J Comp Physiol A (2015) 201:817–825
DOI 10.1007/s00359-015-1025-4

ORIGINAL PAPER

Facultative thermogenesis during brooding is not the norm among pythons

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Received: 8 February 2015 / Revised: 2 May 2015 / Accepted: 12 June 2015 / Published online: 27 June 2015

Abstract Facultative thermogenesis is often attributed to pythons in general despite limited comparative data available for the family. While all species within Pythonidae brood their eggs, only two species are known to produce heat to enhance embryonic thermal regulation. By contrast, a few python species have been reported to have insignificant thermogenic capabilities. To provide insight into potential phylogenetic, morphological, and ecological factors influencing thermogenic capability among pythons, we measured metabolic rates and clutch-environment temperature differentials at two environmental temperatures—python preferred brooding temperature (31.5 °C) and a sub-optimal temperature (25.5 °C)—in six species of pythons, including members of two major phylogenetic branches currently devoid of data on the subject. We found no evidence of facultative thermogenesis in five species: *Aspidites melanocephalus*, *A. ramsayi*, *Morelia viridis*, *M. spilota cheynei*, and *Python regius*. However, we found that *Bothrochilus boa* had a thermal metabolic sensitivity indicative of facultative thermogenesis (i.e., a higher metabolic rate at the lower temperature). However, its metabolic rate was quite low and technical challenges prevented us from measuring temperature differential to make conclusions about facultative endothermy in this species. Regardless, our data combined with existing literature demonstrate that facultative thermogenesis is not as widespread among pythons as previously thought.

Keywords Evolution of endothermy · Metabolic rate · Parental care · Reptile · Snake

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Introduction

Although it often requires a substantial expenditure of resources on the part of the parent, parental care is widespread because it confers numerous fitness benefits to offspring (e.g., increased survival and growth) (Clutton-Brock 1991). A common benefit of parental care is thermoregulation of the developing offspring (Shine and Harlow 1996; Ashmore and Janzen 2003). In fact, thermoregulation of the developmental environment has been proposed as an initial driving factor in the evolution of endothermy (Farmer 2000).

Pythons (Squamata: Pythonidae) are rare among reptiles in providing ubiquitous parental care that provides thermal, hydric, and predator defense benefits (Shine 2004; Stahlschmidt and DeNardo 2010). Pythons are ectothermic throughout most of life, but during brooding the females of some species are known to increase their metabolic rate and exhibit muscular twitching, termed facultative thermogenesis. While widely accepted as a trait of pythons in general, of the 44 extant python species (Barker et al. 2015), facultative thermogenesis has been confirmed in only two species: the Burmese python (*Python molurus*) (Vinegar et al. 1970), which has since been broken into two species (*P. molurus* and *P. bivitattus*, Jacobs et al. 2009) and two subspecies of the carpet python (*Morelia spilota spilota*) (Slip and Shine 1988); (*M. s. imbricata*) (Pearson et al. 2003). In

these species, facultative thermogenesis provides substantial heat to the clutch, supplementing the insulatory benefits associated with coiling around the eggs (Stahlschmidt et al. 2008). Female heat production is inversely proportional to environmental temperature (T_{env}), with metabolic rate and muscular twitching increasing as temperature decreases below optimal developmental temperature (approximately 31.5 °C for most species) (Brashears and DeNardo 2013). In *P. molurus*, maximum heat production occurs when T_{env} approaches 24 °C (Van Mierop and Barnard 1978).

Currently, the occurrence of facultative thermogenesis within the Pythonidae remains unclear (Stahlschmidt and DeNardo 2010). This limitation compromises our understanding of the factors that have shaped its evolution within the *pythons* and, as a result, decreases the utility of *python* facultative endothermy in evaluating the driving forces behind the evolution of endothermy. More complete knowledge of the existence of facultative endothermy among *pythons* would provide for a better understanding of the relative importance of phylogenetic constraints, morphological limitations, and environmental conditions on the existence of facultative endothermy, and thus provide insight into driving forces (and constraints) of endothermic capability in general.

