

RESEARCH ARTICLE

Bearded dragons (*Pogona vitticeps*) with reduced scalation lose water faster but do not have substantially different thermal preferences

Nicholas B. Sakich* and Glenn J. Tattersall

ABSTRACT

Whether scales reduce cutaneous evaporative water loss in lepidosaur reptiles (Superorder Lepidosauria) such as lizards and snakes has been a contentious issue for nearly half a century. Furthermore, while many studies have looked at whether dehydration affects thermal preference in lepidosaurs, far fewer have examined whether normally hydrated lepidosaurs can assess their instantaneous rate of evaporative water loss and adjust their thermal preference to compensate in an adaptive manner. We tested both of these hypotheses using three captive-bred phenotypes of bearded dragon (*Pogona vitticeps*) sourced from the pet trade: 'wild-types' with normal scalation, 'leatherbacks' exhibiting scales of reduced prominence, and scaleless bearded dragons referred to as 'silkbaks'. Silkbaks on average lost water evaporatively at about twice the rate that wild-types did. Leatherbacks on average were closer in their rates of evaporative water loss to silkbaks than they were to wild-types. Additionally, very small (at most $\sim 1^{\circ}\text{C}$) differences in thermal preference existed between the three phenotypes that were not statistically significant. This suggests a lack of plasticity in thermal preference in response to an increase in the rate of evaporative water loss, and may be reflective of a thermal 'strategy' as employed by thermoregulating bearded dragons that prioritises immediate thermal benefits over the threat of future dehydration. The results of this study bolster an often-discounted hypothesis regarding the present adaptive function of scales and have implications for the applied fields of animal welfare and conservation.

KEY WORDS: Lepidosauria, Squamata, Reptile, Water, Temperature, Scale

INTRODUCTION

Scales are a ubiquitous feature of wild lepidosaurs (Superorder Lepidosauria: the lizards, snakes, amphisbaenians and tuatara), with numerous postulated adaptive, functional roles. These variously include mechanical protection from the environment (Chang et al., 2009), a thermal radiator (Soulé, 1966), a thermal 'shield' (Regal, 1975), a barrier that reduces cutaneous evaporative water loss (Spearman, 1966), and a barrier that reduces the penetration of ultraviolet light into the tissues (Chang et al., 2009). In some lepidosaurs, scales serve a documented physiological function in that they funnel droplets of water from the body to the mouth to enable drinking of rainwater (Sherbrooke, 1990).

Lepidosaur scales can also serve anti-predatory functions; for example, when they are rubbed together by certain lepidosaurs to produce a noise to deter predators (Gans and Maderson, 1973; Gans and Richmond, 1957; Gholamifard et al., 2015).

Of these scale-related functional hypotheses, that the scale forms a barrier that reduces cutaneous evaporative water loss is probably the most heavily investigated. Cutaneous resistance to evaporative water loss in lepidosaurs is generally speaking higher than in other amniote taxa (Lillywhite, 2006). For example, 'stripping' the scales of lepidosaurs with adhesive tape increases the animals' rate of cutaneous evaporative water loss (Maderson et al., 1978). Additionally, some eco-morphological studies have found interspecific or intraspecific correlations between scale size or scale number and environmental humidity in wild lepidosaurs; lepidosaurs inhabiting drier areas have more or larger (longer and/or wider) scales compared with lepidosaurs inhabiting wetter environments (Calsbeek et al., 2006; Hellmich, 1951; Malhotra and Thorpe, 1994, 1997; Sanders et al., 2004; Soulé and Kerfoot, 1972; Wegener et al., 2014). Additionally, corroborating experimental evolutionary work conducted by releasing animals onto islands suggests that hot and dry conditions exert selection pressure on lepidosaur scale number (Calsbeek et al., 2006). These are all reasonable analyses in light of the fact that the rate of evaporative water loss is correlated with environmental moisture level in squamate lepidosaurs: species and populations from drier habitats tend to have lower rates of evaporative water loss than species and populations from moister habitats (Cox and Cox, 2015; Dmi'el et al., 1997; Mautz, 1982; Perry et al., 1999).

However, the pattern of correlation is not entirely straightforward. Some research has found that environmental aridity is correlated with metrics of scalation in some regions of the body but not in others (Malhotra and Thorpe, 1997; Sanders et al., 2004) or the relationship was different in direction for different areas of the body (Sanders et al., 2004). Certain scalation metrics and habitat aridity are significantly correlated across some habitats but not across others (Calsbeek et al., 2006) or in one sex but not the other (Malhotra and Thorpe, 1997; Sanders et al., 2004). Calsbeek et al. (2006) also found the relationship to be different in direction between intraspecific comparisons and interspecific comparisons. Oufiero et al. (2011) found the opposite trend entirely: lizards in the genus *Sceloporus* had larger and fewer scales in moister areas than in drier areas. Similarly, Malhotra and Thorpe (1994) found lizards in the genus *Anolis* had more scales in drier environments than in moister environments, although it is unclear what connection this had to scale size.

There has been some speculation as to the source of this variation in correlation. Malhotra and Thorpe (1997) contend that in some cases larger scales may increase body surface area from the surface area of the scale itself and thus increase water loss, whereas in other

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cases, larger scales would decrease the surface area of the inter-scale area as a result of scaling effects and thus decrease water loss. However, Horton (1972) rebuts this line of thinking by pointing out there is no reason to think the relationship between scales and water loss should suddenly switch when examining different lizard taxa. There also appear to be sources of variation in scalation that are unrelated to environmental aridity (e.g. Penner et al., 2013; Rand, 1954; Scherz et al., 2017) and there are clearly other physiological mechanisms at play that help lepidosaurs resist cutaneous evaporative water loss (e.g. Mautz, 1982; Roberts and Lillywhite, 1980). Together, these make resolving any relationship between scales and evaporative water loss difficult. The best possible way to ascertain whether or not lepidosaur scales are barriers to evaporative water loss would be using a loss-of-function model.

