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Effects of food and thermal regimes on body condition indices and skin colouration in corn snakes

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Abstract: One of the open problems in evolutionary ecology is signal reliability. While the view that signals need to be costly to be honest has attracted most attention, this type of signals may apply only to some contexts. Also, different views exist about the nature of costs involved in signal honesty, and the classification and examination of these costs remains controversial. Pigmentary-based colouration of animal integuments has received considerable attention among researchers seeking to explain what maintains the honesty of visual signals, but support for existing hypotheses is far from conclusive. Here we use a whole-animal approach and consider a distinct time scale, the period of juvenile growth, to test the effects of different feeding and thermal regimes on different physical parameters and skin colouration in corn snakes *Pantherophis guttatus*. Postnatal body length growth rate and body mass index (BMI) were sensitive to the thermal, but not to the food regime. The length of intervals between skin shedding was shorter and the short-wavelength reflectance of dorsal skin was higher for snakes receiving food more frequently and having an uninterrupted possibility to thermoregulate. This work suggests that if juvenile corn snakes are environmentally constrained, their preferred life history strategy is to grow at faster rates. The study adds to the growing body of evidence in that BMI may not accurately reflect individual condition in reptiles. Instead, this study demonstrates that the length of shedding intervals and the short-wavelength component of skin colouration may be used in this animal system as proxies of the individual's condition or quality, possibly reflecting exposure to environmental stress or an ability to cope with it.

Key words: corn snakes; body condition; colouration

Introduction

The cause of evolutionary stability of animal communication, be it homo- or hetero-specific, is a lively topic in evolutionary ecology (Maynard Smith & Harper 2003; Stevens 2013; Summers et al. 2015). One of the recurrent problems is signal reliability, namely how informational traits can persist if the interests of informers and perceivers diverge (Searcy & Nowicki 2005; Ruxton & Schaefer 2011).

While the view that signals need to be costly to be honest has attracted most attention, it has been realized that this type of signals may apply only to some contexts (Stevens 2013). One context is when signals inform about the signaller's or environmental quality (Cotton et al. 2004). Currently, different views exist about the nature of costs involved in signal honesty (Zahavi 1975; Grafen 1990; Maynard Smith & Harper 2003; Számadó 2011). Two main costs were proposed to explain the stability of honest signals; the strategic and efficacy costs (Guilford & Dawkins 1991; Maynard Smith & Harper 2003). While strategic costs represent costs needed to produce, develop or maintain the signal, efficacy costs involve costs to effectively transmit the signal to perceivers (Searcy & Nowicki 2005).

Still, other authors claim that it is more appropriate to split costs into realized, which are needed for signal production and transmission, and into potential, which entail, e.g., costs of cheating for low quality individuals (Számadó 2011; Higham 2014). Nonetheless, the classification and examination of the costs remains controversial (e.g., Polnaszek & Stephens 2015; Számadó & Penn 2015). For example, even though “index” signals are commonly viewed separately from strategic signals (Maynard Smith & Harper 2003; Számadó & Penn 2015), some authors argue that “index” signals are just a variant of Zahavian honest signals with “index” physical constraints representing developmental costs (Searcy & Nowicki 2005; Biernaskie et al. 2014).

Red, orange or yellow carotenoid-based colouration of animal integuments is used in various communication contexts such as mate selection, male-male competition, or predator avoidance (Blount & McGraw 2008), and it has received considerable attention among researchers seeking to explain what maintains the honesty of visual signals (Lozano 1994; Svensson & Wong 2011; Simons et al. 2012). Simple mechanisms were initially suggested how carotenoids could be involved in honest signalling, including carotenoid environmental scarcity or increased conspicuousness of signallers

to predators (e.g., Endler 1983). Currently, carotenoid-based signals are mainly assumed to reflect the levels of non-integumentary antioxidative carotenoids (Lozano 1994), the level of non-carotenoid antioxidants (Hartley & Kennedy 2004), or both the level of antioxidative carotenoids and non-carotenoids (von Schantz et al. 1999). The support for one or another of these hypotheses is not conclusive (Constantini & Møller 2008; Svensson & Wong 2011; Simons et al. 2012).

An under-explored area of research into red visual signals is the role of non-carotenoid pigments and the multicomponent nature of carotenoid-based signals (Svensson & Wong 2011). For example, in fishes or reptiles, red, orange and yellow colouration is not created solely by carotenoid pigments, but also by pterins, which can be in contrast to carotenoids synthesized by signallers (e.g., Grether et al. 2001; Steffen & McGraw 2007). Interestingly, the levels of pterins and carotenoids correlate positively in male guppies *Poecilia reticulata* Peters, 1859, suggesting that pterins do not function as a “cheaper” way to produce carotenoid-based signals (Grether et al. 2001). In fact, despite their internal origin, pterin-based visual signals may also act as honest signals (Olsson et al. 2013), e.g., owing to their antioxidative potential (McGraw 2005). Finally, it has been shown that the expression of carotenoid-based signals may vary dramatically with respect to their non-pigment-based UV light component (e.g., San-Jose et al. 2013; Kottler et al. 2014). Therefore, it seems imperative to examine visual signals without concentrating on a single pigment, condition index, or part of the body, but to use a whole-animal approach and consider different time scales (Constantini & Møller 2008; Svensson & Wong 2011; Wilgers & Hebets 2015).

In this study, corn snakes *Pantherophis guttatus* (L., 1766) (Colubridae) were subjected to different food and thermal regimes in order to see the effects of two environmental constraints on different physical indices and skin colouration. If colouration is condition-dependent, as predicted by the Zahavian honest signalling hypothesis (Cotton et al. 2004), irrespective of signalling contexts, we predicted that corn snakes reared under restrictive environmental conditions should show impaired body condition and reduced expression of skin colouration.

Material and methods

Study system

The corn snake *Pantherophis guttatus* is a medium-sized colubrid snake distributed in southeastern North America. It is a diurnal, non-venomous constrictor species, which can be found in various habitats from wood edges to agricultural fields (Love & Love 2012). It is a popular species for herpetoculture and increasingly also for research into the causes and mechanisms of colour variation (e.g., Ullate-Agote et al. 2015). Long-wavelength (red, orange, yellow) colouration of corn snakes, similarly to other reptiles, is presumably based on the mixture of pterin and carotenoid pigments contained within erythrophores/xanthophores (Bechtel 1978; Bechtel 1995; Kikuchi et al. 2014; Cuervo et al.

