

RESEARCH ARTICLE

Developmental phenotypic plasticity in a marsupial

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SUMMARY

Climate change is likely to substantially affect the distribution ranges of species. However, little is known about how different mammalian taxa respond morphologically and physiologically to a rapid change of climate. Our objective was to provide the first quantitative data on the effect of continuous cold exposure during development on morphological and functional variables of a marsupial. Fat-tailed dunnarts (*Sminthopsis crassicaudata*, Dasyuridae) were reared at an ambient temperature (T_a) of 16°C [cold-reared (CR)] or 22°C [warm-reared (WR)] until they reached adult age (>200 days). Body and head length of CR animals were significantly longer than in WR animals (mean \pm s.e.m.; body: CR 80.8 \pm 6 mm, WR 76.4 \pm 5 mm; head: CR 29.4 \pm 3 mm, WR 27.5 \pm 2 mm), but other body attributes were not significantly different. Use of torpor was more frequent, torpor bout duration was longer and average daily metabolic rate and percentage of savings when using torpor were significantly higher ($P<0.01$) in CR than in WR animals at 16°C T_a but not at 24°C. Furthermore, resting metabolic rates measured at 16°C T_a were significantly lower in CR than WR animals; at 30°C T_a values were similar. Our results do not conform to Allen's rule, but to some extent they do conform to Bergmann's rule. However, the data demonstrate that a relatively moderate cold exposure from birth until adulthood induces marked changes in the morphology and thermal energetics of small marsupials. Such short-term phenotypic responses without the need for long-term selection are likely important for the ability to cope with different climates over a wide range of distribution, but will also play a crucial role in enhancing the survival of species during climate change.

Key words: body measurement, climate change, marsupial, metabolic rate, rearing temperature, torpor.

INTRODUCTION

Seasonality of weather is increasingly superimposed by global climatic changes (Walther et al., 2002; Williams and Middleton, 2008). However, it remains largely unknown how different endothermic species and conspecifics from different climatic regions respond to climatic changes, or to what extent they possess phenotypic flexibility (Boyles et al., 2011). It is known that endotherms with a wide geographic range encompassing different climatic regions vary in size and body mass. Generally, conspecifics are larger in higher latitudes or in cold regions of the species' range and smaller in lower latitudes or warmer regions (Bergmann's rule). Temperature is correlated with latitude and latitude in turn with body and skull size, which often increase with latitude (Ashton et al., 2000; Storz et al., 2001; Meiri and Dayan, 2003; Meiri et al., 2004). Furthermore, morphological differences in extremities (e.g. ears, tail and limbs) observed within some species occupying different geographical regions, also known as Allen's rule, are suggested to be a response to the environment and not the exclusive result of genetic adaptation or selection for an optimum body type (Siegel et al., 1977). However, little is known about which factors exactly determine these morphological differences. Serrat et al. (Serrat et al. 2009) suggested that the elongated extremities in mice subjected to a warm environment were due to the indirect effect of vascular modification on bone growth.

Marsupials from Australia also appear to conform to these rules. It has been shown that body size, including skull length, in eastern quolls (*Dasyurus viverrinus*) is larger in Tasmania (>41°S) than on the mainland [<39°S (Jones, 1997)]. There is also a positive

correlation between body size and latitude for western (*Macropus fuliginosus*) and eastern grey kangaroos (*Macropus giganteus*) as well as for brush-tail possums (*Trichosurus vulpecula*) (Yom-Tov and Nix, 1986). In contrast, mean annual temperature seems to be negatively correlated with body size in these species (Yom-Tov and Nix, 1986). It has been suggested that this is a response to cooler temperatures at higher latitudes: because of the relationship between surface area and volume, an increased body size is energetically more economical for thermoregulation in the cold (e.g. Schmidt-Nielsen, 1985).

Physiological variables in addition to morphological ones may also differ among individuals of the same species living in areas with different climatic conditions. It has been demonstrated that hibernation and torpor patterns, characterised by pronounced reduction of metabolic rate (MR) and body temperature (T_b), differ among geographic populations of woodchucks and bats (Dunbar and Brigham, 2010; Zervanos et al., 2010; Stawski and Geiser, 2011). Furthermore, in the marsupial feathertail glider (*Acrobatus pygmaeus*), activity patterns and torpor differed between animals raised in captivity and animals captured from the wild, and wild-caught individuals from the subtropics differed from those captured at high elevation (Geiser and Ferguson, 2001). This suggests that torpor and activity patterns are not entirely species-specific traits, but to some extent result from the influence of environmental conditions affecting the development of young. This interpretation is supported by results from controlled laboratory experiments in pigs. Cold-reared (CR) pigs not only had shorter extremities and were generally shorter in size, but also had an increased capacity

for non-shivering thermogenesis compared with warm-reared (WR) pigs of the same mass (Heath and Ingram, 1983; Heath, 1984). However, it remains unknown how different rearing temperatures (T_r) affect the morphology and physiology of marsupials. Furthermore, data on torpor expression in relation to different T_r values are entirely lacking.

