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RESEARCH ARTICLE

Increased homeothermy during reproduction in a basal placental mammal

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ABSTRACT

Homeothermic endothermy, the maintenance of a high and stable body temperature (T_b) using heat produced by elevated metabolism, is energetically expensive. There is increasing evidence that the earliest endotherms were heterotherms that, rather than maintaining strict homeothermy, allowed T_b to fluctuate with large variations between active and rest-phase T_b. The high level of homeothermy observed in modern mammals is therefore likely to have evolved from an ancestral heterothermic state. One of the hypotheses for the evolution of endothermy is that homeothermy allows for greater energetic output during reproduction (parental care model). We tested this hypothesis by measuring metabolic rates over a range of ambient temperatures in both reproductive and non-reproductive greater hedgehog tenrecs (*Setifer setosus*), a physiologically primitive mammal from Madagascar. Tenrecs have some of the lowest metabolic rates and highest levels of T_b variability of any mammal and are therefore good models of the ancestral eutherian state. During pregnancy and lactation, there was an increase in metabolism and T_b below the thermoneutral zone, accompanied by a decrease in T_b variability. The lower critical limit of the thermoneutral zone was estimated at ~25°C. However, whereas increases in resting metabolism were substantial below 20°C (up to 150% higher during reproduction), daytime rest-phase ambient temperatures at the study site rarely reached equivalent low levels. Thus, *S. setosus* provide an example for how relatively low-cost increases in homeothermy could have led to substantial increases in fitness by allowing for the faster production of young. The mechanisms necessary for increases in thermogenesis during reproduction would have further benefited the development of homeothermy in mammals.

KEY WORDS: Evolution of endothermy, Parental care hypothesis, Thermoregulation, Reproduction, Torpor, Madagascar, *Setifer setosus*, Tenrecinae

INTRODUCTION

All extant mammals are endotherms, capable of maintaining core body temperature (T_b) above ambient temperatures (T_a) through the production of heat from metabolism (Bartholomew, 1972; Schmidt-Nielsen, 1997). The precision of T_b regulation varies considerably among modern mammals (Clarke and Pörtner, 2010; Lovegrove, 2012a), ranging from species that maintain a high degree of homeothermy (small circadian variations in T_b), to those that have highly labile T_b (Angilletta et al., 2010; Boyles et al., 2013; Clarke and Pörtner, 2010; Lovegrove, 2012a; Refinetti and Menaker, 1992). In addition to circadian rhythms, in which T_b typically decreases during the rest phase (but see Lovegrove et al., 2014), many mammals also lower T_b and metabolic rate (MR) for extended periods of time during daily torpor and hibernation (Geiser and Ruf, 1995; Lyman et al., 1982).

Strict homeothermy could have evolved as early as 200 million years ago (mya), coincident with increased encephalization and a shift to a nocturnal lifestyle, or as late as 66 mya before the crown placental groups diversified following the mass extinctions at the Cretaceous–Paleogene (K–Pg) boundary (Crompton et al., 1978; Grigg et al., 2004; Lovegrove, 2012b; O'Leary et al., 2013; Rowe et al., 2011). Moreover, there is increasing evidence that endothermy evolved in a tropical environment from an ancestral state in which T_b was highly labile and T_a-dependent (Crompton et al., 1978; Grigg et al., 2004; Lovegrove, 2012a; Lovegrove, 2012b). Under this hypothesis, the ancestral eutherian mammal was small, nocturnal, insectivorous, and likely to have expressed either short- or long-term periods of torpor (Lovegrove, 2012a; Luo, 2007; O'Leary et al., 2013). It is this plesiomorphic heterothermic capacity that is thought to have been the most likely means by which the ancestral eutherian was able to have survived the short- and long-term devastation of the K–Pg boundary asteroid impact (Lovegrove, 2012b).

A number of hypotheses have been proposed in an attempt to explain how and why endothermy, a costly method of thermoregulation and existence in general, evolved in mammals (Bennett and Ruben, 1979; Crompton et al., 1978; Farmer, 2000; Koteja, 2000). In this study, we argue that the study of modern mammals that putatively retained plesiomorphic heterothermic characteristics, that is, physiological characteristics that are thought to have prevailed in Cretaceous eutherian ancestors, should shed light on the transition from ectothermic-like heterothermy to homeothermy (Crompton et al., 1978; Eisentraut, 1960; Grigg et al., 2004; Lovegrove, 2012a). Termed 'protoendotherms' by Grigg, Beard and Augee (Grigg et al., 2004), and 'basoendotherms' by Lovegrove (Lovegrove, 2012a), extant basal eutherians, often found on low-latitude islands with little paleoclimatic history of Cenozoic cooling, are highly heterothermic, with variable T_b and frequent use of torpor. The large amplitudes in the circadian rhythm of T_b in these animals can lead to difficulties in differentiating between torpor and normothermy using T_b alone (Brice et al., 2002; Canale et al., 2012; Kuchel, 2003; Lovegrove and Génin, 2008; Poppitt et al., 1994). Also, the determination of a distinct thermoneutral zone (TNZ), a range of T_a over which MR remains minimal and constant, is highly problematic if T_b is not maintained at a constant level (Brice, 2008; Scholander et al., 1950). The high thermolability of basoendotherms generates a relatively linear relationship between T_b and T_a, with no clear inflection points in MR at the lower and upper critical limits of thermoneutrality that typically define the TNZ in classic homeothermic endotherms (Brice, 2008; Stephenson and Racey, 1993b; Nicoll, personal communication in Stephenson and Racey, 1994).

