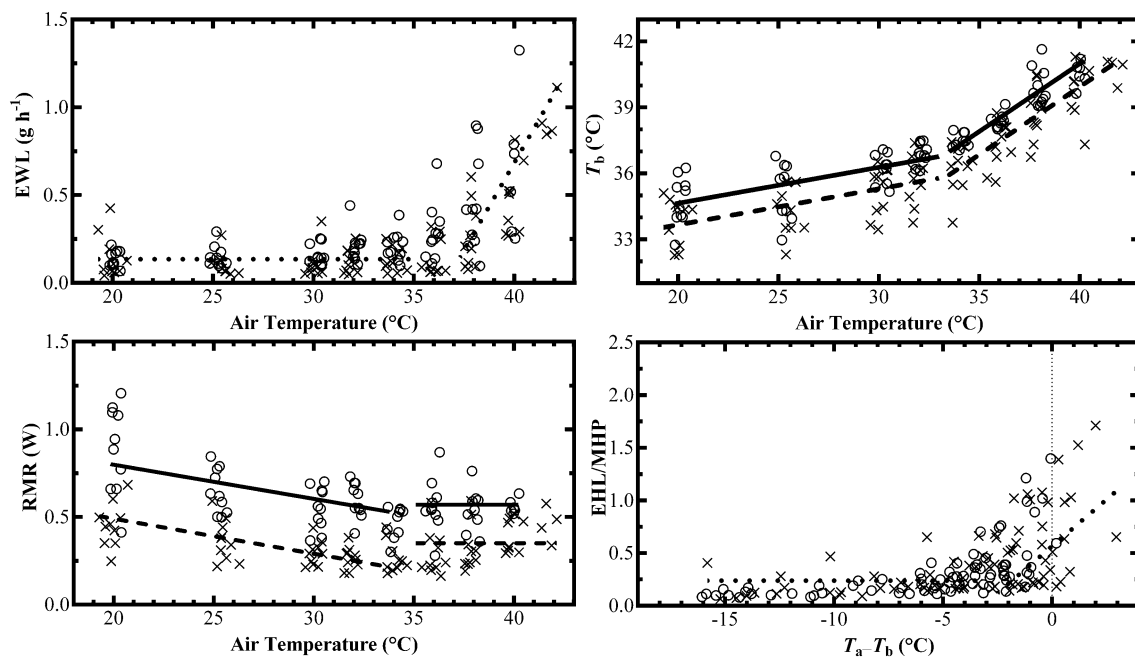

Fig. 5 Relationships between evaporative water loss (EWL), resting metabolic rate (RMR), evaporative heat loss/metabolic heat production (EHL/MHP), core body temperature (T_b) and air temperature (T_a) in two populations of hairy-footed gerbils (*Gerbillurus paeba*). Data were obtained from 20 individuals using open flow-through respirometry. Lines indicate linear mixed-effects regression models fitted to T_a above and below inflection points; regression lines are fitted where there are significant differences between Radnor (solid) and Aggeneys (dashed) individuals At $T_a >$ inflections, EWL $= 0.11 T_a - 3.9$, RMR $= 0.01 T_a - 0.002$, EHL/MHP $= 0.23(T_a - T_b) + 0.89$, $T_b = 0.60 T_a + 16.17$. At $T_a <$ inflections, Radnor: EWL $= 0.08$ and Aggeneys: EWL $= 0.12$, RMR $= -0.02 T_a + 0.78$, Radnor: EHL/MHP $= 0.01(T_a - T_b) + 0.29$ and Aggeneys: EHL/MHP $= 0.01(T_a - T_b) + 0.37$, $T_b = 0.06 T_a + 33.96$. Circles and crosses represent individuals caught at Radnor and Aggeneys, respectively

Fig. 6 Relationships between evaporative water loss (EWL), resting metabolic rate (RMR), evaporative heat loss/metabolic heat production (EHL/MHP), core body temperature (T_b) and air temperature (T_a) in two populations of Namaqua rock rats (*Micaelamys namaquensis*). Data were obtained from 20 individuals using open flow-through respirometry. Lines indicate linear mixed-effects regression models fitted at T_a above and below inflection points; separate regression lines are fitted where there are significant differences between Aggeneys (dashed) and Radnor (solid) populations.