Because morphology and the thermal energetics of species seem at least partly driven by environmental factors, we tested the hypothesis that different T_r values would affect morphological parameters, MR and torpor patterns in fat-tailed dunnarts [*Sminthopsis crassicaudata* (Gould 1844)], a small insectivorous/carnivorous marsupial. We selected this dasyurid as a model species because they are born 12 days after fertilization in an extremely altricial state (average birth mass: 15 mg) into a pouch and are exposed to environmental conditions from about mid-pouch life. Also, this species is widely distributed over the southern half of the continent, including the hot arid centre and cool temperate region in the south (Menkhorst and Knight, 2004). We were especially interested in differences in morphology in relation to energy expenditure and employment of torpor of CR and WR animals.

MATERIALS AND METHODS

Animals

All dunnarts were obtained from a colony maintained at the University of New England, Armidale, New South Wales, Australia. The original founding members of the colony came from various populations in southeastern Australia, but were bred in captivity for several generations. Ten dunnarts from five different litters were kept continuously at a T_r of $16.0 \pm 0.4^\circ\text{C}$ from birth until adult age (CR) and nine dunnarts from seven different litters were kept at an average T_r of $22.0 \pm 2.0^\circ\text{C}$ from birth until adult age (WR). The females of mating pairs were checked weekly for pouch young. Once young were detected (i.e. at <1 week of age), the mother with their pouch young was transferred to a temperature-controlled room at an ambient temperature (T_a) of 16 or 22°C , respectively. When young reached approximately 2 months of age (weaning age for *S. crassicaudata*) they were separated from their mothers and kept individually in cages provided with bedding and nest boxes ($45 \times 30 \times 30$ cm) in the same temperature-controlled rooms. Dunnarts received water and a mix of canned cat food and moistened cat biscuits *ad libitum*, which was provided fresh daily. Measurements commenced when all animals were adults [7–10 months, i.e. >200 days (Morton and Dickman, 2008)]. The study was conducted under an approved protocol from the University of New England Animal Ethics Committee (AEC09/175).

Body mass and body measurements

Body measurements of all adults were taken with calipers to the nearest 0.01 mm for body length (base of the skull to the base of the tail), head length (base of the skull to the tip of the nose), tail length, tail width and pes (or foot) length. Values for body measurements were expressed as absolute and mass-specific values. One animal in the CR group was born without a tail, but because body mass and all the other body measurements did not differ significantly ($P > 0.05$) from the other animals in the same group, values of this individual, except for tail measurements, were included in the analysis.

Metabolic measurements

The MR was measured as the rate of oxygen consumption (\dot{V}_{O_2}) for at least 2 h each at T_a values of 16.0 ± 0.1 , 23.0 ± 0.05 and

$30.0 \pm 0.01^\circ\text{C}$ for both CR and WR animals to cover a wide range of T_a , including the two different T_r values, using open flow respirometry. All resting MR (RMR) measurements were conducted during the daytime, which is the inactive phase for *S. crassicaudata* (nocturnal species), for 7–8 h, which is long enough to obtain reliable minimum values (Cooper and Withers, 2009). The RMR was defined over at least three continuous readings (i.e. over 27 to 36 min) when MR was stable and minimal. These values were considered to represent steady-state conditions and were averaged. Measurements were made once for each adult animal. Body mass was recorded before and after each MR measurement. Mass-specific MR was calculated assuming a linear decrease of body mass during MR measurements. Food and water were not available to the animals during MR measurements, but were (*ad libitum*) at all other times. For all MR measurements, single channel oxygen (FC-10, Sable Systems, Las Vegas, NV, USA; resolution 0.0001%) and CO_2 (CA-10, Sable Systems; resolution 0.0001%) analyzers were used. Variables for either two or three individuals and outside air as a reference were measured in sequence using solenoid valves and recorded in intervals of 3 min with a custom-written program (Visual Basic for DOS). Animals were placed in metabolic chambers (750 ml; flow rate $\sim 450 \text{ ml min}^{-1}$), which were placed into a temperature-controlled cabinet. The T_a in the metabolic chambers was measured to the nearest 0.1°C via calibrated thermocouple probes that were inserted 1 cm into the air space of the chamber. The flow-rate of dry air passing through the metabolic chambers was controlled with rotameters (7908, Aalborg, New York, NY, USA) and measured with mass flowmeters (FMA-5606, Omega, Stamford, CT, USA). Air exiting the chamber passed through a column of Drierite (CaSO_4) to remove water vapour, and a subsample was used for gas analysis.

