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Individual variation in metabolic traits of wild nine-banded armadillos (*Dasypus novemcinctus*), and the aerobic capacity model for the evolution of endothermy

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Summary

A fundamental assumption of the aerobic capacity model for the evolution of endothermy is that basal (BMR) and peak (PMR) metabolic rates are functionally linked at the intraspecific level. The purpose of this study was to use the nine-banded armadillo *Dasypus novemcinctus* as a model to test this assumption. Measurements of BMR, PMR, mass and rectal temperature were obtained over two summers from wild, adult individuals from a population in Louisiana, USA. BMR and PMR were positively correlated (r=0.62), and both were significantly higher (by 46% for BMR and by 35% for PMR) in 1999 than in 1998. Similar results were obtained whether metabolic rates were expressed in whole-animal or mass-independent units. These results suggest the existence of a functional link between BMR and PMR and are therefore consistent with the aerobic capacity model. In addition, this study confirmed that, compared with most eutherian mammals, the nine-banded armadillo exhibits low and highly variable basal and peak metabolic rates (20–60% the predicted values; 23% and 27% coefficients of variation) and rectal temperatures (range 32.7–35.3°C). Such metabolic traits are, however, consistent with the general pattern previously observed for other members of the order Xenarthra and with the hypothesis that low metabolic rates in armored mammals evolved as a result of unbalanced selection in which, because of low predation risks, selection for a high aerobic capacity was much weaker than the opposing selection for energy conservation.

Key words: armadillo, *Dasypus novemcinctus*, endothermy, aerobic capacity, thermoregulation.

Introduction

Endotherms use metabolic heat production to maintain a relatively high and constant body temperature. This metabolic strategy, found primarily in mammals and birds, is associated with high levels of energy expenditure. Indeed, the basal metabolic rate (BMR) of endotherms, which accounts for a large (30–80%) and unavoidable component of daily energy expenditure (Blaxter, 1989), is 5–10 times higher than that of ectotherms, even when differences in body temperature are taken into account (Robinson et al., 1983; Withers, 1992). Because the ability to maintain energy balance is critical to survival and fitness, there must be a substantial selective advantage to endothermy and its associated high BMR for this strategy to have evolved. Not surprisingly, therefore, BMR is one of the most measured physiological variables, and numerous studies have investigated the ecological and evolutionary significance of its variation, either at the inter- or intraspecific level. However, because animals in their natural environments are rarely in a 'basal' state (resting adults in a post-absorptive state and at thermoneutrality), it is unlikely that variation in BMR has a direct impact on the survival or fitness of individuals. Rather, studies that have investigated the ecological or evolutionary significance of variation in BMR have generally relied on the assumption that BMR is correlated with other physiological variables that have a direct impact on fitness.

An important model that explains the evolution of endothermy and its associated elevated BMR is the aerobic capacity model, originally outlined by Bennett and Ruben (1979). This model argues that natural selection favored individuals with a high aerobic capacity because of its associated benefits to predator avoidance, prey capture and other performance characteristics that directly affect fitness. Selection for increased aerobic capacity would indirectly result in increased BMR, reflecting the energetic costs required to sustain the physiological machinery necessary to support a high aerobic capacity. A fundamental assumption of this model is that aerobic capacity and BMR are functionally linked. Previous studies have demonstrated that aerobic capacity and BMR in vertebrates are generally weakly correlated at the intraspecific level, and similar correlation analyses conducted at the interspecific level have provided only mixed support for the model (for a review, see Hayes and Garland, 1995).

The nine-banded armadillo *Dasypus novemcintus* is an excellent subject in which to test the aerobic capacity model because, compared with most eutherian mammals, armadillos and other members of the order Xenarthra exhibit an atypical

endothermy characterized by low and variable metabolic rates and body temperatures (Eisenberg, 1981; Johansen, 1961; Schmidt-Nielsen et al., 1978). The main objective of the present study was to use measurements of BMR and cold-induced peak metabolic rate (PMR) obtained from wild nine-banded armadillos to investigate the assumption that aerobic capacity and BMR are functionally linked at the intraspecific level.

Materials and methods

Animals

Wild nine-banded armadillos *Dasypus novemcinctus* L. were collected using a large dip net at a research station located 35 km south of New Orleans (Louisiana, USA) during June 1998 and from early June to mid-July 1999. Climatic conditions during the sampling period and during the month that preceded sampling differed between the two years; in 1998, air temperature was warmer and rainfall was lower than in 1999 and compared with normal values (Table 1). On the evening preceding an experimental day, two individuals were captured between 18:00 h and 20:00 h and isolated overnight at 25°C in plastic dog crates containing bedding material and water; no food was provided to ensure a post-absorptive state for the next morning. The animals were calm in the crates and appeared to be sleeping most of the time. After the completion of the experiments, the animals were sexed, weighed to the nearest 50 g, ear-clipped for identification and for the purpose of another study, and released at the site of capture. Animals were used and cared for in accordance with Institutional Animal Care and Use Committee guidelines of the University of New Orleans, following modified recommendations of Storrs (1987).