The Pythonidae is divided between two primary phylogenetic clades, an Afro-Asian clade with two major lineages and an Indo-Australian clade with eight major lineages (Reynolds et al. 2014; for recent review, see Barker et al. 2015). The two species with confirmed facultative thermogenesis are in the different clades—*P. molurus* within the Afro-Asian group and *M. spilota* within the Indo-Australian group. Conversely, both primary clades are also known to contain species where the absence of facultative thermogenesis has been confirmed—the rock python (*P. sebae*) (Vinegar et al. 1970) and the ball *python* (*P. regius*) (Ellis and Chappell 1987) within the Afro-Asian clade and the reticulated python (*P. reticulatus*) (Vinegar et al. 1970), the water python (*Liasis fuscus*) (Stahlschmidt et al. 2012), and the Children's python (*Antaresia childreni*) (Stahlschmidt and DeNardo 2009) in the Indo-Australian clade (Fig. 1). Brooding metabolic data are currently absent for all other species, including no representation from four of the ten major lineages.

The aim of this study was to assess the presence of brooding facultative thermogenesis in four previously unstudied species of python, including representatives from two of the four lineages where no data exist—the black-headed python (*Aspidites melanocephalus*), the woma (*A. ramsayi*), and the bismarck ringed python (*Bothrochilus boa*). The addition of these species provides insight into possible phylogenetic influence on endothermic capability. We also evaluated two other pythons. The jungle carpet python (*M. spilota cheynei*) is a member of the same species as the diamond python (*M. s. spilota*) and the southwestern carpet python (*M. s. imbricata*), both of which have been shown to use facultative thermogenesis (Slip and Shine 1988; Pearson et al. 2003). *Morelia spilota* is widely distributed throughout Australia and thus experiences a great range with considerable variation in environmental temperature. *Morelia s. spilota* and *M. s. imbricata* are both from high latitudes, while *M. s. cheynei* is more tropical in distribution. Examining *M. s. cheynei* provides insight into environmental influences on facultative thermogenesis. Lastly, we examined the ball pythons (*Python regius*) to clarify somewhat ambiguous prior results from this species. Together, these species combined with the previous species studied provide a considerable variation in adult size and thus may reveal any influence that size may have on the use of endothermy.

Materials and methods

Animals

Over two breeding seasons (2007 and 2008), data were collected from 18 brooding females representing six species of python: 3 *A. melanocephalus*, 3 *A. ramsayi*, 2 *B. boa*, 3 *M. spilota cheynei*, 1 *M. viridis*, and 6 *P. regius*. Snakes were either borrowed from private breeders and housed at Arizona State University (ASU) during the two-year period or were part of a long-term captive breeding colony at ASU.

Animals were housed individually in temperature-controlled rooms (27 ± 1 °C) under a 12:12 h photoperiod with supplemental heat provided at one of the cage by a subsurface heating element (Flexwatt, Flexwatt Corp., Wareham, MA, USA). During the non-breeding season (June to November), animals were fed weekly and provided water ad libitum. In November, animals underwent a 6-week cooling period in which we turned off the subsurface heating elements and ceased feeding. In January, subsurface heating and feeding resumed. After 2 weeks, we began rotating males through the females' cages. In February, we began weekly ultrasound scans (Concept/MCV, Dynamic Imaging, Livingston, Scotland) of females to assess their reproductive progress.

We weighed gravid females 1 week prior to oviposition. For three of the species (*M. viridis*, *M. s. cheynei*, *P. regius*), pre-oviposition females were placed in triple-ported, cylindrical containers (7.0–12.0 l), which we housed within a temperature-controlled walk-in environmental chamber (T = 30.5 ± 0.3 °C; 14:10 L:D photoperiod). The containers were tightly sealed and supplied with humidified air (20–40 ml/min; relative humidity (RH) = 80–85 %). For the other three species (*A. melanocephalus*, *A. ramsayi*, *B. boa*), because of their higher activity levels, we

