Increased homeothermy during reproduction in a basal placental mammal

Danielle L. Levesque*, and Barry G. Lovegrove

School of Life Sciences, University of KwaZulu-Natal, P/Bag X01, Scottsville, 3209, South Africa

*Correspondence: Danielle L. Levesque, School of Biological and Conservation Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, 3209, South Africa
e-mail: danielle.l.levesque@gmail.com
tel: +27-33-26-5113; fax: +27-33-2605105

Running Header: Tenrec reproductive homeothermy

Keywords- Evolution of endothermy, Parental Care Hypothesis, thermoregulation, reproduction, torpor, Madagascar, Setifer setosus, Tenrecinae
Summary

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.
**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 which maintain a high degree of homeothermy (small circadian variations in $T_b$), to those which have highly labile $T_b$s (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 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 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 which 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 which 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 (2004), and
‘basoendotherms’ by Lovegrove (2012a), extant basal eutherians, often found on low-latitude islands with little paleoclimatic history of Cenozoic cooling, are highly heterothermic, with variable Tb's and frequent use of torpor. The large amplitudes in the circadian rhythm of Tb in these animals can lead to difficulties in differentiating between torpor and normothermy using Tb 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 Ta over which MR remains minimal and constant, is highly problematic if Tb is not maintained at a constant level (Brice, 2008; Scholander et al., 1950). The high thermolability of basoendotherms generates a relatively linear relationship between Tb and Ta, with no clear inflection points in MR at the lower and upper critical limits of thermoneutrality which typically define the TNZ in classic homeothermic endotherms (Brice, 2008; Stephenson and Racey, 1993b; Nicoll pers. comms. in Stephenson and Racey, 1994).

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 Tb's of any extant mammal and the spiny tenrecs, members of the sub-family Tenrecinae, have some of the lowest basal metabolic rates (BMR, Lovegrove, 2000; Symonds, 1999). They have also retained the ancestral diet (insectivory), 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 Tb, as well as frequent (daily in the case of Echinops telfari) 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 Tb 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. (1991) to denote that resting measurements of metabolism occurred at thermoneutrality despite failing to meet all of the requirements for basal metabolism.
No study has yet to characterise changes in thermoregulatory profiles during reproduction in a basoendotherm.

Brice, Levesque and Grigg (in prep, see also 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 $T_a$'s 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), they have been shown to enter into torpor over a wide range of $T_a$'s 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 (4 females, and 5 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 of 2012.

Model selection using Akaike weights (AICcWt, 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, see Levesque et al., 2013), ‘reproductive status’ (male 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.,
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 \texttt{varFixed (~ ‘day’)} as the variance structure. The model with the highest AICcWt (0.61) included ‘day’ only with a parameter estimate of 0.95 ± 0.16 g per day (Table 2). The inclusion of ‘reproductive status’ in the model resulted in an additional AICcWt of 0.31. The remaining AICcWt was contributed by adding ‘season’ to the model. A Tukey post-hoc test on the model containing ‘day’ and ‘status’, using the \texttt{glht} function in package \textit{multcomp} (Hothorn, Bretz & Westfall 2008), provided parameter estimate sizes for differences between ‘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 (differences > 25 g).

\textit{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 $\Delta T$ (T$_b$ – T$_a$) was less than 5°C, and thermoregulating if it was greater (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$_a$s 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 (BCI, Levesque et al., 2013) and torpor expression ($F_{1,45} = 0.52$, $p = 0.48$).

Resting metabolic rate (RMR), measured as oxygen consumption ($\bar{V}O_2$), and T$_b$ were highly variable (Table 1; Fig. 1). Piecewise linear regression indicated an inflection point in the slope of $\bar{V}O_2$ versus T$_a$ at approximately 24.6 - 24.8°C in the model including all individuals (N = 31, n = 133), 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 thermoneutral zone (TNZ) was therefore estimated to be around 25°C. Initial results indicated that models containing
an inflection point at 25°C had lower AICc scores, and therefore it was justified to analyse the
data above (within the TNZ: \( T_a \geq 25°C \)) 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, which 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 and 3) at the whole-animal level. However, the best
model predicting TRMR (AICcWt = 0.96) contained ‘mass’ (parameter estimate = 0.37 ± 0.04
mLO₂.hr⁻¹) only as a fixed factor, and ‘day’ and ‘animal ID’, as well as their interaction (in the
form ∼‘day|animal ID’), as random factors (Table 2). A model containing ‘reproductive status’
only, as well as one with ‘reproductive status’ and ‘\( T_a \)’, explained the remaining AICcWt (0.02,
and 0.01 respectively).