Experimental procedures

On the morning following capture, at approximately 07:30 h, the two animals were restrained with a towel while a calibrated thermocouple was inserted into the rectum to a depth of 15 cm and secured in place by taping it to the tail. Animals were placed individually in rectangular Plexiglas chambers (25 cm wide × 25 cm high × 50 cm long) in which a small fan on the lid prevented gas stratification. Chambers were connected to an open-flow respirometry system (described by Knopper and Boily, 2000) and placed in a modified freezer. The freezer had an electrical heater controlled by a computerized data-acquisition system (Sable Systems) that maintained the air temperature inside the chambers at 30±0.5°C, which is within the thermoneutral zone of armadillos (Johansen, 1961; McNab, 1980). A system of solenoid valves alternated the flow pattern of the respirometry system so that each chamber was sampled for 45 min. Each chamber was sampled three times, yielding 2.25 h of observations per individual. There was no significant difference between the two chambers for any of the measurements. Animals were allowed to acclimate to the chamber for 1 h before starting the measurements. Data (gas concentrations, rectal temperatures, chamber temperatures) were averaged (10 readings) and recorded at 10 s intervals. Airflow (dried, CO_2-free ambient air) through the system was controlled with a mass-flow controller and ranged from 2 to 4.5 l min^{-1} STPD, depending on animal size. The animals were visually monitored at regular intervals, and they always appeared to be resting or sleeping. Once BMR measurements had been completed, animals were returned to their crates until measurements of PMR.

A cold-exposure protocol using a heliox mixture (21% O_2:79% He) as the environmental gas was selected to measure PMR. Heliox was used to accelerate heat loss from the animal while reducing the risk of peripheral cold injuries (Rosenmann and Morrison, 1974). Although cold-induced protocols may underestimate PMR in some mammals (Seeherman et al., 1981; but see Chappell and Bachman, 1995), they do not require the repetitive training associated with exercise protocols, which is impractical for sampling numerous wild individuals. Cold-induced PMR is therefore a method widely used to estimate the aerobic capacity of endotherms (e.g. Dutenhoffer and Swanson, 1996; Sparti, 1992). For many mammalian species, measurements of PMR using cold-exposure protocols are highly correlated with measurements obtained using exercise protocols (Chappell and Bachman, 1995; Hayes and Chappell, 1990). At approximately 14:00 h, the first animal was placed in a chamber after securing a rectal thermocouple, as described above. Heliox was mixed from pressurized tanks of pure gases using a proportional gas mixer calibrated with an electronic bubble flow meter (model 730, Humonics Inc., Rancho Cordova, CA, USA). The chamber was flushed with heliox for approximately 10 min at a rate of 15 l min^{-1} (STPD) at room temperature (20–25°C) before being transferred to a cold (–20°C) freezer. Data were averaged (five readings) and recorded at 5 s intervals until the animal's metabolic rate was obviously declining or when its rectal temperature fell below 28°C. The animal was then returned to its crate for recovery, and the procedure was repeated on the second animal. The order in which the animals were used had no significant effect on the results.

Table 1. *Temperature and rainfall during the sampling periods*

	Month	Normal value	1998	1999
Air temperature (°C)	May	23.8	26.0	24.8
	June	26.7	28.7	27.4
Rainfall (cm)	May	11.6	1.1	8.6
	June	14.8	8.6	31.0

Normal values refer to long-term averages.

Air temperature is given as the monthly average of daily temperature averages.

Rainfall is given as total rainfall for each month, measured at New Orleans International Airport.

Source: National Oceanographic and Atmospheric Agency (http://www.srh.noaa.gov/ftproot/lix/html/new/climate.htm).

Data analyses

Because armadillos are seasonal breeders, producing one litter

a year during the spring, the body mass of the individuals originally sampled (N=87) was not normally distributed, forming three distinct groups probably related to age (Loughry and McDonough, 1996): 0.5–1 kg (<1 year old), 2–3.35 kg (1 year old) and more than 3.5 kg (adults). All analyses were limited to adults because they formed the majority (80%) of individuals sampled, and all measured traits were normally distributed within this group. Of these adults, reliable measurements of metabolic rates and body temperature were obtained from 47 individuals (N=22 for 1998, N=25 for 1999), seven of which had clipped ears in 1999 and therefore may also have been used in 1998; these animals were removed from the dataset, leaving a sample size of 18 individuals for 1999 and of 40 for the two years combined (15 males, 25 females). The reproductive biology of armadillos of the genus *Dasypus* is unique among vertebrates because females give birth to sets of monozygotic siblings, quadruplets in the case of *D. novemcinctus* (McBee and Baker, 1982). The likelihood that some of the individuals sampled were clonal siblings is small, as a parallel study (P. Boily, P. A. Prodöhl and P. G. O'Neil, unpublished data), conducted on 75% of the 87 individuals initially sampled, identified only three sets of twins, none of which was part of the final sample used for the purpose of the present study.