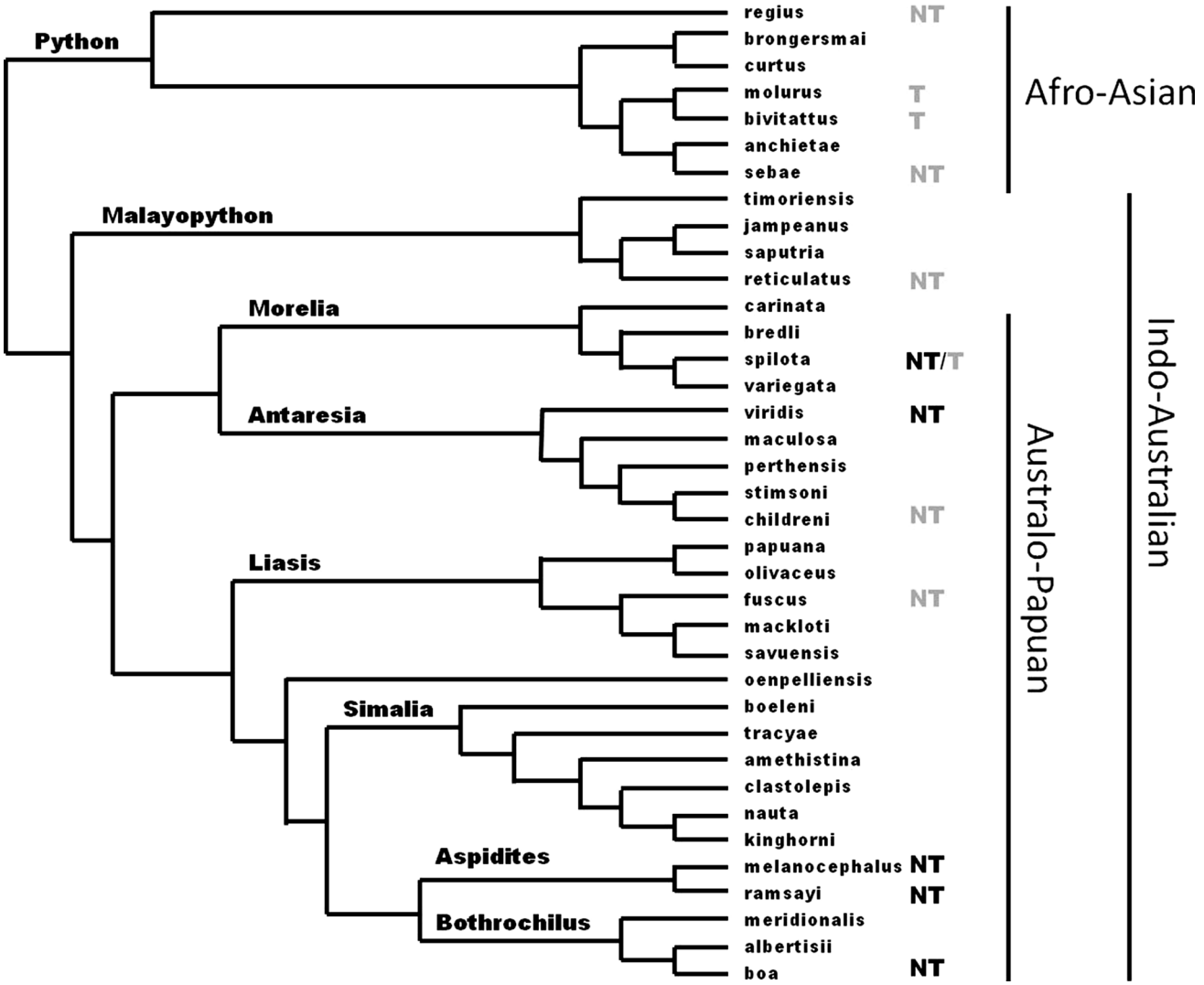

Fig. 1 Phylogeny of pythons (adapted from Reynolds et al. 2014) showing the presence (T) or absence (NT) of facultative thermogenesis in pythons. The results from previous studies are shown in gray (see text for references), while results from the present study are shown in black. The addition of *B. boa*, *A. melanocephalus*, *A. ramsayi*, *M. viridis*, and *M. s. cheynei* now provides data for species within eight of the ten major phylogenetic branches of *Pythonidae*. T/NT for *M. spilota* represents differing results among three subspecies

placed similar brooding containers inside the pre-oviposition females' cages. These containers had openings bored into the lids that allowed females to enter the container to oviposit. Following oviposition, the container holding the brooding female had its bored lid replaced with a solid lid and was then moved to the environmental chamber and provided air as described above. All females oviposited in the containers between April and June. The oviposition containers served as metabolic containers during the study.

Measurements

Rates of O_2 consumption ($\dot{V}_{O_2}$; ml/h) and CO_2 production ($\dot{V}_{CO_2}$; ml/h) were collected from brooding females using a flow-through respirometry system within 7 days of oviposition. Supply air was scrubbed of CO_2 and water (CDA 1112, PureGas, Broomfield, CO), then routed through a mass flow controller (Unit Instruments, Inc., Yorba Linda, CA, USA) before entering the metabolic chamber containing the brooding female. Efflux air from the metabolic chamber sequentially passed through a hygrometer (R2300, Sable Systems, Las Vegas, NV, USA), a drying vial of $CaSO_4$, a CO_2 analyzer (LI-6252, Li-Cor Biosciences, Lincoln, NE, USA), and an O_2 analyzer (FC-1B, Sable Systems, Las Vegas, NV, USA). The entire system was plumbed with minimally hygroscopic tubing (Bev-A-Line IV, Cole-Parmer, Vernon Hills, IL, USA). Air flow was adjusted for each metabolic chamber to replace 99 % of its

air in less than 20 min using the 99 % equilibration equation of Lasiewski et al. (1966). Flow rates ranged from 0.75 to 2.0 l/min. Gas analyzers were calibrated weekly, and data were recorded using a datalogger (23X, Campbell Scientific Instruments, Logan, UT, USA).