To assess torpor patterns we measured MR for both CR and WR animals over a 24 h period. We quantified the occurrence, duration and depth of torpor bouts (TMR) as well as average daily MR (ADMR) at 16.0 ± 0.1 and $24.0 \pm 0.1^\circ\text{C}$ T_a . Torpor was defined as a reduction in MR below 75% of RMR and torpor bout duration was calculated as the time MR remained below 75% RMR (Geiser et al., 2006). TMR was defined as the lowest of at least three continuous readings (i.e. over 27 to 36 min) when TMR was stable and minimal. The ADMR was calculated by integrating the data over the entire 24 h and converted to Joules [$20.083 \text{ J ml}^{-1} \text{ O}_2$ (Schmidt-Nielsen, 1990)]. We also calculated the normothermic ADMR (ADMR_n) of torpid animals by replacing the total MR during torpor, from entry to arousal, with RMR.

Statistical analysis

Statistical analyses were performed with SAS version 9.01 (SAS, 2001). We used mixed modelling (PROC MIXED) to control for repeated measurements from the same individuals, with sex, T_r (CR at 16°C vs WR at 22°C) and T_a (for MR measurements at T_a values of 16, 23 and 30°C) as fixed effects and the animal as a random effect. Summary statistics are given as means \pm s.e.m., where N is the number of individuals.

RESULTS

Body mass and body measurements

Gender had no significant effect ($P > 0.05$) on body mass or any of the body measurements. Furthermore, body mass did not differ between CR and WR animals (15.97 ± 0.43 vs 16.20 ± 0.47 g, respectively; $F_{1,16} = 0.24$, $P > 0.05$; Table 1). However, T_r had a significant effect on the absolute body length ($F_{1,16} = 18.13$, $P < 0.001$), with CR animals being on average significantly longer (80.8 ± 6 mm)

Table 1. Body mass and body measurements of adult *Sminthopsis crassicaudata* reared at either 16°C (cold reared) or 22°C (warm reared)

Body measurements	Cold reared (N=10)	Warm reared (N=9)
Body mass (g)	15.97±0.43	16.20±0.47
Body length (mm)	80.8±6 ^a	76.4±5 ^b
Mass-specific body length (mm g ⁻¹)	5.1±0.01 ^a	4.7±0.01 ^b
Head length (mm)	29.4±3 ^a	27.5±2 ^b
Mass-specific head length (mm g ⁻¹)	1.8±0 ^a	1.6±0.01 ^b
Tail length (mm)	5.76±0.07*	5.60±0.11
Mass-specific tail length (mm g ⁻¹)	0.36±0.01*	0.35±0.01
Tail width (mm)	0.51±0.01*	0.50±0.02
Mass-specific tail width (mm g ⁻¹)	0.03±0*	0.03±0
Pes length (mm)	1.65±0.01	1.62±0.02
Mass-specific pes length (mm g ⁻¹)	0.10±0	0.10±0

Data are means ± s.e.m.; different superscripted letters among values in the same row indicate a significant difference ($P < 0.05$).
*N=9 (see Materials and methods, 'Body mass and body measurements' for details).

than WR animals (76.4±5 mm). The same is true when body length was expressed on a mass-specific basis (CR 5.1±0.01 mm g⁻¹, WR 4.7±0.01 mm g⁻¹, $F_{1,16}=4.97$, $P < 0.05$; Table 1). Head length also was significantly longer ($F_{1,16}=18.03$, $P < 0.001$) in CR (29.4±3 mm) versus WR animals (27.5±2 mm), and likewise for mass-specific head length (CR 1.8 mm g⁻¹, WR 1.6±0.01 mm g⁻¹; $F_{1,16}=4.97$, $P < 0.05$). Both body and head length were positively correlated with body mass (Fig. 1; see legend for regression equations). There was no relationship between T_r and any other body measurement (tail length, tail width or pes length, $P > 0.05$; Table 1).

Thermal energetics

Resting metabolic rate

RMR was significantly related to T_a ($F_{2,29}=500.65$, $P < 0.001$), but not to gender ($F_{1,29}=4.26$, $P=0.092$). Moreover, T_r had a significant effect on RMR ($F_{2,29}=27.26$, $P < 0.001$). Regressions for each animal separately (RMR against T_a) produced slopes for the individuals within each rearing group that did not differ (CR, $F_{4,9}=2.58$, $P=0.384$; WR, $F_{3,8}=2.58$, $P=0.296$), hence averages were calculated. The common regression lines for CR animals were $\text{RMR}=10.58-0.290T_a$ (where RMR is in ml O₂ g⁻¹ h⁻¹ and T_a is in °C; $R^2=0.99$, $N=10$, $P < 0.001$; Fig. 2) and for WR animals, $\text{RMR}=12.79-0.369T_a$ ($R^2=0.99$, $N=9$, $P < 0.001$; Fig. 2). Data on \dot{V}_{CO_2} followed the same pattern as \dot{V}_{O_2} and thus are not presented separately here.