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List of symbols and abbreviations

AIC_c	Akaike's information criterion scores corrected for small sample size
C_{wet}	wet thermal conductance (ml O_2 h^{-1} $°C^{-1}$ g^{-1})
K–Pg	Cretaceous–Paleogene
MR	metabolic rate
T_a	ambient temperature (respirometer temperature)
T_b	core body temperature
T_{lc}	lower critical limit of the TNZ
T_{sk}	skin temperature
TNZ	thermoneutral zone
TRMR	$\dot{V}_{O_2}$ measured in the TNZ
$\dot{V}_{O_2}$	volumetric rate of oxygen consumed by the animal (ml O_2 h^{-1})
ΔT	temperature differential (T_b–T_a)

Malagasy tenrecs, members of the Afrotherian order Afrosoricida, are perhaps one of the best examples of eutherian basoendotherms (Crompton et al., 1978; Eisentraut, 1960; Lovegrove and Génin, 2008). Tenrecs display some of lowest T_b of any extant mammal and the spiny tenrecs, members of the sub-family Tenrecinae, have some of the lowest basal metabolic rates (BMRs) (Lovegrove, 2000; Symonds, 1999). They have also retained the ancestral diet (insectivory) and nocturnal activity patterns, and have inhabited the relatively warm climate of Madagascar throughout their evolutionary history (Olson and Goodman, 2003). Studies on free-ranging and captive Tenrecinae have indicated that these animals are highly heterothermic, with large rhythms in circadian T_b, as well as frequent (daily, in the case of *Echinops telfairi*) torpor bouts (Lovegrove and Génin, 2008; Nicoll, 1986; Oelkrug et al., 2013; Stephenson and Racey, 1994). However, periods of homeothermy, indicated by an increase in the level and precision of T_b as well as a decrease in torpor use, have been observed in a number of tenrec species during both gestation and lactation (Poppitt et al., 1994; Stephenson and Racey, 1993a; Stephenson and Racey, 1993b; Thompson and Nicoll, 1986). These observations provide strong support for the hypotheses that endothermy evolved in mammals to benefit parental care (Farmer, 2000; Farmer, 2003; Koteja, 2000). However, to date, these studies, all on captive animals, have focused on changes in BMR or, to be more precise, the thermoneutral resting metabolic rate (TRMR) as defined by Lovegrove et al. (Lovegrove et al., 1991) to denote that resting measurements of metabolism occurred at thermoneutrality despite failing to meet all of the requirements for basal metabolism (Stephenson and Racey, 1995; Symonds, 1999). No study has yet to characterise changes in thermoregulatory profiles during reproduction in a basoendotherm.

Brice, Levesque and Grigg [see chapter 3 in Brice (Brice, 2008)] predicted that the higher level of homeothermy observed during reproduction in basoendotherms would result in thermal profiles that conformed more closely to classic mammalian patterns. This study provides the first test of this prediction. We sought to fully characterize the thermoregulatory patterns during reproduction in a basoendotherm by measuring RMR over a range of T_a in a free-ranging population of greater hedgehog tenrecs [*Setifer setosus* (Schreber 1778)] in the dry deciduous forest of western Madagascar. Although *S. setosus* is known to use torpor less than its sister species *E. telfairi* (Eisenberg and Gould, 1969; Eisenberg and Muckenhirn, 1968), it has been shown to enter into torpor over a wide range of T_a and to express a high degree of thermolability when not torpid (Levesque et al., 2012; Lovegrove et al., 2014). In addition, *S. setosus* is both larger and more abundant than *E. telfairi* and, unlike the larger common tenrec (*Tenrec ecaudatus*), is not readily consumed by humans at the study site, making it an ideal candidate for a long-term study on the energetics in a free-ranging population (Randrianjafy, 2003).

RESULTS

Reproductive status and sample size

From October 2010 to April 2011, metabolic measurements were obtained from 22 individuals (10 females, 12 males) for a total of 92 measures. Fewer animals (four females and five males; 43 measures in total) were captured during the second season (October 2011–February 2012). Individuals were also caught for the first time later in the year and therefore no recordings were obtained from non-reproductive females during the second season. High rates of mortality (Levesque et al., 2013) precluded the collection of data after January 2012.

Model selection using corrected Akaike's information criterion (AIC_c) weights (Burnham and Anderson, 2002) was performed to assess the influence of time since emergence from hibernation [day: calculated as number of days since 1 September (Levesque et al., 2013)], reproductive status (males and gestating, lactating and non-reproductive females) and season on body mass (Table 1). Only the values from the first day of measurements per individual were used and, as these included multiple measures per individual, a random factor [in the form ~1|animalID (see Zuur et al., 2009)] was included in all models. Mass was ln-transformed to ensure a normal distribution of the model residuals, and heteroscedasticity within the factor day was controlled by using varFixed (~day) as the variance structure. The model with the highest Akaike weight (0.61) included day only, with a parameter estimate of 0.95±0.16 g day^{-1} (Table 2). The inclusion of reproductive status in the model resulted in an additional Akaike weight of 0.31. The remaining Akaike weight was contributed by adding season to the model. A Tukey *post hoc* test on the model containing day and status, using the 'glht' function in R package 'multcomp' (Hothorn, Bretz and Westfall 2008), provided parameter estimate sizes for differences in status. There was little difference between gestating and lactating females as well as between males and non-reproductive females (parameter estimate of less than 5 g), whereas both of the latter groups were smaller than the former groups (differences >25 g).