Females were acclimated for 12 h before beginning data collection. Steady-state metabolic data were collected from each female over 12 h at each of the two temperatures: 31.5 °C which approximates optimal developmental temperature of pythons (Ross and Marzec 1990) and 25.5 °C, a sub-optimal temperature at which *P. molurus* is known to generate substantive heat (Brashears and DeNardo 2013; Van Mierop and Barnard 1978).

We collected temperature data from the clutch (T_c), the metabolic chamber (T_n), and the environmental chamber (T_e) using type-T thermocouples feeding into the 23X datalogger. We measured T_c by inserting a thermocouple into the center of the clutch through a sealed port in the floor of the metabolic chamber. T_n was measured using a thermocouple that passed through the influx port in the side of the chamber so that it protruded ~5 cm into the metabolic chamber. T_e was measured by securing a thermocouple ~10 cm from the metabolic chamber.

After each 24-h trial (12 h acclimation and 12-h data collection) to collect brooding data, the female was temporarily separated from its clutch to collect clutch metabolic data at the same temperatures using closed respirometry to preserve the hydric state of the unprotected clutch. Single-ported, closed respirometry containers were air tight and ranged in size from 1.2 to 6.7 l. We used 140 ml plastic syringes to draw initial and final gas samples, then dried the air using $CaSO_4$ before passing it through an O_2 analyzer (S-3A, Applied Electrochemistry, Inc., Sunnyvale, CA, USA). Durations of the trials were adjusted to allow for sufficient oxygen consumption to occur. Oxygen consumption of the clutch (ml O_2/h) was calculated as (O_2 $\%_{\text{final}}$ − O_2 $\%_{\text{initial}}$) X functional chamber volume/time. The $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated for the brooding unit (female plus clutch) using the equations supplied by Walsberg and Hoffman (2006) by taking the 20 min interval with the lowest metabolic values. Taking the 20 min interval with the lowest metabolic values eliminated periods of female activity and was indicative of the average trial values. Female alone $\dot{V}_{O_2}$ was calculated by subtracting clutch $\dot{V}_{O_2}$ from the brooding unit $\dot{V}_{O_2}$.

Statistics

We used a repeated measures Generalized Linear Model with temperature as a within-subjects factor (two levels: 25.5, 31.5 °C), species as a between-subjects factor (six species), and mass as a covariate to test for differences in metabolic rate. Within-species comparisons of metabolic rate at the two different temperatures were completed by applying a single post hoc paired T test. All statistics were performed in SPSS (SPSS Inc., Chicago, IL, USA). All values are presented as mean ± SEM.

Table 1 Female and clutch metrics of six species of python

Species, *n*	Female mass (g)	Clutch size (eggs)	Clutch mass (g)
Aspidites melanocephalus, 3	2116	5	627.5
	1724	6	644.0
	3157	7	648.5
Mean ± SEM	2332 ± 427	6 ± 1	640.0 ± 6.4
Aspidites ramsayi, 3	950	9	348.0
	1008	10	439.7
	866	2	384.2
Mean ± SEM	942 ± 41	10 ± 1	390.6 ± 26.7
Bothrochilus boa, 2	1563	18	351.0
	1780	13	243.0
Mean ± SEM	1671	16	297.0
Morelia spilota cheynii, 3	2100	16	636.0
	2200	19	760.3
	1422	11	479.5
Mean ± SEM	1907 ± 244	15 ± 2	625.3 ± 81.2
Morelia viridis, 1	1000	13	126.0
Python regius, 6	1017	5	323.7
	641	3	121.7
	734	4	212.0
	934	4	241.2
	1137	7	722.0
	1593	6	303.2
Mean ± SEM	1009 ± 138	5 ± 1	294.1 ± 95.7

Female mass, clutch size, and clutch mass are presented for each individual in the experiment

Results

All females maintained a tight coil around their clutches during the experiment until the clutch was removed for determination of clutch metabolism. The coils of most females covered the majority of the side and dorsal surfaces of the clutch; however, female *B. boa* displayed an unusual coil in that they wrapped completely around their clutches, including the underside of the clutches. We did not observe twitching by any female during or after the experiment. Female mass, clutch size, and clutch mass are presented in Table 1.