Extrapolation of RMR to zero (Scholander et al., 1950) gave a theoretical T_b of 36.5°C for CR animals, and a theoretical T_b of 34.6°C for WR animals, which is only 0.5°C higher and 1.4°C lower, respectively, than the normothermic T_b [36.0°C (Schmidt-Nielsen, 1985)] in marsupials.

CR animals had significantly lower ($F_{1,18}=15.29$, $P < 0.001$) RMRs when measured at 16°C T_a [5.89±0.11 ml O₂ g⁻¹ h⁻¹ or 86.36±1.94 ml O₂ h⁻¹; body mass (M_b)=14.85±0.52 g] than WR animals (6.92±0.24 ml O₂ g⁻¹ h⁻¹ or 103.10±1.75 ml O₂ h⁻¹; M_b =15.01±0.42 g; Fig. 2). Mass-specific RMR (ml O₂ g⁻¹ h⁻¹) measured at 16°C T_a was also negatively correlated with body mass at both values of T_r , with the slope for WR animals steeper ($\text{RMR}=14.58-0.480T_a$; $R^2=0.74$, $N=9$, $P < 0.001$) than that for CR animals ($\text{RMR}=8.94-0.206T_a$; $R^2=0.90$, $N=10$, $P < 0.001$). At 23°C T_a , RMR did not differ between CR (4.00±0.06 ml O₂ g⁻¹ h⁻¹ or 61.76±2.89 ml O₂ h⁻¹; M_b =15.45±0.61) and WR animals

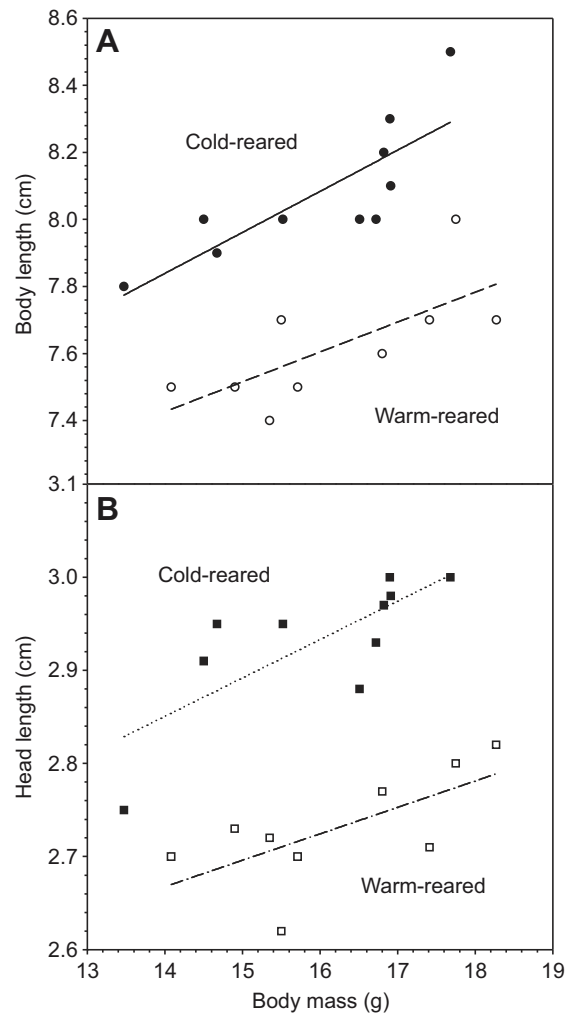


Fig. 1. Body length (A) and head length (B) as a function of body mass in cold- (CR; 16°C, $N=10$) and warm-reared (WR; 22°C, $N=9$) adult *Sminthopsis crassicaudata*. Regression equations are as follows: (A) CR, $y=6.12+0.123x$, $F^2=0.66$; WR, $y=6.18+0.089x$, $F^2=0.50$; (B) CR, $y=2.27+0.041x$, $F^2=0.56$; WR, $y=2.27+0.028x$, $F^2=0.45$. All regressions were significant at $P < 0.001$.