At $T_a >$ inflections, $EWL = 0.20T_a - 7.282$, Radnor: $RMR = 0.57$ and Aggeneys: $RMR = 0.35$, $EHL/MHP = 0.18(T_a - T_b) + 0.65$, Radnor: $T_b = 0.62T_a + 16.34$ and Aggeneys: $T_b = 0.62T_a + 15.29$. Circles and crosses represent individuals caught at Radnor and Aggeneys, respectively. At $T_a <$ inflections, $EWL = 0.135$, Radnor: $RMR = -0.02T_a + 0.89$ and Aggeneys: $RMR = -0.02T_a - 1.19$, $EHL/MHP = 0.24$, Radnor: $T_b = 0.16T_a + 31.37$ and Aggeneys: $T_b = 0.16T_a + 30.39$
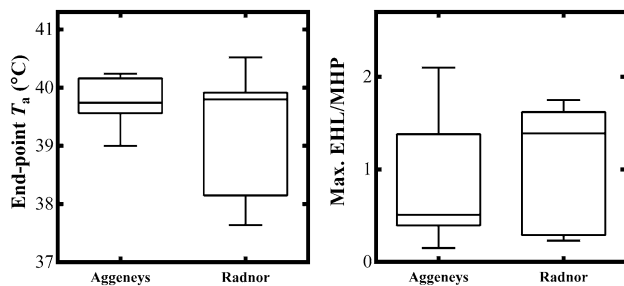

Fig. 7 Maximum air temperature (T_a) at which individuals reached thermal endpoints and maximum evaporative heat loss/metabolic heat production (EHL/MHP) in two populations of hairy-footed gerbils (*Gerbillurus paeba*) at Aggeneys and Radnor, two sites in southern Africa differing in daily maximum T_a. Data were obtained from 20 individuals using open flow-through respirometry. Thermal endpoints are the T_a at which $T_b = 41$ ℃. Asterisks represent significance at $P < 0.05$

$T_a - T_b = -1.5$ ℃ and $T_a - T_b = -3.0$ ℃ at Radnor and Aggeneys, respectively (Fig. 6). Above these inflection points, EHL/MHP increased linearly and significantly with $T_a - T_b$ in both species (*G. paeba*: $t = 3.307$, $df = 49$, $P = 0.002$; *M. namaquensis*: $t = 3.903$, $df = 25$, $P < 0.001$;

Figs. 5, 6); EHL/MHP was similar between sites (*G. paeba*: $t = 0.256$, $df = 15$, $P = 0.801$; *M. namaquensis*: $t = 1.059$, $df = 12$, $P = 0.309$; Figs. 5, 6). The linear regression for $T_a - T_b$ values above the inflection has a y-intercept of 0.9 and 0.7 for *G. paeba* and *M. namaquensis*, respectively, meaning that 90% and 70% of MHP was dissipated evaporatively when $T_b = T_a$.

Below this inflection point, EHL/MHP increased linearly and significantly with $T_a - T_b$ in *G. paeba*: $t = 7.749$, $df = 63$, $P < 0.001$; Fig. 5). However, for *M. namaquensis* $T_a - T_b$ was not a significant explanatory variable for EHL/MHP (Fig. 6 and Electronic supplementary file). There was no site effect for *M. namaquensis* ($t = 0.581$, $df = 17$, $P = 0.569$; Fig. 6), whereas for *G. paeba*, EHL/MHP below the inflection point was greater for Aggeneys individuals compared to those caught at Radnor ($t = 2.357$, $df = 17$, $P = 0.031$; Fig. 5); 0.29 ± 0.07 ($n = 10$) compared to 0.23 ± 0.11 ($n = 10$) at $T_a = 30$ ℃. There was no significant difference in maximum EHL/MHP values between *G. paeba* individuals from Aggeneys and Radnor (t-test: $t = 0.856$, $df = 16$, $P = 0.404$; Fig. 7). Maximum EHL/MHP values were significantly greater (t-test: $t = 17.910$, $df = 18$, $P = 0.012$; Fig. 8) in *M.*

namaquensis from Aggeneys ($\bar{x} = 1.07 \pm 0.11$, $n = 10$) than Radnor ($\bar{x} = 0.60 \pm 0.12$, $n = 10$).

Body temperature

At T_a values above the inflection point, T_b increased linearly and significantly with T_a in both species (*G. paeba*: $t = 20.612$, $df = 54$, $P < 0.001$; *M. namaquensis*: $t = 22.576$, $df = 59$, $P < 0.001$; Figs. 5, 6). For *G. paeba*, individuals at the two sites had indistinguishable T_b values ($t = 0.295$, $df = 18$, $P = 0.771$; Fig. 5). Conversely, *M. namaquensis* individuals caught at Aggeneys exhibited significantly lower T_b values compared to Radnor individuals ($t = 3.124$, $df = 18$, $P = 0.006$; Fig. 6).