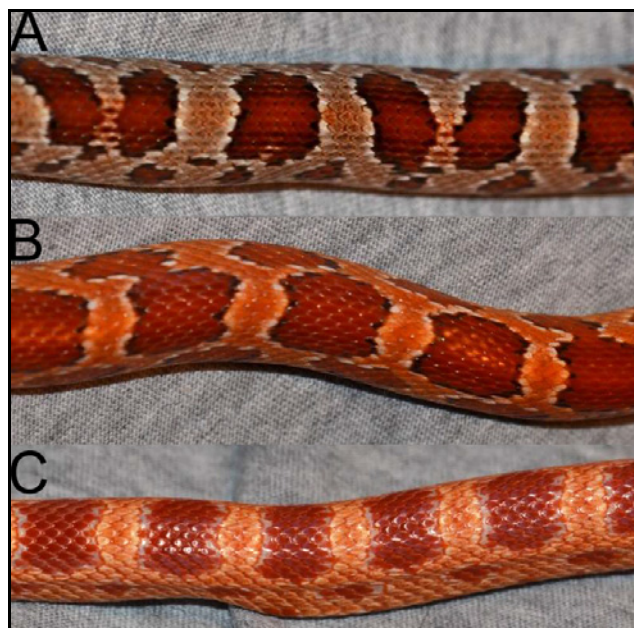


Fig. 1. Colour variants of corn snakes *Pantherophis guttatus* used in this study. Normal (Classic) (A), hypomelanistic (Hypo A) (B), and amelanistic (Amel) (C) variants.

2016). Dietary carotenoids are particularly thought to cause a distinct yellow skin colouration of corn snakes, accumulating progressively from the head towards the tail as snakes mature (Bechtel 1995; Love & Love 2012).

The study was conducted between February and December 2013, using 19 juvenile corn snakes originated from three different broods. Each brood, obtained from a different snake breeder based in Slovakia, was represented by a different colour variant: ten individuals (8 males and 2 females) of the normal (Classic) variant, four individuals (4 females) of the hypomelanistic (Hypo A) variant, and five individuals (3 males and 2 females) of the amelanistic (Amel) variant (Fig. 1). The reduction of melanin pigment in both the amelanistic and the hypomelanistic variant is known to be based on autosomal recessive inheritance of independent genes (Bechtel 1995; Love & Love 2012).

Snakes were obtained when they were three to four months of age. Morphological and skin spectral reflectance data for these individuals were subsequently collected during ten months, i.e., snakes were 13 to 14 months of age at the end of study. Each snake was kept in a transparent plastic container (55 × 39 × 18 cm), containing a newspaper substrate, two hides made of cardboard boxes and placed in the warm and cool corners, a bowl with water, a climbing branch, and the thermometer and hygrometer. Humidity was maintained at 50–60%, but it was increased to 70–80% before skin shedding. Containers were ventilated through holes drilled at the sides and the lid of containers. Temperature was maintained by the thermostat and the 32W heating foil (35 °C), which covered 1/3 of the container's floor. Corn snakes were fed with pre-killed thawed nestling rodents (ca. 2–9 g).

Experimental manipulation

Corn snakes were reared under two food and two thermal regimes, considering that food and temperature represent two major environmental factors for ectothermic animals (Bajer et al. 2012). Snakes were fed every five and seven

days in the high food and low food treatments, respectively, following pre-established feeding protocols (e.g., Love & Love 2012). The length of the feeding intervals was chosen as a compromise to reveal an effect of food abundance on body condition indices while avoiding negative effects of food restriction on health and ontogenetic development of juvenile corn snakes. With respect to the thermal conditions, snakes in the optimal thermal group were kept under optimal thermal conditions with continuous access to the cool corner (25°C), the warm corner (27°C), and the heating foil (35°C). Similarly as for feeding, we followed recommendations on thermal conditions in this species (e.g., Love & Love 2012). In contrast, snakes in the suboptimal thermal treatment were kept under ambient temperature (21–25°C) and had access to the heating foil daily only between 18:00 and 22:00 hrs. We used a split-plot design to assign corn snakes into the two treatments. Consequently, 5, 5, 5 and 4 individual corn snakes were assigned to suboptimal temperature–low food, suboptimal temperature–high food, optimal temperature–low food, and optimal temperature–high food treatments, respectively. Snakes were assigned into the four treatments randomly with respect to their melanin-based colour variant.

Morphological, behavioural and colour measurements

In order to obtain multiple indices of physical condition, we measured body length in terms of snout–vent length (SVL, to the nearest mm) and body mass (to the nearest g) immediately after each skin shedding. In total, depending on the length of the shedding interval, 8 to 12 measurements for each of the two parameters were obtained for each snake during the study period. Subsequently, body mass and SVL were used to determine body mass index (BMI, see below). In turn, SVL increases (to the nearest mm) were calculated between successive skin-shedding events to establish body length growth rates. Finally, the length of the skin-shedding interval (in days) was used as an additional measure of physical condition because it is assumed to depend on food abundance (e.g., Vitt & Caldwell 2009).

Thermoregulatory behaviour was studied in terms of the latency time taking to reach the heating foil and the basking time at the heating foil, respectively. The latency time was recorded during the first hour after switching the heating foil and the basking time was recorded during three hours after snakes reached the heating foil. Thermoregulatory behaviour was studied only for snakes in the suboptimal thermal treatment because it was not possible to study this parameter for the optimal thermal treatment with the heating foil remaining permanently switched on. In total, latency time and basking time were recorded during 228 and 13 days, respectively, obtaining 1138 and 103 measurements, respectively.

Skin colour measurements were collected for each snake and colour patch four times (February, April, October, and December), with each measurement taking place within one week after skin shedding. Skin reflectance was measured in the proximal part (first third) of the body for two colour patches – red saddles (blotches) and the yellow ground. Three to six measurements were collected for each colour patch and colour measurement event. The spectrometer was calibrated to a white standard (OceanOptics WS-1-SS) and the dark current (light source turned-off and optic fibre blocked) for each individual. Spectral reflectance at 300–700 nm was measured with an OceanOptics USB2000+ spectrometer, a DT-Mini-2GS light source, and a QR400-

7-SR-BX bifurcated optic fibre. The spectral reflectance of each skin patch was assessed with the probe fixed in a RHP-1 holder at an angle of 45° to the skin surface.