(4.22±0.25 ml O₂ g⁻¹ h⁻¹ or 64.89±1.68 ml O₂ h⁻¹; M_b =15.39±0.41 g). However, mass-specific RMR measured at 23°C T_a in WR animals declined significantly ($P < 0.001$) with increasing body mass from 5.08 ml O₂ g⁻¹ h⁻¹ at 13.97 g to 3.30 ml O₂ g⁻¹ h⁻¹ at 17.31 g ($\text{RMR}=11.52-0.475T_a$; $R^2=0.86$, $N=9$, $P < 0.001$), whereas in CR animals mass-specific RMR remained constant irrespective of body mass ($\text{RMR}=4.62-0.011T_a$; $R^2=0.01$, $N=10$, $P=0.67$). At 30°C T_a , which is close to being within the thermal neutral zone for *S. crassicaudata* [31.5–35.5°C (MacMillen and Nelson, 1969)], RMR also did not differ between CR (1.82±0.11 ml O₂ g⁻¹ h⁻¹ or 28.84±2.45 ml O₂ h⁻¹; M_b =15.79±0.65 g) and WR animals (1.74±0.11 ml O₂ g⁻¹ h⁻¹ or 27.46±1.78 ml O₂ h⁻¹; M_b =15.85±0.42 g; Fig. 2). For animals from both T_r groups, mass-specific RMR was not affected by body mass at 30°C T_a .

Torpor

Body mass before and after torpor measurements as well as body mass loss during the 24 h measurement periods did not differ ($P > 0.05$) between CR and WR animals at 16 and 24°C T_a .

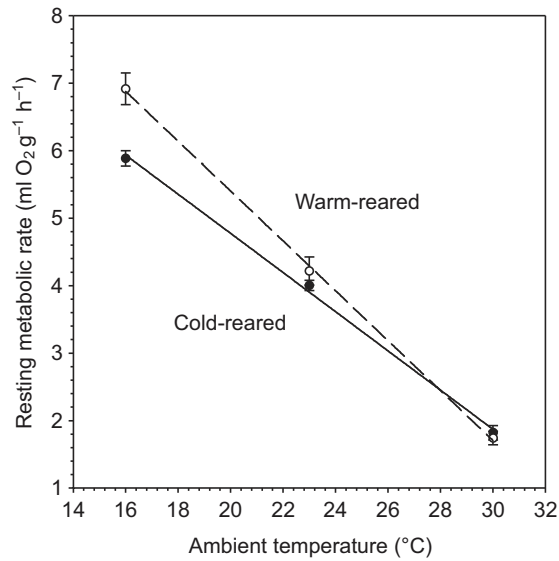


Fig. 2. Resting metabolic rate (RMR) measured as the rate of oxygen consumption in relation to ambient temperature in cold- (CR; 16°C) and warm-reared (WR; 22°C) adult *S. crassicaudata*. Regression equations are as follows: CR, $RMR=10.58-0.290T_a$ (where RMR is in $ml\ O_2\ g^{-1}\ h^{-1}$ and T_a is in °C), $R^2=0.99$, $N=10$, $P<0.001$; WR, $RMR=12.79-0.369T_a$, $R^2=0.99$, $N=9$, $P<0.001$.

Furthermore, values for torpor variables did not differ between sexes. At 16°C T_a , torpor occurred in all CR animals (100%) and in six out of nine WR animals (67%). At 24°C T_a , torpor occurred in six CR animals (60%) and in four WR animals (44%; Table 2). Torpor bout duration differed significantly ($F_{1,17}=7.73$, $P<0.05$) between CR and WR animals at 16°C T_a , but not at 24°C T_a . Furthermore, T_a had a significant effect ($F_{1,17}=36.95$, $P<0.001$) on torpor bout length, with 10.43 ± 0.71 h (range: 7.00–13.40 h) being the longest in CR animals measured at 16°C T_a and lowest in WR animals measured at 24°C T_a (3.20 ± 0.21 h; range: 4.91–2.01 h). TMR was not affected by T_r ($F_{1,17}=2.50$, $P=0.173$) but was significantly affected by T_a ($F_{1,17}=46.56$, $P<0.001$), with approximately 30% lower values at 16°C than at 24°C T_a .

Average daily metabolic rate

ADMR, whether expressed as $kJ\ day^{-1}$ or on a mass-specific basis, differed significantly ($P<0.01$) between CR and WR animals measured at 16°C but not at 24°C T_a (Table 2). Furthermore, torpor

bout duration was negatively correlated with ADMR at both T_a values for the two groups (Fig. 3; see figure legend for regression equations). Even when it is assumed that animals remained normothermic, CR animals had a significantly lower ($P<0.05$) ADMR_n than WR animals at 16°C, but not at 24°C T_a . However, ADMR_n differed ($P<0.01$) between 16 and 24°C T_a in both CR and WR animals (Table 2). Energetic savings per day by animals employing torpor by comparing measured ADMR with predicted ADMR_n revealed that CR animals employing torpor saved on average nearly 10% more energy per day than WR animals employing torpor ($P<0.01$; Table 2). Furthermore, daily savings were positively correlated with torpor bout duration at both T_a values for animals from both T_r groups ($T_r=16^\circ C$, $T_a=16^\circ C$, $y=-7.02+4.09x$, $R^2=0.94$, $N=10$; $T_r=22^\circ C$, $T_a=16^\circ C$, $y=-5.44+4.17x$, $R^2=0.98$, $N=6$; $T_r=16^\circ C$, $T_a=24^\circ C$, $y=-2.42+2.89x$, $R^2=0.95$, $N=6$; $T_r=22^\circ C$, $T_a=24^\circ C$, $y=2.56+1.58x$, $R^2=0.43$, $N=4$; all $P<0.01$).