Instantaneous O_2 and CO_2 concentrations were calculated according to Bartholomew et al. (1981). Rates of O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) were calculated according to Withers (2001). For each individual, the lowest continuous 10 min periods of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were used as measurements of BMR, and they were significantly correlated with each other (r=0.98, P<0.0001), with a mean respiratory quotient (RQ) of 0.81±0.05 (mean ± S.D., N=40). The highest continuous 2 min periods of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were used as measurements of PMR, and they were significantly correlated with each other (r=0.79, P<0.0001), with a mean RQ of 1.00±0.20 (N=40). Large errors in measurements of $\dot{V}_{O_2}$ obtained by using heliox mixed on-site as an environmental gas can occur as a result of minute changes in the composition of the gas entering the chamber (see Appendix). However, measurements of $\dot{V}_{CO_2}$ during periods of high metabolic demand can overestimate PMR because the rate of CO_2 excretion can exceed the rate of CO_2 production as a result of the buffering of lactic acid by bicarbonates (Seeherman et al., 1981; McArdle et al., 1986). Despite these independent potential sources of error associated with each measurement of gas exchange, statistical analyses yielded consistent results whether $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$ was used as a measure of PMR. For simplicity and ease of comparison with previous studies, all statistical analyses presented are those using $\dot{V}_{O_2}$ as a measure of BMR and PMR.

Analyses of correlation were used to test for relationships between physiological variables. Mass-independent BMR and PMR were obtained by performing regression analyses for each variable as a function of body mass, calculating the residual for each data point, and adding the overall mean to the residual of each data point. This method, described in detail by Packard and Boardman (1988), yields mass-independent variables that have the same mean as their whole-animal values. Analyses of variance were used to test for differences in whole-animal and mass-independent physiological variables between years and sexes; identical results were obtained when mass was used as a covariate in analyses of covariance (ANCOVA). All values are presented as the mean ± 1 S.D.

Results

Body mass ranged from 3.52 to 4.98 kg and was 8% larger (F=10.97, P=0.002) for individuals sampled in 1999 (4.36±0.33 kg, N=18) than for those sampled in 1998 (4.03±0.29 kg, N=22); body mass did not differ between sexes (P=0.74). BMR ranged from 8.6 to 19.5 ml O_2 min^{-1} and was 46% higher (F=93.52, P<0.001) for individuals sampled in 1999 (15.8±2.0 ml O_2 min^{-1}) than for those sampled in 1998 (10.7±1.21 ml O_2 min^{-1}). BMR was positively correlated with body mass (Fig. 1; r=0.64, P<0.001, N=40), and the slope of this relationship did not differ between sampling years (ANCOVA, P=0.45). Mass-independent BMR was 29% higher (F=38.01, P<0.001) for individuals sampled in 1999 (14.8±1.86 ml O_2 min^{-1}) than for those sampled in 1998 (11.5±1.44 ml O_2 min^{-1}). Neither whole-animal nor mass-independent BMR differed between sexes (P=0.71). PMR ranged from 47.3 to 147.3 ml O_2 min^{-1} and was 35% higher

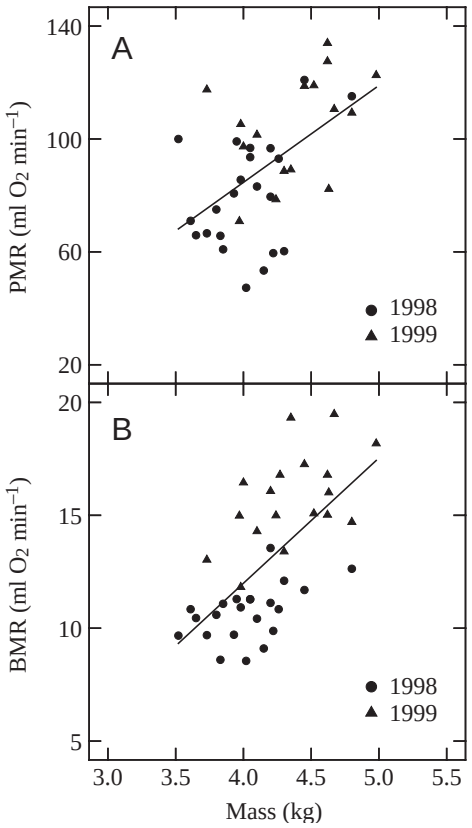

Fig. 1. Relationship between peak metabolic rate (PMR; A) or basal (BMR; B) and body mass. Both BMR (r=0.64, P<0.001) and PMR (r=0.50, P<0.005) were significantly related to mass and differed significantly between sampling years (P<0.001).