Data analysis

Linear mixed models (LMM) were used to examine the longitudinal effects of experimental manipulation on body condition parameters, thermoregulatory behaviour, and skin colouration. The length of the shedding interval, basking and latency time, and skin colouration parameters (see below) were examined as explicit response variables (i.e., variables were not conditional on confounders). In turn, skeletal growth rate and BMI were examined employing a covariate-adjusted approach as a regression of SVL increase on the length of the shedding interval and as a regression of body mass on SVL, respectively. That is to say, skeletal growth rate and BMI refer to body length increases adjusted for the length of the growth period, i.e., the length of the shedding interval, and body mass adjusted for body size, i.e., SVL, respectively.

The following variables were log-transformed to satisfy the linearity assumption of LMM: SVL, SVL increase, body mass, and latency and basking time. Moreover, all continuous response variables, fixed covariates and random terms were centred and z-transformed (scaled) to allow interpretability of main effects that were involved in interaction terms and to allow comparisons of parameter estimates within models and between studies (Schielzeth 2010). The exceptions were principal components of spectral reflectance that were obtained by PCA as centred and scaled. All LMMs were constructed including random intercepts to address the random effects of snake identity, colour variant/brood identity, and snake sex. In addition, the effect of random slopes was considered in LMMs to address the effect of between-group variation in response variables (Schielzeth & Forstmeier 2009). Specifically, we accounted for between-individual variation in the slope between response variables and the measurement sequence and for between-brood variation in the slope between the length of the shedding interval and initial SVL (i.e., SVL at the beginning of the experiment). Modelling for each response variable started with choosing an optimal random-effects structure for the saturated model while examining the diagnostic plots to assess the model's assumptions (Zuur et al. 2009). In order to avoid convergence problems and to satisfy the homogeneity assumption, it was necessary to consider a cubic transformation of a random variable for LMM on growth rate and to use multiple random-effect terms for LMM on the length of the shedding interval. Finally, an optimal fixed-effects structure was established for each model, eliminating the terms that did not importantly improve the model's fit and following the procedure by Zuur et al. (2009). LMMs were conducted with the lme4 package 1.1-12 and an lmer function (Bates et al. 2015) within the R software 3.2.1 (R Core Team 2016). Fixed-effect terms were examined with Type 3 and post-hoc tests with the lmerTest package 2.0-30 (Kuznetsova et al. 2016) and the multcomp package 1.4-4 (Hothorn et al. 2008). While Tables 1–3 show model parameter estimates, Type 3 tests for fixed-effect terms are reported in the Results section.

PCA was used to capture variation in spectral reflectance at wavelengths from 300 to 700 nm for red and yellow skin colour patches (Endler 1990; Cuthill et al. 1999). This statistical method extracts multiple independent components corresponding to brightness, saturation and hue aspects of a patch's colour (Endler 1990), and it is use-

Table 1. Linear mixed models (LMM) examining the effects of thermal and food regimes on selected body condition indices in juvenile corn snakes *Pantherophis guttatus*. All continuous terms were centred and scaled. Parameter estimates for fixed categorical terms are shown for the high food and optimal thermal groups. Continuous random terms (random slopes) are shown in italics with the suffix referring to the categorical random term (random intercept) at which the slopes were assessed. The food regime, the thermal regime, and their interaction were considered for each model, but dropped if they significantly did not improve the model's fit.

Fixed effects Parameter	Estimate	SE	df	<i>t</i>	<i>P</i>	Random effects Parameter	SD
(a) Skin-shedding interval							
Intercept	0.11	0.19	6.97	0.61	0.560	Snake ID	0.04
Thermal regime (T)	0.42	0.22	122.72	1.90	0.055	<i>Date_Snake ID</i>	0.15
Food regime (F)	-0.37	0.19	120.27	-1.94	0.060	Brood ID	0.26
T × F	-0.63	0.29	128.24	-2.16	0.032	<i>Date_Brood ID</i>	0.06
(b) log-SVL increase							
Intercept	0.32	0.17	16.28	1.89	0.077	Snake ID	0.64
Thermal regime (T)	-0.47	0.25	15.76	-1.90	0.076	<i>Date^2_Snake ID</i>	0.85
Skin-shedding interval (S)	-0.14	0.11	139.60	-1.27	0.206		
T × S	0.31	0.15	144.58	2.07	0.040		
(c) log-Body mass							
Intercept	-0.11	0.06	8.31	-1.90	0.092	Snake ID	0.14
Thermal regime (T)	-0.17	0.07	14.75	-2.26	0.039	<i>Date_Snake ID</i>	0.06
log-SVL (SVL)	0.57	0.05	16.13	11.64	< 0.001	Brood ID	0.09
T × SVL	-0.23	0.04	15.22	-5.21	< 0.001	<i>Date_Brood ID</i>	0.12
						Sex	0.15
						<i>Date_Sex</i>	0.16

ful for spectral examination of both unimodal and multimodal colours (e.g., Martín & López 2009). The spectral data of each reading was grouped in 10 nm bins and mean reflectance was calculated for all (3–6) readings per colour patch, individual and measurement event (e.g., Václav et al. 2007). PCA was conducted with Statistica 7 (StatSoft, Tulsa, OK, USA), setting the lowest limit for eigenvalues to 2 and using the Varimax normalization as the rotation method.

Results

Skin-shedding rate, growth rate, and body mass index

The length of skin-shedding interval was generally shorter for snakes in the high food than the low food treatment ($F_{1,73.74} = 21.38$, $P < 0.001$). Yet, the interaction effect of food and thermal treatments revealed that overall skin-shedding intervals were shortest for snakes under both high food and optimal thermal treatments (thermal treatment, $F_{1,83.79} = 0.49$, $P = 0.487$; food treatment × thermal treatment, $F_{1,128.24} = 4.68$, $P = 0.032$; Table 1a; Fig. 2).