This very approach was tried by Licht and Bennett (1972) and Bennett and Licht (1975): the evaporative water loss rates of partially scaleless snakes was comparable to or even lower than that of the wild-type snakes (Bennett and Licht, 1975; Licht and Bennett, 1972). However, these two studies are constrained by their sample sizes: one partially scaleless and one wild-type snake (Licht and Bennett, 1972) and two partially scaleless snakes and six wild-type snakes (Bennett and Licht, 1975), respectively. Roberts and Lillywhite (1980) compared evaporative water loss across *in vitro* patches of naturally shed skin between wild-type and scaleless snakes from the same litter, and found that the rates were comparable and actually slightly lower for the scaleless samples. However, it is unclear whether using the portion of the integument that is naturally sloughed off represents the actual whole-animal response.

Many ectothermic terrestrial tetrapods respond to dehydration by lowering their thermal preference (Anderson and Andrade, 2017; Bradshaw et al., 2007; Crowley, 1987; Ladyman and Bradshaw, 2003; Sannolo and Carretero, 2019; Shoemaker et al., 1989; Tracy et al., 1993), including lepidosaurs (Bradshaw et al., 2007; Crowley, 1987; Ladyman and Bradshaw, 2003; Sannolo and Carretero, 2019). Lower body temperatures reduce the rate of evaporative water loss by affecting the water vapour deficit across the external surfaces of the body. Evidence suggests that thermal preference in hydrated lepidosaurs is also connected to their rate of evaporative water loss, but in a different way: in two closely related European viper species (genus *Vipera*) in an area where their ranges overlap, the species with the higher rate of evaporative water loss prefers cooler microhabitats than the species with the lower rate of evaporative water loss (Guillon et al., 2014). A similar study with wall lizards (genus *Podarcis*) had opposite findings (Sannolo et al., 2018), but this may have to do with differing windiness of the habitats in question (Sannolo et al., 2018), as higher winds would also result in the microhabitats being cooler as a consequence of convective cooling (Kreith and Bohn, 1997).

The proximate mechanism linking evaporative water loss and thermal preference is not precisely known; in other words, it is unclear whether lepidosaurs can detect their instantaneous rate of evaporative water loss and adjust their thermal preference accordingly to compensate, thus preventing eventual dehydration. Plausible sensory mechanisms that could drive such behaviour exist. For instance, the TRPV4 receptor has been demonstrated to respond to fluid flow rate (Wu et al., 2007) and it is present in the skin (Suzuki et al., 2003). Alternatively, preferred temperature and evaporative water loss might be traits that have co-evolved. Is the thermal preference of species with higher rates of evaporative water loss a result of phenotypic plasticity in the face of a higher rate of evaporative water loss, or have species with a certain thermal preference evolved a certain rate of evaporative water loss?

A uniquely suitable model organism for addressing both questions regarding evaporative water loss and questions regarding thermal preference is the bearded dragon (*Pogona vitticeps*). The bearded dragon is a lizard in the family Agamidae native to the eastern interior of Australia (Wilson and Swan, 2017). Bearded dragons have been bred in captivity since at least the 1980s (Sherriff, 1989; Stahl, 1999) and today are one of the most popular species of pet lizard (Howell and Bennett, 2017; Prestridge et al., 2011; Wakao et al., 2018). Additionally, bearded dragons have been used for years as model organisms for studies of how terrestrial ectotherms can cope with a complex physical environment and changing physiological states. These studies include those on physiological gaping behaviour (da Silveira Scarpellini et al., 2015; Tattersall and Gerlach, 2005), behavioural thermoregulation (Black and Tattersall, 2017; Cadena and Tattersall, 2009a,b; da Silveira Scarpellini et al., 2015; Khan et al., 2010) and colour change (Cadena et al., 2017, 2018; de Velasco and Tattersall, 2008; Fan et al., 2014; Smith et al., 2016a,b). Furthermore, a mutation has arisen in the bearded dragon captive breeding population that affects the animal's scalation (de Vosjoli et al., 2017; Di-Poi and Milinkovitch, 2016). The mutant allele exhibits incomplete dominance over the wild-type allele. One copy of the mutant allele (genotype *Sca/sca*) results in an animal termed a 'leatherback', exhibiting scales of reduced prominence compared with the wild-type phenotype (de Vosjoli et al., 2017). Two copies of the mutant allele (genotype *Sca/Sca*) results in a completely scaleless animal (de Vosjoli et al., 2017; Di-Poi and Milinkovitch, 2016), termed a 'silkbac' (de Vosjoli et al., 2017).

In light of the existence of the silkbac and leatherback phenotypes in the bearded dragon, the following hypotheses were tested. (1) The lepidosaur scale is a barrier that reduces cutaneous evaporative water loss. (2) Lepidosaurs experiencing higher rates of evaporative water loss as the result of a single gene mutation will choose cooler temperatures, which will lower their rate of evaporative water loss and thus compensate for this effect. This hypothesis presumes that lepidosaurs have some physiological mechanism that allows them to detect their rate of evaporative water loss.

MATERIALS AND METHODS

Animals

Thirty-four bearded dragons (*Pogona vitticeps* Ahl 1926) were used in these experiments: 13 wild-types, 12 leatherbacks and 9 silkbacs (see Fig. 1 for representative individuals). An *a priori* power analysis that assumed a large effect size and used variances from Khan et al. (2010) was performed to inform sample size. Animals were acquired from private pet industry breeders and sellers. All animals were acquired at a small (juvenile) size with the exception of three silkbacs, which were acquired at a larger (sub-adult) size. The animals came from nine different clutches. All animals within a clutch were full siblings to all the other animals from that same clutch. There was phenotypic crossover within two clutches, such that there were leatherbacks and wild-types that were full siblings to one another in this study.