Brooding metabolic rates varied considerably among species ($F_{5,\ 12} = 4.47$, $p = 0.016$; Table 2), and there was a strong interaction effect between species and temperature ($F_{5,\ 12} = 8.73$, $p = 0.001$; Table 2). Within a species,

Table 2 Metabolic metrics in six species of python

Species, *n*	Q_{10}	25.5 °C		
		RER	$\dot{V}_{O_2}$ (ml/h)	$\dot{V}_{O_2}$ (ml/h/kg)
Aspidites melanocephalus, 3	4.8	0.7	28.0	13.2
	1.2	0.6	50.8	29.5
	1.4	0.7	38.8	12.3
Mean ± SEM	2.5 ± 1.2	0.6 ± 0.1	39.2 ± 6.6	18.3 ± 5.5
Aspidites ramsayi, 3	3.6	0.7	24.4	25.7
	2.1	0.8	14.8	14.6
	2.3	0.7	19.0	21.9
Mean ± SEM	2.7 ± 0.5	0.7 ± 0.1	19.4 ± 2.8	20.8 ± 3.2
Bothrochilus boa, 2	0.7	0.7	64.3	41.1
	0.6	0.7	182.3	102.4
Mean	0.6	0.7	123.3	71.8
Morelia spilota cheynii, 3	1.0	0.8	122.1	58.1
	3.8	0.7	66.5	30.2
	5.5	0.7	25.1	17.7
Mean ± SEM	3.4 ± 1.3	0.7 ± 0.1	71.2 ± 28.1	35.3 ± 12.0
Morelia viridis, 1	3.5	0.9	18.2	18.2
Python regius, 6	1.4	0.6	12.3	12.1
	1.6	0.8	13.6	21.1
	0.4	0.4	25.1	34.2
	1.0	1.4	28.3	30.3
	1.6	0.9	29.2	25.7
	8.9	1.6	14.0	8.8
Mean ± SEM	2.5 ± 1.3	0.9 ± 0.2	20.4 ± 3.3	22.0 ± 4.1
Species, *n*	Q_{10}	31.5 °C		
		RER	$\dot{V}_{O_2}$ (ml/h)	$\dot{V}_{O_2}$ (ml/h/kg)
Aspidites melanocephalus, 3		0.8	71.5	33.8
		0.8	56.1	32.5
		1.3	47.8	15.1
Mean ± SEM		1.0 ± 0.2	58.5 ± 7.0	27.2 ± 6.0
Aspidites ramsayi, 3		0.4	52.8	55.5
		0.9	23.2	23.0
		0.7	31.1	35.9
Mean ± SEM		0.7 ± 0.1	35.7 ± 8.8	38.1 ± 9.4
Bothrochilus boa, 2		0.7	53.0	33.9
		0.8	128.7	72.3
Mean		*0.7*	*90.8*	*53.1*
Morelia spilota cheynii, 3		0.7	125.3	59.6
		0.7	148.8	67.6
		0.8	69.7	49.0
Mean ± SEM		0.7 ± 0.1	114.6 ± 23.4	58.7 ± 5.4
Morelia viridis, 1		0.7	38.7	38.7
Python regius, 6		1.1	15.0	14.7
		0.9	17.9	27.9
		1.1	13.8	18.8
		1.8	28.0	30.0
		0.7	38.2	33.6
		0.7	51.8	32.5

Table 2 continued

Species, *n*	Q_{10}	31.5 °C		
		RER	$\dot{V}_{O_2}$ (ml/h)	$\dot{V}_{O_2}$ (ml/h/kg)
Mean ± SEM		1.0 ± 0.2	27.5 ± 6.5	26.3 ± 3.2

