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Regulation of Exposure to Ultraviolet Light in Bearded Dragons (*Pogona vitticeps*) in Relation to Temperature and Scalation Phenotype

Nicholas B. Sakich[1] and Glenn J. Tattersall[1]

Exposure to ultraviolet (UV) light has both physiological benefits as well as costs. Many lepidosaur reptiles can behaviorally self-regulate their exposure to UV light in order to take advantage of the benefits of UV light while minimizing the costs. Furthermore, lepidosaur scales have been conceptualized by some as a barrier to the penetration of UV light. Here we examine regulation of self-exposure to UV light in three different phenotypes of Bearded Dragon (*Pogona vitticeps*): wild type, animals exhibiting scales of reduced prominence ('Leatherback'), and scaleless animals ('Silkback'). Silkbacks on average chose to expose themselves to lower levels of UV light irradiation than Leatherbacks or wild types did. Bearded Dragons of all scalation phenotypes on average received higher UV irradiation when they were in the cold section of a UV gradient apparatus compared to when they were in the hot section of the apparatus. This either demonstrates that Bearded Dragons under higher UV irradiances choose cooler temperatures or demonstrates that Bearded Dragons at cooler temperatures choose higher UV irradiances. The relationship between chosen temperature and chosen UV light irradiance was not affected by scalation phenotype. This study highlights external influences on the mechanism that regulates UV self-exposure behavior in lepidosaur reptiles.

NUMEROUS adaptive functions have been postulated or empirically demonstrated for the scales of lepidosaurs (superorder Lepidosauria: the lizards, snakes, amphisbaenians, and Tuatara; Gans and Richmond, 1957; Soulé, 1966; Spearman, 1966; Gans and Maderson, 1973; Regal, 1975; Sherbrooke, 1990; Chang et al., 2009; Gholamifard et al., 2015). Prominent among these is the hypothesis that scales reduce the penetration of ultraviolet (UV) light through the skin (Chang et al., 2009). Diurnal lepidosaurs will routinely be exposed to wavelengths of light in the UV spectrum in the wild (Ferguson et al., 2010, 2013, 2014, 2015a; Edmonds et al., 2018). UV light catalyzes a reaction in the skin of lepidosaurs that converts provitamin D_3 into previtamin D_3 (Klaphake, 2010). Previtamin D_3 is then isomerized into cholecalciferol, which is hydroxylated into calcidiol, which is finally hydroxylated into calcitriol (Klaphake, 2010). Calcitriol, the active form of vitamin D_3, then promotes physiological processes like the uptake of ingested calcium from the gut (Klaphake, 2010). However, UV light can also be harmful to lepidosaurs, causing tissue damage (Chang and Zheng, 2003). Therefore, it would be adaptive for a lepidosaur to maintain its level of exposure to UV light within a certain safe range, where a mechanism allowing for such exists. The keratinized skin of lepidosaurs provides a physical block to some wavelengths of UV light: only about 5% of the incident light penetrates the keratinized layer, and this ratio remains constant across various incident light irradiances (Chang and Zheng, 2003). This physical defense may be supplemented by a behavioral defense, where a mechanism for such exists.

There are two ranges of wavelengths of UV light that animals encounter to a biologically relevant degree in the wild: UVA (315–400 nm) and UVB (280–315 nm; Cronin and Bok, 2016). Evidence suggests that different wavelength categories of UV light have different biological effects. For instance, human experiments indicate that UVB is better at catalyzing the aforementioned conversion of provitamin D_3 into previtamin D_3 than UVA is (Sallander et al., 2013). UVB is also more damaging to biological structures than UVA is (Rouzaud et al., 2005). Furthermore, UVA is likely the only UV light that animals can see, with most vertebrates being able to see it (Cronin and Bok, 2016), because vertebrate ocular photoreceptors are sensitive to UVA, with comparatively low sensitivity to UVB (Cronin and Bok, 2016). It is only in a minority of species, such as humans, where the eye's lens filters out UVA before it can hit the photoreceptors (Cronin and Bok, 2016). However, the lack of ocular sensitivity to UVB does not necessarily mean that lepidosaurs are unable to detect UVB using some other mechanism, such as vitamin D receptors in the brain (Ferguson et al., 2003). Vitamin D receptors in the brain could allow a lepidosaur to assess the effect that their photic environment is having on their internal vitamin D status and thus indirectly detect UVB. Furthermore, vitamin D receptors have been found in the brain of at least one species of lepidosaur (the Green Anole, *Anolis carolinensis*; Bidmon and Stumpf, 1996).

In humans, UVB is better at catalyzing the aforementioned conversion of provitamin D_3 into previtamin D_3 than UVA is (Sallander et al., 2013). UVB is also more damaging to biological structures than UVA is (Rouzaud et al., 2005). Lepidosaur species that are heliothermic (sun-seeking, or basking) are able to adjust their levels of UV self-exposure in response to their vitamin D status, exposing themselves to UV light more if their vitamin D levels are low than if their vitamin D levels are high (Ferguson et al., 2003, 2013, 2015b). However, it is unclear to what degree various species distinguish between UVA, UVB, and visible light and respond behaviorally accordingly to maximize photobiosynthesis of vitamin D_3 (Ferguson et al., 2003, 2013).

An ectothermic organism's interactions with light can also be modulated by environmental temperature. For instance, in some lepidosaurs cooler temperatures result in increased melanosome dispersion, which darkens the skin (Hadley and Goldman, 1969; Walton and Bennett, 1993; de Velasco and Tattersall, 2008; Langkilde and Boronow, 2012; Smith et al., 2016a, 2016b; Cadena et al., 2018). Warmer temperatures in

[1] Department of Biological Sciences, Brock University, 500 Glenridge Avenue, St. Catharines, Ontario, Canada L2S 3A1; Email: (NBS) sakichnick@gmail.com; and (GJT) gtattersall@brocku.ca. Send correspondence to NBS.
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these same species result in increased melanosome aggregation, which has the opposite effect (Hadley and Goldman, 1969; Walton and Bennett, 1993; de Velasco and Tattersall, 2008; Langkilde and Boronow, 2012; Smith et al., 2016a, 2016b; Cadena et al., 2018). Experiments in tadpoles indicate that ectotherms at cooler temperatures both accumulate more UV light-induced DNA damage and take longer to repair said damage than ectotherms at warmer temperatures (Morison et al., 2020). Darker skin reduces the penetration of potentially mutagenic light into deeper tissues (Porter and Norris, 1969). However, black peritoneums, as possessed by some lepidosaurs, provide a secondary block to light (Porter and Norris, 1969) and, therefore, the adaptive significance of melanosomes as a barrier to light is unclear. Temperature can have effects on other non-melanosome-related aspects of skin color in lepidosaurs as well (Morrison et al., 1996; Langkilde and Boronow, 2012; Stephenson et al., 2017).