The animals were housed individually in black PVC cages with dimensions of 61×61×40.6 cm l×w×h. At the front of each cage there was a clear acrylic door measuring 50.8 cm long by 25.4 cm high. A 30×30 cm tile was securely wedged in one of the rear corners of each cage such that there was a gap under each tile that the animal could use as a retreat. Above each tile was suspended a light fixture with an incandescent bulb which created a basking spot on the upper surface of the tile. Each cage also contained a piece of disposable paper pulp

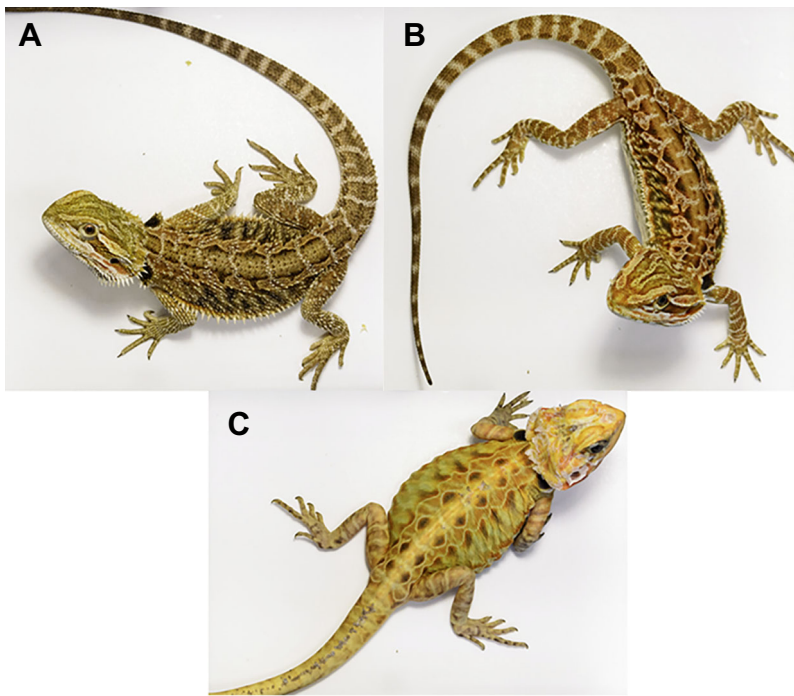


Fig. 1. The three phenotypes of bearded dragon (*Pogona vitticeps*). (A) Wild-type animals, (B) animals exhibiting scales of reduced prominence ('leatherbacks') and (C) scaleless animals ('silkbacs'). Note the flakes of shedding skin on the head of the silkback (C), which are characteristic of this phenotype. On the tail of the silkback there are the irregular epidermal structures that are sometimes present in this phenotype; when present, they cover approximately 5–10% of the external surface of the body.

packaging for the animals to hide under or perch on. Basking tile upper surface temperatures during the light photoperiod reached 35–45°C, as measured periodically using a FLIR TG165 Spot Thermal Camera (FLIR Systems, Inc., Wilsonville, OR, USA). Also suspended in the cage was another light fixture containing an Exo Terra Reptile UVB200 13 W bulb (Rolf C. Hagen Inc., Baie d'Urfé, QC, Canada) to provide continuous access to UVB radiation. Animals were kept on paper towel substrate, except for the three large silkbacs, which were kept on ground coconut husk substrate. Animals were randomly allotted to cages in the housing room. Photoperiod was set to 12 h light:12 h dark.

The animals were fed daily on chopped fruits and vegetables, a 1:1 mixture of Mazuri[®] Insectivore Diet and Mazuri[®] Herbivorous Reptile LS Diet-Small (Mazuri Exotic Animal Nutrition, St Louis, MO, USA), and live insects. The insects were dusted 5 days a week with Rep-Cal Calcium with Vitamin D₃ and twice a week with Rep-Cal Herptivite Multivitamin (Rep-Cal Research Labs, Los Gatos, CA, USA). The animals were misted with water daily.

After the first three silkbacs of this study were maintained in the lab it became evident that silkbacs suffered from shedding issues. From then on, silkbacs were provided with constant access to a large, shallow dish of water that was cleaned and refilled daily. Because of this *ad libitum* silkback water access and the fact that none of the phenotypes were observed to drink at a particularly high frequency (data not shown), we are confident that hydration state did not differ between the three phenotypes.

All procedures were approved by the Animal Care Committee of Brock University (AUP 17-12-02). Data from the following experiments are available in Table S1.

Evaporative water loss

The evaporative water loss rates of these animals were determined using flow-through open respirometry. To achieve this, outside air was drawn through tubing into the lab by an electronic air pump. The air was then passed through an analog flow meter, humidified, and then passed through a dewpoint generator (Model DG-4, Sable Systems International, North Las Vegas, NV, USA) set to 1.5°C.

This meant that the air excurrent from the dewpoint generator had a water vapour pressure of 680.9 Pa and a water vapour density of 5.4 $\mu\text{g ml}^{-1}$. From there, the air was split into two channels, each running through one of the twin controllers of a Sable Systems MFC-2 Mass Flow Controller, typically set to a flow rate of 150 ml min^{-1} , except for during the trials of the three large silkbacs, when the flow rates were set to 400 ml min^{-1} . Channel 1 went through another analog flow meter and then into the glass jar (of appropriate size for the individual animal in question) that contained the animal. The jar was sealed with a rubber stopper, with two syringe barrels used as air-ports for incurrent and excurrent gas, respectively. A long piece of tubing was wedged into the intake port. The excurrent air vented to an open manifold chamber connected to a sub-sample gas line, controlled by a solenoid switch. The subsampler drew air (100 ml min^{-1}) through a Sable Systems RH-300 Water Vapor Analyzer and then a Sable Systems FoxBox Respirometry System, which measured the O₂ and CO₂ content of the air. Gas values were recorded at a rate of 1 Hz using BIOPAC AcqKnowledge[®] Data Acquisition and Analysis Software (BIOPAC Systems Inc., Goleta, CA, USA). When the solenoid switch was triggered, the gas being passed through the RH-300 Water Vapor Analyzer and the FoxBox Respirometry System would switch from Channel 1 (gas from the animal chamber) to Channel 2 ('baseline' gas). This provided mostly continuous, precise measurements of incurrent and excurrent gases. Except for saturation/pre-conditioning of the air, all tubing used in the system was Bev-A-Line[®] IV Tubing (Thermoplastic Processes, Georgetown, DE, USA). The RH-300 Water Vapor Analyzer was periodically calibrated, with nitrogen gas as a 'zero' value, and air bubbled through an aquarium air stone inside a sealed Erlenmeyer flask partially filled with water of known temperature used as the saturated 'span' value. The O₂ value on the FoxBox Respirometry System was periodically 'spanned' to a value of 20.95% O₂ concentration prior to a trial to adjust for potential sensor drift.

During the trial, the chamber containing the animal was held inside an incubator with a pulse-proportional thermostat set to 35°C. This has previously been determined to be approximately the

preferred temperature of wild-type bearded dragons (Cadena and Tattersall, 2009b). The incubator had its own internal fan to circulate air to help maintain equal temperature throughout the incubator and independent light source so that the animal was not in the dark. Trials were run for approximately 3 h. Animals were monitored using a webcam inside the incubator and actual disturbance of the incubator itself was kept to a bare minimum.