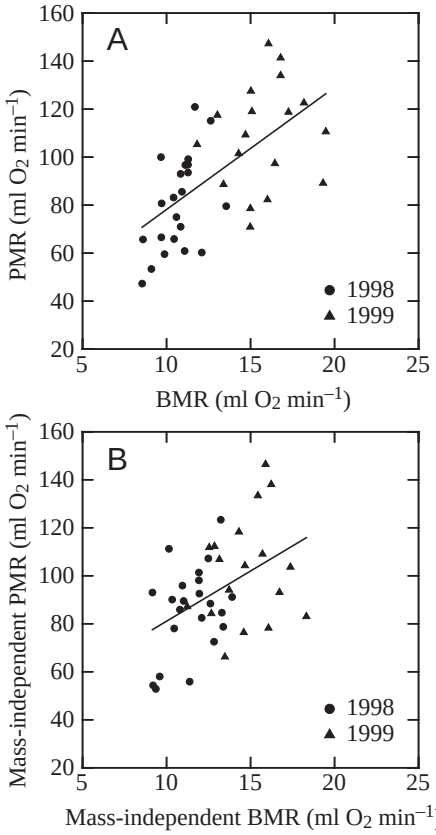

Fig. 2. Correlation between peak (PMR) and basal (BMR) metabolic rates represented as whole-animal (A; r=0.62, P<0.001) and mass-independent (B; r=0.45, P<0.005) values.

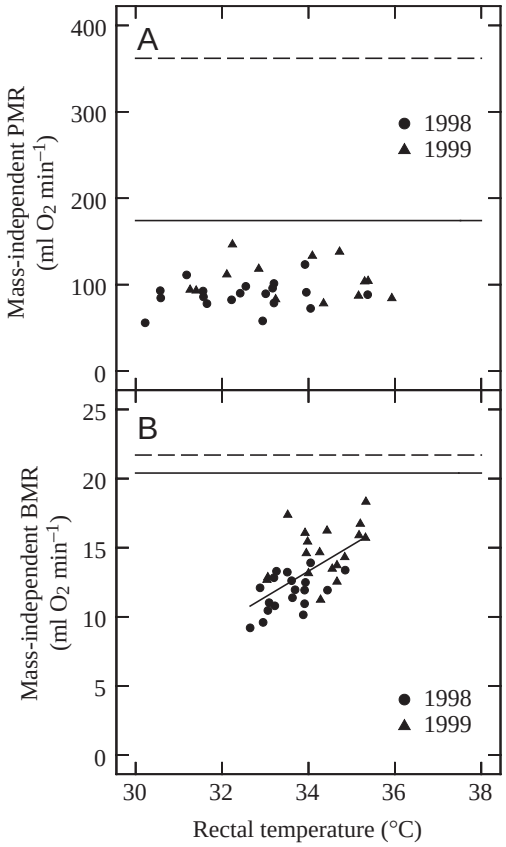

Fig. 3. Relationship between mass-independent peak (A; PMR) or basal metabolic rates (B; BMR) and rectal temperature. Rectal temperatures were measured at the same time as BMR or PMR. Mass-independent BMR was significantly (r=0.81, P<0.001) correlated with rectal temperature, but mass-independent PMR was not (P=0.13). Solid horizontal lines represent mean metabolic rate calculated (assuming a Q_{10} of 3) if rectal temperatures were 38°C instead of the observed average of 33.9°C for mass-independent BMR and 32.5 for mass-independent PMR. Dashed horizontal lines represent predicted values for eutherian mammals of similar size (mean 4.2 kg) according to Lovegrove (2000) for mass-independent BMR and Taylor et al. (1981) for mass-independent PMR.

(F=18.76, P<0.001) for individuals sampled in 1999 (109.0±21.7 ml O_2 min^{-1}) than for those sampled in 1998 (80.5±19.8 ml O_2 min^{-1}) and 28% higher (F=10.38, P<0.005) in males (108.0±21.8 ml O_2 min^{-1}, N=15) than in females (84.4±22.8 ml O_2 min^{-1}, N=25). PMR was positively correlated with body mass (Fig. 1; r=0.50, P<0.005), and the slope of this relationship did not differ between sampling years (ANCOVA, P=0.62). Mass-independent PMR was 20% higher (F=6.89, P=0.012) for individuals sampled in 1999 (102.6±22.2 ml O_2 min^{-1}) than for those sampled in 1998 (85.7±18.5 ml O_2 min^{-1}) and 28% higher (F=12.87, P<0.005) in males (107.2±18.5 ml O_2 min^{-1}) than in females (84.9±19.3 ml O_2 min^{-1}); there was no significant interaction between sex and sampling year (P=0.65).

PMR was significantly correlated with BMR (Fig. 2), both as whole-animal (r=0.62, P<0.001) and as mass-independent (r=0.45, P<0.005) values. The aerobic scope, calculated as the ratio of PMR to BMR, ranged from 4.6 to 10.3 (7.3±1.5) and was higher (F=19.86, P<0.001) for males (8.4±1.2) than for females (6.6±1.9) but did not differ significantly between sampling years and was not related to body mass.