Snout-to-vent length (SVL) increase was affected by the interaction effect of the thermal treatment and the length of skin-shedding interval (thermal treatment × skin-shedding interval, $F_{1,144.58} = 4.29$, $P = 0.040$; Table 1b; Fig. 3). Specifically, while for the optimal thermal treatment SVL increase tended to increase with the length of skin-shedding interval (estimate ± SE = 0.18 ± 0.11 , $t_{148.17} = 1.66$, $P = 0.099$), SVL increase was not associated with the length of skin-shedding interval for the suboptimal thermal treatment (estimate ± SE = -0.14 ± 0.11 , $t_{139.60} = -1.27$, $P = 0.206$). Importantly, while SVL increase for snakes in the suboptimal thermal treatment was generally higher

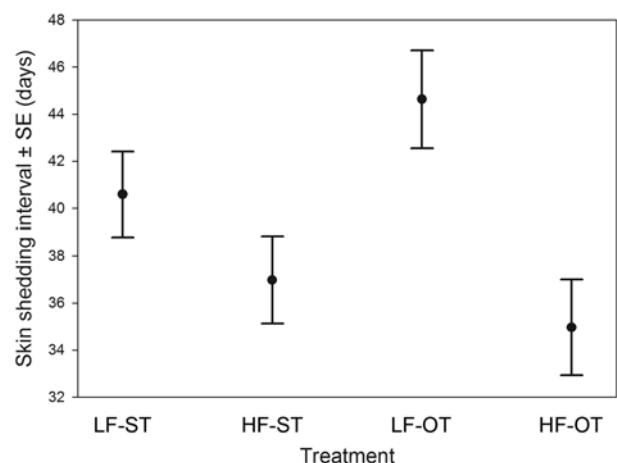


Fig. 2. The effect of food and thermal regimes on the length of the skin-shedding interval in juvenile corn snakes *Pantherophis guttatus*. The abbreviations refer to treatment combinations: sub-optimal thermal (ST), optimal thermal (OT), low food (LF) and high food (HF) treatments. The response variable was centred and scaled for analysis, but it is graphed on the original scale (days) for illustrative purposes.

than in the optimal thermal treatment, this feature was masked by high SVL increase shown by snakes in the optimal thermal treatment with the longest skin-shedding intervals (thermal treatment, $F_{1,15.76} = 3.61$, $P = 0.076$; skin-shedding interval, $F_{1,144.58} = 0.07$, $P = 0.798$; Table 1b; Fig. 3).

Compared to snakes in the optimal thermal treatment, snakes were relatively heavier for their SVL when exposed to the treatment without continuous access to the heat element (SVL, $F_{1,16.91} = 105.94$, $P < 0.001$; thermal treatment, $F_{1,14.75} = 5.12$, $P = 0.039$; SVL × thermal treatment, $F_{1,15.22} = 27.16$, $P < 0.001$; Table 1c; Fig. 4).

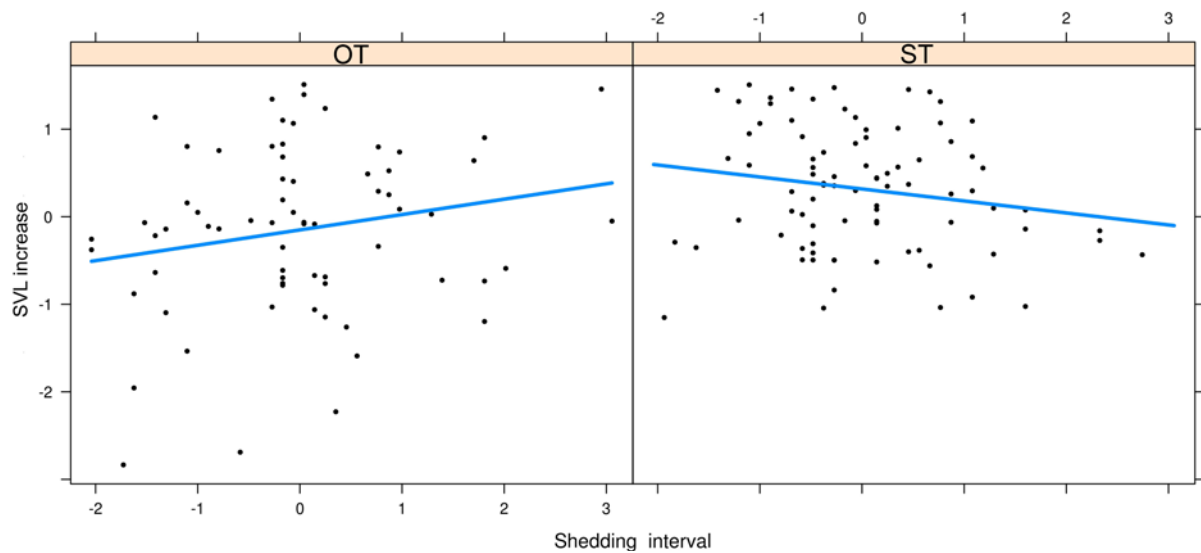


Fig. 3. The effect of the thermal regime on skeletal growth rate in juvenile corn snakes *Pantherophis guttatus*. The interaction effect of the thermal regime and the length of the skin-shedding interval points out that growth rate tended to increase with the length of the shedding interval for snakes in the optimal thermal treatment (OT), but it was dissociated with skin-shedding rate for snakes in the suboptimal thermal treatment (ST). SVL increase was log-transformed to conform to the linearity assumption.