Table 1. Mean body mass, resting metabolic rates at thermoneutrality [TRMR; ambient temperature (T_a)=25–33.5°C] and minimum thermal conductance (C_{wet}; T_a<30.5°C) of reproductive and non-reproductive *Setifer setosus*

		Female		
	Male	Non-reproductive	Gestating	Lactating
Mass (g)	172.7±46.2 (21)	147.1±17.7 (9)	272.3±7.3 (11)	222.1±74.8 (2)
TRMR (ml O_2 h^{-1})	60.7±17 (41)	53±14.6 (19)	99.9±34.4 (29)	81.4±19.8 (3)
TRMR (ml O_2 h^{-1} g^{-1})	0.36±0.11 (41)	0.36±0.11 (19)	0.40±0.07 (29)	0.30±0.08 (3)
Minimum C_{wet} (ml O_2 hr^{-1} $°C^{-1}$ g^{-1})	0.10±0.05 (14)	0.12±0.11 (7)	0.10±0.02 (7)	0.08 (1)
	N=17	*N*=8	*N*=7	*N*=2

Values in parentheses indicate the total number of measures obtained.

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Table 2. Best-fitting linear mixed models evaluating the effect of various factors on the physiological parameters of reproductive and non-reproductive *Setifer setosus*

Fixed factors	Random factors	Variance structure	*k*	AIC$_c$	Akaike weights
Body mass					
Day	~1|animalID	varFixed (~day)	4	431.12	0.57
Day + status	~1|animalID	varFixed (~day)	7	432.10	0.35
Day + status + season	~1|animalID	varFixed (~day)	8	435.07	0.08
$\dot{V}_{O_2}$ in TNZ (T_a≥25°C)					
Mass	~day|animalID	varFixed (~day)	6	745.47	0.96
Status + mass	~day|animalID	varFixed (~day)	9	752.84	0.02
T_a + status + mass	~day|animalID	varFixed (~day)	10	754.43	0.01
$\dot{V}_{O_2}$ below TNZ (T_a<25°C)					
T_a + status + mass	n/a	varFixed (~day)	5	139.75	0.96
T_a + status + mass + day	n/a	varFixed (~day)	6	147.17	0.02
Mass	n/a	varFixed (~day)	3	148.52	0.01
Body temperature					
T_a × status + day	~1|animalID	varIdent (~1|status)	11	263.97	0.51
T_a × status + mass + day	~1|animalID	varIdent (~1|status)	12	265.11	0.29
T_a × status + mass	~1|animalID	varIdent (~1|status)	11	266.11	0.18
T_a × status	~1|animalID	varIdent (~1|status)	10	270.99	0.02
Wet thermal conductance (T_a<32.5°C)					
T_a × status + mass	~1|animalID	varFixed (~T_a)	9	66.97	0.72
T_a × status + mass + day	~1|animalID	varFixed (~T_a)	10	68.89	0.28

Ranking was performed using corrected Akaike's information criterion (AIC$_c$) scores and Akaike weights. All models with an Akaike weight >0 are presented and the number of parameters contained in the model are included (*k*). Status refers to the reproductive status of the individual (male and gestating, lactating and non-reproductive female). Day is the number of days since 1 September, a proxy for the start of the active season. TNZ, thermoneutral zone; $\dot{V}_{O_2}$, oxygen consumption.

Oxygen consumption, body temperature and thermal conductance

Although torpor and normothermy are difficult to distinguish in species with a high degree of thermolability (Canale et al., 2012; Stephenson and Racey, 1993b), it was necessary to consider 'torpid' animals separately from those that thermoregulated. Individuals were therefore classed as torpid if the ΔT (T_b–T_a) was less than 5°C, and thermoregulating if ΔT was greater than 5°C (Hosken and Withers, 1999). Data from thermoregulating animals only were included in the analyses. Data from three data measurement sessions, two on males at 22°C and one on a male at 32°C, were excluded from the analysis because the animals remained active throughout the duration of the recording. In the first season, non-reproductive individuals entered torpor at all T_a except for a male that maintained a T_b of 29.8°C at T_a=22.1°C and a female with a T_b of 30.1°C at a T_a of 25°C. In contrast, in 2011–2012, only two of the five males entered torpor during data measurements: one at all temperatures and the other at the coldest temperature (22°C) only. There was no correlation, however, between body condition index (see Levesque et al., 2013) and torpor expression ($F_{1,45}$=0.52, P=0.48).

RMR, measured as oxygen consumption ($\dot{V}_{O_2}$), and T_b were highly variable (Table 1, Fig. 1). Piecewise linear regression indicated an inflection point in the slope of $\dot{V}_{O_2}$ versus T_a at ~24.6–24.8°C in the model including all individuals (N=31 individuals, n=133 measurements), at 24.8–24.9°C for reproductive females only (N=8, n=41), and at 24.9°C for non-reproductive individuals (N=27, n=85). The lower critical limit (T_{lc}) of the TNZ was therefore estimated to be around 25°C. Initial results indicated that models containing an inflection point at 25°C had lower AIC$_c$ scores, and therefore it was justified to analyse the data above (within the TNZ: T_a≥25°C) and below the T_{lc} (T_a<25°C) separately.

Preliminary analysis of $\dot{V}_{O_2}$ in the TNZ (TRMR, T_a>24.5°C) indicated a single outlier, a reproductive female 33 days from parturition who expended twice as much energy per gram while defending a similar T_b as any other individual at 25°C. This datum was excluded from all subsequent analyses. Mean values for TRMR appeared to differ between reproductive and non-reproductive individuals (Table 1, Figs 1, 2) at the whole-animal level. However, the best model predicting TRMR (Akaike weight=0.96) contained mass (parameter estimate=0.37±0.04 ml O_2 h^{-1}) only as a fixed factor, and day and animal ID, as well as their interaction (in the form ~day|animalID), as random factors (Table 2). A model containing reproductive status only, as well as one with reproductive status and T_a, explained the remaining Akaike weight (0.02 and 0.01, respectively).