At T_a below the inflection point, T_b increased linearly and significantly with T_a in both species (*G. paeba*: $t = 5.842$, $df = 56$, $P < 0.001$; *M. namaquensis*: $t = 12.157$, $df = 58$, $P < 0.027$; Figs. 5, 6). *Gerbillurus paeba* did not display site specific T_b values below the inflection point ($t = 1.563$, $df = 18$, $P = 0.135$; Fig. 5). However, *M. namaquensis* at Aggeneys displayed lower T_b values compared to individuals at Radnor ($t = 2.412$, $df = 18$, $P = 0.027$; Fig. 6). For *G. paeba*, at $T_a = {\sim}25$ °C the mean normothermic T_b value was 35.2 ± 1.0 °C ($n = 20$). For *M. namaquensis*, the mean normothermic T_b value was $T_b = 33.8 \pm 1.1$ °C ($n = 10$ for individuals at Aggeneys; whereas at Radnor, the mean value was 34.8 ± 1.1 °C ($n = 10$) at $T_a = {\sim}20$ °C.

For *G. paeba*, individuals from Aggeneys and Radnor reached thermal endpoints at similar T_a (*t* test: $t = 1.252$, $df = 12$, $P = 0.076$; Fig. 7). At Aggeneys, *G. paeba* individuals reached thermal endpoints at $T_a = 41.5 \pm 1.8$ °C ($n = 10$) and conspecifics at Radnor reached thermal endpoints at $T_a = 40.5 \pm 1.0$ °C ($n = 10$). For *M. namaquensis*, individuals from Aggeneys reached thermal endpoints at higher T_a values compared to Radnor individuals (*t* test:

Fig. 8 Maximum air temperature (T_a) at which individuals reached thermal endpoints and maximum evaporative heat loss/metabolic heat production (EHL/MHP) in two populations of Namaqua rock rats (*Micaelamys namaquensis*) at Aggeneys and Radnor, two sites in southern Africa differing in daily maximum T_a. Data were obtained from 20 individuals using open flow-through respirometry. Thermal endpoints are the T_a at which $T_b = 41$ °C. Asterisks represent significance at $P < 0.05$

$t = 2.566$, $df = 17.19$, $P < 0.001$; Fig. 8). For *M. namaquensis* at Aggeneys, individuals reached thermal endpoints at $T_a = 41.9 \pm 1.5$ °C ($n = 9$); whereas, individuals from Radnor reached thermal endpoints at $T_a = 40.4 \pm 1.3$ °C ($n = 9$).

Discussion

Inter- and intraspecific variation in heat tolerance and evaporative cooling capacity of nocturnal rodents was correlated with modelled thermal conditions expected within diurnal refugia. At the interspecific level, the arboreal *T. nigricauda* exhibited higher heat tolerance than sympatric burrow-dwelling *M. namaquensis*; *T. nigricauda* reached their thermal endpoints ($T_b = 41$ °C) at $T_a {\sim} 3$ °C higher than *M. namaquensis*. At the intraspecific level, population differences in heat tolerance were evident in *M. namaquensis* but not in *G. paeba*. The lack of population differences in *G. paeba* is potentially due to the microclimates within *G. paeba* refugia varying by only ${\sim} 1.5$ °C, a difference that is statistically significant but perhaps not large enough to elicit a response. Moreover, the direction of variation is consistent with the microclimatic conditions and not broad-scale climate (Fig. 2). These findings provide support for the hypothesis that heat tolerance limits and evaporative cooling capacity are related to microclimate within diurnal refugia in nocturnal desert-adapted rodents. Differences in heat tolerance were attributable to maximum efficiency of thermoregulatory heat dissipation by evaporation (i.e., EHL/MHP). At the interspecific level, increased maximum efficiency of thermoregulatory heat dissipation by evaporation appeared to be functionally related to greater ability to increase rates of evaporative water loss in *T. nigricauda* when compared to *M. namaquensis*. Intraspecific variation in *M. namaquensis* heat tolerance, on the other hand, appeared to be attributable to reduced resting metabolic rate, thus reduced metabolic heat production, and consequently lower core body temperatures in the population inhabiting more thermally challenging diurnal refugia.