The highest ranking model describing the \( \dot{V}O_2 \) of non-torpid animals (5 males, 9 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 of 186.53 ± 35.4 mLO₂.hr⁻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 mLO₂.hr⁻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 similar temperatures to
those for \( \dot{V}O_2 \) (∼ 25°C), but their inclusion in the model produced no improvement in AICc
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 °C change in \( T_a \). Males and non-reproductive females had
lower \( T_b \)s 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).

An initial inflection point in measures of wet thermal conductance (\( C_{wet} \); Fig. 2) 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 \( T_a < 32.5^\circ C \) was located at \( T_a = 30.5^\circ C \) under which point \( C_{\text{wet}} \) was at a minimum (Table 1). Values for \( C_{\text{wet}} \) were at a minimum at the lowest temperatures, increased slightly with increases in \( T_a \) below the TNZ, and dramatically above \( 32.5^\circ C \) (Fig. 2).

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 \( C_{\text{wet}} \) below \( 32.5^\circ C \), along with ‘animal ID’ as a random factor (Table 2). Gestating females had the highest \( C_{\text{wet}} \): 7.3 ± 1.5 mLO₂.h⁻¹.°C⁻¹ greater than males and 12.5 ± 1.5 mLO₂.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 in Stephenson and Racey, 1994). The results from this study, however, are mixed. Although TRMR was constant over a wide range of \( T_a \)s in both reproductive and non-reproductive individuals, indicative of a TNZ between \(-25^\circ C \) and \( 32.5^\circ C \), \( T_b \) 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 \( T_k \) meant that the \( \dot{V}O_2 \) would never intersect with \( T_a \) within biologically viable \( T_b \)s. 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 \( C_{\text{wet}} \) also indicates some level of deviation from the classic mammalian model. In most homeothermic mammals \( C_{\text{wet}} \) reaches a minimum at the \( T_k \), and increases with increasing \( T_a \) within and above the TNZ (Brice, 2008; Scholander et al., 1950). In this study \( C_{\text{wet}} \) 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 Lovegrove et al. (2014) would indicate that high \(T_b\)s can be reached during torpor, although this is not always the case. The high levels of \(C_{\text{wet}}\) observed in a few individuals (Fig. 3) indicates 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 since this \(T_b\) was maintained for the length of the measurement period. The maintenance of such intermediate rest-phase \(T_b\)s appears to be characteristic of tenrecs. Similar observations have been made on a shrew tenrec, \(Microgale\ dobsoni\) (Stephenson and Racey, 1993b) and on \(S.\ setosus\) McNab (1980a), observed defending \(T_b\)s ranging between 23 - 29°C. However, the large and very likely obese (530 g) animals used in the latter study render those results unreliable. Nevertheless, as shown in Levesque et al. (2013), there were no differences in body condition index (BCI) between seasons, or between torpid versus thermoregulating males in this current 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 \(T_b\)s 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, make 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 $T_a$ ($\Delta T$, 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 $\Delta T$ 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 current 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 at all stages of reproduction in this study 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 mLO$_2$.hr$^{-1}$.g$^{-1}$ for non-reproductive individuals) falls within the reported values for this species (0.34-0.46 mLO$_2$.hr$^{-1}$.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 both in 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 current 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 & Thompson 1987, Stephenson & Racey 1993a). This was not observed during either the constant environment trials (this study) or in free-ranging animals (Levesque and Lovegrove in prep), 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 hrs (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 due the reproductive condition per se. Increases in homeothermy are therefore likely to have higher effects on foetal development than increases in TRMR. Presumably, the reliance upon homeothermy can at least partly be attributed to a relationship between Tₚ and foetal 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 $21.9 \pm 2.3^\circ C$ ($n = 3572$) and daytime (rest-phase) $T_a$ decreased below $20^\circ C$ 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 which 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, 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 foetal 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, either via 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 cold $T_a$s, 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_{bs} 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).

**Methods and Techniques**

*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 by Levesque et al. (2013; 2012). 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 five 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 in Levesque et al. (2012). Females with radio-transmitters were captured once a week to determine reproductive status, and males once every two or three 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 to 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.

**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
$T_a < 10^\circ C$ were not attempted (Lachiver pers. comms. in Kayser, 1961). Measurements were
conducted between 04:30 and 18:00, to coincide with the natural rest phase, and were made over
a period of 4-6 hours. 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, RSA). 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), 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, Las Vegas, NV). 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 two seconds. The precise temperatures experienced
by the animals were monitored using pre-calibrated iButtons taped to the sides of the
respirometer and programed 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 (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 two days, allowing for measurements to take place at 4 - 5 different $T_a$s. 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 (two 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., 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 minute 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 two hours 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 (2001). $\dot{V}O_2$ and the $T_b$ measured at the end of the experiment were used to calculate $C_{wet}$ using Equation 3 from 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 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 $T_l_c$. 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 Information Criterion scores corrected for small sample size and Akaike weights (AICc and AICcWt, 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.