Q_{10} thermal sensitivity, *RER* respiratory exchange ratio, total $\dot{V}_{O_2}$ (ml/h), and mass specific $\dot{V}_{O_2}$ (ml/h/kg) are presented for each female in the experiment. Clutch $\dot{V}_{O_2}$ was subtracted from total $\dot{V}_{O_2}$ prior to calculations

the mass-adjusted $\dot{V}_{O_2}$ was significantly increased at the higher temperature for *A. melanocephalus* [t (2) = 4.47, p = 0.046], *A. ramsayi* [t (2) = 5.72, p = 0.029], and *M. s. spilota* [t (2) = 4.56, p = 0.044]. Mean Q_{10} values for these three species were 2.5 ± 1.2, 2.7 ± 0.5, and 3.4 ± 1.3, respectively (Table 2). Similarly, the single *M. viridis* increased its mass-adjusted $\dot{V}_{O_2}$ from 18.2 to 38.7 ml/kg/h (Q_{10} = 3.5; Table 2). *Python regius* had substantial intraspecific variation in mass-adjusted $\dot{V}_{O_2}$ at each temperature, which was reflected in the lack of significant difference in mean mass-adjusted $\dot{V}_{O_2}$ between the two temperatures [t (5) = 2.23, p = 0.155; Table 2]. *Python regius* had a mean Q_{10} value of 2.5 ± 1.3 (Table 2). In contrast to the other species, the mass-adjusted $\dot{V}_{O_2}$ of both female *B. boa* decreased at the higher temperature, from 41.1 to 102.4 ml/kg/h at 25.5 °C to 33.9 and 72.3 ml/kg/h at 31.5 °C, respectively (Q_{10} = 0.8 and 0.6; Table 2).

We were able to collect temperature data from all species except *B. boa* (Table 3). Due to the unusual nature of their coiling, it was impossible to insert thermocouples into their clutches. Additionally, these females are extremely sensitive to any disturbance of their nesting environment and quickly abandon their clutches if disturbed. There were no significant differences between T_c and T_e within any of the other five species (Table 3). Calculated temperature differentials from these five species showed that ΔT was not significantly different from zero (Table 3).

Discussion

With the addition of our brooding metabolic rate measurements in *A. melanocephalus*, *A. ramsayi*, *B. boa*, *M. s. cheynei*, and *M. viridis*, there now exist data on facultative thermogenesis for representatives within eight of the ten branches of Pythonidae. While our study was limited by low sample size, the lack of comparative data on brooding python metabolic rates and the difficulty in obtaining reproductive animals for experimental purposes make the data presented here valuable in furthering the understanding of what factors influence the existence of facultative endothermy among pythons. Based on both metabolic (Table 2) and thermal (Table 3) assessments, our results strongly suggest that five of the species are not thermogenic during brooding. While the data from *B. boa* are less conclusive, in general, our results in combination with previous studies strengthen the growing realization that facultative thermogenesis among pythons is not as widespread as previously thought.

The higher metabolic rate at 25 °C compared to 31.5 °C in *B. boa* reflects a pattern seen in facultatively endothermic species (Vinegar et al. 1970; Ellis and Chappell 1987). Unfortunately, we were unable to collect temperature data from the clutches of *B. boa* to definitely demonstrate a temperature differential between the clutch and the chamber. No twitches were observed at any time during reproduction in either female, but this does not necessarily imply that heat was not produced muscularly or metabolically. Muscle contractions that produce heat do not necessarily produce visible muscle tremors (e.g., birds) (Hohtola 2004). Regardless, despite being higher at 25.5 °C, metabolism of *B. boa* was quite limited, and the resulting low power production (brooding max = 1 mW/kg at 25.5 °C assuming lipid catabolism) seems insufficient to sustain a temperature differential between the clutch and chamber environment. In comparison, both facultatively thermogenic species, *P. m. bivittatus* and *M. spilota*, have a calculated maximum power production of approximately 1 W/kg (Van Mierop and Barnard 1978; Harlow and Grigg 1984). As a result of the low proportional increase in metabolic rate at 25 °C as well as the low power output relative to that of species with effective brooding thermogenesis, we suspect that the heat production by *B. boa* would have little effect on the developmental environment unless the nest was extremely well insulated.

Work by Ellis and Chappell (1987) demonstrated that, unlike non-reproductive females, brooding *P. regius* have a $\dot{V}_{O_2}$ that is insensitive to temperature (i.e., Q_{10} not significantly different from 0) and, as a result, the metabolic rate at lower temperature is 3–4 times higher in brooding compared to non-reproductive females (Ellis and Chappell 1987). Our data from *P. regius* support this previous work in that our calculated mean Q_{10} value for *P. regius*, while relatively high at 2.5 ± 1.3, was also not significantly different from zero. The high mean value was largely due to the unusually high thermal sensitivity of a single female (Table 2). With this female excluded, the mean Q_{10} for this species would be 1.2 ± 0.2, matching the previous finding that this species is metabolically uncoupled from