DISCUSSION

Our study provides the first quantitative data on developmental phenotypic flexibility of torpor expression in mammals and of morphology and thermal energetics for a marsupial. We show that even a small difference in rearing temperature of 6°C (16 vs 22°C) significantly affected morphology, thermal physiology, energy expenditure and torpor patterns in adult *S. crassicaudata*.

Body measurements

Our results on body measurements do not conform with Allen's rule, but conform to some extent to Bergmann's rule. Although Allen's rule postulates that animals living in cold climates have shorter extremities (e.g. tail, limbs and ears) than their conspecifics in warmer climates to prevent heat loss (Allaby, 1985), Bergmann's rule suggests that animals living in cooler environments are larger in size than the same species living in warmer environments. Instead, we found that tail length and width as well as pes length did not differ between CR and WR animals. Contrarily, body and head length were significantly longer in CR than in WR animals (Table 1, Fig. 1) whereas body mass remained the same irrespective of T_r .

Allen's rule is often used to explain the morphological differences of animals living in cold or warm environments. And indeed the morphological differences described by Allen's rule have been experimentally reproduced by rearing animals at cold or warm temperatures during their growth period in a few placental species [e.g. mice (Serrat et al., 2009), rats (Riesenfeld, 1973), rabbits (Ogle and Mills, 1933) and pigs (Heath, 1984)]. In pigs, for example, WR animals had a larger surface area and were longer than CR animals of the same body mass (Heath, 1984), which is exactly the opposite

Table 2. Torpor variables measured over 24 h at ambient temperatures (T_a) of 16 and 24°C in adult *S. crassicaudata* reared at either 16°C (cold reared) or 22°C (warm reared)

Torpor variables	Cold reared (N=10)		Warm reared (N=9)	
	T_a 16°C	T_a 24°C	T_a 16°C	T_a 24°C
Torpor occurrence (%)	100	60	67	44
Mean body mass (g)	14.43±0.23	14.06±0.31	15.06±0.53	14.40±0.61
Torpor bout duration (h)	10.43±0.71 ^a	4.53±0.74 ^{bd}	8.23±1.55 ^c	3.20±0.21 ^d
TMR ($ml\ O_2\ g^{-1}\ h^{-1}$)	0.20±0.01 ^a	0.58±0.09 ^b	0.25±0.05 ^a	0.80±0.01 ^b
ADMR ($kJ\ d^{-1}$)	24.41±0.73 ^a	24.95±0.65 ^a	35.90±1.12 ^b	26.95±0.91 ^a
ADMR ($kJ\ g^{-1}\ d^{-1}$)	1.77±0.10 ^a	1.76±0.07 ^a	2.33±0.16 ^b	1.95±0.07 ^a
ADMR _n ($kJ\ d^{-1}$)	39.27±0.94 ^a	26.62±1.13 ^{bd}	44.52±1.28 ^c	28.25±0.82 ^d
Savings (%)	38.04±2.69 ^a	10.69±2.19 ^{bd}	28.92±6.56 ^c	6.29±2.07 ^d

ADMR, average daily metabolic rate; ADMR_n, calculated normothermic ADMR; TMR, torpor metabolic rate.

Data are means ± s.e.m. Different superscripted letters among values in the same row indicate a significant difference ($P<0.05$).