We assumed RER = 1.0 (i.e., carbohydrate metabolism) when converting $\dot{V}_{CO2}$ to metabolic rate. The EHL/MHP values when $T_a = T_b$ were ${\sim} 1.0$ and ${\sim} 0.9$ in the inter- and intraspecific comparison, respectively; EHL/MHP = 1 is expected on theoretical grounds, and it appears the assumption of carbohydrate metabolism was correct. Uncertainty regarding the metabolic substrate illustrates a limitation inherent in the approach we used here; the high flow rates needed to maintain low chamber humidity so as not to impede evaporative heat dissipation precluded accurate measurements of oxygen consumption and hence empirical determination of RER (McKechnie et al. 2016).

The greater heat tolerance in *T. nigricauda* compared to *M. namaquensis* in the present study confirms earlier
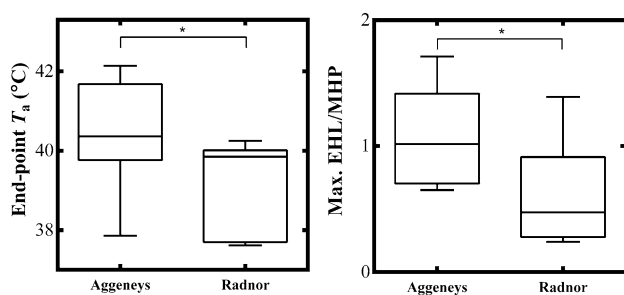
findings by Lovegrove et al. (1991), who quantified RMR and T_b (but not EWL) in these two species at T_a up to 42 ℃. However, maximum T_a tolerated in the latter study were substantially lower: $T_a = 38$ ℃ and 40 ℃ in *M. namaquensis* and *T. nigricauda*, respectively, compared to 42 ℃ and 45 ℃ in this study. Moreover, whereas RMR increased linearly at T_a above the upper critical limit of thermoneutrality (Lovegrove et al. 1991), in this study, RMR was independent of T_a at temperatures above the lower limit of thermoneutrality and no upper critical limit of thermoneutrality was evident. We suspect the differences between our results and those of Lovegrove et al. (1991) are attributable to two factors: acclimation to laboratory conditions and differences in flow rates and, hence, chamber humidities. We used much higher flow rates (up to 14 L min^{-1}) than Lovegrove et al. (1991; maximum 1.2 L min^{-1}). Higher flow rates maintain lower chamber humidity, ensuring negligible impedance of evaporative heat dissipation and that animals remain calm (Gerson et al. 2014; Whitfield et al. 2015; van Dyk et al. 2019). In addition, the individuals used by Lovegrove et al. (1991) were caught in the Kalahari and then transported to Germany where they remained in captivity at $T_a = 30$ ℃ until measurements ~ 6 months later, raising the possibility that the differences in acclimation/acclimatisation status may also be driving some of the differences in heat tolerance between the two studies.

Our data provide more general insights into rodent thermoregulation in the heat, most notably the relationship between metabolic rate and T_b. The notion of active suppression of metabolic rates (i.e., facultative hypometabolism) under conditions of high heat loads has received increased attention in light of recent evidence for 'hyperthermic daily torpor' and an absence of an Arrhenius effect on RMR (i.e., $Q_{10} = 2$–3) in some small tropical mammals (Lovegrove et al. 2014; Reher et al. 2018). Assuming $Q_{10} = 2.5$, we would expect a 51% increase in metabolic rate in *T. nigricauda* and *M. namaquensis* from 0.5 W to ~ 0.8 W associated with the observed ~ 4.5 ℃ increase in T_b between the inflections for T_b at $T_a = 32$ ℃ and maximum values (Figs. 3, 7). However, the average RMR in *M. namaquensis* and *T. nigricauda* increased by only ~ 6% across this range of T_a, an increase that was not statistically significant (Figs. 2, 7). The much smaller increases in RMR than expected on the basis of an Arrhenius effect suggest active down-regulation of metabolic rate in both these species when hyperthermic, supporting recent arguments that metabolic suppression is an important aspect of mammalian thermoregulation in the heat (Welman et al. 2018). Hyperthermic hypometabolism is thought to aid in negating increased energy expenditure associated with the Arrhenius effect (Lovegrove et al. 2014). Previous studies have reported increasing core T_b in the absence of corresponding increases in RMR in both birds and mammals (Weathers 1981; Tieleman and Williams 1999; Lovegrove et al. 2014; O'Connor et al. 2017; Reher et al. 2018).