The highest ranking model describing the $\dot{V}_{O_2}$ of non-torpid animals (five males, nine gestating females) below the T_{lc} contained T_a, reproductive status and mass as fixed factors, and animal ID as the sole random factor (Table 2). Of the fixed effects, reproductive status had the largest impact on $\dot{V}_{O_2}$, with gestation resulting in an increase to a value 186.53±35.4 ml O_2 h^{-1} higher than that of males. The RMR of reproductive females also increased as T_a decreased below T_{lc} at a rate of 14 ml O_2 h^{-1} °C^{-1} (Fig. 1). The relationship between T_a and RMR in thermoregulating males was not significant (t_3=–0.39, P=0.72), although sample size was very small.

Inflection points in the regression of T_b against T_a were located at temperatures similar to those for $\dot{V}_{O_2}$ (~25°C), but their inclusion in the model produced no improvement in AIC$_c$ scores; one set of models only, containing all data from non-torpid individuals, was used in the T_b analysis. Fixed factors showing the most influence on T_b were reproductive status, T_a and day (Table 2, Fig. 1), although the effect of the latter was slight (<0.01°C day^{-1}). Animal ID was also included as a random effect. Contrary to the patterns seen in $\dot{V}_{O_2}$, T_b was dependent on T_a: T_b increased by 0.4±0.02°C per 1°C change in T_a. Males and non-reproductive females had lower T_b than reproductive females (by 13.2±2.9 and 4.8±1.5°C, respectively), although this difference decreased with increasing T_a. The second best fitting model replaced day with mass, although the effect of this was once again small (<0.001°C g^{-1}).

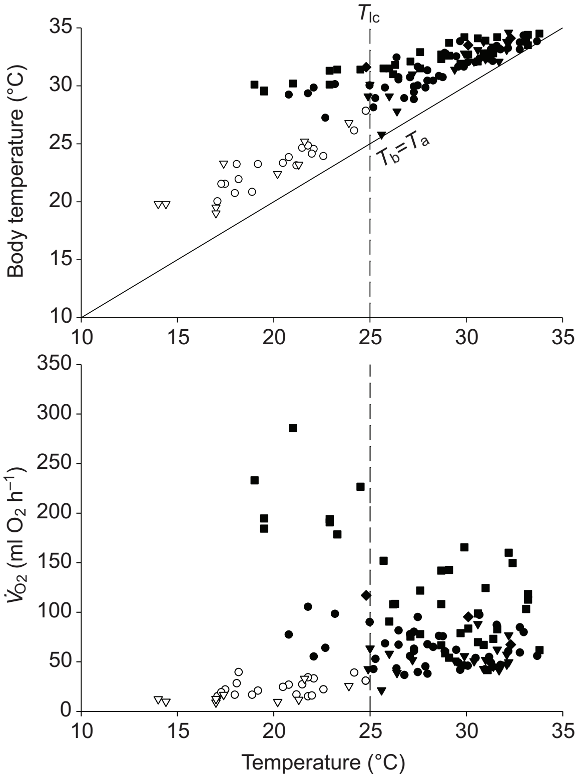

Fig. 1. Body temperature and resting metabolic rates of *Setifer setosus* over two reproductive seasons in Ankarafantsika National Park. Reproductive females (squares, gestating; diamonds, lactating), males (circles) and non-reproductive females (triangles) that thermoregulated are plotted in black; torpid [T_b–T_a (ΔT)<5°C, where T_b is core body temperature and T_a is ambient temperature] individuals in white. The solid line indicates T_b=T_a and the dashed line the approximate lower critical limit (T_{lc}) of the thermoneutral zone. Significant differences were found between torpid and normothermic animals for both oxygen consumption ($\dot{V}_{O_2}$) and T_b. Despite defending similar T_b, there was also a significant difference in $\dot{V}_{O_2}$ between reproductive females and normothermic males.

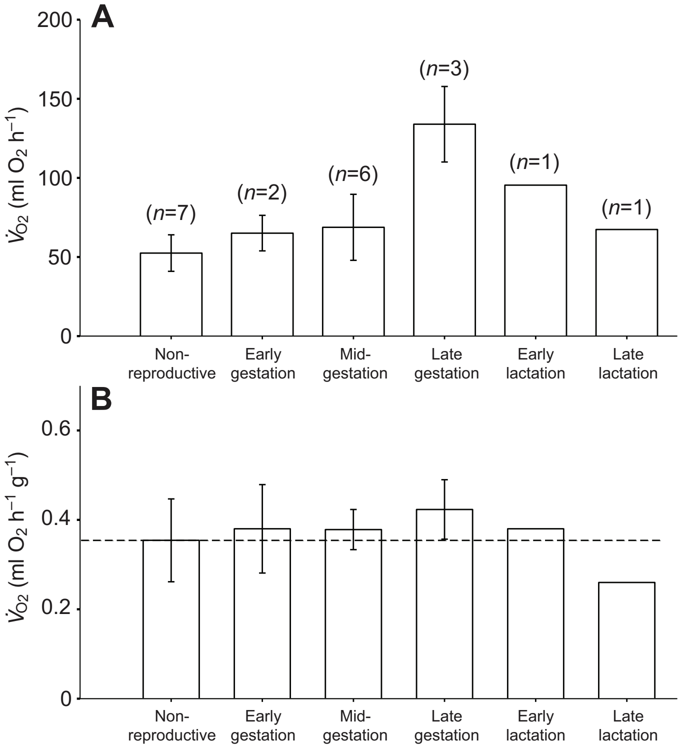

Fig. 2. Resting metabolic rate in the thermoneutral zone (T_a>25°C) for female *Setifer setosus* according to reproductive status. (A) Whole-animal $\dot{V}_{O_2}$. (B) Mass-specific $\dot{V}_{O_2}$. Categories describe the number of days prior to parturition (early=60–35 days, mid=35–10 days, late=10–0 days). The female measured during late lactation was also in mid-gestation. Repeated measures were available for some, but not all individuals. There were no differences in $\dot{V}_{O_2}$ between groups when mass was used as a covariate. The dashed line in B indicates the mean $\dot{V}_{O_2}$ of non-reproductive females.