A useful model for addressing questions about photoregulatory behavior in lepidosaurs is the Bearded Dragon (*Pogona vitticeps*). Bearded Dragons have been bred in captivity since at least the 1980s (Sherriff, 1989; Stahl, 1999) and are one of the most popular species of pet lizard (Prestridge et al., 2011; Howell and Bennett, 2017; Wakao et al., 2018). They have also been extensively used as physiological model organisms (Tattersall and Gerlach, 2005; de Velasco and Tattersall, 2008; Cadena and Tattersall, 2009a, 2009b; Khan et al., 2010; Fan et al., 2014; da Silveira Scarpellini et al., 2015; Smith et al., 2016a, 2016b; Black and Tattersall, 2017; Cadena et al., 2017, 2018). A spontaneous scalation-related mutation has arisen in breeding colonies of Bearded Dragon (Di-Poï and Milinkovitch, 2016; de Vosjoli et al., 2017). One copy of the mutant allele (genotype *Sca*/*sca*) results in a 'Leatherback,' an animal with scales of reduced prominence (de Vosjoli et al., 2017). Two copies of the mutant allele (genotype *Sca*/*Sca*) results in a scaleless animal (Di-Poï and Milinkovitch, 2016; de Vosjoli et al., 2017), termed a 'Silkback' (de Vosjoli et al., 2017). The Silkback phenotype was characterized thoroughly by Di-Poï and Milinkovitch (2016). Histologically, the β-keratin-composed layers of Silkback skin are reduced compared to wild type skin, as is the superficial loose dermis (Di-Poï and Milinkovitch, 2016). Indeed, the skin of Silkbacks is similar to the skin that wild types have in their 'hinge regions' (the regions of skin in between scales; Di-Poï and Milinkovitch, 2016). Di-Poï and Milinkovitch (2016) did not characterize the Leatherback phenotype, but its skin phenotype is presumably intermediate between that of Silkbacks and that of wild types. The *Sca* mutation occurs at the locus that encodes for the protein ectodysplasin-A (EDA), which is a ligand of the EDA receptor (Di-Poï and Milinkovitch, 2016). No evidence of non-ectodermal effects of the mutant *Sca* allele has been observed (M. Milinkovitch, pers. comm.).

The following experiments were undertaken to explore the hypothesis that the lepidosaur scale is a barrier that reduces the penetration of UV light through the skin. Thicker integument would logically reduce the penetration of UV light through the integument. Wild types should therefore choose to expose themselves to higher UV irradiances than either Leatherbacks or Silkbacks, as they have thicker integument than Leatherbacks or Silkbacks do. Silkbacks should choose to expose themselves to lower UV irradiances than either Leatherbacks or wild types, as their integument is thinner than that of Leatherbacks or wild types. Having an intermediate integumentary phenotype, the UV self-exposure levels of Leatherbacks should be intermediate between those of wild types and Silkbacks. Although the mechanism of UV irradiance level detection was not directly tested in our experiments, *a priori* we considered visual detection of UV irradiance level to be the most plausible mechanism for facilitating UV light-related behavioral photoregulation. No *a priori* hypotheses were made with regard to the effect that temperature would have on photoregulatory behavior, as this portion of the study was inherent to the study design to promote basking behavior and thus is purely exploratory.

MATERIALS AND METHODS

The animals.—Thirty-four Bearded Dragons (*Pogona vitticeps*) were acquired from private pet industry breeders and sellers. Phenotypic breakdown of the sample was as follows: 13 wild types, 12 Leatherbacks, and 9 Silkbacks. An *a priori* power analysis was performed. This power analysis assumed a large effect size and used variances taken from Khan et al. (2010). The animals were acquired at a small (juvenile) size (approximately 3–20 g in mass) with the exception of three Silkbacks that were acquired at a larger (sub-adult) size (approximately 100–160 g in mass). Clutch information was provided by the suppliers. Animals within each clutch were full siblings. Two clutches supplied both Leatherback and wild type individuals to this study as a result of phenotypic crossover. Despite the lack of histological data on the integument of Leatherbacks, due to the fact that genetically they are the heterozygous intermediate between wild types and Silkbacks (de Vosjoli et al., 2017) and the fact that from a visual and tactile standpoint they are also intermediate between wild types and Silkbacks, we have chosen to treat them as the phenotypic intermediate between wild types and Silkbacks in this study.

The animals were housed individually in black PVC cages measuring 61 cm long by 61 cm wide by 40.6 cm high with clear acrylic doors measuring 50.8 cm long by 25.4 cm high. Each cage had a 30 cm by 30 cm basking tile as well as a piece of disposable paper pulp packaging as enrichment; there was a gap under each tile that the animal could use as a retreat. The basking tile was heated with an incandescent bulb mounted in a light fixture above it. This resulted in upper surface temperatures during the light photoperiod reaching 35–45°C, as measured periodically using a FLIR TG165 Spot Thermal Camera (Teledyne FLIR LLC, Wilsonville, OR). Another light fixture contained an Exo Terra Reptile UVB200 13W bulb (Rolf C. Hagen Inc., Baie d'Urfé, QC, Canada) to provide continuous UVB. Animals were kept on paper towel substrate or ground coconut husk substrate in the case of the three large Silkbacks. Cage allotment in the housing room was randomized. Photoperiod was set to 12L:12D.

Feedings were daily and consisted of 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), and live insects dusted five days a week with Rep-Cal Calcium with vitamin D_3 and twice a week with Rep-Cal Herptivite Multivitamin (Rep-Cal Research Labs, Los Gatos, CA). The cages were misted with water on a daily basis. Due to apparent shedding issues, with the exception of the first three Silkbacks housed in the lab, all

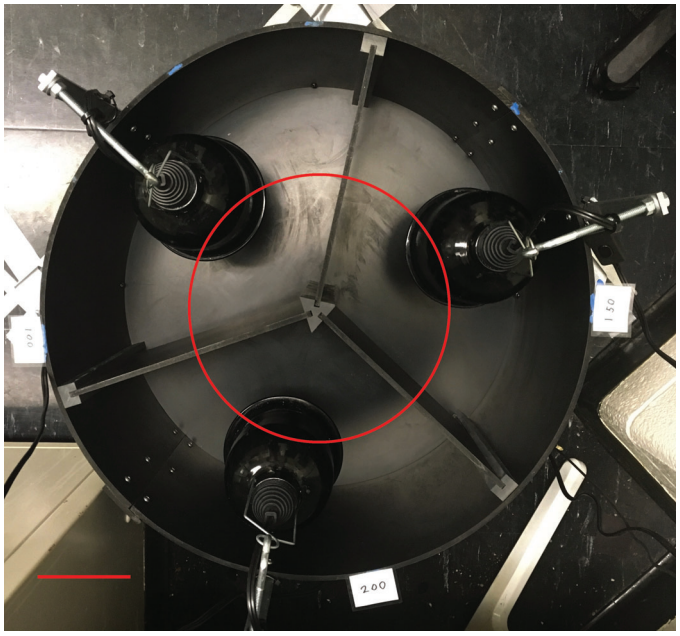

Fig. 1. The ultraviolet light irradiance gradient apparatus used in this study. The labels on the sides of the apparatus indicate that at this time the Exo Terra Reptile UVB100 26W bulb (Rolf C. Hagen Inc., Baie d'Urfé, QC, Canada) is in the light fixture in the compartment on the upper left, followed clockwise by the Exo Terra Reptile UVB150 26W bulb and the Exo Terra Reptile UVB200 26W bulb. The circle indicates the approximate boundaries of the 'hot' portion of the apparatus generated by coils of heat cable underneath the apparatus. Beyond this is the ambient temperature 'cold' portion. Lizards were free to walk beneath a large gap underlying each divider. Scale bar is 10 cm.