Abbreviations

$C_{wet}$ = wet thermal conductance (mLO$_2$.°C$^{-1}$.h$^{-1}$)

$T_a$ = ambient temperature (respirometer temperature)

$T_b$ = core body temperature

$T_{sk}$ = skin temperature

$T_l_c$= lower critical limit of the TNZ

$\Delta T$ = temperature differential ($T_b$-$T_a$)

TNZ= thermoneutral zone

TRMR= $\dot{V}O_2$ measured in the TNZ

$\dot{V}O_2$ = volumetric rate of oxygen consumed by the animal (mLO$_2$.h$^{-1}$)

Acknowledgments

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. We wish to thank the following individuals and institutions for their assistance: Prof. D. Rakotondravony (Département de Biologie Animale, Université d’Antananarivo), the local guides (Tosy, Alpha and Ndrema),
Kerileigh Lobban, Oliva Malala Loavasoa Andriambola, Sophie Nirina Rakotoharimala, Christophe Bouvier, the Malagasy National Parks, and the Malagasy Institute for the Conservation of Tropical Environments and Andrew McKechnie and an anonymous reviewer for comments on the paper.

**Funding**

The research was funded through an *Ad Hoc* Travel Grant, an incentive grant, and a competitive grant, from the National Research Foundation (South Africa), and incentive grants from the University of KwaZulu-Natal, to BGL. Idea Wild provided a donation for the purchase of GPS units. DLL was funded by a National Science and Engineering Research Council (Canada) postgraduate scholarship.

**Author Contributions**

DLL and BGL conceived and designed the experiments. DLL performed the data collection, analyses and drafted the manuscript. BGL contributed to, and approved of the manuscript.
Table 1 Mean body mass, resting metabolic rates at thermoneutrality (TRMR, $T_a = 25 - 33.5^\circ C$) and minimum thermal conductance ($C_{\text{wet}}, T_a < 30.5^\circ C$) of reproductive, and non-reproductive *Setifer setosus*.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Non-reproductive</th>
<th>Female</th>
<th>Gestating</th>
<th>Lactating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td>172.7 ± 46.2</td>
<td>147.1 ± 17.7</td>
<td>272.3 ± 7.3</td>
<td>222.1 ± 74.8</td>
<td></td>
</tr>
<tr>
<td>(g)</td>
<td>(21)</td>
<td>(9)</td>
<td>(11)</td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td>TRMR</td>
<td>60.7 ± 17</td>
<td>53 ± 14.6</td>
<td>99.9 ± 34.4</td>
<td>81.4 ± 19.8</td>
<td></td>
</tr>
<tr>
<td>(mLO$_2$.hr$^{-1}$)</td>
<td>(41)</td>
<td>(19)</td>
<td>(29)</td>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td>TRMR</td>
<td>0.36 ± 0.11</td>
<td>0.36 ± 0.11</td>
<td>0.40 ± 0.07</td>
<td>0.30 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>(mLO$_2$.g$^{-1}$.hr$^{-1}$)</td>
<td>(41)</td>
<td>(19)</td>
<td>(29)</td>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td>Min $C_{\text{wet}}$</td>
<td>0.10 ± 0.05</td>
<td>0.12 ± 0.11</td>
<td>0.10 ± 0.02</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>(mLO$_2$.hr$^{-1}$.°C$^{-1}$.g$^{-1}$)</td>
<td>(14)</td>
<td>(7)</td>
<td>(7)</td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>17</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

The number in parentheses indicates the total number of measures obtained.
Table 2 Best fitting linear mixed models evaluating the effect of various factors on the physiological parameters of reproductive and non-reproductive *Setifer setosus*. Ranking was performed using corrected Akaike Information Criterion (AICc) scores and Akaike weights (AICcWt). All models with an AICcWt > 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 September 1, a proxy for the start of the active season.