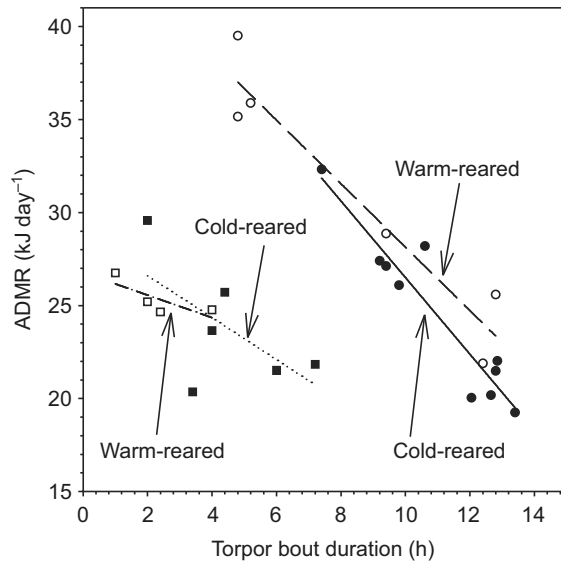


Fig. 3. Effect of torpor bout duration on average daily metabolic rate (ADMIR) in adult *S. crassicaudata* at two different rearing temperatures (T_r) and two different ambient temperatures (T_a). Regression equations are as follows: filled circles, solid line: $T_r=16^\circ\text{C}$, $T_a=16^\circ\text{C}$, $y=47.09-2.06x$, $R^2=0.89$, $N=10$; open circles, dashed line: $T_r=22^\circ\text{C}$, $T_a=16^\circ\text{C}$, $y=45.20-1.71x$, $R^2=0.92$, $N=6$; filled squares, dotted line: $T_r=16^\circ\text{C}$, $T_a=24^\circ\text{C}$, $y=28.88-1.13x$, $R^2=0.36$, $N=6$; open squares, dot-dashed line: $T_r=22^\circ\text{C}$, $T_a=24^\circ\text{C}$, $y=26.77-0.61x$, $R^2=0.62$, $N=4$. All regressions were significant at $P<0.01$ (see Results for details).

to our results. However, to our knowledge no such experiments have been conducted with marsupials. Hence our results are the first suggesting that the dasyurid *S. crassicaudata* does not strongly follow either Allen's or Bergmann's rule. Our results rather suggest that CR *S. crassicaudata* grow larger in size relative to WR animals at the same body mass, which could be an adaptation to the ecological constraints faced by this species. This agrees with data from a detailed study on Bergmann's rule and body size in mammals (Freckleton et al., 2003). In this study, the authors argue that, contrary to previous theoretical work, Bergmann's rule is exhibited most strongly in larger species and is weaker or non-existent in small species (<0.16 kg), suggesting that temperature is not the only factor exerting selective pressure on body size in small mammals. Dunnarts, for example, are known to use basking to lower arousal costs from daily torpor and thus save large amounts of energy as compared with active, endothermic arousal where animals have to substantially increase their MR (Warnecke et al., 2008; Warnecke and Geiser, 2010). A slightly longer body and head would therefore enable *S. crassicaudata* to bask more efficiently because of the larger surface area available.

Resting metabolic rate

In our study, RMR at 30°C did not differ between WR and CR animals (Fig. 2). Furthermore, in both rearing groups, RMR at 30°C was independent of body mass. Therefore, assuming that RMR at 30°C approximates BMR, the BMR of WR and CR animals (1.74 and $1.82\text{ ml O}_2\text{ g}^{-1}\text{ h}^{-1}$, respectively) was in the range of reported values for *S. crassicaudata* [$1.67\text{ ml O}_2\text{ g}^{-1}\text{ h}^{-1}$ (MacMillen and Nelson, 1969)]. Interestingly, RMR measured at 23°C T_a was independent of body mass in CR but not in WR animals, i.e. RMR in animals reared at 16°C did not change with body mass whereas the RMR in animals reared at 22°C increased with decreasing body mass.

RMR in CR animals at 16°C T_a was significantly lower compared with WR animals, which differs from acclimation experiments in adult *S. macroura* (Geiser et al., 2003), where cold-acclimated animals mainly increased maximum MR during cold exposure compared with warm-acclimated animals. However, during acclimation, animals are subjected only for a short period to a cold or warm environment whereas our study animals were reared from early postnatal age at constant T_a , possibly resulting in an improved non-shivering thermogenic capacity. RMR at 16°C T_a was only approximately 3.1 times BMR in CR animals compared with 4.0 times BMR in WR animals, indicating only moderate thermoregulatory costs above BMR for CR animals. The reduced RMR values at 16°C T_a in CR animals compared with WR animals in our study are most likely due to the improved defense to cold exposure in CR animals because thermoregulatory mechanisms are susceptible to modification by early postnatal experience (Hahn, 1956; Cooper et al., 1980; Young, 1985; Young and Shimano, 1998). It has been demonstrated in rats that continuous early life exposure to a cold environment induces a permanent and irreversible increase in brown adipose tissue (BAT) thermogenesis (Morrison et al., 2000), whereas when adult animals are exposed to cold (cold acclimation) the changes that occur in BAT are reversible when the animals are returned to normal temperatures (Vollmer and Skött, 2002). In small placental mammals, BAT is the main source for non-shivering thermogenesis (Andrew, 1978; Himms-Hagen, 1984; Wunder and Gettinger, 1996; Jastroch et al., 2008). In marsupials however the role of BAT is controversial. Although some studies have shown that BAT in marsupials is absent or non-functional (Nicol et al., 1997; Rose et al., 1999), others have shown that BAT is present in some marsupials (e.g. Hope et al., 1997). A recent study by Jastroch et al. (Jastroch et al., 2008) found that in some marsupials (*Monodelphis domestica* and *Antechinus flavipes*) BAT expressing uncoupling protein 1 may be recruited transiently during early life and is lost in later life stages, whereas in *S. crassicaudata* it remains also during adult life stages. We found that body mass and specifically tail width, which is an indicator of fat storage in *S. crassicaudata*, did not differ between both rearing groups (Table 1). Therefore, it could be hypothesised that *S. crassicaudata* evolved the ability to generate BAT during the postnatal exposure to the cold and retained this ability in later life stages. Furthermore, marsupials are also able to use vasoconstrictor-induced non-shivering thermogenesis in skeletal muscles (Eldershaw et al., 1996), which may have been enhanced during development in cold-exposed animals. However, these explanations remain speculative until more data on non-shivering thermogenesis for marsupials in general and *S. crassicaudata* in particular become available. Nevertheless, our results clearly show that the continuous exposure to a cold environment starting from the early postnatal phase until adulthood induces significant changes in thermal energetics, resulting in an improved thermogenic capacity or insulation evidenced by reduced energy expenditure.