Compared with allometric predictions, the mean mass-independent BMR (13.0±2.3 ml O_2 min^{-1}) was 60% of that predicted for eutherian mammals of similar mass (4.2 kg; 21.7 ml O_2 min^{-1}; Lovegrove, 2000). Assuming a Q_{10} of 3, mass-independent BMR would rise to 20.4 ml O_2 min^{-1}, or 94% of the predicted value, if body temperature were 38°C instead of the observed mean of 33.9±0.74°C (Fig. 3). The mean mass-independent PMR (93.3±21.7 ml O_2 min^{-1}) was 26% of the value predicted for eutherian mammals of similar mass (4.2 kg; 362 ml O_2 min^{-1}; Taylor et al., 1981). Assuming a Q_{10} of 3, mass-independent PMR would rise to 174 ml O_2 min^{-1}, or 57% of the predicted value, if body temperature were 38°C instead of the observed mean of 32.5±1.93°C (Fig. 3). Rectal temperatures obtained during BMR measurements ranged from 32.7 to 35.3°C and were higher (F=13.89, P<0.001) in 1999 (34.1±0.69°C) than in 1998 (33.3±0.84°C). These rectal temperatures were positively correlated with whole-animal (r=0.81, P<0.001) and mass-independent (r=0.63; P<0.001) BMR and with body mass (r=0.56, P<0.001).

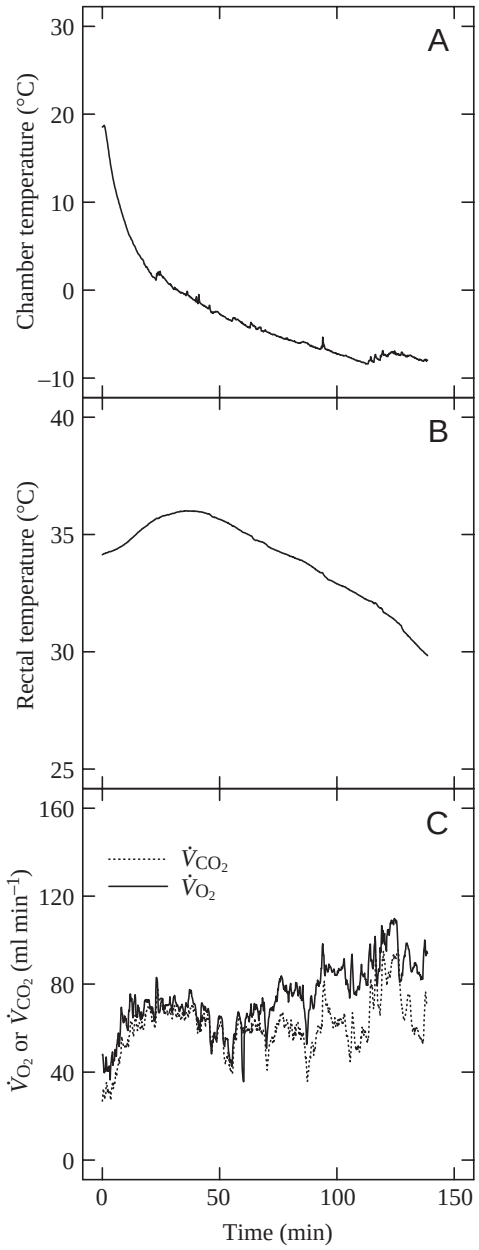

Fig. 4. Typical example of temporal changes in chamber temperature (A), rectal temperature (B) and rates of gas exchange ($\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$; C) of an armadillo during cold-exposure. Time zero indicates the start of cold-exposure. In this example, it took 35 min for peak rectal temperature (36.0°C) to occur and 123 min for peak $\dot{V}_{CO_2}$ (93.6 ml min^{-1}) and $\dot{V}_{O_2}$ (110.6 ml min^{-1}) to occur.

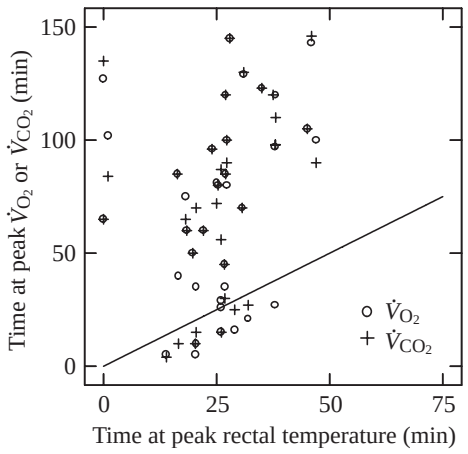

Fig. 5. Relationship between the time at which peak $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$ occurred and the time at which peak rectal temperature occurred in armadillos following the start of cold-exposure. Data points above the solid line represent experimental trials in which the peak rectal temperature occurred before the peak $\dot{V}_{O_2}$ and *vice versa*. Individuals (N=3) for which the time at peak rectal temperature has a value of zero did not exhibit the typical cold-induced increase in rectal temperature.