Silkbacks were provided with constant access to a large, shallow dish of water, cleaned and refilled daily.

Data handling and statistical analysis for the following experiments was performed in either Excel 2011 for Mac Version 14.7.7 (Microsoft Corporation, Redmond, WA; https://www.microsoft.com) or R Version 3.5.1 (R Core Team, 2018) or Version 4.0.2 (R Core Team, 2020). Plots were made with the ggplot2 package in R (Wickham, 2016). Data from our experiments are available as supplementary material (Table S1; see Data Accessibility).

Experiments.—UV irradiance choice experiments were conducted in a circular apparatus measuring 60 cm outer diameter by 30.1 cm internal height, constructed of black expanded PVC plastic with a thickness of 6 mm (Fig. 1). At the center of the apparatus was a central triangular column with a groove in each side. Along the wall at the periphery of the apparatus were three rectangular black supporting columns, each with a central groove. A light baffle measuring 23 cm high made of the same material as the rest of the apparatus was wedged and secured between each of the pairs of grooves at a height of 7 cm above the floor of the apparatus, to ensure overhead lights cast onto only a fixed portion of the apparatus floor. The end effect was to create three roughly triangular compartments (numbered one, two, and three) within the apparatus with unfettered movement capabilities (Fig. 1).

Suspended centered in each compartment 19.3 cm above the floor of the apparatus (measured from the upper surface of the bottom lip of the light fixture) was an Exo Terra 15 cm Reptile Dome light fixture. An Exo Terra 25W 4.5 m Heat Cable was taped to the underside of the apparatus's floor using electrical tape. The heat cable was secured at the center of the underside of the apparatus and then spiraled out from the center in concentric circles spaced approximately 3 cm apart. The end effect was a spiral of heat cable with a maximum outer diameter of 33 cm. This created two 'temperature zones' in the apparatus: the 'hot' center of the apparatus that was over top of the heat cable and the 'cold' periphery of the apparatus where the temperature was equal to the ambient temperature (Fig. 1). The heat cable was connected to an Inkbird ITC-308 Temperature Controller (Inkbird Tech. Co., Ltd., Shenzhen, Guangdong, China). The entire apparatus sat on top of a piece of foam, and, for safety, was spaced from this foam by rubber 'feet.' The end effect was that the upper surface of the floor of the apparatus within the hot central zone was maintained at approximately 40°C, and floor upper surface temperature sharply dropped off to ambient temperature (~21°C) when the threshold between the hot zone and the cold zone was crossed (Fig. 1). These temperatures were verified using a FLIR TG165 Spot Thermal Camera. The design of the apparatus was such that the animals were able to regulate their body temperatures independently of their chosen UV light irradiance, while the dual choice in temperature encouraged continual, active behavioral thermoregulation.

Animals were exposed to the apparatus individually. During a trial, each of the three light fixtures contained one of three possible fluorescent bulbs: an Exo Terra Reptile UVB100 26W bulb, an Exo Terra Reptile UVB150 26W bulb, or an Exo Terra Reptile UVB200 26W bulb. These bulbs produce light in the human visible spectrum as well as both UVA and UVB light. Of the three bulbs, the 'UVB200′ bulb emits UV light at the highest intensity, followed in descending order by the 'UVB150′ bulb and the 'UVB100′ bulb. These bulbs also vary in the illuminance of the light they emit, but only very slightly. Based on data provided by the manufacturer, this difference would be a 60 Lux difference (an approximately 6% difference) in illuminance between the most illuminating ('UVB200′) and least illuminating ('UVB100′) of the three bulbs as measured at a distance of 20 cm from the bulbs. Furthermore, based on spectrographs provided by the manufacturer, the spectral profile of light emitted by these three bulbs is very similar, differing mostly in the UV portion of the spectrum, and the emissions in the 690–740 nm range were very low for all three bulbs (<10% relative spectral power). The three bulbs were randomized among the three fixtures before each trial.

In order to rule out the possibility of higher temperature under the bulbs of higher intensity being a confounding factor, at the beginning of each trial a probe-based digital thermometer was placed under each light. The temperature under each light was then recorded to the nearest 0.1°C. A one-way repeated measures ANOVA was run on these temperature values to test for any difference in temperature between the areas under each bulb. No statistically significant difference was found between the temperatures in the areas under the three bulbs ($F_{2,66} = 0.20$, $P = 0.82$). Examination of the residuals of this ANOVA using a Q-Q plot revealed no obvious deviation from normality.

At the beginning of a trial, an animal was placed in the apparatus under the light in compartment two. A Raspberry Pi 3 camera (Raspberry Pi Foundation, Cambridge, UK)