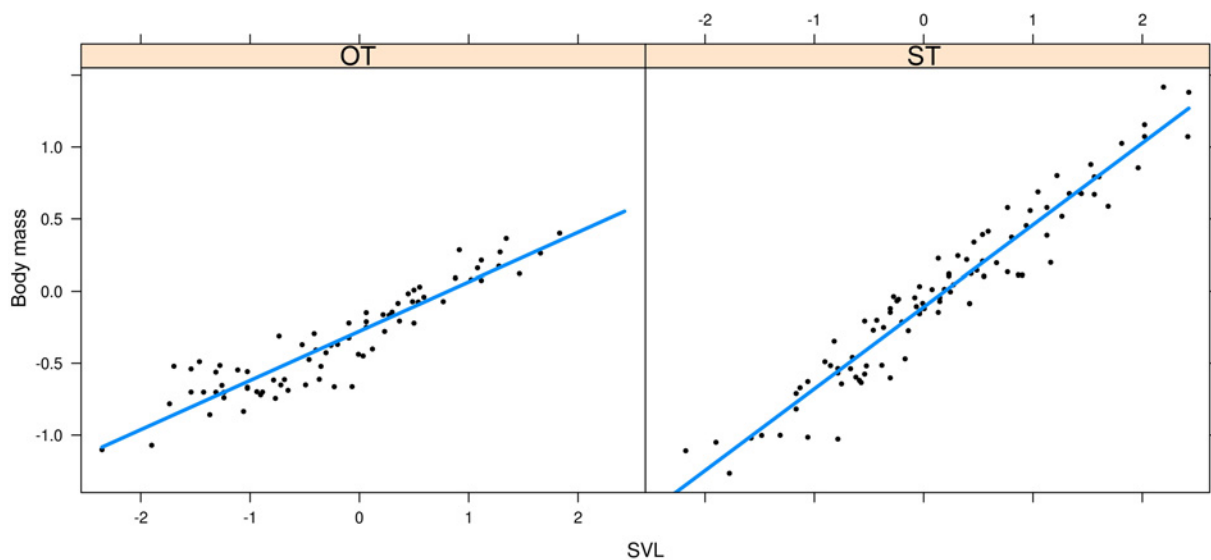


Fig. 4. The effect of the thermal regime on body mass in juvenile corn snakes *Pantherophis guttatus*. The interaction effect of the thermal regime and SVL shows that body mass in snakes in the suboptimal thermal treatment (ST) increased with body length more sharply than in snakes in the optimal thermal treatment (OT). BMI and SVL were log-transformed for analysis to conform to the linearity assumption.

Thermoregulatory behaviour

Considering only the snakes without continuous access to the heat element, individuals receiving food at longer intervals spent significantly longer time basking on the heat element compared to the snakes receiving food at shorter intervals ($F_{1,56.91} = 85.08$, $P < 0.001$; Table 2a). Snakes receiving food at longer intervals showed higher latency to reach the heat element compared to more frequently fed snakes (food treatment, $F_{1,119.59} = 122.77$, $P < 0.001$; Table 2b).

Skin colouration

PCA identified three principal components (PCs), which described 97.54 and 96.65% of variation in spec-

tral reflectance of red saddles and the yellow ground colour, respectively (Figs 5A, B). Examining the factor loadings for individual PCs, each PC appears to inform about variation in the relative amount of reflectance for specific sections of the spectrum. Namely, for the red patches, PC1, 2, and 3 inform about variation in the relative amount of reflectance for the wavelengths of 300–430 nm, 560–700 nm and 440–550 nm, respectively (Fig. 5A). In turn, for the yellow patches, PC1, 2 and 3 inform about variation in the amount of reflectance for the wavelengths of 340–550 nm, 560–700 nm and 300–330 nm, respectively (Fig. 5B).

While controlling for random effects, food and thermal treatments did not have an effect on PC2 and

Table 2. Linear mixed models (LMM) examining the effects of thermal and food regimes on selected thermoregulatory behaviours in juvenile corn snakes *Pantherophis guttatus*. The response variables for the LMM models are the log-time to reach the heat element and the log-time spent on basking at the heat element, respectively. All continuous terms were centred and scaled. Parameter estimates for fixed categorical terms are shown for the high food and optimal thermal groups. Random continuous terms (random slopes) are shown in *italics*, with the suffix referring to the random categorical term (random intercept) at which the random slopes were assessed. The food regime, the thermal regime, and their interaction were considered for each model, but dropped if they significantly did not improve the model's fit.

Fixed effects Parameter	Estimate	SE	df	<i>t</i>	<i>P</i>	Random effects Parameter	SD
(a) Basking time							
Intercept	4.81	0.06	59.70	81.62	< 0.001	Snake ID	0.25
Food regime	-0.83	0.09	57.52	-9.22	< 0.001	<i>Date_Snake ID</i>	0.01
(b) Latency to bask							
Intercept	2.71	0.04	82.14	68.69	< 0.001	Snake ID	0.03
Food regime	-0.63	0.06	119.89	-11.08	< 0.001	<i>Date_Snake ID</i>	0.09