A typical example of temporal changes in chamber temperature, rectal temperature, $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ of an armadillo during cold-exposure for measurements of PMR is depicted in Fig. 4. Shortly after the chamber was transferred to the pre-cooled freezer, the gas (heliox) temperature inside the metabolic chamber quickly dropped below 0°C and gradually leveled to temperatures between –5 and –10°C. The rectal temperature of the animal increased, reaching a peak value 25.6±10.8 min following the start of cold-exposure, and then gradually declined. Values of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ also increased quickly, but their peak values generally occurred after the peak in rectal temperature, on average (for $\dot{V}_{O_2}$) 71.7±43.1 min (N=40) following the start of cold-exposure and up to 127 min after the peak rectal temperature occurred (Fig. 5). A few individuals (N=3) did not exhibit an increase in rectal temperature during cold-exposure but had a constant rectal temperature that eventually declined, indicating a state of hypothermia. Rectal temperatures measured when PMR occurred were lower (paired t-test) by 2.54±1.77°C than their peak values (t=8.51, P<0.001) and by 1.30±2.02°C than their thermoneutral values measured at BMR (t=3.74, P<0.001). Rectal temperatures measured when PMR occurred ranged from 28.8 to 35.9°C, did not differ between sampling years and were not correlated with whole-animal (P=0.55) or mass-independent (P=0.13, N=37, Fig. 3) PMR. However, peak rectal temperatures were significantly correlated with whole-animal (r=0.66, P<0.001) and mass-independent (r=0.60, P<0.001) PMR. Rectal temperatures obtained during BMR measurements were positively correlated with peak rectal temperatures during cold-exposure (r=0.63, P<0.001), but not with rectal temperatures measured when PMR occurred during cold-exposure (P=0.98).

Discussion

Basal and peak metabolic rates of wild nine-banded armadillos, either as whole-animal or as mass-independent values, were positively correlated with each other and were both significantly higher in 1999 than in 1998, suggesting the existence of a functional link between the two variables. The results of this study therefore support the aerobic capacity model and are consistent with previous studies that

demonstrated an intraspecific correlation between basal and peak metabolic rates in mammals (Chappell and Bachman, 1995; Hayes, 1989), although similar studies conducted at the interspecific level provided only mixed support for the model (for a review, see Hayes and Garland, 1995). Measurements of metabolic rates and of rectal temperature obtained in the present study were also consistent with previous observations indicating that armadillos have low and variable metabolic rates compared with most eutherian mammals. The coefficients of variation of BMR and PMR were of 23 and 27%, respectively, which is substantially larger than generally observed in other mammals (10%) (e.g. Chappell and Bachman, 1995). Interestingly, there was also considerable individual variation in rectal temperatures (range 32.7–35.3°C), even when the animals were resting under thermoneutral conditions during measurements of BMR. This individual variation in rectal temperature appears to have a functional significance, because rectal temperature at thermoneutrality was positively correlated with BMR (r=0.81), with peak rectal temperatures during cold-exposure (r=0.63) and with PMR (r=0.64). Such correlations are not evidence of cause and effect, because it is impossible to say whether low metabolic rates resulted in low body temperatures or *vice versa*. In addition, the low body temperatures alone cannot fully explain the low metabolic rates of armadillos compared with those of other mammals. Indeed, although mean BMR would rise to 94% of the value predicted for eutherian mammals at a rectal temperature of 38°C, PMR would only rise to 57% of the predicted value using the same assumptions.

The investigation of the selective forces that led to the evolution of species with metabolic rates that deviate substantially from interspecific allometric predictions has been the subject of numerous studies. For instance, species with low metabolic rates have been associated with low (Mueller and Diamond, 2001) or unpredictable (Lovegrove, 2000) environmental productivity, with burrowing or fossorial habits (McNab, 1979) and with low food quality or availability (McNab, 1986). Although the last two associations apply directly to armadillos, they could be the result of the confounding effect of phylogeny (e.g. Elgar and Harvey, 1987). Using phylogenetically independent analyses, Lovegrove (2001) observed that armored mammals, such as armadillos, have low metabolic rates compared with non-armored mammals and proposed that their slow metabolic physiology results from a weak selective pressure to increase locomotor performance (and thus for a high aerobic capacity) because body armor substantially reduces predation risk without the need for fast escape mechanisms. The appeal of this hypothesis is its relationship to the aerobic capacity model (Bennett and Ruben, 1979), which proposes that BMR is subject to two opposing selection forces, one to increase aerobic capacity, which increases BMR, the other to maximize energy conservation, which reduces BMR. Thus, for mammals that do not require a high aerobic capacity to escape predators or to catch prey, the selective force for energy conservation would dominate and lead to low metabolic rates (BMR and PMR). This is entirely consistent with the biology of the nine-banded armadillo, which feeds mostly on invertebrates and has a very low predation risk (McBee and Baker, 1982). The hypothesis of Lovegrove (2001) can also be expanded explain the low metabolic rates of non-armored mammals that have other predation defense mechanisms and do not require a high locomotor performance to catch prey. For instance, spotted (*Spilogale putorius*) and striped (*Mephitis mephitis*) skunks use a musk spray for defense, feed mostly on invertebrates and plants and have BMRs 30% lower than predicted (Knudsen and Kilgore, 1990).