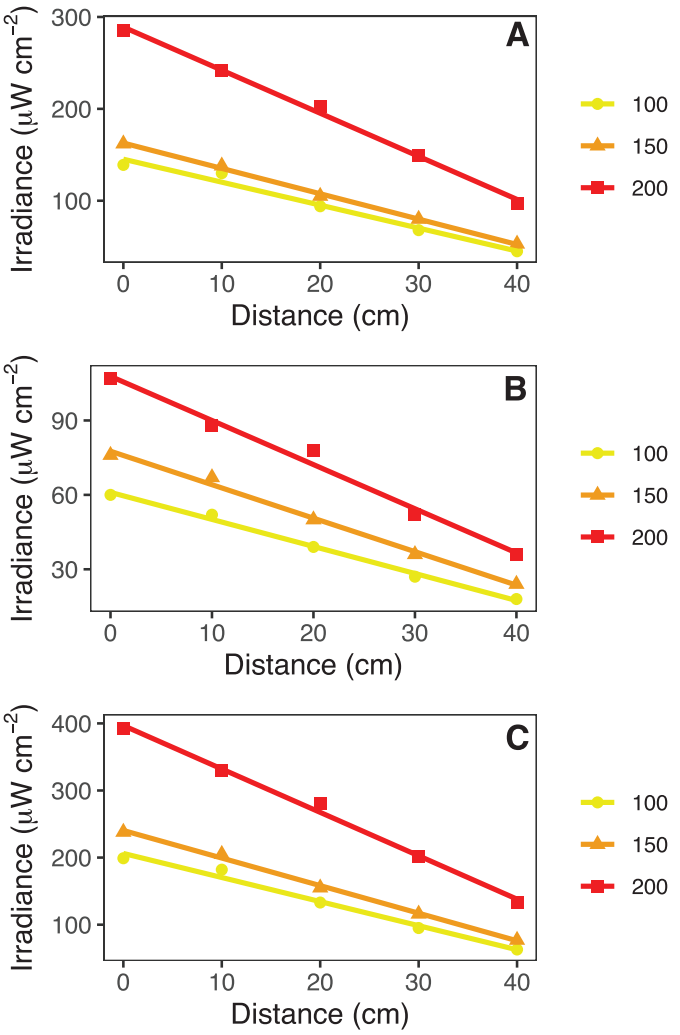

Fig. 2. The regressions of ultraviolet (UV) light irradiance used in this study to infer an animal's UV light exposure level at each time point of its trial. The regressions model how UV light irradiance changes as a function of horizontal bench top distance from the center of the area underneath the bulb. Regressions were modeled for three different bulbs: an Exo Terra Reptile UVB100 26W bulb, an Exo Terra Reptile UVB150 26W bulb, and an Exo Terra Reptile UVB200 26W bulb (Rolf C. Hagen Inc., Baie d'Urfé, QC, Canada). Regressions were modeled for three different wavelength categories: (A) ultraviolet-A (UVA), (B) ultraviolet-B (UVB), and (C) combined UVA+UVB.

mounted directly above the apparatus was set to take a photo of the apparatus every ten seconds, using image capture software (motionEyeOS; https://github.com/ccrisan/motioneyeos). Trials were run for 4–6 hours. After each trial, the animal was weighed to the nearest 0.01 g and measured for total length to the nearest mm by placing it on top of either a ruler or a tape measure and measuring from above. The bulbs were periodically checked to make sure they had not degraded in their UV output to any substantial degree using either a Solarmeter® Model 6.2 UVB Meter or a Solarmeter® Model 5.7 Total UV (A+B) Meter (Solar Light Company, Inc., Glenside, PA) or both.

In order to infer an animal's chosen level of UV exposure at any given time point, regressions were produced which mathematically represented how measured UV irradiance changed as a function of the linear floor distance from each bulb. An Exo Terra 15 cm Reptile Dome was suspended 29.8 cm from the laboratory bench as measured from the top of the bottom lip of the light fixture. This height was used because the light fixtures in the apparatus were suspended 19.3 cm from the floor of the apparatus, with 10.5 cm added to compensate for the height of the UV measuring devices. The fixture was loaded with fresh versions of the three different bulbs used in the trials, one after the other. Two different devices were used to measure how UV irradiance varied with distance from the bulb: a Solarmeter® Model 6.2 UVB Meter and a Solarmeter® Model 5.7 Total UV (A+B) Meter. Output was measured with each device at four different linear floor distances radiating away from the center point of the space directly under the light fixture: 0 cm, 10 cm, 20 cm, 30 cm, and 40 cm. At each distance from the bulb, the UV meters were tilted so that their sensors were pointed at the light source. The exception to this was at 0 cm where no tilting needed to occur and so the UV meters were held perpendicular to the bench top.

In order to infer how UVA exposure varied with distance from the bulb, the values produced by the UVB meter were subtracted from the values produced by the UVA+UVB meter for a given bulb for each distance point from the bulb. A linear regression of UV irradiance by distance was then performed on each of the six data sets produced (three different bulbs, two different wavelength categories; Fig. 2). It is noteworthy that the lowest instantaneous irradiance that a Bearded Dragon could choose in the apparatus was much lower than the instantaneous irradiances measured in the shade of trees in Toowoomba, Queensland, Australia for both UVA and UVB (Figs. 1, 2; Parisi and Kimlin, 1999; Parisi et al., 2001), although Parisi and Kimlin's (1999) respective definitions of UVA and UVB each differ from ours by 5 nm increments. Examining the results of Parisi et al. (2001), which gathered UVA data over multiple seasons, reveals that for UVA at least this difference holds even in winter. Toowoomba is at the same latitude as portions of the Bearded Dragon's geographic range in the wild (Wilson and Swan, 2017).

Images from the trials were loaded into FIJI/ImageJ (National Institutes of Health, Bethesda, MD; https://fiji.sc), and each animal's position in the apparatus was tracked over time using the manual tracking function. The approximate center of the animal's head was selected. If the center of the animal's head was obscured by something other than the dome of a light fixture itself, then the visible portion of the body closest to the center of the head was selected instead. If the center of the animal's head was obscured by the dome of a light fixture itself, or it was inferred from previous photos that the animal was under the dome of a particular light fixture, then the center of the light fixture in question was selected instead.

In order to determine which bulb the animal was closest to at any given time, the animal's x and y coordinates from its tracking were matched with the x and y coordinates of the center point of each light fixture. Pythagorean theorem was used to determine the linear distance between the animal's x and y coordinates and the x and y coordinates of each bulb. At each time point, the animal was deemed at that time point to be under the bulb that corresponded to the shortest of these three linear distances. The equation for the bulb that the animal was under was used to infer an animal's chosen level of UV exposure at that time point for each of the three

wavelength categories. This process was repeated for each image. The pixel-to-cm relationship was calibrated for each trial based on the diameter of the apparatus being of known length. Only the last three hours of any individual animal's time in the apparatus were used, both to standardize the length of the time period assessed and to allow the animal an exploration period.

Statistical analysis: effect of phenotype.—After converting each position to a chosen UV bulb position, a UV light irradiation dose was calculated for each animal. The irradiances experienced by the animal at each measured time point (based on the values inferred for each photo as described above) were converted into W cm^{-2}. Each of these time point values were then multiplied by ten to interpolate irradiance experienced over the course of each ten-second interval, as one photo was taken every ten seconds. These multiplied values were then summed for each animal to produce a final irradiation dose for each animal in J cm^{-2}. These irradiation values were then used for subsequent statistical analysis of how UV exposure level varied by phenotype. Bartlett's tests for unequal variance were performed using the olsrr package in R (Hebbali, 2020). There was no evidence of unequal variance for UVB ($\chi^2 = 1.60$, df = 2, $P = 0.45$), but the variance was unequal for UVA ($\chi^2 = 6.42$, df = 2, $P = 0.04$) and marginal for combined UVA+UVB ($\chi^2 = 4.92$, df = 2, $P = 0.08$). Due to the unequal variances for UVA and marginally unequal variances for UVA+UVB, phenotype effects were tested using robust linear models made with the MASS package in R (Venables and Ripley, 2002) instead of using standard linear models. Although UVB data did not exhibit evidence of unequal variance, in order to facilitate comparison between the models fitted on the three different wavelength categories, robust linear models were used for all three wavelength categories, including UVB.

