RESEARCH ARTICLE

High muscle mitochondrial volume and aerobic capacity in a small marsupial (*Sminthopsis crassicaudata*) reveals flexible links between energy-use levels in mammals

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SUMMARY

We investigated the muscle structure–function relationships that underlie the aerobic capacity of an insectivorous, small (~15 g) marsupial, *Sminthopsis crassicaudata* (Family: Dasyuridae), to obtain further insight into energy use patterns in marsupials relative to those in placentals, their sister clade within the Theria (advanced mammals). Disparate hopping marsupials (Suborder Macropodiformes), a kangaroo (*Macropus rufus*) and a rat-kangaroo (*Bettongia penicillata*), show aerobic capabilities as high as those of 'athletic' placentals. Equivalent muscle mitochondrial volumes and cardiovascular features support these capabilities. We examined *S. crassicaudata* to determine whether highly developed aerobic capabilities occur elsewhere in marsupials, rather than being restricted to the more recently evolved Macropodiformes. This was the case. Treadmill-trained *S. crassicaudata* attained a maximal aerobic metabolic rate ($\dot{V}_{O_2,max}$ or MMR) of 272 ml O_2 min^{-1} kg^{-1} (*N*=8), similar to that reported for a small (~20 g), 'athletic' placental, *Apodemus sylvaticus*, 264 ml O_2 min^{-1} kg^{-1}. Hopping marsupials have comparable aerobic levels when body mass variation is considered. *Sminthopsis crassicaudata* has a basal metabolic rate (BMR) about 75% of placental values but it has a notably large factorial aerobic scope (fAS) of 13; elevated fAS also features in hopping marsupials. The $\dot{V}_{O_2,max}$ of *S. crassicaudata* was supported by an elevated total muscle mitochondrial volume, which was largely achieved through high muscle mitochondrial volume densities, $V_v(mt,f)$, the mean value being 14.0±1.33%. These data were considered in relation to energy use levels in mammals, particularly field metabolic rate (FMR). BMR is consistently lower in marsupials, but this is balanced by a high fAS, such that marsupial MMR matches that of placentals. However, FMR shows different mass relationships in the two clades, with the FMR of small (<125 g) marsupials, such as *S. crassicaudata*, being higher than that in comparably sized placentals, with the reverse applying for larger marsupials. The flexibility of energy output in marsupials provides explanations for this pattern. Overall, our data refute widely held notions of mechanistically closely linked relationships between body mass, BMR, FMR and MMR in mammals generally.

Key words: aerobic scope, basal metabolism, exercise, field metabolism, maximum metabolism, mitochondria.

INTRODUCTION

Marsupials (Metatheria) are the sister clade of placentals (Eutheria). Together they comprise the Theria or advanced mammals and they have many anatomical and physiological characteristics in common, which largely reflect ancestral traits that evolved prior to the divergence of these two groups about 148 million years ago (Bininda-Emonds et al., 2007). Differences between the two groups, such as their distinct reproductive features, reflect divergent evolutionary trajectories during their long, separate histories. Another area where differences seem to have occurred concerns metabolic patterns. Marsupials have relatively low basal metabolic rates (BMRs), generally about 75% of placental values, and historically were seen as being less competent at thermoregulation and also as 'low energy' mammals (Martin, 1902; Dawson, 1973). While distinctions regarding the thermal biology of these clades have been long discarded (Dawson, 1973; Dawson, 1989), debate about differences in metabolic capabilities of marsupials has persisted in some disciplines (e.g. McNab, 1980; McNab, 2005).

Here, we expand on investigations of the aerobic capacities of marsupials and also focus on the functional structures that underlie the capacity for oxygen use in their muscles. Our aim was to put these into perspective relative to the features that have recently been established for the structure–function relationships underlying the aerobic capacities of the placental mammals (Weibel et al., 2004).