The observed differences in metabolic rates and rectal temperatures between sampling years may be related to rainfall. Nine-banded armadillos generally feed on soil invertebrates and, during periods of drought, food availability may be substantially limited by a lower abundance of soil invertebrates and by soil that is harder to dig into. Thus, a reduced food supply could lead to a temporary metabolic depression, which would be consistent with the observation that metabolic rates, body masses and rectal temperatures were lower in 1998, when rainfall was considerably below normal values, than in 1999. Such a decrease in food availability caused by a drought can be further amplified by the fact that low water availability can directly cause a decrease in food intake and in body mass in the nine-banded armadillo (Greegor, 1975).

Basal metabolic rates did not differ between male and female armadillos, but males had a significantly higher PMR and, as a consequence, a higher aerobic scope. Typically, female nine-banded armadillos give birth in the early spring (around March), nurse until late spring (around May) and mate in the summer (July), but gestation does not start until late fall because the embryo remains in a state of suspended development until November, when implantation occurs (McBee and Baker, 1982). Because they were sampled in the summer, it is unlikely that any female was either lactating or gestating, and these potentially confounding factors can therefore be eliminated. The ecological significance of this observation or the mechanism involved in generating this difference between sexes is unclear. The only comparable observation of which I am aware is that of Chappell and Bachman (1995), who observed that BMR of the Belding's ground squirrels (*Spermophilus beldingi*) did not differ between sexes but that females had a higher PMR than males (by approximately 7%); the authors did not discuss the potential significance of their observation.

The observation that the nine-banded armadillo exhibits an increase in body temperature during cold-exposure is not new; Johansen (1961) originally described this unusual response over 40 years ago. In a detailed study using direct and indirect calorimetry, Mercer and Hammel (1989) concluded the armadillo actively regulates its core temperature at elevated levels during cold-exposure. A possible adaptive advantage of this unusual response is that regulating core temperature at an elevated level during cold-exposure could lead to an increase in PMR because of the Q_{10} effect and, thus, increase cold-tolerance. If this is the case, then PMR should occur when core temperatures are at or near their peak, but this was not the case.

Indeed, PMR occurred up to 100 min after peak rectal temperature, when rectal temperatures had dropped on average by 2.4°C from peak values. The other puzzling observation was that PMR was correlated with peak rectal temperature but not with rectal temperature measured when PMR occurred. This apparent disassociation between the timing of peak core temperature and peak metabolic rate could be the result of three errors. First, rectal temperature can be misleading if there is regional heterothermy. This is unlikely because rectal temperatures in the armadillo are highly correlated with core temperatures measured at other sites (Mercer and Hammel, 1989; F. M. Knight and P. Boily, unpublished data). Second, errors in measurements of gas exchange rates could have been involved. The use of heliox as a respiratory gas can cause errors in $\dot{V}_{O_2}$ if baseline O_2 concentration changes during an experiment (see Appendix), and $\dot{V}_{CO_2}$ during periods of high metabolic demand can overestimate metabolic rates because of the excess CO_2 excretion rate resulting from the buffering of lactic acid. This is also unlikely because the sources of errors for measurements of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are independent and yet nearly identical results were obtained for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. Third, the effects of exercise may have caused these results, because armadillos can become very active during sustained cold-exposure (Johansen, 1961), and maximum exercise metabolic rates can exceed peak rates of thermogenesis (Seeherman et al., 1981; but see Chappell and Bachman, 1995). This is the most likely hypothesis, but cannot be tested with the data collected during this study. While I have no evidence to provide a satisfactory explanation for the apparent disassociation between the timing of peak core temperature and metabolic rates, it should be noted that the animals were not necessarily hypothermic at the time when PMR was measured because rectal temperatures were, on average, only 1.2°C below thermoneutral levels at the time when PMRs were observed.

In conclusion, the results obtained during this study are consistent with the existence of a functional relationship between basal and peak metabolic rates and, therefore, support the aerobic capacity model for the evolution of endothermy. In addition, this study confirmed that the nine-banded armadillo exhibits low and variable metabolic rates that may have evolved because the presence of a body armor reduced predation risk and thus the need for a high aerobic capacity, resulting in a unbalanced selection strongly favoring energy-conservation mechanisms.