Every 20–30 min during a respirometry trial, the solenoid switch was triggered so that the air being delivered to the RH-300 Water Vapor Analyzer and the FoxBox Respirometry System was air from Channel 2 ('baseline' air) instead of air from the animal chamber. These 'baseline' measurements were run for 3–5 min. The first 90 s after the switch from animal chamber air to baseline air or vice versa was discarded because of dead-space gas transit time. A time-lag correlation function was used to determine the appropriate time lag (8–20 s) between the water vapour density, O₂ concentration and CO₂ concentration readings. The mean of the time-aligned water vapour density, O₂ concentration or CO₂ concentration was taken for each baseline. A linear regression was then fitted between each baseline for each of the three measured parameters to interpolate baseline values throughout each respirometry trial.

The following equations were used to calculate the rate of O₂ consumption (\dot{V}_{O_2}), rate of CO₂ production (\dot{V}_{CO_2}) and rate of water vapour production (evaporative water loss, EWL), respectively; these equations were sourced from Lighton (2008):

$$\dot{V}_{O_2} = FR_i \left\{ F_{I_{O_2}} - \left[\frac{F_{E_{O_2}}(1 - F_{I_{O_2}} - F_{I_{CO_2}} - F_{I_{H_2O}})}{(1 - F_{E_{O_2}} - F_{E_{CO_2}} - F_{E_{H_2O}})} \right] \right\}, \quad (1)$$

$$\dot{V}_{CO_2} = FR_i \left\{ \left[\frac{F_{E_{CO_2}}(1 - F_{I_{O_2}} - F_{I_{CO_2}} - F_{I_{H_2O}})}{(1 - F_{E_{O_2}} - F_{E_{CO_2}} - F_{E_{H_2O}})} \right] - F_{I_{CO_2}} \right\}, \quad (2)$$

$$EWL = FR_i \{ WVD_e - WVD_i \}, \quad (3)$$

where FR_i is the flow rate of the incurrent air, $F_{I_{O_2}}$ is the fractional O₂ concentration of the incurrent air, $F_{I_{CO_2}}$ is the fractional CO₂ concentration of the incurrent air, $F_{I_{H_2O}}$ is the fractional water vapour concentration of the incurrent air, $F_{E_{O_2}}$ is the fractional O₂ concentration of the excurrent air, $F_{E_{CO_2}}$ is the fractional CO₂ concentration of the excurrent air, $F_{E_{H_2O}}$ is the fractional water vapour concentration of the excurrent air, WVD_i is the water vapour density of the incurrent air and WVD_e is the water vapour density of the excurrent air.

The data traces of \dot{V}_{O_2} , \dot{V}_{CO_2} and EWL produced by each animal were examined visually. A period of time from each animal's trial from which to use data for the purposes of statistical analysis was then selected based on examining all three data traces and choosing a period of time with readings as flat and stable as possible, as close to the end of the trial as possible. The chosen time periods were 807±192 s long (mean±s.d., range 401–1191 s). The median value for \dot{V}_{O_2} , \dot{V}_{CO_2} and EWL was taken for each animal over this period to minimise the influence of extreme data points. These median values were used as each animal's \dot{V}_{O_2} , \dot{V}_{CO_2} or EWL value, respectively, for subsequent statistical analysis.

The external body surface area of animals was estimated following the method of Belasen et al. (2017); snout–vent length and tail length were measured to the nearest millimetre using a ruler, and mid-body girth and pelvic girth were measured using a length of string that was then measured to the nearest millimetre using a ruler. These surface area estimates (in mm²) correlated very highly with animal mass (in g) ($r^2=0.96$).

For statistical analysis, EWL and metabolic parameters were log-transformed because doing so was found to increase the normality of the distribution of the residuals of the model. Six linear models were created with phenotype as the predictor variable and log of rate of evaporative water loss (mg h⁻¹) as the response variable, each with one of six possible covariates: body surface area (mm²), \dot{V}_{O_2} (ml h⁻¹) or \dot{V}_{CO_2} (ml h⁻¹), each either log-transformed or not log-transformed, respectively. The model including log-transformed \dot{V}_{CO_2} as a covariate was chosen as the final model over the other models based on comparison of ΔAIC_c values using the AICcmodavg package in R (<https://CRAN.R-project.org/package=AICcmodavg>). Furthermore, use of \dot{V}_{CO_2} as a proxy for metabolic rate in subsequent statistical analysis instead of \dot{V}_{O_2} avoided any concerns with the lower detection limit of the O₂ analyser. \dot{V}_{O_2} and \dot{V}_{CO_2} data were highly correlated (raw data $r^2=0.86$, r^2 when both are log-transformed=0.96), so analysing only one metabolic metric was justified.

Examination of the residuals of the final model using a $Q-Q$ plot revealed no excessive deviation from the assumption of normality. Comparison of the final linear model with a linear mixed effects model made using the lme4 package in R (Bates et al., 2015) including clutch as a random effect revealed no evidence of a clutch effect ($\chi^2=0$, d.f.=1, $P=1$). *Post hoc* tests (Tukey's HSD) using the multcomp package in R (Hothorn et al., 2008) were employed to isolate specific phenotype differences. Data handling and statistical analysis were performed in either Excel 2011 for Mac version 14.7.7 (Microsoft Corporation, Redmond, WA, USA) or R version 3.5.1 (<http://www.R-project.org/>). The effects package in R (Fox and Weisberg, 2019) was used to extract model fits from the linear model. Plots were made with the ggplot2 package in R (Wickham, 2016).

Thermal preference

To determine their thermal preference, the animals were tested in thermal gradients. Most animals were tested in a small thermal gradient, except for the three large silkbacks, which were tested in a large thermal gradient. The small thermal gradient consisted of two parallel apparatuses. The apparatuses had internal dimensions of 53×24.3×15 cm l×w×h and were constructed of white plastic sides with a copper sheet for the bottom. The upper surface of the copper sheet was painted white. Under the copper sheet, copper pipes were connected to either a hot water bath or a cold bath containing an antifreeze solution, which generated a temperature gradient along the apparatus floor. Each apparatus was divided down the centre with a white plastic divider, creating four long sections (i.e. lanes), two in each apparatus. On top of each apparatus was a mesh lid.