For model selection, first the models were created, each with phenotype as a predictor variable and each with one of the three measures of chosen UV irradiation (UVA, UVB, or combined UVA+UVB) as a response variable. The other predictor variables that were included in the complete models as covariates were animal mass (g), animal total length (cm), trial start time (converted to seconds elapsed since midnight on the day that the trial occurred, factoring in the fact that only the last three hours of an animal's trial were used), and the total distance an animal moved during the last three hours of its trial (cm). Weighting of the residuals of these models did not change drastically when bisquare weighting was used instead of Huber weighting, so the default (Huber) weighting was used. Next, three linear mixed effects models were created using the lme4 package in R (Bates et al., 2015), each with phenotype as a predictor variable, one of the three measures of chosen UV irradiation (UVA, UVB, or combined UVA+UVB) as a response variable, and clutch included as a random effect. These were each then compared to a corresponding robust linear model with phenotype as a predictor variable and the relevant measure of chosen UV irradiation as a response variable, to test for evidence of a clutch effect. The full models were not used for these comparisons as they produced poor fits that were not ameliorated by our attempts at rescaling. The linear mixed effects models with clutch included as a random effect also produced poor fits, but there was no way to ameliorate this. There was no evidence of a clutch effect for UVA ($\chi^2 = 2.11$, df = 1, $P = 0.14$), UVB ($\chi^2 = 1.53$, df = 1, $P = 0.22$), or combined UVA+UVB ($\chi^2 = 1.95$, df = 1, $P = 0.16$).

Using the MuMIn package in R (Barton, 2020), ΔAIC_c values were computed and used to decide which of the other predictor variables besides phenotype would be included in the final models. The best-supported model for each of the wavelength categories was that with no other predictor variables besides phenotype. Significance of these final models was tested for using the sfsmisc package in R (Maechler, 2020).

Statistical analysis: UV and temperature.—In order to address the question as to whether or not thermoregulatory decisions are influenced by photoregulatory decisions (or vice versa), we exploited the fact that the center of the UV gradient apparatus was 'hot' and the perimeter of the UV gradient apparatus was 'cold.' The maximum diameter of the hot portion of the apparatus was 33 cm. Therefore, if an animal was less than 16.5 cm from the center point of the apparatus, at a particular time point it was determined to be 'hot,' (~40°C). By the same token, if an animal's coordinates were more than 16.5 cm from the center point of the apparatus, in that photo it was determined to be 'cold.' This linear distance from the center point of the apparatus was determined using Pythagorean theorem. Mean UV exposure level was calculated for each animal using only photos where the animal was less than 16.5 cm from the center point, and then again for each animal using only photos where the animal was more than 16.5 cm from the center point. This was done for each of the three wavelength categories, resulting in a mean for each animal when it was 'hot' and a mean for each animal when it was 'cold,' for each of the three wavelength categories. These means were used as 'hot' and 'cold' UV values, respectively, for each animal for subsequent statistical analysis. The means of the instantaneous UV irradiances chosen by each animal were used instead of calculating an irradiation dose in J cm^{-2} as for the phenotype effect statistical analysis. This is because time spent in the 'hot' and 'cold' portions of the apparatus respectively differed within individual animals' trials.

As an exploratory measure to look for evidence of an interaction between phenotype and temperature, three separate linear mixed effects models were created with the lme4 package in R (Bates et al., 2015). These models each had the floor temperature interacting with phenotype as the explanatory variables and one of the three measures of chosen UV light irradiance as a response variable. Each of these models included individual animal ID as a random effect. Significance of these models was assessed using the lmerTest package in R (Kuznetsova et al., 2017). There was no statistically significant interaction between temperature and phenotype (UVA: $P = 0.54$; UVB: $P = 0.69$; combined UVA+UVB: $P = 0.58$). There was also no evidence of a clutch effect (UVA: $\chi^2 = 0$, df = 1, $P = 1$; UVB: $\chi^2 = 0$, df = 1 $P = 1$; combined UVA+UVB: $\chi^2 = 0$, df = 1, $P = 1$). As there was no evidence of an interaction between temperature and phenotype, paired t-tests were instead used to compare UV exposure level between hot and cold animals for each of the three wavelength categories. Examination of the differences between each animal's hot and cold value using Q-Q plots revealed no excessive deviation from the assumptions of normality and equal variance.

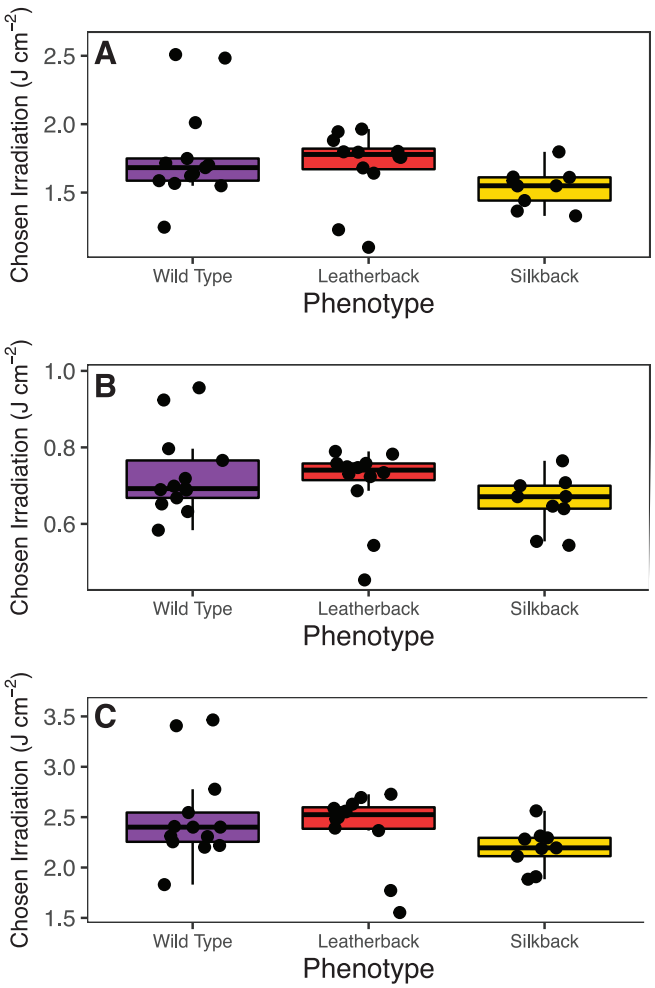

Fig. 3. Ultraviolet (UV) light irradiations chosen in a UV light gradient apparatus by Bearded Dragons (*Pogona vitticeps*) of three different phenotypes: Wild Type ($n = 13$), animals exhibiting scales of reduced prominence ('Leatherback'; $n = 12$), and scaleless animals ('Silkback'; $n = 9$). Data are displayed for three different wavelength categories: (A) ultraviolet-A (UVA), (B) ultraviolet-B (UVB), and (C) combined UVA+UVB. Boxes display the median and the first and third quartiles, and whiskers display at maximum 1.5 times the inter-quartile range.

RESULTS

Effect of phenotype.—There were statistically significant differences in chosen UV irradiation between the three phenotypes for UVA (Fig. 3A; Table 1; $F = 4.86$, $P = 0.01$). There was no evidence of differences in chosen UV irradiation for UVB (Fig. 3B; Table 2; $F = 2.41$, $P = 0.11$), but there was for combined UVA+UVB (Fig. 3C; Table 3; $F = 4.55$, $P = 0.02$).