Appendix

Potential errors associated with measurements of the rate of gas exchange using gas mixtures mixed 'on-site'

The use of heliox (21% O_2, 79% He) as a respiratory gas is a common method of measuring peak metabolic rates in endotherms. While the use of pre-mixed heliox (O_2 and He are mixed in advance in a single tank) is a reliable and economical method for small endotherms, it becomes excessively expensive for studies using mid-size or large animals, for which high flow rates are required. An economical alternative to using pre-mixed heliox is to mix the heliox on-site, during the experiments, using separate O_2 and He gas tanks and flow meters calibrated for each gas. The validity of this procedure depends on the stability of the gas mixture during the course of experiments because small fluctuations in the flow rate of either gas used in the mixture can result in changes in the O_2 concentration of the gas entering the chamber ($[O_2]_{in}$) that can lead to large errors in $\dot{V}_{O_2}$ estimates. The following calculations demonstrate this potential problem using flow rates and O_2 deflection values similar to those observed during the experiments described in the present study. For this purpose, it was assumed that the animal's true $\dot{V}_{O_2}$ is 100 ml min^{-1} and that the flow rate of either O_2 (FR_{O_2}) or He (FR_{He}) entering the chamber increases by 0.25% during the course of an experiment. Calculations of observed $\dot{V}_{O_2}$ ($\dot{V}_{O_2}'$) were made according to:

$$\dot{V}_{O_2}' = \frac{FR_T \times ([O_2]_{in} - [O_2]_{out})}{1 - [O_2]_{out}}, \quad \text{(A1)}$$

where FR_T is the total flow rate through the chamber and $[O_2]_{out}$ is the O_2 concentration of the gas leaving the chamber. The value of $[O_2]_{out}$ depends on the animal's true $\dot{V}_{O_2}$ (100 ml min^{-1}), on FR_T and on $[O_2]_{in}$, and was calculated by rearranging Equation 1 as:

$$[O_2]_{out} = \frac{FR_T \times [O_2]_{in} - \dot{V}_{O_2}}{FR_T - \dot{V}_{O_2}}. \quad \text{(A2)}$$

The results show that minute changes (0.25%) in either of the gas flow rates during an experiment can lead to large errors (10%) in the observed $\dot{V}_{O_2}$ (Table 2). Such errors are proportional to the changes occurring in the flow rates of either of the gases during an experiment; if the flow rate of one gas changes by 1% during the course of an experiment, the error

Table 2. *Parameters used to estimate potential errors in the calculation of observed* $\dot{V}_{O_2}$, $\dot{V}_{O_2}'$

Conditions	FR_{He} (ml min^{-1})	FR_{O_2} (ml min^{-1})	FR_T (ml min^{-1})	$[O_2]_{in}$ (%)	$[O_2]_{out}$ (%)	$\dot{V}_{O_2}'$ (ml min^{-1})
Original conditions	15 000	3975	18 975	20.95	20.53	100
Flow rate of He up 0.25%	15 038	3975	19 013	20.91	20.49	110
Flow rate of O_2 up 0.25%	15 000	3985	18 985	20.99	20.57	90

FR_{He}, flow rate of He; FR_{O2}, flow rate of O_2; FR_T, total flow rate; $\dot{V}_{O_2}$, observed rate of oxygen uptake; $[O_2]_{in}$, $[O_2]$ of gases entering the chamber; $[O_2]_{out}$, $[O_2]$ of gases leaving the chamber.

Up 0.25% indicates that the flow rate increases by 0.25% during the course of the experiment.

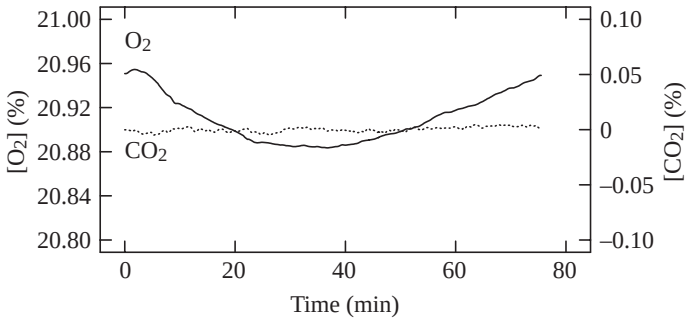

Fig. 6. Typical example of changes in baseline O_2 and CO_2 concentration occurring while heliox was circulated through an empty metabolic chamber. Five other trials were conducted, and all yielded similar results.

in observed $\dot{V}_{O_2}$ will be approximately 40%. The critical factor involved is not the accuracy of flow rate measurements, but rather the stability of flow rates over time. Further, while a linear drift in O_2 concentration during an experiment can be corrected by measuring the O_2 concentration of the circulating gas at the beginning and end of an experimental trial, non-linear fluctuations cannot be corrected unless the O_2 concentration of the inflowing gas is continuously measured. Preliminary trials using the same procedures as those used in the present study, in which heliox was circulated through an empty chamber, gas pressures were regulated at 345 kPa using dual-stage regulators and gas temperatures varied by less than 0.1°C, indicated that baseline O_2 concentration typically fluctuated non-linearly by 0.06% over a 80 min interval (Fig. 6). Such fluctuations would lead to errors in $\dot{V}_{O_2}$ calculations of up to 16%. However, because the baseline concentration of CO_2 in the gas mixture entering the chamber is constant (0%), errors in $\dot{V}_{CO_2}$ are equal to errors in FR_T, i.e. the potential error in $\dot{V}_{CO_2}$ is 0.25% if the flow rate of either gas varies by 0.25% during an experiment.

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