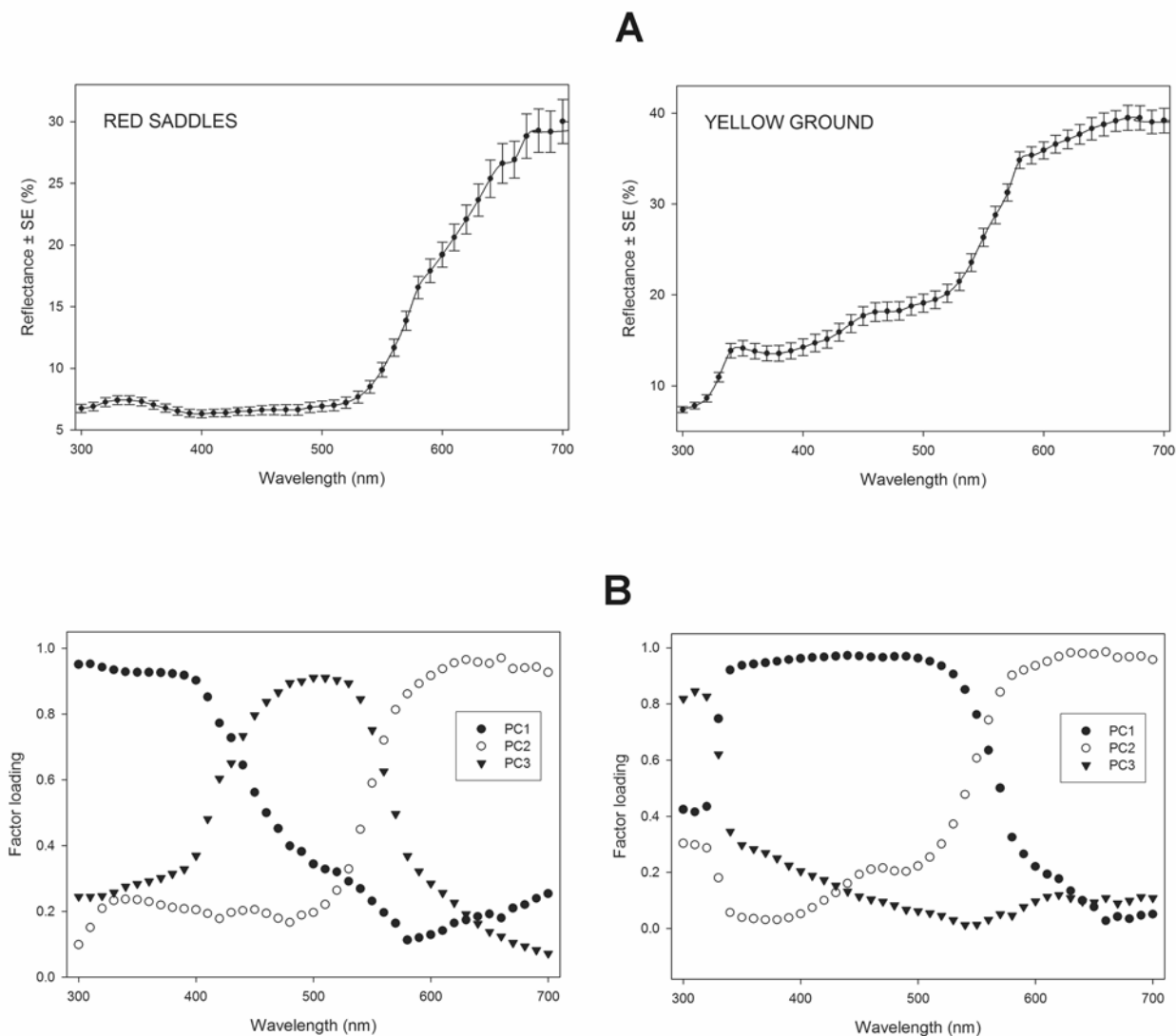


Fig. 5. Spectral reflectance of two dorsal skin colour patches in juvenile corn snakes *Pantherophis guttatus*. A – Skin reflectance of red saddles and the yellow ground. B – The relationship between factor loadings and wavelengths for skin reflectances of the two colour patches. Factor loadings for each colour patch were extracted with PCA involving reflectances at the 300–700 nm range. Eigenvalues corresponding to PC1, 2, and 3, respectively, are 27.84, 8.31, and 3.84 for red saddles and 26.89, 10.70, and 2.04 for the yellow ground, respectively.

PC3 of the red patches and on PC1 and PC2 of the yellow patches (Table 3). However, our analyses consis-

tently point to an interaction effect of food and thermal treatments on skin reflectance at short wavelengths, i.e.,

Table 3. Linear mixed models (LMM) examining the effects of thermal and food regimes on dorsal skin colouration (red saddles and the yellow ground colour) in juvenile corn snakes *Pantherophis guttatus*. The response variables for the LMM models are principal components (PCs) that were extracted from spectral reflectance – data (300–700 nm) and inform about different colour qualities. All continuous terms were centred and scaled. Parameter estimates for fixed categorical terms are shown for the high food and optimal thermal groups. Random continuous terms (random slopes) are shown in italics, with the suffix referring to the random categorical term (random intercept) at which the random slopes were assessed. The food regime, the thermal regime, and their interaction were considered for each model, but dropped if they significantly did not improve the model's fit.

Fixed effects Parameter	Estimate	SE	df	<i>t</i>	<i>P</i>	Random effects Parameter	SD
PC1 – red saddles							
Intercept	0.22	0.19	13.51	1.13	0.277	Snake ID	0.26
Thermal regime (T)	–0.06	0.26	54.21	–0.23	0.822	<i>Date_Snake ID</i>	0.08
Food regime (F)	–0.28	0.25	52.74	–1.13	0.265	Brood ID	0.38
T × F	0.89	0.38	54.20	2.37	0.021	<i>Date_Brood ID</i>	0.13
						Sex	0.89
						<i>Date_Sex</i>	0.46
PC2 – red saddles							
Intercept	–0.62	0.24	25.54	–2.64	0.014	Snake ID	0.65
						<i>Date_Snake ID</i>	0.01
						Sex	0.00
						<i>Date_Sex</i>	0.68
PC3 – red saddles							
Intercept	–0.01	0.13	22.95	–0.07	0.948	Snake ID	0.47
						<i>Date_Snake ID</i>	0.08
PC1 – yellow ground							
Intercept	–0.63	0.11	5.41	–5.87	0.002	Snake ID	0.32
						<i>Date_Snake ID</i>	0.10
						Brood ID	0.42
						<i>Date_Brood ID</i>	0.08
						Sex	2.29
						<i>Date_Sex</i>	0.69
PC2 – yellow ground							
Intercept	0.13	0.25	3.17	0.51	0.641	Snake ID	0.18
						<i>Date_Snake ID</i>	0.17
						Brood ID	0.44
						<i>Date_Brood ID</i>	0.02
PC3 – yellow ground							
Intercept	0.27	0.48	1.13	0.56	0.667	Snake ID	0.31
Thermal regime (T)	–0.09	0.33	63.68	–0.27	0.789	<i>Date_Snake ID</i>	0.04
Food regime (F)	0.10	0.27	44.79	0.36	0.717	Brood ID	1.08
T × F	0.81	0.43	53.79	1.87	0.067	<i>Date_Brood ID</i>	0.43

PC1 of the red patches and PC3 of the yellow patches (PC1 – red patches, food treatment × thermal treatment, $F_{1,54.20} = 5.62$, $P = 0.021$; PC3 – yellow patches, food treatment × thermal treatment, $F_{1,53.79} = 3.49$, $P = 0.067$; Table 3). The relative amount of reflectance for the wavelengths of 300–430 nm and 300–330 nm for red and yellow patches, respectively, was highest for snakes in the high food–optimal thermal treatment (Figs 6A, B).

Discussion

Postnatal body length growth rate and body mass index (BMI) were sensitive to the thermal, but not to the food regime. The length of intervals between skin shedding was shorter for snakes receiving food more frequently and even shorter if snakes also had an uninterrupted possibility to thermoregulate freely. The effect of experiment on variation in skin spectral reflectance of two colour patches was detected for short wavelengths only. Relative spectral reflectance at these wavelengths was highest when snakes received food more frequently and had a chance to thermoregulate freely, though this

combined effect was significant at $\alpha = 0.05$ for red skin patches only.