Table 1. The results of a robust linear model comparing chosen ultraviolet-A (UVA) light irradiation between three different phenotypes of Bearded Dragon (*Pogona vitticeps*): Wild Type, animals exhibiting scales of reduced prominence ('Leatherback'), and scaleless animals ('Silkback').

Coefficient	Value	SE	t-value
Intercept (Wild Type)	1.6843	0.0489	34.4730
Leatherback	0.0802	0.0705	1.1370
Silkback	–0.1535	0.0764	–2.0088

Table 2. The results of a robust linear model comparing chosen ultraviolet-B (UVB) light irradiation between three different phenotypes of Bearded Dragon (*Pogona vitticeps*): Wild Type, animals exhibiting scales of reduced prominence ('Leatherback'), and scaleless animals ('Silkback').

Coefficient	Value	SE	t-value
Intercept (Wild Type)	0.7072	0.0206	34.3552
Leatherback	0.0228	0.0297	0.7690
Silkback	–0.0477	0.0322	–1.4828

UV and temperature.—There were significant differences between the chosen UV irradiances of hot and cold animals for UVA ($t = -4.85$, $df = 33$, $P < 0.0001$), UVB ($t = -5.23$, $df = 33$, $P < 0.0001$), and combined UVA+UVB ($t = -4.97$, $df = 33$, $P < 0.0001$; Fig. 4). Animals experienced on average higher UV irradiances when they were in the cold portion of the apparatus than when they were in the hot portion of the apparatus (Fig. 4).

DISCUSSION

Effect of phenotype.—Three primary mechanisms exist in animals to combat damage to biological structures caused by UV light: molecular mechanisms (e.g., Blaustein and Belden, 2003; Londero et al., 2019), morphological mechanisms (e.g., Blaustein and Belden, 2003; Chang and Zheng, 2003), and behavioral mechanisms (e.g., Blaustein and Belden, 2003). Our results demonstrate that the Bearded Dragon possesses a behavioral mechanism to combat damage caused by UV light. This is in addition to the other two mechanisms (molecular and morphological), which analogy to other animal taxa suggests the Bearded Dragon has as well. This behavior is directly influenced by the thickness of a Bearded Dragon's integument, as the lepidosaur integument is a barrier to the penetration of UV light (Chang and Zheng, 2003).

Our study supports previous research demonstrating some lepidosaurs self-regulate their exposure to UV light (Ferguson et al., 2003, 2013, 2015b). Furthermore, this behavior is plastic enough that Bearded Dragons can adjust it in response to a single gene mutation affecting the thickness of their integument. *A priori,* we considered visual cues to be the most plausible mechanism for a lepidosaur to use to behaviorally regulate its UV light irradiation. Other lizards in the family Agamidae have visual sensitivity into the UV portion of the photic spectrum (Barbour et al., 2002; Yewers et al., 2015). Bearded Dragons could be using either the eyes proper, or the pineal-gland-associated eye-like structure known as the 'parietal eye' (Gundy and Wurst, 1976a), or both. While there is perhaps no reason to think that the

Table 3. The results of a robust linear model comparing chosen combined ultraviolet-A (UVA) and ultraviolet-B (UVB) light irradiation between three different phenotypes of Bearded Dragon (*Pogona vitticeps*): Wild Type, animals exhibiting scales of reduced prominence ('Leatherback'), and scaleless animals ('Silkback').

Coefficient	Value	SE	t-value
Intercept (Wild Type)	2.3953	0.0664	36.0504
Leatherback	0.1022	0.0959	1.0659
Silkback	–0.2065	0.1039	–1.9874

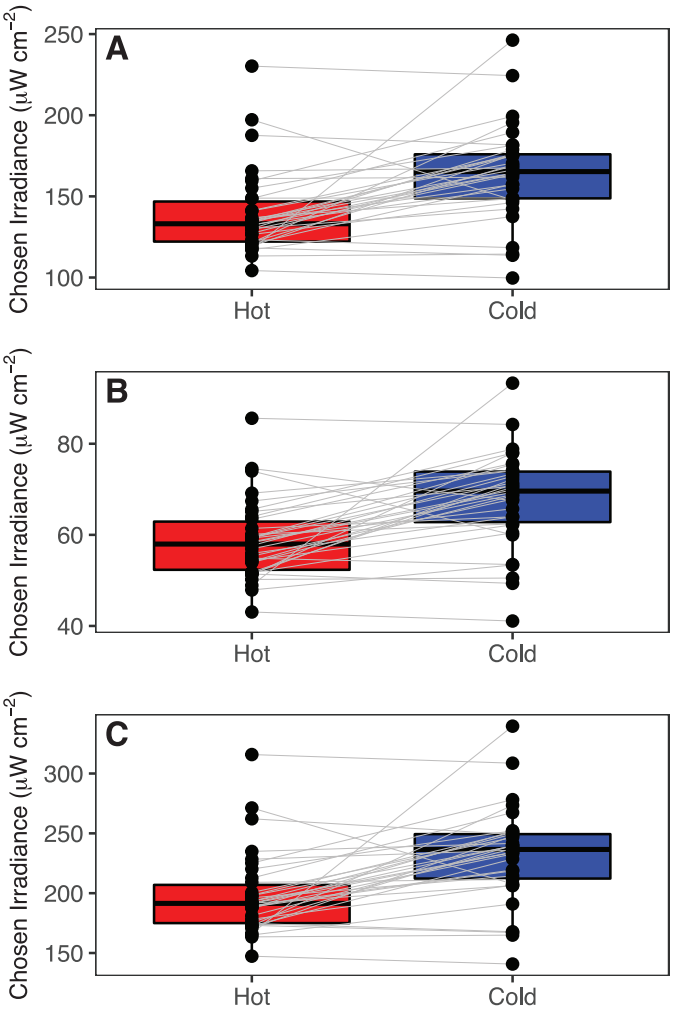

Fig. 4. The difference in ultraviolet (UV) light irradiance chosen in a UV light gradient apparatus by Bearded Dragons (*Pogona vitticeps*; $n = 34$) when they were in the heated ('Hot') portion of the apparatus compared to when they were in the ambient temperature ('Cold') portion of the apparatus. Data are displayed for three different wavelength categories within the UV portion of the photic spectrum: (A) ultraviolet-A (UVA), (B) ultraviolet-B (UVB), and (C) combined UVA+UVB. Boxes display the median and the first and third quartiles, and whiskers display at maximum 1.5 times the inter-quartile range. Lines connect data points from the same individual animal.