An initial inflection point in measures of wet thermal conductance (C_{wet}; Fig. 3) was found at 32.5–32.8°C, after which C_{wet} increased dramatically. All analyses of C_{wet}, ln-transformed to conform to model assumptions, included data below this inflection point only. An additional inflection point in the data at T_a<32.5°C was located at T_a=30.5°C, under which point C_{wet} was at a minimum (Table 1). Values for C_{wet} were at a minimum at the lowest temperatures, increased slightly with increases in T_a below the TNZ, and dramatically above 32.5°C (Fig. 3). Similar to the T_b dataset, reproductive status, T_a and their interaction as well as mass were the fixed factors in the best model describing C_{wet} below 32.5°C, along with animal ID as a random factor (Table 2). Gestating females had the highest C_{wet}: 7.3±1.5 ml O_2 h^{-1} °C^{-1} greater than males and 12.5±1.5 ml O_2 h^{-1} °C^{-1} greater than non-reproductive females, although, similar to T_b, the difference was less at higher T_a.

DISCUSSION

Previous studies on the thermoregulation of basoendotherms found thermal profiles that significantly deviated from the Scholander–Irving model (Brice, 2008; Stephenson and Racey, 1993b; Nicoll, personal communication in Stephenson and Racey, 1994). The results from the present study, however, are mixed. Although TRMR was constant over a wide range of T_a in both reproductive and non-reproductive individuals, indicative of a TNZ between ~25°C and 32.5°C, T_b was highly correlated with T_a at all temperatures. This observation deviates from the classical Scholander–Irving model, which assumes that normothermic T_b is maintained within a narrow range. Similarly, high levels of variability in T_b seen in both reproductive females and the small number of thermoregulating males led to unusual relationships between $\dot{V}_{O_2}$ and T_a below the TNZ. The classical model predicts that the slope of $\dot{V}_{O_2}$ below the TNZ should intersect with the *y*-axis ($\dot{V}_{O_2}$) at T_a=T_b (Scholander et al., 1950). This was not the case in either group of non-torpid animals. For the thermoregulating non-reproductive individuals, a lack of correlation between T_a and $\dot{V}_{O_2}$ below the T_{lc} meant that the $\dot{V}_{O_2}$ would never intersect with T_a within a biologically viable T_b. Similarly, reproductive females would have to have a T_b of >36°C for the classical model to be an accurate representation of their thermoregulation.

The pattern of C_{wet} also indicates some level of deviation from the classic mammalian model. In most homeothermic mammals, C_{wet} reaches a minimum at the T_{lc}, and increases with increasing T_a within and above the TNZ (Brice, 2008; Scholander et al., 1950). In the present study, C_{wet} never reached a minimum and started to increase only well into the TNZ, above around 32.5–32.8°C. Such a delayed deployment of heat loss mechanisms would indicate that T_b is flexible until a certain point, after which it is defended against potential hyperthermia, and efforts are made by the animal to offload stored heat. The T_b measurements from *S. setosus* presented in our previous study (Lovegrove et al., 2014) indicate that high T_b can be

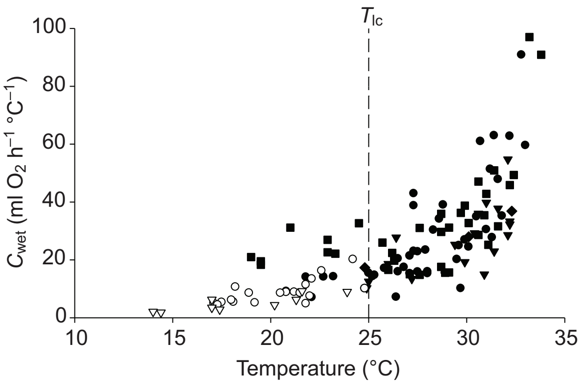

Fig. 3. Wet thermal conductance of *Setifer setosus* over a range of ambient temperatures. Reproductive females (squares, gestating; diamonds, lactating), males (circles) and non-reproductive females (triangles) that thermoregulated are plotted in black; torpid [T_b–T_a (ΔT)<5°C] individuals in white. The straight line indicates the lower critical limit of the thermoneutral zone.

reached during torpor, although this is not always the case. The high levels of C_{wet} observed in a few individuals (Fig. 3) indicate a capacity to withstand high temperatures; however, the tolerance of high temperatures by tenrecs is not currently known. Furthermore, evaporative water loss was not measured in this study. It would be of interest for future studies to measure this species' capacity for evaporative cooling, as well as its reliance upon these mechanisms for thermoregulation. Water loss considerations may be especially important in tropical species whose entire period of activity coincides with periods of high T_a and simultaneous high levels of ambient humidity (Krockenberger et al., 2012; Levesque et al., 2013; Lovegrove et al., 2014).