Table 3 Temperature metrics in six species of python

Species, *n*	25.5 °C			
	T_e (°C)	T_n (°C)	T_c (°C)	ΔT ($T_c - T_e$)
Aspidites melanocephalus, 3	25.5	25.7	25.5	−0.07
	26.3	26.3	26.2	−0.08
	25.7	25.4	25.7	−0.07
Mean ± SEM	25.8 ± 0.2	25.8 ± 0.3	25.8 ± 0.2	−0.1 ± 0.3
Aspidites ramsayi, 3	26.1	27.1	26.2	0.17
	25.4	25.8	26.6	1.19
	25.4	24.9	25.5	0.04
Mean ± SEM	25.6 ± 0.2	26.0 ± 0.6	26.1 ± 0.3	0.5 ± 0.6
Morelia spilota cheynei, 3	25.5	25.9	25.6	0.07
	25.8	26.3	26.0	0.20
	25.5	25.7	25.3	0.67
Mean ± SEM	25.6 ± 0.1	26.0 ± 0.2	25.6 ± 0.2	0.3 ± 0.2
Morelia viridis, 1	26.0	26.0	26.3	0.27
Python regius, 6	24.4	25.7	26.1	1.77
	26.3	25.7	25.8	−0.47
	26.3	26.1	26.1	−0.20
	25.9	26.0	26.4	0.45
	26.2	25.8	25.7	−0.59
	25.0	25.8	25.3	0.32
Mean ± SEM	25.7 ± 0.3	25.9 ± 0.1	25.9 ± 0.1	0.2 ± 0.4
Species, *n*	31.5 °C			
	T_e (°C)	T_n (°C)	T_c (°C)	ΔT ($T_c - T_e$)
Aspidites melanocephalus, 3	31.9	31.9	31.6	−0.28
	32.4	32.2	32.2	−0.18
	31.5	32.3	31.3	−0.14
Mean ± SEM	31.9 ± 0.3	32.1 ± 0.1	31.7 ± 0.3	−0.2 ± 0.3
Aspidites ramsayi, 3	32.9	31.5	32.2	−0.69
	31.1	33.9	32.7	1.56
	31.4	33.9	32.3	0.86
Mean ± SEM	31.8 ± 0.6	33.1 ± 0.8	32.4 ± 0.1	0.6 ± 0.8
Morelia spilota cheynii, 3	31.3	31.9	31.6	0.31
	31.6	31.7	31.7	0.08
	31.3	31.6	31.4	0.10
Mean ± SEM	31.4 ± 0.1	31.7 ± 0.1	31.6 ± 0.1	0.2 ± 0.1
Morelia viridis, 1	31.8	31.4	31.9	0.13
Python regius, 6	31.3	31.5	31.7	0.41
	31.3	31.4	31.3	0.09
	31.2	31.3	31.4	0.17
	31.5	31.8	31.5	−0.02
	31.9	31.3	31.9	−0.08
	31.8	32.5	32.1	0.33
Mean ± SEM	31.5 ± 0.1	31.6 ± 0.2	31.7 ± 0.1	0.2 ± 0.2

T_e environmental temperature, T_n metabolic chamber temperature, T_c clutch temperature, and ΔT are presented for each female in the experiment

temperature during brooding (Ellis and Chappell 1987). We also found no evidence that female *P. regius* are able to sustain a temperature differential (Table 3).

The value of limited but higher than expected metabolic rate at lower temperatures for brooding *B. boa* and *P. regius* is currently uncertain, and would require further study. In

both Ellis and Chappell (1987) and our studies, the females brooded in an environment where there was considerable air flow through the brooding chamber to enable respirometric measurements. The two species (*P. molurus* and *M. spilota*) with demonstrated dramatic increases in metabolic rate at cooler temperatures are both known to brood on the surface (Slip and Shine 1988; Ramesh and Bhupathy 2010). However, *P. regius* is known to brood in burrows and the brooding location for *B. boa* is unknown. If *B. boa* also broods in a more insulated environment, the less impressive metabolic response of these two species to low temperatures may be ample to enhance the thermal conditions of development under natural conditions. Future studies should look at the impact of insulation on power demands of brooding female pythons in thermoregulating the developmental environment of their offspring.