The apparatus for the large thermal gradient consisted of one long lane with internal dimensions of 158×18×9.7 cm l×w×h. The sides were wooden and the ends were metal grilles and, as for the small thermal gradient, the bottom was a copper sheet with its upper surface painted white. Copper pipes were connected to the same baths and arranged in the same way as for the small thermal gradient. Pieces of clear acrylic plastic were used as a lid to keep the animals in. The hot and cold baths were adjusted such that the temperature at the warm end of each of the gradients was approximately 45°C and the temperature at the cold end of each of the gradients was approximately 20°C.

Animals were randomly allotted to lanes in the small thermal gradient. In the large thermal gradient, only one animal could be run at a time. Animals were started approximately at the centre of their lane. In the small thermal gradient, animals in lanes one and two were started facing the warm end of the gradient, whereas animals in

lanes three and four were started facing the cold end of the gradient. In the large thermal gradient, whether the animal started facing the warm or the cold end of the gradient was randomised. The purpose of some animals starting facing a different end of the gradient from other animals was to account for thigmotaxis towards the end of the gradient that an animal faces at the start. Trials were run for approximately 6 h. During the trials, the animals' positions in their lanes were recorded by a Raspberry Pi 3 camera (Raspberry Pi Foundation, Cambridge, UK) mounted directly above the gradient. The Raspberry Pi 3 was set to take a photo of the gradient every 30 s. Air temperature was measured to the nearest 0.01°C at each trial. Animals were weighed to the nearest 0.01 g and measured for total length to the nearest millimetre by placing them on a ruler or tape measure and measuring from above.

Each sequence of images was imported into the computer program FIJI (National Institutes of Health, Bethesda, MD, USA) and images were cropped to show just up to the warm and cold ends of the floor of each apparatus. Each animal's position in the gradient was tracked over time using the manual tracking function. The approximate centre of the animal's head was selected in each photo or, if the centre of the head was obscured, the visible portion of the body nearest to the centre of the head was selected. Photos where the animal was climbing on the mesh lid were excluded from the analysis.

Using a thermocouple thermometer, the surface temperature in each apparatus was systematically measured to the nearest 0.01°C at intervals of 10% of the floor length of the given apparatus from one end to the other during a test run. A quadratic function was then generated which modelled how temperature changed as a function of position in each of the apparatuses. The relevant function was then applied to each animal's track to infer the animal's chosen temperature at each time point over the course of its entire thermal gradient trial. The length of the gradient, being of known distance, was used to calibrate the pixel-to-cm relationship during each manual tracking session.

The first 15 min of each trial was discarded to allow for exploration of the gradient. Over the rest of each trial, each animal's median chosen temperature was inferred as its chosen temperature for subsequent statistical analysis. Median values were used instead of mean values as median values are less influenced by the skew typically present in individual thermal preference data (DeWitt, 1967). Multiple linear models were constructed to compare chosen temperature among the three phenotypes. These models all included phenotype as a predictor variable and chosen temperature (°C) as a response variable, but variously contained all possible combinations of mass (g), total length (cm), air temperature (°C), start time of the trial (in seconds elapsed since midnight on the day of the trial), and cumulative distance moved in the apparatus by an animal during its trial (cm, determined from the animal's tracked

Table 1. Results of a linear model comparing rate of evaporative water loss between three different phenotypes of bearded dragon (*Pogona vitticeps*)

Coefficient	Estimate	s.e.	t-value	P-value
Intercept (wild-type)	0.18827	0.08719	2.159	–
Leatherback	0.21852	0.06802	3.213	0.003136
Silkback	0.29727	0.07376	4.030	0.000352
log(\dot{V}_{CO_2})	1.04004	0.07320	14.208	<0.0001

The three phenotypes were: wild-type; leatherback, animals exhibiting scales of reduced prominence; and silkback, scaleless animals. \dot{V}_{CO_2} , rate of CO₂ production.

coordinates) as covariates. The complete linear model was compared with a linear mixed effects model made using the lme4 package in R (Bates et al., 2015) including clutch as a random effect. There was no evidence of a clutch effect ($\chi^2=0$, d.f.=1, $P=1$). ΔAIC_c values, as compared using the MuMIn package in R (<https://CRAN.R-project.org/package=MuMIn>), indicated that the best-supported model was the model with phenotype alone, without any covariates. This model was used for all subsequent statistical analysis.

If rate of evaporative water loss affected a bearded dragon's thermoregulatory decisions, then not only should there be a phenotype effect but also there should be an inter-individual variation effect. In other words, individual bearded dragons that have higher rates of evaporative water loss should choose cooler temperatures than individual bearded dragons that have lower rates of evaporative water loss. To test this, the residuals of the linear model from the evaporative water loss study were extracted from that model. These were then compared with the residuals of the thermal preference linear model described here, with residuals paired by individual animal ID. Using residuals in this manner instead of using raw evaporative water loss or raw thermal preference data removes any \dot{V}_{CO_2} effect from the evaporative water loss data and removes any phenotype effect from both the evaporative water loss and thermal preference data. A linear model was created with evaporative water loss [$\log(\text{mg h}^{-1})$] residual as the predictor variable and chosen temperature (°C) residual as the response variable.

Examination of the residuals of the two models using $Q-Q$ plots revealed no evidence of excessive deviation from normality. Data handling and statistical analysis were performed as for evaporative water loss.

RESULTS

Water loss

Evaporative water loss differed among the phenotypes (Fig. 2, Table 1; $F_{3,30}=75.55$, $P<0.0001$). Silkbacks on average lost water at a slightly higher rate than leatherbacks and at a rate nearly double that of wild-types (Fig. 2). *Post hoc* tests found a statistically significant difference between wild-types and silkbacks ($t=4.03$, $P<0.001$) and between wild-types and leatherbacks ($t=3.21$, $P=0.008$), but the difference between silkbacks and leatherbacks was not statistically significant ($t=1.05$, $P=0.55$).

Thermal preference

There were small ($\leq 1^\circ\text{C}$) differences between the medians of temperatures chosen by the three phenotypes, with highest temperatures for wild-types being, intermediate temperatures for leatherbacks, and lowest temperatures for silkbacks (Fig. 3). However, these differences were not statistically significant (Fig. 3, Table 2; $F_{2,31}=1.02$, $P=0.37$).