Even though our study did not aim at identifying the mechanisms of food or temperature effects on growth rates or the length of shedding intervals, it adds to the knowledge on the evolution of life-history traits. Juvenile corn snakes that were exposed to more favourable environmental conditions did not consistently outperform other snakes with respect to skeletal growth rate, BMI, and skin-shedding rate. In fact, enhanced skeletal growth and BMI were detected for snakes reared under thermally constrained conditions. This suggests that accelerated skeletal growth may entail fitness costs in corn snakes and it is not favoured under thermally favourable conditions. Thus, our results do not lend support to the maximization hypothesis predicting the positive effect of superior environmental conditions on growth rates and other life-history traits (c.f. Bronikowski 2000).

Our findings are consistent with the idea that in some organisms it may be beneficial for juveniles to accelerate skeletal growth and fat deposition under certain socio-ecological conditions (Vitt & Caldwell 2009).

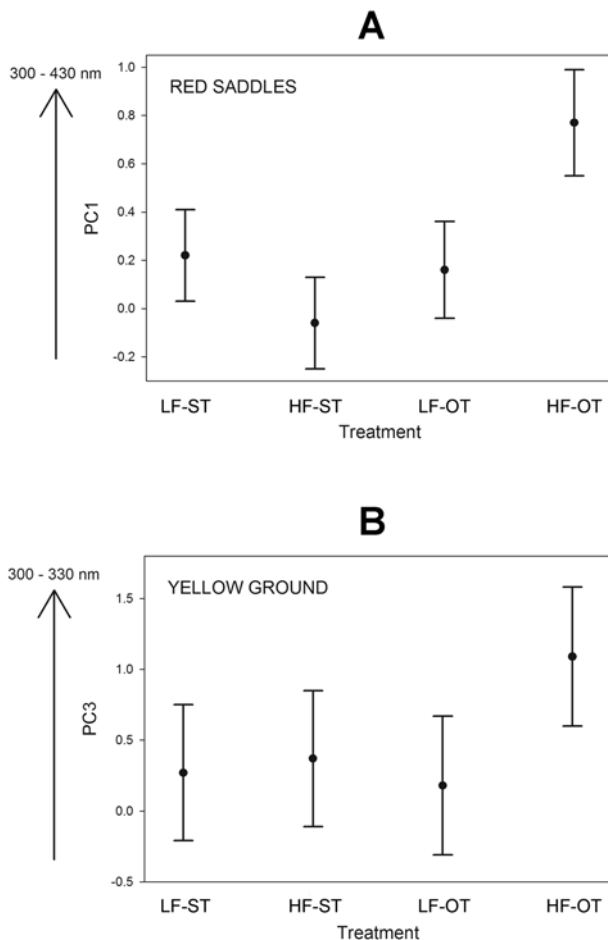


Fig. 6. The effect of food and thermal regimes on dorsal skin colouration in juvenile corn snakes *Pantherophis guttatus*. Skin colouration was analysed in terms of principal components (PCs) of spectral reflectance data, with PCs informing about different colour aspects of (A) red saddles and (B) the yellow ground. The abbreviations refer to treatment combinations: suboptimal thermal (ST), optimal thermal (OT), low food (LF) and high food (HF) treatments. Arrows indicate how PCs relate to relative reflectance for the given spectral range. Shown are parameter estimates \pm SE.

Such a life-history strategy to prioritize juvenile growth and fat deposition can be beneficial if the outcome of predatory or competitive interactions depends on body size (Werner & Gilliam 1984), if larger or heavier individuals better face environmental stress (Getty 2002), or if age at first reproduction depends on the length of favourable environmental conditions (Kozłowski & Teriokhin 1999). Importantly, this study highlights that variation in growth rates in reptiles can be attributed to temporal limitations rather than to the possibility to thermoregulate optimally.

The idea by Burkett (1966) and Smith (1976) that in snakes skeletal growth and fat accumulation rates are energetically traded-off against skin-shedding frequency is not supported by this study. While variation in both skeletal growth rate and BMI points to differential temperature-dependent food conversion efficiency, skin-shedding frequency was affected by the absolute level of food resources as well as the thermal regime.

Nonetheless, our results do not rule out the optimality hypothesis stating that somatic growth is traded-off against other life history traits (see Bronikowski 2000). Further work is necessary to address if the enhanced skeletal growth of thermally constrained juvenile corn snakes is associated with the physiological costs of increased risk of disease, decreased immunocompetence or lower muscular performance later in life (Arendt 1997; Johnsson & Bohlin 2006; Royle et al. 2006). Importantly, even without potential long-term costs, enhanced juvenile growth via altered thermoregulatory behaviour may incur short-term costs. These costs may involve physiological costs of thermal stress or a higher risk of predation mortality as a result of prolonged basking (e.g., Duellman 1978; Angilletta 2009). We propose that both short- and long-term costs of enhanced skeletal growth be considered in studies on the evolution of growth rates in organisms with indeterminate growth.

Our results corroborate findings that, within the normal range of thermal and food conditions, ambient thermal conditions play a key role in the rate of skeletal growth and fat deposition in ectothermic vertebrates (Duellman & Trueb 1986; Vitt & Caldwell 2009). Importantly, corn snakes in this study grew faster and showed higher BMI when they did not have continuous access to the heat element (4 hours vs. 24 hours/day). This seemingly paradoxical result of the thermoregulation experiment indicates that thermally constrained snakes invested more time than snakes without thermal constraints in basking on the heat element. Behavioural data revealed that basking behaviour was altered even within the group of thermally constrained snakes. Specifically, snakes that were fed less frequently basked on the heat element significantly longer than snakes fed at shorter intervals, even though it took longer time to the former snake group to reach the heat element. Thus, it appears that food- but mainly thermally-constrained juvenile corn snakes altered their thermoregulatory behaviour so as to enhance their food conversion efficiency and, consequently, skeletal growth and BMI. These results are consistent with the view that BMI in reptiles may not be an accurate index of individual quality or dominance (see Pérez i de Lanuza et al. 2014). Rather than reflecting higher access to food resources, enhanced skeletal growth and higher BMI in corn snakes may be the best of a bad job when growing under thermally stressful conditions. Consequently, higher BMI frequently reported in reptiles for unpaired, more highly infested or autotomized individuals (e.g., Václav et al. 2007; Molnár et al. 2013; Maia et al. 2014; Megía-Palma et al. 2016) may not be due to higher energetic demands by dominant individuals (e.g., Whiting et al. 2006), but due to altered thermoregulatory behaviour by environmentally constrained individuals.