Facultative thermogenesis does not have a clear phylogenetic signal in Pythonidae. Of the now 11 species of pythons that have been examined for facultative thermogenesis, only two have been shown to have considerable heat production, two others have shown atypical temperature response curves suggestive of limited thermogenic potential, and seven fail to show any indication of thermogenesis (Fig. 1). Thus, we conclude that strict ectothermy, not facultative thermogenesis, may be the norm for brooding pythons. Furthermore, the occurrence of facultative thermogenesis, whether pronounced or limited, is phylogenetically widely dispersed among the family. Both the Afro-Asian and Indo-Australian lineages have species that have either pronounced thermogenesis, limited thermogenesis, or no detectable thermogenesis (Fig. 1). Given current data, it is not possible to determine whether facultative thermogenesis during brooding has evolved multiple times or whether it has been lost multiple times within the Pythonidae. However, the lack of a phylogenetic pattern to the presence and absence of thermogenesis within the pythons suggests a lack of any phylogenetic constraint on thermogenesis within the group.

Size appears to act as a limiting factor in thermogenesis. To sustain the energetic demands of an elevated metabolism, the female must be able to possess considerable energy storage to support thermogenesis after investing 30 % or more of its body mass into egg production (Angilletta and Sears 2003). Additionally, larger size provides greater energetic potential (Shine 1992; Aubret et al. 2002) as well as greater clutch insulation. Thus, it is reasonable to suspect that small or thin species could not be facultatively thermogenic during brooding. This is supported by the fact that neither *A. childreni*, a member of a genus of small species, nor *M. viridis*, a thin arboreal species, are thermogenic (Stahlschmidt and DeNardo 2009; and this paper, respectively). However, large size alone does not guarantee facultative thermogenesis by females. *Python reticulatus* and *P. sebae* are two of the three largest python species, yet both of them are non-thermogenic during brooding (Vinegar et al. 1970).

Geographical range appears to be a stronger predictor of facultative thermogenesis in pythons, but further analyses are necessary to support this preliminary conclusion. While large size is likely necessary to support facultative endothermy, cooler environments are likely necessary for the developmental advantages to offset the energetic costs. The three species that express pronounced facultative thermogenesis, *P. molurus, P. bivittatus,* and *M. spilota*, have distributions at the northern and southern latitudinal limits, respectively, of the family (Vinegar et al. 1970; Slip and Shine 1988). Facultative thermogenesis also appears linked to geographic distribution across the subspecies of *Morelia spilota*. While its presence has been demonstrated in *M. s. spilota* and *M. s. imbricata*, we show that *M. s. cheynei* is not facultatively thermogenic. *Morelia s. spilota* and *M. s. imbricata* reside in southeastern and southwestern Australia, respectively, and thus occur at the southern latitudinal extreme of pythons. However, *M. s. cheynei* lives in the sub-tropical northeast part of the continent (Wilson and Swan 2008). Because body size and shape are similar among the three subspecies (in fact, *M. s. spilota* is relatively arboreal and slimmer than the other two sub-species), it is likely that the lack of facultative thermogenesis in *M. s. cheynei* is more a reflection of the relatively warm climate it inhabits more so than an inherent physiological or morphological limitation preventing the evolution of facultative thermogenesis in this race.

The question of whether facultative endothermy is ancestral to Pythonidae and has been maintained in large-bodied species inhabiting cooler climates, or whether it has evolved separately in multiple lineages is beyond the scope of this study. However, the high within-species metabolic variation, particularly within *P. regius* (Table 2), in the absence of detectable heat production (Table 3), is further evidence that metabolic rate may be particularly labile within Pythonidae. Several python species have been shown to increase metabolic rate 10- to 15-fold when digesting a meal (Secor and Diamond 1995; Ott and Secor 2007). Such variation could have provided the substrate for the evolution of facultative thermogenesis in species where the female can sustain the energetic costs and considerable developmental benefits can be realized because of environmental conditions.

Finally, the existence of facultative thermogenesis in pythons has been used to support the reproductive model of the evolution of endothermy, whereby the initial driving force for the evolution of endothermy was the benefits that heat production provided to the developmental environment of offspring (Farmer 2000). However, it has been pointed out that the substantial costs of thermogenesis

may be prohibitive (Angilletta and Sears 2003). The rarity of python facultative thermogenesis supports this caution, but as yet there has been no consideration of strategies brooding females use to reduce these costs. Brooding females can reduce thermal conductance and thus energy expenditures via the selection of thermally favorable nest sites (Shine and Harlow 1996) and can supplement thermogenesis with heat acquired through basking (Slip and Shine 1988). These factors need to be quantitatively evaluated if python facultative thermogenesis is to be useful in discussions regarding the evolution of endothermy.

Acknowledgments This study was funded by the National Science Foundation, IOS-0543979 to DFD. All procedures performed in this study were in accordance with the ethical standards of the Institutional Animal Care and Use Committee at Arizona State University.

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