Previous studies on the thermal profiles of basoendotherms found the delineation between torpid and non-torpid animals problematic (Brice, 2008; Stephenson and Racey, 1994). Although the distinction between the two states were clear at T_a<T_{lc}, three males, removed from the full data analysis, defended a lower body temperature (~26°C). These males were in a steady state as this T_b was maintained for the length of the measurement period. The maintenance of such intermediate rest-phase T_b appears to be characteristic of tenrecs. Similar observations have been made on a shrew tenrec, *Microgale dobsoni* (Stephenson and Racey, 1993b); in addition, McNab observed *S. setosus* defending T_b ranging between 23 and 29°C (McNab, 1980a). However, the large and very likely obese (530 g) animals used in the latter study render those results unreliable. Nevertheless, as shown in a concurrent study (Levesque et al., 2013), there were no differences in body condition index between seasons, or between torpid versus thermoregulating males in the present study. It is therefore unlikely that differences in body mass alone can account for the different thermoregulatory states of non-reproductive individuals.

In accordance with previous studies on *T. ecaudatus* (Eisentraut, 1960), lethargy and unresponsiveness, usually requirements for the diagnosis of torpor (IUPS Thermal Commission, 2003), were not a characteristic of torpor in tenrecs. Similarly, a number of species of tenrec have been found to be active at T_b as low as 25°C (Crompton et al., 1978; Eisenberg and Gould, 1969; Poppitt et al., 1994). Activity at low T_b has similarly been observed in monotremes (Kuchel, 2003), marsupials (Rojas et al., 2012; Turner et al., 2012), placental mammals (Wooden and Walsberg, 2004) and birds (Merola-Zwartjes and Ligon, 2000). As a consequence, differentiating torpor from normothermy in these species using T_b alone is complicated (Brice et al., 2002; Canale et al., 2012). Without a discernible pattern in the thermal profiles of the reproductive females, we would be reluctant to accept the inflection point found at 24.9°C in the non-reproductive individuals as a valid T_{lc} as prescribed by the Scholander–Irving model. The consistent relationship observed between T_b and T_a, and a lack of corresponding data on activity or responsiveness, makes any distinction between torpid and thermoregulating individuals in this context arbitrary. In addition, the Scholander–Irving model was formulated to describe a mammal defending a narrow T_b setpoint, which is evidently not the case for non-reproductive *S. setosus*. As has been reported in previous studies on tenrecs (Lovegrove and Génin, 2008; Poppitt et al., 1994), there is no threshold normothermic T_b under which all animals can be considered to be torpid. However, a coarse definition of torpor using the difference between T_b and T_a (ΔT) (Arlettaz et al., 2000; Canale et al., 2012) was supported somewhat by the $\dot{V}_{O_2}$ data. As an approximation, an animal with a ΔT of less than 5°C could be considered torpid, but only reliably at T_a less than 25°C, and only if other methods for diagnosis (such as activity, metabolic rate or heart rate) are unavailable.

Previous studies on captive tenrecs (Nicoll and Thompson, 1987; Poppitt et al., 1994; Racey and Stephenson, 1996) have shown increases in TRMR during reproduction. This was not the case in the present study. One explanation for the observed differences is that none of the recordings in this study can be considered to be truly basal because the population was both reproductively active and in the process of accumulating fat stores for hibernation (Levesque et al., 2013). During early pregnancy, as well as during lactation, the captive tenrecs of previous studies did not show significant gains in mass (Poppitt et al., 1994; Stephenson and Racey, 1993a; Stephenson and Racey, 1994), whereas individuals from this population at all stages of reproduction showed steady increases in body mass (Levesque et al., 2013). Potential increases in $\dot{V}_{O_2}$ during reproduction were possibly masked by simultaneous increases in mass. However, the average TRMR (0.36±0.09 ml O_2 h^{-1} g^{-1} for non-reproductive individuals) falls within the reported values for this species [0.34–0.46 ml O_2 h^{-1} g^{-1} (Eisenberg and Gould, 1969; Kayser, 1960; Stephenson and Racey, 1995)].

Interestingly, one of the lowest TRMR values measured in a reproductive female was from an individual who was in both late lactation and mid-gestation (Fig. 2). This low value is similar to those found during a study on reproduction in a species of shrew tenrec, *Geogale aurita* (Stephenson and Racey, 1993a), where simultaneous gestation and lactation did not result in combined increases in TRMR. However, sample sizes of the different reproductive stages in the present study were too small to determine the fine-grain relationship between reproduction and TRMR. The earlier studies on captive individuals also indicated that tenrecs can enter torpor during pregnancy (Nicoll and Thompson, 1987; Stephenson and Racey, 1993a). This was not observed either during the constant environment trials (present study) or in free-ranging animals (D.L.L. and B.G.L., unpublished), with the exception of a single female 46–49 days prior to parturition. This individual was in very poor body condition, having recently emerged from hibernation, and entered torpor at the lowest temperatures (14–21°C). However, despite poor body condition early on in the season, a single young was raised successfully [SF14 (Levesque et al., 2013)] and a body condition comparable to that of the population was attained within a few weeks. Laboratory-based studies on the shrew *Crocidura russula monacha* (Mover et al., 1989; Mover et al.,

1988) also found that TRMR during gestation and lactation, as well as during simultaneous gestation and lactation, showed little change. There was, however, a significant increase in food intake, and therefore in daily energy expenditure. This was also the case in reproductive *E. telfairi*, where increases in TRMR during gestation and lactation were less than in those in which daily energy expenditure was measured over 24 h (Poppitt et al., 1994). It is therefore possible that the costs of maintaining a higher degree of homeothermy during reproduction in *S. setosus* were underestimated by the methods used in this study.