mutant *Sca* allele would have any effect on a Bearded Dragon's eyes proper, there is indeed a reason to think it might affect the parietal eye. The Bearded Dragon's parietal eye is in close association with a scale (Nishimura et al., 2010). Furthermore, in lizards, the pineal gland, to which the parietal eye connects, may also directly detect light itself through somewhat transparent cartilage deposits that bridge the gap between it and the dorsal external surface of the lizard's head (Gundy and Wurst, 1976a). Therefore it is plausible that the reduced scalation of Leatherbacks and Silkbacks alters their UV light-related behavior by directly influencing how much and how light is detected by the parietal eye and the pineal gland through the skin on the head. Whether or not this is a maladaptive or adaptive behavioral change remains to be seen and is, in fact, somewhat a matter of perspective. It is possible that as integument has evolved in lizard lineages, the parietal eye and pineal gland have responded plastically to this by increasing or decreasing a species' behavioral exposure to UV light. This hypothetically could have occurred without any changes to parietal eye or pineal-gland-associated genes occurring. If this is the case, then the parietal eye and pineal gland's response to the mutant *Sca* allele may be analogous, and this behavioral shift might be termed adaptive. However, it is also possible that the behavioral shift in Leatherbacks and Silkbacks is a result of interference in the parietal eye and pineal gland's normal detection pathways caused by the reduced scalation phenotype. This is perhaps the result of altering some lens-like properties of the scales directly over the parietal eye. However, it seems reasonable to assume that more UV light penetrates the integument of Silkbacks and Leatherbacks than penetrates that of wild types. Therefore, even if this reduction in self-exposure to UV light is the result of interference with a sensory system, it still is, in a sense, adaptive.

There is also the possibility that a feedback mechanism within the vitamin D-related calcium metabolism pathway is in play in Bearded Dragon photoregulatory behavior. This mechanism would entail Bearded Dragons using changes in their vitamin D status to indirectly detect the UV light irradiance of their current environment and modify their behavior accordingly (Ferguson et al., 2003). Our husbandry methods for the animals in this study were adequate to maintain their vitamin D status (Oonincx et al., 2010); therefore, they were not vitamin D stressed. As their bodies had adequate vitamin D_3 concentrations, no additional vitamin D_3 was produced cutaneously when they were in the UV light irradiance gradient apparatus. Therefore, they would not have been able to use a change in their circulating levels of vitamin D_3 per se to detect the irradiance that they were exposed to.

However, human research indicates that the cutaneous vitamin D_3 photobiosynthetic pathway does not halt when an animal's vitamin D status is adequate. Under those circumstances, instead of thermally isomerizing previtamin D_3 to cholecalciferol, previtamin D_3 is instead photoisomerized to tachysterol$_3$ and lumisterol$_3$ (Holick et al., 1981). Although tachysterol$_3$ cutaneous concentration quickly levels off as UV light exposure continues, lumisterol$_3$ cutaneous concentration continues to climb the longer skin is exposed to UV light (Holick et al., 1981). This reaction is furthermore reversible: if previtamin D_3 stores become depleted, tachysterol$_3$ and lumisterol$_3$ are photoisomerized back into previtamin D_3 (Holick et al., 1981). Furthermore, when vitamin D_3 itself is exposed to UV light it is photoisomerized into 5,6-transvitamin D_3, suprasterol I, and suprasterol II (Webb et al., 1989). This reaction occurs in at least human skin, and it also occurs in serum should any UV light penetrate into the bloodstream (Webb et al., 1989). Therefore, there is the possibility that this not only affects photobiosynthetically produced vitamin D_3, but it also affects diet-derived vitamin D (Webb et al., 1989). 5,6-Transvitamin D_3 is hydroxylated to 25-hydroxy-5,6-trans-vitamin D_3, a vitamin D_3 analog, but there remains the possibility of as yet unknown biological roles for suprasterol I and suprasterol II (Webb et al., 1989). If Bearded Dragons possess receptors for lumisterol$_3$, suprasterol I, or suprasterol II in their skin or elsewhere in their bodies, concentrations of these compounds could provide a negative feedback mechanism that Bearded Dragons could use to indirectly detect UV

light and behaviorally respond to their photic environment (vis a vis UV light) in an adaptive manner. This is speculative since, to our knowledge, no receptors for these compounds are known to exist. However, their existence in Bearded Dragons or other lepidosaurs is not outside the realm of possibility.

Nevertheless, these receptor-based hypotheses seem far less likely mechanisms than the use of the parietal eye or the eyes proper to detect UV light irradiance. This is because in adult female Bearded Dragons no statistically significant decrease was found in circulating blood concentrations of either $25(OH)D_3$ or $1,25(OH)_2D_3$ after 83 days of UV light deprivation and being fed a diet low in vitamin D_3 (Oonincx et al., 2013). Admittedly, over the course of this same study, average blood total calcium concentration decreased, average total blood potassium concentration decreased, and average blood uric acid concentration increased to a statistically significant degree (Oonincx et al., 2013). However, a detection of any or all of these changes is still not a very plausible mechanism for UV light irradiance detection. This is because after 83 days average blood total calcium concentration only decreased by approximately 22%, average total blood potassium concentration decreased by approximately 61%, and average blood uric acid concentration increased by only approximately 36% (Oonincx et al., 2013). Therefore, in Bearded Dragons, the half-lives of these changes in blood chemistry are so long that one basking session or one experience in a UV light irradiance gradient apparatus probably causes negligible change in these parameters.

It is noteworthy that we found only a statistically significant difference in the chosen irradiation for UVA and for combined UVA+UVB, but not for UVB when considered alone without UVA. This suggests that whatever mechanism it is that Bearded Dragons are using to detect their UV light exposure level is sensitive to UVA but not to UVB. This cannot, however, be conclusively verified without biochemical or electrophysiological studies on the sensitivities of various Bearded Dragon physiological processes to different wavelengths of light.

UV and temperature.—The fact that animals in the cold portion of the apparatus experienced higher UV irradiances on average than animals in the hot portion of the apparatus has one of two possible explanations. One is that when Bearded Dragons are at lower temperatures, they choose higher UV light irradiances than animals at higher temperatures. The other is that Bearded Dragons under higher UV light irradiances choose cooler temperatures than Bearded Dragons under lower UV light irradiances. In other words, the choice is driven either by an animal's body temperature, or by the UV light irradiance it is experiencing.