Torpor

Torpor bout duration was significantly longer at 16°C than at 24°C T_a for both CR and WR animals (Table 2). In contrast, ADMIR did not differ between 16 and 24°C T_a within either rearing group, suggesting that irrespective of environmental T_a , animals use torpor for balancing daily energy expenditure, which accords with earlier results for this species (Holloway and Geiser, 1995). Torpor bouts were longer in CR than WR animals at 16°C T_a , with ADMIR showing the reverse: a lower energy expenditure for CR than for WR animals. This suggests that animals raised in a cooler

environment employ more torpor than animals not used to cold environments, resulting in lower daily energy expenditures. This likely explains why this species has such a large distribution range in Australia from cool temperate to hot arid regions, which also appears to be the case for other opportunistic heterotherms (Geiser et al., 2011). However, our results are in contrast to short-term acclimation experiments in adult stripe-faced dunnarts, *Sminthopsis macroura*, where no differences in torpor bout length were found between animals subjected to a cold or warm environment for 4 weeks (Geiser et al., 2003). Acclimation is known to induce only temporal changes in thermoregulation whereas rearing animals at different T_a values can induce irreversible changes that are maintained throughout life (Morrison et al., 2000; Vollmer and Skøtt, 2002).

To assess how much energy was saved by using torpor, we calculated $ADMR_n$ by replacing the MR during torpor from entry to arousal with RMR, assuming the animal would have stayed normothermic. By relating the actual measured ADMR to the hypothetical calculated $ADMR_n$, we estimated how much the daily energy expenditure of the two rearing groups was reduced by entering torpor. Our results show that CR animals save on average 10% more than WR animals per day at 16°C T_a , indicating that CR animals by employing longer bouts of torpor are more efficient in their use of energy than animals not used to these environmental conditions.

CONCLUSIONS

Our data on developmental plasticity have implications for understanding the geographic range of mammals as well as how mammals could respond rapidly to changes in climate by altering body shape and thermal energetics without the evolutionary changes. Several studies have already indicated that climate change influences the geographic range of many taxa (e.g. Walther et al., 2002; Parmesan and Yohe, 2003). However, the capacity of a species to respond to climate change depends on its rate of plastic and developmental adjustments. If these adjustments are slower than the change in environmental conditions (i.e. climate change) or are physiologically impossible, the species will likely go extinct (Chown et al., 2010). Thus, a rapid adjustment to environmental conditions, such as developmental plasticity, could be a major determinant in a species' ability to cope with climate change. The rate of adjustment, however, depends largely on the physiological capacity of the species. Therefore, we argue that mammals employing some form of energy-saving mechanism, such as torpor or hibernation, exhibit a higher physiological and morphological developmental plasticity, thus allowing them to respond to rapid changing environmental conditions more quickly (in our case just one generation) than species that do not employ such energy-saving mechanisms. This argument is supported by a recent study on the extinction rate of mammals (Geiser and Turbill, 2009), where it has been demonstrated that of the 61 recently extinct species, although most were small, only 6.5% were heterothermic, thus suggesting that animals employing some form of energy-saving mechanism stand a better chance of surviving during adverse climatic or environmental conditions.

Our study presents the first long-term experiment on differences in body shape, metabolic rate and torpor pattern in a CR and WR mammal. Our data show that the marsupial *S. crassicaudata* grows longer in size while retaining the same body mass when reared in a cold environment. Furthermore, CR animals exhibited an improved defense to cold exposure compared with WR animals, as evidenced by longer torpor bouts, lower RMR at low T_a values and higher

energy savings. Our results indicate that permanent exposure to warm or cold T_a values from early life influences thermoregulatory mechanisms irreversibly and appropriately.

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