Conclusions; reproduction and the evolution of homeothermy via heterothermy

This study provides further evidence that homeothermy is sustained in otherwise heterothermic mammals during reproduction. Interestingly, unlike in laboratory populations fed *ad libitum*, the only increases in TRMR observed during reproduction in *S. setosus* were due to increases in mass (therefore, in total, whole-animal metabolic rate), and not the reproductive condition per se. Increases in homeothermy are therefore likely to have higher effects on fetal development than increases in TRMR. Presumably, the reliance upon homeothermy can be at least partly attributed to a relationship between T_b and fetal growth rate (Farmer, 2000). As an illustration, a captive population of *S. setosus* held at higher-than-average ambient temperatures (>25°C) had shorter gestation lengths than those housed in colder conditions (Mallinson, 1974). This shortened gestation period matched those observed in the current study population (Levesque et al., 2013), where environmental temperatures were similarly warm. The mean minimum daily T_a during the active season (October–May) since 1997 was 21.9±2.3°C (n=3572) and daytime (rest-phase) T_a decreased below 20°C only on 0.01% of study days during the second season. The costs of maintaining homeothermy in this environment are therefore relatively low, which is, presumably, how females in this population can give birth to up to three litters per year while also accumulating fat stores for hibernation.

Assuming that the climate of the tropical forests of Madagascar have changed very little since the time when the placental mammal radiations occurred (~65 mya) (Jury, 2003; Lovegrove, 2012a; O'Leary et al., 2013), *S. setosus* provide a useful model to test a crucial trade-off associated with the evolution of endothermy in general. The trade-off balances the fitness benefits of small increments in homeothermy during reproduction with the relatively low fitness costs involved in minimal thermoregulatory energy demands. Such a trade-off can occur in tropical environments only, which is the climate that prevailed broadly across the globe from the Jurassic ~200 mya at the putative establishment of endothermy in small, nocturnal mammaliaformes (Rowe et al., 2011), until at least the Eocene Thermal Maximum ~50 mya (Zachos et al., 2001). Continental global cooling occurred thereafter in the late Cenozoic, but the climates of the current tropics remained much the same as they were in the Late Eocene. Thus in both ancestral and contemporary small, tropical mammals, low-cost increments in homeothermy provide(d) a stable environment for fetal development which, by increasing the rate of development, would have increased fitness (Farmer, 2003) and allowed for greater energy reserves to be used in provisioning the young, via either milk production or fat storage (Koteja, 2004). Thus apart from the support for the parental care hypothesis, our data also support the predictions of the plesiomorphic–apomorphic endothermy hypothesis, which argues that basoendotherms such as *S. setosus* display plesiomorphic endothermic traits that implicate stabilizing selection (Lovegrove, 2012a).

The presence of fully functional uncoupling proteins (especially UCP1) in the brown adipose tissue of tenrecs (Oelkrug et al., 2013) indicates that the physiological capacity to maintain homeothermy has existed in the eutherian mammal lineage for at least 66 million years. UCP1, found in mammalian mitochondrial membranes, allows increases in heat production by increasing membrane leakiness, and constitutes an important part of heat produced by non-shivering thermogenesis, necessary for rewarming from hibernation in eutherian mammals (Jastroch et al., 2005). When housed at a cold T_a, tenrecs periodically maintain high T_b similar to periods of normothermy observed in all mammalian hibernators (Oelkrug et al., 2013; Willis, 1982). They therefore have the capacity to maintain high and relatively stable T_b when necessary. However, the fact that homeothermy is observed solely during reproduction indicates that, at least in warm climates, it is not necessary for a day-to-day existence, especially in tropical Madagascar. Increased homeothermy during reproduction has also been observed in a monotreme (Beard and Grigg, 2000; Nicol and Andersen, 2006), as well as in a number of eutherian and marsupial mammals (Audet and Fenton, 1988; Geiser et al., 1998; Morrison, 1945), indicating that it is likely to have been one of the first steps in the evolution of homeothermy in ancestral heterothermic mammals (Farmer, 2000).

MATERIALS AND METHODS

Study site, capture and surgical methods

The study was conducted over two rainy seasons, from September 2010 to April 2011 and from September 2011 to February 2012, in Ankarafantsika National Park (16°19′S, 46°48′E), Madagascar. A detailed description of the study site and general methods is provided in two previous studies (Levesque et al., 2012; Levesque et al., 2013). Briefly, all animals were caught by hand, in the Jardin Botanique A research area adjacent to the Ampijoroa Forestry Station, by walking the established trails in the area at night with local guides. Upon capture, individuals were transported to the research camp where they were housed in plastic containers lined with paper towel and provided with live insects and tinned sardines. At initial capture, all animals were anesthetized using isoflurane in oxygen (induction: 1–2%; maintenance, 0.5%) and morphometric measurements were taken. Each animal was marked with a small distinctive clip in the ear and injected with a transponder (Small Animal Marking System, Trovan Ltd, UK) to allow for identification at recapture. They were kept for a maximum of 5 days for the collection of metabolic data, after which selected animals had a combination of radio-transmitter and body temperature data logger (DS1922L Thermochron iButtons, Dallas Semiconductor, Dallas, TX, USA) implanted into the peritoneal cavity as described previously (Levesque et al., 2012). Females with radio-transmitters were captured once a week to determine reproductive status, and males once every 2 or 3 weeks to assess body condition. In addition, the rest sites of the females were located every day as repeated use of a single nest site would indicate parturition (Levesque et al., 2012), and males every couple of days. Pregnant females (within 10 days of parturition) were captured in the field and brought back to the laboratory for a repeat of the metabolic measurements. If both the lactating female and her pups were accessible, they were caught on the morning of the experiment and released back into the nest by sunset the same day. Ambient temperatures for the study site were obtained from the Durrell Wildlife Conservation Trust.

All procedures involving the use of animals were approved by the Madagascar National Parks (Permit 218/09/MEF/SG/DGF/DCB.SAP/SLRSE and 158/10/MEF/SG/DGF/DCB.SAP/ SCBSE) and comply with all national Malagasy laws.