The relationship between the residuals of the evaporative water loss model and the residuals of the thermal preference model was not statistically significant (Fig. 4, Table 3; $r^2=0.009$, d.f.=32, $P=0.60$).

Table 2. Results of a linear model comparing temperatures chosen in a thermal gradient between three different phenotypes of bearded dragon

Coefficient	Estimate	s.e.	t-value	P-value
Intercept (wild-type)	37.7071	0.5306	71.064	–
Leatherback	–0.5331	0.7659	–0.696	0.492
Silkback	–1.1804	0.8296	–1.423	0.165

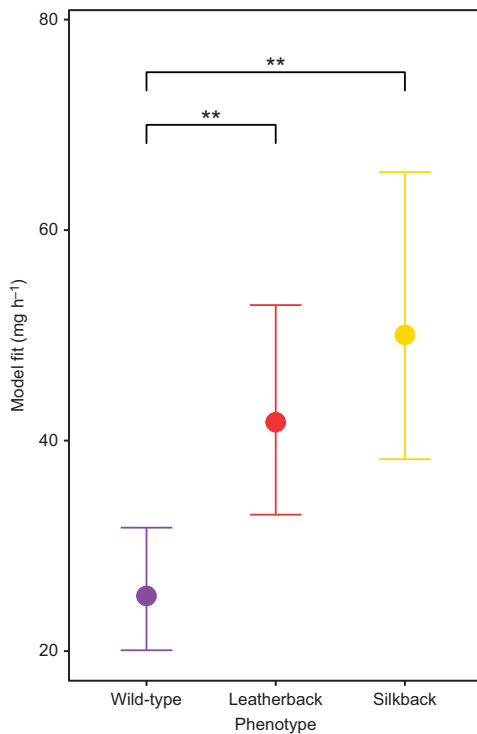


Fig. 2. Evaporative water loss across the three different phenotypes of bearded dragon. Model fits ($\pm 95\%$ confidence intervals) were taken from a linear model with phenotype as a predictor variable, rate of evaporative water loss [$\log(\text{mg h}^{-1})$] as a response variable, and \dot{V}_{CO_2} [$\log(\text{ml h}^{-1})$] as a covariate. There was a statistically significant difference between phenotypes ($F_{3,30}=75.55$, $P<0.0001$). *Post hoc* tests indicated statistically significant differences (as indicated by the asterisks) between wild-types and silkbacks ($t=4.03$, $**P<0.001$) and between wild-types and leatherbacks ($t=3.21$, $**P=0.008$), but not between silkbacks and leatherbacks ($t=1.05$, $P=0.55$). Sample size was 13 wild-types, 12 leatherbacks and 9 silkbacks.

DISCUSSION

Our study demonstrates that the scales of lepidosaurs do indeed function as a barrier to cutaneous evaporative water loss. This runs counter to what has been the predominant dogma for nearly half a century of comparative physiology. However, we found no support for the hypothesis that lepidosaurs can detect their instantaneous rate of evaporative water loss to effect homeostatic adjustments in thermal preference. In contrast to our evaporative water loss findings, this lends further support to the traditional view of the relationship between evaporative water loss and thermal preference in ectothermic tetrapods. In other words, previously observed decreases in thermal preference associated with increased water loss are due to the physiological effects of dehydration and not a compensatory response to detection of the rate of water loss itself.

Water loss

The fact that silkbacks lose water evaporatively at a much higher rate than wild-types is unremarkable in light of anecdotal reports from the reptile-keeping hobby that silkbacks dehydrate if not kept moist (A. Liendo, personal communication) and the fact that silkbacks were observed drinking more often than the other two phenotypes (data not shown). What is remarkable, however, is that these results differ so drastically from the results of the studies of Licht and Bennett (1972) and Bennett and Licht (1975). However, these studies had very low sample sizes of one (Licht and Bennett, 1972) and two (Bennett and Licht, 1975) partially scaleless snakes,

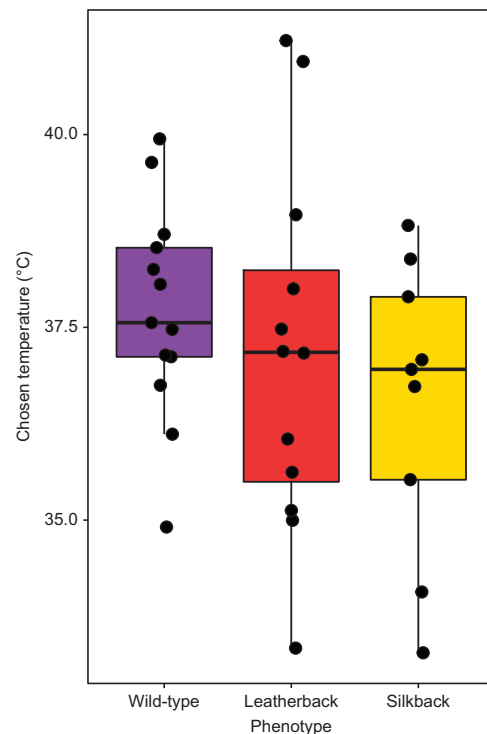


Fig. 3. Temperature chosen in a thermal gradient by the three different phenotypes of bearded dragon. Boxes display the median and the first and third quartiles, and whiskers display at maximum 1.5 times the inter-quartile range. There was no statistically significant difference in chosen temperature between the three phenotypes ($F_{2,31}=1.02$, $P=0.37$). Sample size was 13 wild-types, 12 leatherbacks and 9 silkbacks.

respectively. This is probably the main reason for the difference between our respective results. We cannot rule out the possibility that silkbacks and leatherbacks differ from wild-types in their degree of respiratory water loss. However, as we included a proxy for respiration (\dot{V}_{CO_2}) as a covariate in our statistical model, we feel confident that the remaining phenotype effect on the rate of evaporative water loss can mostly be attributed to differences in cutaneous evaporative water loss.