If it is the former (i.e., thermal choice alters UV exposure), this may be because Bearded Dragons' dorsal surfaces darken in response to lower temperatures (de Velasco and Tattersall, 2008; Smith et al., 2016b). In theory, this should decrease the penetration of UV light through the integument into vulnerable tissues deeper in the body, and thus make Bearded Dragons more resistant to UV-induced damage. This would perhaps negate their need to regulate their UV exposure levels to a lower set point than the levels potentially available in the apparatus. However, Bearded Dragon skin reflectivity in the 'UV-visible' portion of the photic spectrum (300–700 nm) actually increases at higher temperatures (de Velasco and Tattersall, 2008; Smith et al., 2016b). If looked at in a simplistic fashion, the hypothesis that the animals were choosing cooler temperatures when under higher UV irradiance as a protective measure against higher UV irradiance only makes sense if reflectivity in the UV portion of the photic spectrum increases at lower temperatures, and it does not make sense if it instead increases at higher temperatures. However, the darkening of the skin at cooler temperatures is caused by increased dispersion of the melanosomes in the dermis (Taylor and Hadley, 1970; Sherbrooke et al., 1994). Melanin absorbs UV radiation, and thus prevents damage to more sensitive structures underneath (Rouzaud et al., 2005). Therefore, somewhat counterintuitively, moving to a cooler location and thus absorbing more UV radiation may actually be a more adaptive response to higher UV irradiance, compared to moving to a hotter location and thus increasing skin reflectivity of UV light and protecting against damage. During physiological darkening, the melanosomes move up through the dendritic processes of the melanophores and into the uppermost layer of the dermis (Taylor and Hadley, 1970). The upper layer of the dermis of at least the Silkback phenotype is known to be reduced in comparison to the wild type (Di-Poï and Milinkovitch, 2016). Therefore, if the difference in UV light irradiances chosen by 'hot' and 'cold' animals respectively is due to the effect of temperature on melanosome aggregation or dispersion in the dermis, one might plausibly expect to see a statistical interaction with phenotype. However, our statistical analysis failed to find any such interaction effect. At the very least, this implies that there is no feedback mechanism between melanosome aggregation and behavioral response to UV light. If there were such a feedback mechanism, one would expect to see a difference in the relationship between temperature choice and UV light irradiance choice between the phenotypes.

If temperature does indeed alter UV-related behavioral photoregulatory decisions, as is posited by this first hypothesis, then there is also another way of explaining this behavior in adaptive terms. The relationship between floor temperature and experienced UV conditions is not necessarily a result of behavior that has evolved to cause dispersal of melanosomes and thus protect the animal from UV-related tissue damage, as is posited above. Instead, the fact that 'cold' Bearded Dragons on average experienced higher UV irradiances than 'hot' Bearded Dragons could plausibly be due to 'confusion.' In the wild, heliothermic lepidosaurs, like Bearded Dragons, would usually experience higher temperatures in conjunction with higher light irradiances, and lower temperatures in conjunction with lower light irradiances. If they have therefore evolved to use light irradiance as a proximate cue to assist in precise thermoregulation, they may exhibit phototaxis when engaging in thermoregulatory behavior. An animal circling the perimeter of the apparatus, as they were sometimes observed to do, will eventually equilibrate in temperature with the 'cold' portion of the apparatus. It will plausibly then attempt to behaviorally thermoregulate in order to raise its body temperature back to a more physiologically optimum temperature. This may result in the animal spending more time in the higher irradiance areas of the apparatus than would be expected by chance, due to instinctive phototaxis. As mentioned previously, Bearded Dragons likely have UV-sensitive retinal

photoreceptors (Barbour et al., 2002; Yewers et al., 2015; Cronin and Bok, 2016). These receptors, or other, less conventional photoreceptors (e.g., hypothetically, cutaneous photoreceptors), could serve as a proximate mechanism to facilitate this phototaxis.

The exception to the rule that Bearded Dragons in the wild would encounter high UV irradiances in concert with high temperatures and low UV irradiances in concert with low temperatures would be in the early morning of cloudless days, when the sun is bright and basking surfaces are still cold. Therefore, a pre-existing early morning basking behavior may be triggered when an animal equilibrates in temperature with the 'cold' portion of the apparatus, triggering it to seek higher UV irradiances. This still falls under the umbrella hypothesis that the Bearded Dragons are using UV irradiance as a proximate cue for thermoregulation. There is some somewhat equivocal evidence that Bearded Dragons indeed exhibit phototaxis independently from thermotaxis (Khan et al., 2010); this would support this hypothesis.

The latter hypothesis, that higher UV light irradiances cause Bearded Dragons to choose cooler temperatures (i.e., UV choice alters thermoregulatory decisions), has an unclear physiological mechanism. There are some limited data to suggest that heliothermic lepidosaurs will bask less often under lights that produce UV light than under lights that do not (Dickinson and Fa, 1997). This observation could perhaps be congruent with increased UV light irradiance lowering thermal preference. However, the study in question possessed the confounds of the animals being able to choose between the non-UV light-producing and UV light-producing light, and of temperature being different under the two bulbs (Dickinson and Fa, 1997). In light of all of this, the driving force behind this difference in UV light irradiances experienced by hot and cold animals remains unclear.

Conclusions.—Remaining questions regarding the proximate influences on behavior in response to UV light in lepidosaurs can be best answered by taking a comparative approach. Snakes do not possess parietal eyes (Bradshaw and Holzapfel, 2007), and neither do some other lepidosaurs, which are exceptions to the rule (Gundy and Wurst, 1976a). Partially scaleless Texas Rat Snakes (*Pantherophis obsoletus lindheimeri*) mostly lacking scales on the dorsal surface of the body have been produced in captivity (Bechtel and Bechtel, 1991). This trait has a genetic origin that allows replicable production of this phenotype via captive breeding (Bechtel and Bechtel, 1991). Future studies looking at these animals' response to UV light in comparison to that of wild type Texas Rat Snakes would help identify whether or not the parietal eye is the sole facilitator of UV-related photoregulation in lepidosaurs. To our knowledge all of the species of lepidosaur whose behavior in response to UV light has heretofore been examined (Ferguson et al., 2003, 2013, 2015b) either have been confirmed to possess parietal eyes or can be said to plausibly possess parietal eyes based on the presence of the parietal eye in a congener (Gundy and Wurst, 1976a, 1976b), although those of Panther Chameleons (*Furcifer pardalis*) are somewhat rudimentary (Gundy and Wurst, 1976a). It has been demonstrated that the eyes proper of some lizards can see into the UV portion of the photic spectrum (Yewers et al., 2015). Future research would do well to compare behavior in a UV irradiance gradient apparatus of some of those lepidosaur species that lack parietal eyes to a statistical null model. This would help elucidate the mechanism or mechanisms at play that allow lepidosaurs to photoregulate in response to UV light. Indeed, the results of one behavioral study suggest Common Wall Lizards (*Podarcis muralis*) may have photoreceptors in their skin (Tosini and Avery, 1996). Furthermore, experiments suggest some species of sea snake in the genus *Aipysurus* have photoreceptors on their tails, whereas other species of sea snake lack them (Zimmerman and Heatwole, 1990; Crowe-Riddell et al., 2019). One species, the Arafura Sea Snake (*Aipysurus tenuis*), may have photoreceptors in the skin elsewhere on its body as well (Crowe-Riddell et al., 2019). Studies of lepidosaurs lacking parietal eyes would therefore help determine the degree to which parietal eyes are necessary for lepidosaur photoregulation.

With regard to the relationship between temperature and chosen UV light irradiance, future studies would do well to separately manipulate animal body temperature and UV light irradiance, and measure the effect that each has on chosen UV light irradiation and chosen temperature. This would provide answers as to what drives the increase in chosen UV light irradiances associated with the decreases in chosen temperature observed in the present study.

DATA ACCESSIBILITY

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