Respirometry data

Flow-through respirometry was used to obtain values for $\dot{V}_{O_2}$ over a range of ambient temperatures (15–34°C). To avoid injury through pathological hypothermia, measurements at T_a<10°C were not attempted (Lachiver, personal communication in Kayser, 1961). Measurements were conducted between 04:30 and 18:00 h, to coincide with the natural rest phase, and were made over a period of 4–6 h. The animal was placed in a 700 ml respirometer consisting of an air-tight plastic container (Komax Industry Co, Seoul, Korea) with three small air holes at the base and an outlet near the top. The respirometer was placed inside a modified refrigerator in which the temperature was controlled using heat lamps activated via a programmable temperature controller (TC410, Rhomberg Instruments, Johannesburg, South Africa). Ambient air, partially dried using silica gel, was pulled through the chamber at a rate of 500 ml min^{-1}. The air was pulled through the chamber into a mass flow meter and pump (MFS Mass Flow System, Sable Systems, Las Vegas, NV, USA), dried using Drierite, and pushed into a manifold. A subsample of the air from the respirometer was pulled at 150 ml min^{-1} through scrubbers containing soda lime to remove CO_2 and Drierite to remove water released by the soda lime, and a mass flow meter before entering the pump and being pushed through an O_2 analyser (FoxBox-C Field Gas Analysis System, Sable Systems). Channels were configured in Sable System’s data acquisition software, Expedata (v 1.1.15), to record the fractional concentrations of O_2, the flow rate, and the barometric pressure every 2 s. The precise temperatures experienced by the animals were monitored using pre-calibrated iButtons taped to the insides of the respirometer and programmed to record T_a once every minute with a resolution of 0.0625°C. To control for O_2 analyser drift, a baseline measurement from an empty reference respirometer was used at regular intervals (5 min every 20–40 min) throughout the experiment. Before each measurement, the animal was weighed and T_b was measured by inserting a calibrated Cu–Cn thermocouple 2 cm into the cloaca. At the end of the $\dot{V}_{O_2}$ measurement period, the position of the animal within the chamber was recorded and the T_b measurement was repeated. During the second season, skin temperature (T_{sk}) was measured by securing an iButton to the stomach of the animal using surgical tape. Core T_b data, obtained from implanted iButtons, was only available for a small number of individuals (N=4, n=16). A mixed model, using measurement type as a fixed factor and animal ID as a random effect, indicated that T_{sk} was significantly different from both core T_b (t_{30}=3.02, P=0.005) and T_b at the end of the experiment (t_{30}=2.30, P=0.028), whereas the two methods of T_b did not differ from each other (t_{30}=0.72, P=0.47). T_b at the end of data measurement was therefore used in all subsequent analyses.

Most experiments were conducted during the initial capture period of each year (October–November) and for each individual usually consisted of a single measurement at a low temperature (18–25°C) and one in the TNZ estimated at around 30–33.5°C based on values used by Nicoll and Thompson (Nicoll and Thompson, 1987) for *E. telfairi*. If possible, individuals with radio-transmitters were recaptured later in the season (December–April) for additional measurements. Upon recapture the animals were kept in captivity for a total of 2 days, allowing for measurements to take place at four to five different T_a. Lactating females were measured only if it was possible to capture both the mother and the pups. Thus measurements were obtained from two females only, both in the first season: one with three pups 13–14 days old (2 weeks from weaning) the other with a single pup aged 30–33 days old (within days of weaning). The latter female was also gestating [38 days from parturition: SF14 in Levesque et al. (Levesque et al., 2013)]. Measurements on these females were conducted at two temperatures and the mother was returned to the pups for at least an hour between measurements.

Data analysis

To prepare the raw data files for analysis, O_2 concentrations were corrected for analyser drift throughout the experimental period using the recorded baselines and the drift correction function in Expedata. To obtain steady-state values, a pre-recorded macro was used to locate multiple 10 min sections (300 samples) of data with the most stable trace. The lowest of these values was used as the RMR for that temperature. T_{sk} values were used to confirm that the animal was in a steady state at this time. Only values after the first 2 h of measurements were used to ensure that the animals were fully acclimated to the temperature. $\dot{V}_{O_2}$ was calculated using the proportion of O_2 entering and leaving the respirometer, flow rate, chamber temperature and T_b and equations modified from Withers (Withers, 2001). $\dot{V}_{O_2}$ and the T_b measured at the end of the experiment were used to calculate C_{wet} using eqn 3 from McNab (McNab, 1980b).

Statistical analysis

All statistics were performed using R version 3.0.1 (R Development Core Team, 2011) and linear mixed modelling was implemented using the R package ‘nlme’ (Pinheiro et al., 2013). Piecewise linear regression [p. 425 in Crawley (Crawley, 2007)], with mass as a covariate, was used to determine inflection points in the slope of $\dot{V}_{O_2}$ versus T_a, which would typically identify the T_{lc}. This analysis was repeated for the T_b and C_{wet} data.

To quantify the importance of various factors on $\dot{V}_{O_2}$, T_b, C_{wet} and body mass, model selection was performed using Akaike’s information criterion scores corrected for small sample size (AIC$_c$) and Akaike weights (Burnham and Anderson, 2002) implemented in the R package ‘AICcmodavg’ (Mazerolle, 2013), with respirometer temperature (T_a), season, day and reproductive status as fixed factors, and animal ID as a random factor. The assumptions of the models, i.e. normally distributed residuals with a mean of zero, were verified by observing qq-plots and histograms of the residuals.

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Competing interests

The authors declare no competing financial interests.

Author contributions

D.L.L. and B.G.L. conceived and designed the experiments. D.L.L. performed the data collection, analyses and drafted the manuscript. B.G.L. contributed to and approved of the manuscript.

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