Previous studies of inter- and intraspecific variation in rodent thermoregulation have revealed differences in RMR and rates of EWL, but have generally not involved T_a values approaching or exceeding normothermic T_b. For instance, Tracy and Walsberg (2000) reported reduced rates of evaporative water loss in xeric, compared to more mesic, populations of Merriam's kangaroo rat (*Dipodomys merriami*) at 30 ℃. Reduced EWL or RMR at thermoneutral T_a likely reflects enhanced water conservation; whereas at $T_a > T_b$, higher EWL and/or reduced RMR may be expected to evolve to increase the efficiency of thermoregulatory heat dissipation by evaporation. That relatively little is known about the upper limits to heat tolerance and evaporative cooling in nocturnal rodents is unsurprising, as they are widely thought to avoid exposure to high T_a by occupying thermally buffered refugia during the day (Chappell and Bartholomew 1981; Lovegrove et al. 1991). Most evidence for inter- or intraspecific variation in heat tolerance has been provided by studies on birds (Smit et al. 2013; Noakes et al. 2016; Czenze et al. 2020b) or bats (Cory Toussaint and McKechnie 2012; Czenze et al. 2020a), taxa that regularly experience high environmental temperatures on account of being diurnally activity or roosting in hot microsites.

Intra- and interspecific variation in thermal physiology such as that we report here may arise from phenotypic flexibility, developmental plasticity (*sensu* Piersma and Drent 2003), epigenetic transmission or genotypic adaptation (Merilä and Hendry 2014). For example, among-population differences in heat tolerance in wild populations of white-browed sparrow-weavers (*Plocepasser mahali*), whereby the heat tolerance limit of a desert population was significantly higher than that of two mesic populations (Noakes et al. 2016), was shown via acclimation experiments to reflect short-term, reversible phenotypic flexibility (Noakes and McKechnie 2019). Conversely, population differences in EWL in Merriam's kangaroo rats (*D. merriami*) persisted after acclimation, raising the possibility of genotypic adaptation (Tracy and Walsberg 2000). Although our limited sample sizes in terms of species and populations preclude rigorous testing (e.g., Garland and Adolph 1994) and broader, multi-species analyses are needed to confirm the generality of our conclusions, our data suggest that fine-scale variation in thermal physiology correlated with microclimate may be more widespread than currently appreciated. Future studies should employ common-garden and thermal acclimation experiments to elucidate whether these differences are attributable to genetic adaptation, irreversible phenotypic variation induced during development (i.e., developmental plasticity) or reversible phenotypic variation expressed within an

individual's lifetime (i.e., phenotypic flexibility) (Forsman 2015).

The correlation between thermoregulatory performance and the modelled microclimates of diurnal refugia of desert rodents supports the view that microclimate effects, in addition to broad-scale climatic conditions, play a major role in shaping endotherm thermal physiology. This is particularly relevant given the recent advances in identifying the importance of high heat loads in determining the persistence of small endotherms under global heating (Mathewson et al. 2017; Conradie et al. 2019; Riddell et al. 2019; Kemp et al. 2020). These findings also have important implications for modelling the effects of climate change on the physiology and ecology of endotherms (Sears et al. 2016) and emphasis the need for detailed habitat and microclimate information. Studies investigating thermal adaptation in relation to past climate and future climate change should incorporate microclimatic effects using the new set of modelling techniques capable of downscaling broad-scale climatic conditions to scales relevant to organisms (Kearney 2020).

In conclusion, fine-scale microclimatic conditions in daily refugia of rodents are important in shaping thermoregulatory patterns under high heat loads. The arboreal *T. nigricauda* exhibits higher heat tolerance compared to the burrow-dwelling *M. namaquensis*, presumably arising from an increased ability to dissipate heat brought about by a greater capacity to lose heat evaporatively. At the intraspecific level, population-level variation in modelled microclimate experienced by *M. namaquensis*, and the lack thereof in *G. paeba*, within diurnal refugia is mirrored by variation in thermoregulatory processes. In *M. namaquensis*, the population using the more thermally challenging diurnal refugia exhibited higher heat tolerance, attributable to reduced metabolic heat production, whereas no significant differences were detected between populations of *G. paeba*. Thus, microclimatic conditions within rodent diurnal refugia are important when considering eco-evolutionary processes and future studies are encouraged to incorporate these.

Compliance with ethical standards

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