In contrast to skeletal growth rate and BMI, the length of the shedding interval was moulded dominantly by the food and not by the thermal regime. Skin shedding closely matches the period of epidermal growth in lepidosaurs (Vitt & Caldwell 2009). Importantly,

skin shedding does not only impose the costs of tissue growth, but also the production of food-derived compounds such as skin lipids, which are important in water loss and infection prevention as well as in chemosensory communication (López & Martín 2005; Kopena et al. 2011; Martín & López 2014). Our results corroborate the previous studies since higher food availability, combined with favourable thermal conditions, and not thermally dependent food conversion efficiency, resulted in faster skin shedding in juvenile corn snakes.

We found that skin reflectance at short wavelengths was affected by the combined effect of food availability and thermal conditions. It is well established that in reptiles the magnitude of short wavelength reflectance depends on the properties of deeper skin cell layers, i.e., iridophores and melanophores (e.g., Olsson et al. 2013). Consequently, together with the results on skin shedding, it appears that juvenile corn snakes receiving food more frequently and having a continuous possibility to thermoregulate not only develop their skin faster but iridophore nanocrystals in their skin also are more regularly spaced.

Our study reveals that, unlike structurally-based skin coloration, pigment-based coloration may not be energetically costly because long-wavelength reflectance was not affected by the food and/or thermal regime (c.f. Pérez i de Lanuza et al. 2014). Moreover, this study is in accord with the suggestion by San-Jose et al. (2013) that pigmentary-based skin colour patches in reptiles may be multicomponent signals with the non-pigmentary based UV component reflecting body condition and the pigmentary-based component reflecting the genetic context.

Compared to previous studies involving reptiles where specific micronutrients were supplemented to individuals in the experimental group to see their effect on skin colouration (San-Jose et al. 2013; Kopena et al. 2014), corn snakes in this study differed in the frequency with which they received whole food including both micro- and macro-nutrients. Thus, it is possible that our experiment affected iridophore properties through a higher availability of some limiting nutrients, such as antioxidants. However, this idea requires further testing because corn snakes in both food treatments were expected to receive sufficient amounts of nutrients for normal development and, indeed, none of the snakes was found to show a sign of malnutrition. Moreover, snakes receiving food more frequently did not show higher short wavelength reflectance if they were exposed to the restrictive thermal regime. Alternatively, the interaction effect of food and thermal regimes on short-wavelength reflectance may point to the importance of environmental stress in skin UV colouration (Martín & López 2009; Bajer et al. 2011; Molnár et al. 2013; Pérez i de Lanuza et al. 2014; Megía-Palma et al. 2016). Juvenile corn snakes reared in high food and optimal thermal conditions showed higher UV reflectance than snakes in other treatments subjected to thermal and/or food limitations. Therefore, it is possible that short-wavelength skin reflectance in corn snakes can be

a surrogate of environmental stress or an individual ability to handle this stress during skin development. This scenario is consistent with recent findings on the positive effect of non-pigmentary antioxidants on skin UV reflectance, suggesting that some antioxidants such as vitamins E and A1 affect iridophore nanostructure by decreasing the level of physiological stress within deeper skin layers (San-Jose et al. 2013; Kopena et al. 2014). Given that pterins can have similar antioxidative potential as carotenoids (McGraw 2005), our results tentatively support the hypothesis by Hartley & Kennedy (2004) that carotenoid-based signals serve to reflect the true levels of antioxidants, and not the level of carotenoids *per se*. Namely, it is possible that we reduced short- but not long-wavelength reflectance of pigmentary-based red and yellow colour patches by imposing higher physiological stress within deeper skin layers on food- and/or thermally-stressed snakes compared to snakes reared under more permissive conditions.

The results of this study are relevant in three other respects. First, to assess the effect of melanin on skin colouration, we carried out the experiment using different genetically based melanin colour variants of corn snakes, i.e., classic, hypomelanistic and amelanistic variants. Consequently, our study suggests that it is the dominant effect of iridophore properties that is crucial for variation in short-wavelength reflectance in juvenile corn snakes because snakes from the high food and optimal thermal treatments showed consistently higher short-wavelength skin reflectance than other snakes independent of the melanin-based colour variant. Yet, as the colour variant was confounded by brood identity, future studies with larger sample sizes should assess the skin colouration effects of melanin. Second, considering the sex of juvenile corn snakes as a random effect did not reveal significant differences in short-wavelength reflectance between sexes. Even though this result should also be confirmed for adult individuals, this study does not provide evidence that the UV component of dorsal skin colouration could act as a sexual signal in corn snakes. Third, variation in short-wavelength skin reflectance was particularly high for red saddles, with UV reflectance accounting for most spectral variation of these patches (i.e., PC1). Moreover, the interaction effect of food and thermal regimes was statistically significant for the short-wavelength reflectance of red saddles (300–430 nm; PC1), whereas this effect only tended to be significant for the short-wavelength reflectance of the yellow ground colour (300–330 nm; PC3). These results suggest that environmental conditions mediated by the properties of iridophore nanostructure may be more reliably reflected by red than yellow skin colour patches. Intriguingly, while red colouration has a strong genetic basis in corn snakes and other snakes, yellowish colouration, which is assumed to be based on food carotenoids, increases progressively with ontogeny (Bechtel & Bechtel 1978; Bechtel 1995). Future work should identify the pigments contained in the two colour patches, because different pigments (i.e., carotenoids and pterins) may

play different roles in iridophore development in corn snakes.

In conclusion, this work suggests that if juvenile corn snakes are environmentally constrained, their preferred life history strategy is to grow at faster rates. The study adds to the growing body of evidence in that BMI may not accurately reflect individual condition in reptiles. Instead, the length of shedding intervals and short-wavelength skin reflectance may be used in this animal system as surrogates of the individual's condition or quality, possibly reflecting exposure to environmental stress or an ability to handle it. Recent findings on feather moult rate and feather quality (Moreno-Rueda 2010; Vágási et al. 2012) suggest that signals, whose honesty is mediated by physiological constraints during growth, may be common in animals (Royle et al. 2015).

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