<table>
<thead>
<tr>
<th>Fixed Factors</th>
<th>Random Factors</th>
<th>Variance Structure</th>
<th>k</th>
<th>AICc</th>
<th>AICcWt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body mass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘day’</td>
<td>~ 1</td>
<td>animalID’</td>
<td>varFixed (~ ‘day’)</td>
<td>4</td>
<td>431.12</td>
</tr>
<tr>
<td>‘day’ + ‘status’</td>
<td>~ 1</td>
<td>animalID’</td>
<td>varFixed (~ ‘day’)</td>
<td>7</td>
<td>432.10</td>
</tr>
<tr>
<td>‘day’ + ‘status’ + ‘season’</td>
<td>~ 1</td>
<td>animalID’</td>
<td>varFixed (~ ‘day’)</td>
<td>8</td>
<td>435.07</td>
</tr>
<tr>
<td><strong>*O*2 in TNZ (Ta ≥ 25°C)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘mass’</td>
<td>~ ‘day’</td>
<td>animalID’</td>
<td>varFixed (~ ‘day’)</td>
<td>6</td>
<td>745.47</td>
</tr>
<tr>
<td>‘status’+‘mass’</td>
<td>~ ‘day’</td>
<td>animalID’</td>
<td>varFixed (~ ‘day’)</td>
<td>9</td>
<td>752.84</td>
</tr>
<tr>
<td>‘Ta’+‘status’+‘mass’</td>
<td>~ ‘day’</td>
<td>animalID’</td>
<td>varFixed (~ ‘day’)</td>
<td>10</td>
<td>754.43</td>
</tr>
<tr>
<td><strong>*O*2 below TNZ (Ta &lt; 25°C)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Ta’+‘status’+‘mass’</td>
<td>n/a</td>
<td>varFixed (~ ‘day’)</td>
<td>5</td>
<td>139.75</td>
<td>0.96</td>
</tr>
<tr>
<td>‘Ta’+‘status’+‘mass’+‘day’</td>
<td>n/a</td>
<td>varFixed (~ ‘day’)</td>
<td>6</td>
<td>147.17</td>
<td>0.02</td>
</tr>
<tr>
<td>‘mass’</td>
<td>n/a</td>
<td>varFixed (~ ‘day’)</td>
<td>3</td>
<td>148.52</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Body temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Ta’ x ‘status’ + ‘day’</td>
<td>~ 1</td>
<td>animalID’</td>
<td>varIdent (~ 1</td>
<td>status’)</td>
<td>11</td>
</tr>
<tr>
<td>‘Ta’ x ‘status’ + ‘mass’+‘day’</td>
<td>~ 1</td>
<td>animalID’</td>
<td>varIdent (~ 1</td>
<td>status’)</td>
<td>12</td>
</tr>
<tr>
<td>‘Ta’ x ‘status’ + ‘mass’</td>
<td>~ 1</td>
<td>animalID’</td>
<td>varIdent (~ 1</td>
<td>status’)</td>
<td>11</td>
</tr>
<tr>
<td>‘Ta’ x ‘status’</td>
<td>~ 1</td>
<td>animalID’</td>
<td>varIdent (~ 1</td>
<td>status’)</td>
<td>10</td>
</tr>
<tr>
<td><strong>Wet thermal conductance (Ta &lt; 32.5 °C)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Ta’ x ‘status’ + ‘mass’</td>
<td>~ 1</td>
<td>animalID’</td>
<td>varFixed (~ ‘Ta’)</td>
<td>9</td>
<td>66.97</td>
</tr>
<tr>
<td>‘Ta’ x ‘status’ + ‘mass’+‘day’</td>
<td>~ 1</td>
<td>animalID’</td>
<td>varFixed (~ ‘Ta’)</td>
<td>10</td>
<td>68.89</td>
</tr>
</tbody>
</table>
Figure Legends

**Figure 1** Body temperature and resting metabolic rates of *S. setosus* over two reproductive seasons in Ankarafantsika National Park. Reproductive females (gestating = squares and lactating = diamonds), males (circles) and non-reproductive females (triangles) which thermoregulated are plotted in grey, torpid (T_b - T_a [ΔT] < 5°C) individuals in white. The straight line indicates T_b = T_a and the dashed line the approximate lower critical limit (Tlc) of the thermoneutral zone. Significant differences were found between torpid and normothermic animals for both \( \dot{V}/gO_2 \) and T_b. Despite defending similar T_b's there was also a significant difference in \( \dot{V}/gO_2 \) between reproductive females and normothermic males.

**Figure 2** Wet thermal conductance of *Setifer setosus* over a range of ambient temperatures. Reproductive females (gestating = squares and lactating = diamonds), males (circles) and non-reproductive females (triangles) which thermoregulated are plotted in grey, torpid (T_b - T_a [ΔT] < 5°C) individuals in white. The straight line indicates the lower critical limit of the thermoneutral zone.

**Figure 3** Resting metabolic rate in the thermoneutral zone (T_a > 25°C) for female *Setifer setosus* according to reproductive status. 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 \( \dot{I}O_2 \) between groups when mass was used as a covariate.
References


Canale, C. I., Levesque, D. L. and Lovegrove, B. G. (2012). Tropical heterothermy: Does the exception prove the rule or force a re-definition? In Living in a Seasonal World:


Fig. 1
Fig. 3