After accounting for metabolic rate, silkbacks lost on average about 25 mg more water per hour than wild-types did (Fig. 2). For small lizards weighing 10 g, assuming this trend continued unabated and was not halted by the animal consuming water, it would take only 3 days for the difference in water lost to be equivalent to about 18% of animal body mass. Lepidosaur dehydration experiments have shown that an 18% change in mass due to lost water is enough to cause physiological effects. These include changes in thermal preference (Ladyman and Bradshaw, 2003), elevation of physiological response to stressors (Dupoué et al., 2014) and elevation of thermal panting threshold (Parmenter and Heatwole, 1975). These sorts of comparison are crude because of the possibility of allometric scaling of the biological significance of percentage water lost. However, in general terms this illustrates that the amount of extra water lost as a result of the lack of scales is enough to have maladaptive effects on a wild lepidosaur. This supports our central thesis of the water-conserving adaptive function of scales and explains the rarity of scaleless lepidosaurs in the wild.

The view that scales have no functional role in reduction of evaporative water loss typically ascribes the role of a cutaneous water loss barrier primarily to lipids (Lillywhite, 2006; Roberts and

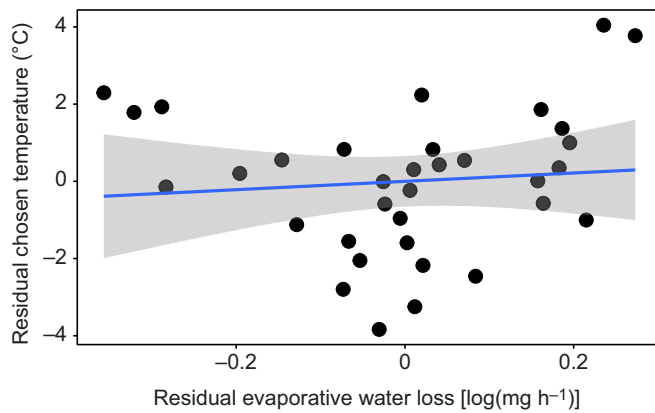


Fig. 4. Residual preferred temperature as a function of residual evaporative water loss in bearded dragons. The shaded area denotes the 95% confidence interval. The relationship between the evaporative water loss residuals and the chosen temperature residuals was not statistically significant ($r^2=0.009$, d.f.=32, $P=0.60$). Sample size was 13 wild-types, 12 leatherbacks and 9 silkbacks.

Lillywhite, 1980). However, silkback skin is reduced in comparison to wild-type skin in two regions: the β -layer and the superficial loose dermis (Di-Poi and Milinkovitch, 2016). The β -layer is not known to contain lipids (Landmann, 1986). Research on human skin indicates that the superficial loose dermis does contain lipids, but primarily those associated with cellular membranes (e.g. phospholipids) (Sjövall et al., 2018). Therefore, a reduction in the loose superficial dermis only results in a decrease in lipids in the same sense that a reduction in any living tissue results in a decrease in lipids. The superficial loose dermis of reptiles in general is made up largely of an extracellular matrix (Alibardi, 2011; Alibardi and DeNardo, 2013) made of proteoglycans (Alibardi, 2011) interspersed with loose bundles of collagen (Alibardi, 2011; Alibardi and DeNardo, 2013). We have no data for the histology of leatherback skin, but it is presumably intermediate between the histology of silkback skin and the histology of wild-type skin. In light of all of this, the increase in the rate of water loss associated with the leatherback and silkback phenotype is probably not attributable to differences in skin lipid content.

Silkbacks can be considered a complete loss-of-function model for scales in that they entirely lack them. In other words, silkbacks are a model for what the null expectation for a lepidosaur reptile without scales should be. Leatherbacks, in contrast, are only a partial loss-of-function model for scales, in that their scales are merely of reduced prominence compared with the wild-type. The difference in thickness between leatherback scales and wild-type scales has not been quantified and has only been qualified. Interspecific studies relating lepidosaur scale thickness to empirically measured rate of evaporative water loss have not been done. Quantification of the relative thickness of leatherback and wild-type scales will allow extrapolation of the relationship between scale thickness and rate of evaporative water loss in wild lepidosaurs.

Thermal preference

In absolute terms, the bearded dragons typically chose temperatures of about 37°C, albeit with some individuals deviating from this by up to ~4°C. This value compares favourably with the bearded dragon's thermal preference of 35.7°C (mean of individual animal median chosen temperatures) as inferred by Cadena and Tattersall (2009b). However, this value of 37°C compares somewhat less favourably with past research on bearded dragons by Black and

Table 3. Results of a linear model with the residual of an evaporative water loss [$\log(\text{mg h}^{-1})$] linear model as the predictor variable and the residual of a chosen temperature (°C) linear model as the response variable for data derived from a group of bearded dragons

Coefficient	Estimate	s.e.	t-value	P-value
Intercept	<0.0001	0.3215	0.000	–
Residual evaporative water loss	1.081	2.015	0.537	0.595

Tattersall (2017), who found that their preferred temperature (mean of individual animal median chosen temperatures) was 34.1°C for adults and 33.7°C for neonates. One very plausible explanation for the difference between the data in the present study and those of Black and Tattersall (2017) is differing genetic or environmental backgrounds of the animals used in our two studies. Early life (e.g. maternal and developmental) environmental effects can influence thermal preference in lepidosaurs (Blouin-Demers et al., 2000; Blumberg et al., 2002; Paranjpe et al., 2013).

It is well known that dehydrated ectothermic tetrapods will often choose cooler temperatures than hydrated ones (Anderson and Andrade, 2017; Bradshaw et al., 2007; Crowley, 1987; Ladyman and Bradshaw, 2003; Sannolo and Carretero, 2019; Shoemaker et al., 1989; Tracy et al., 1993), which reduces their rate of evaporative water loss. In lepidosaurs, one mechanism that triggers this decrease in thermal preference is the neuropeptide arginine vasotocin (AVT), secreted by the pars nervosa of the brain in response to high blood sodium ion concentration (Bradshaw et al., 2007). There has even been past work on bearded dragons specifically to suggest that they thermoregulate differently when they are dehydrated compared with when they are hydrated (da Silveira Scarpellini et al., 2015). However, da Silveira Scarpellini et al. (2015) demonstrated that dehydrated bearded dragons do not strictly exhibit an anapyrexia (i.e. lowered thermal preference) in response to dehydration.

This dehydration hypothesis is, however, not the hypothesis explored by the present study. With the exception of the first three silkbacks used in this study, the silkbacks were provided with *ad libitum* access to water. Therefore, the hypothesis being explored was whether bearded dragons have an internal mechanism to assess their rate of evaporative water loss and shift their thermal preference accordingly. There are limited data in the literature that suggest that lepidosaur species with higher rates of evaporative water loss than their congeners will choose hotter or cooler temperatures compared with their congeners (Guillon et al., 2014; Sannolo et al., 2018). However, the thermal preference data presented here in the present study suggest that this is ultimately controlled and not proximately controlled. Thermal preference appears to be an insufficiently plastic trait to be reduced by a single gene mutation that increases the rate of evaporative water loss. Furthermore, as demonstrated here, residual sources of variation in evaporative water loss unrelated to scalation phenotype are not statistically correlated with thermal preference. There is some equivocal evidence that lepidosaurs may be able to adjust their thermal preference in response to entering physiological states that increase their rate of evaporative water loss (e.g. pregnancy) (Dupoué et al., 2015). It is noteworthy, though, that this preference actually seems to increase as opposed to decrease, possibly because of the metabolic implications of states like pregnancy (Dupoué et al., 2015). However, if this is indeed the case, one must keep in mind that these are states that will have been experienced by a lepidosaur's ancestors repeatedly over the course of evolutionary time. The lack of statistical significance in the present study's thermal preference data could indicate that the ability to adjust thermal preference in response to routinely

entered physiological states is under ultimate and not proximate control. This would mean it has co-evolved with the routine entering of these states over scales of evolutionary time. This would also mean it is not triggered directly by the increase in evaporative water loss itself.

Assuming a sensory mechanism for detecting instantaneous rate of evaporative water loss does exist (which, of course, it may not), the reason a bearded dragon in a physiologically 'normal' state (e.g. non-breeding) does not adjust its thermal preference downwards in response to an increase in instantaneous rate of evaporative water loss can also be explained in terms of thermal 'strategy'. The physiology of a given species of ectotherm adapting to a particular thermal environment results in a physiological optimum existing for that species in that particular thermal environment, and likewise results in thermal environments outside of that particular thermal environment being physiologically sub-optimal (Huey and Stevenson, 1979). Adaptively speaking, the benefits of a bearded dragon reducing its rate of evaporative water loss by moving to a cooler area may be outweighed by the benefits of remaining at its thermal optimum, as the animal has not yet entered a dehydrated state. If lepidosaurs can adjust their thermal preference in response to physiological states such as pregnancy, this may weigh differently in the strategic thermoregulation calculus compared with an increase in the rate of evaporative water loss in a physiologically 'normal' animal. If this is the case, this still supports our conclusion that thermal preference is insufficiently plastic to be affected by a single gene mutation that increases the rate of evaporative water loss. In this scenario, thermal preference would be insufficiently plastic as a result of the balance of a physiological trade-off weighing against this plasticity.

There is another potential explanation for the lack of phenotypic differences in thermal preference that merits brief discussion. Differences in thermal preference between different phenotypes and genotypes of lepidosaurs are sometimes very small (i.e. $\leq 1^\circ\text{C}$) (Sinervo, 1990), and we may have been unable to resolve a difference this small given the effect size. This 'small difference hypothesis', however, is not congruent with the fact that there was essentially no correlation between an animal's evaporative water loss residual and its thermal preference residual. Therefore, the balance of evidence still points towards there being no proximate effect of instantaneous rate of evaporative water loss on thermal preference in lepidosaurs.

Conclusions

The evaporative water loss data from the present study are not merely a physiological curiosity: they have relevance to conservation efforts. Many of the world's described lepidosaurs are poorly known, often only from preserved museum specimens collected at a single encounter with the scientific collector in question (Meiri et al., 2018). However, these specimens do allow researchers to determine the scale phenotype exhibited by a certain species of lepidosaur. One of the current observed climate change trends is an increase in aridity (i.e. environments becoming drier) (Zarch et al., 2015). These are localised trends: only some regions of the world are increasing in aridity, which is evidenced by the empirical data (Zarch et al., 2015). The present study has demonstrated that scale phenotype affects the rate of cutaneous evaporative water loss, and future work examining the relationship between scale phenotype and evaporative water loss could assist in predicting the effects of climate change on little-studied wild lepidosaurs.

Furthermore, the evaporative water loss data presented here have relevance to the fields of animal welfare research and animal welfare application, respectively. Industry reports suggest that the reptile-

keeping hobby is growing rapidly (Collis and Fenili, 2011). The proportion of pet reptiles that are captive bred as opposed to wild caught is also increasing, at least in some countries (Herrel and van der Meijden, 2014) and at least when two outlier species are excluded (Robinson et al., 2015). The production and sale of unusual phenotypes (or 'morphs' as they are termed in the reptile-keeping hobby) has become a major source of revenue for the reptile industry (Tapley et al., 2011). Although there has been much concern in the academic community over the welfare of other captive-bred animals that are highly derived in relation to their 'wild-type' (e.g. bulldogs) (Aromaa et al., 2019; Hopkins, 2015; O'Neill et al., 2018; Packer et al., 2012; Palmer, 2012; Sandøe et al., 2017), there has been less focus on the welfare of unusual reptile phenotypes (Rose and Williams, 2014). The silkback and leatherback data from the present study will hopefully form a scaffold to promote best practice for the care of lepidosaurs with reduced scalation for dissemination among reptile keepers, veterinarians and animal welfare groups.

Future work would do well to examine the effects of other scale-like structures on evaporative water loss. Technologies such as CRISPR/Cas9 now allow the on-demand production of loss-of-function models (Rasys et al., 2019). Loss-of-function models, such as the leatherbacks and silkbacks studied here, may prove to be powerful tools to change our understanding of the functional role of scale-like structures in animals.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: N.B.S., G.J.T.; Methodology: N.B.S., G.J.T.; Software: N.B.S., G.J.T.; Formal analysis: N.B.S., G.J.T.; Investigation: N.B.S.; Resources: N.B.S., G.J.T.; Data curation: N.B.S., G.J.T.; Writing - original draft: N.B.S.; Writing - review & editing: N.B.S., G.J.T.; Visualization: N.B.S., G.J.T.; Supervision: G.J.T.; Project administration: N.B.S.; Funding acquisition: G.J.T.

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