It has become apparent that some marsupials have substantial aerobic capabilities that result from a relatively large factorial aerobic scope (fAS), such that they achieve levels of maximum oxygen consumption ($\dot{V}_{O_2,max}$) similar to those seen in placentals (Dawson and Dawson, 1982; Hinds et al., 1993). Recent data (Kram and Dawson, 1998; Dawson et al., 2004) for *Macropus rufus* (Family Macropodidae), the red kangaroo, are notable because, despite a relatively low BMR, its extreme fAS of 54 results in a $\dot{V}_{O_2,max}$ or maximum metabolic rate (MMR) equivalent to the high levels reported in a group of placental mammals, such as dogs and horses, that were categorised as 'athletic' (Taylor et al., 1987; Weibel et al., 2004). Underlying this capability in *M. rufus* is a large mass of

locomotor muscles that have comparatively high mitochondrial and capillary volumes (Dawson et al., 2004). Another hopping marsupial, *Bettongia penicillata*, a rat-kangaroo (Family Potoroidae), though much smaller (body mass, M_b, 1 kg), also shows an elevated fAS of 23 and a markedly high MMR (Seeherman et al., 1981; Webster and Dawson, 2012). Again, this is associated with a large skeletal muscle mass that has relatively high muscle mitochondrial volume densities and both a large total capillary volume and a large total capillary erythrocyte volume (Webster and Dawson, 2012). Overall, the muscle and cardio-respiratory structural features of *M. rufus* and *B. penicillata* are identical to those previously reported for 'athletic' placental mammals of equivalent sizes (Weibel et al., 2004). Notably, the ratio between MMR and total muscle mitochondrial volume ($\sim$5 ml O_2 min^{-1} ml^{-1}) is, as initially proposed (Hoppeler and Lindstedt, 1985), consistent in placentals (Weibel et al., 2004) and macropodiform marsupials (Webster and Dawson, 2012).

Macropus rufus and *B. penicillata* belong to the specialised monophyletic suborder Macropodiformes (kangaroos, wallabies and rat-kangaroos) (Meredith et al., 2008), but do they differ aerobically from other marsupials? Evidence for 'athletic' level aerobic capacities in other marsupials is strong and comes from disparate studies of their cardio-respiratory features (e.g. Dawson and Needham, 1981; Hallam et al., 1989; Hallam et al., 1995; Agar et al., 2000; Dawson et al., 2003). The generality of this assertion is not certain because, while Hinds and colleagues (Hinds et al., 1993) measured comparatively high fAS during locomotion for several smaller species of marsupial, their reported MMR values did not reach 'athletic' placental levels. However, the MMR value obtained (Hinds et al., 1993) for *B. penicillata* was lower than those obtained on more extensively trained animals (Seeherman et al., 1981; Webster and Dawson, 2012). The only other marsupial for which comparable data are available is a South American opossum, *Monodelphis domestica* (Family: Didelphidae) ($M_b \approx$90 g). It also has a comparatively large fAS (Dawson and Olson, 1988; Schaeffer et al., 2003) but its MMR does not reach an 'athletic' level, though the mitochondrial volume densities of its skeletal muscles are not lower than generally seen in similar sized placentals (Schaeffer et al., 2003). However, there is support for relatively high aerobic capabilities extending to smaller marsupials. This comes from studies of field metabolic rate (FMR). Marsupials and placentals show distinctly different patterns of variation of FMR with M_b but, intriguingly, small marsupials (M_b<125 g) have higher FMRs than placentals of similar size (Nagy et al., 1999; Capellini et al., 2010). These differences are particularly marked among the smallest marsupials; at a M_b of $\sim$15 g, the FMR of *S. crassicaudata* is almost double that of the predicted placental value (Nagy, 1988).

To clarify the factors underlying this disparity and to further our understanding of the metabolic patterns and aerobic capabilities among small marsupial species from different phylogenetic clades, we studied *S. crassicaudata* (Family: Dasyuridae). This species ($M_b \approx$15 g) is an active, quadrupedal insectivore that has often been used as a model for Australian dasyurid marsupials and was among the species examined by Hinds and colleagues (Hinds et al., 1993). We focused first on gaining an accurate determination of its MMR and then ascertained how MMR related to its muscle content and muscle mitochondrial features such as volume density. Because of its high MMR levels, we predicted that it would share characteristics that match what has previously been found in the Macropodiformes, thereby indicating a generally higher aerobic capability among marsupials, matching that seen in placentals designated as 'athletic' (see Weibel et al., 2004). If so, it would point to a fundamental structure–function relationship for oxygen delivery to muscles

evolving in or before the earliest mammals. Further, such information provides the opportunity to examine the presumed relationships between BMR, MMR and FMR in mammals. This is significant in view of the idea that BMR is a good predictor of energy budgets, which is based on the notion that the allometric relationship between M_b and BMR locks in other metabolic levels (West et al., 1997; West et al., 1999; Brown et al., 2004).

MATERIALS AND METHODS
Animals and animal care

Fat-tailed dunnarts, *S. crassicaudata* (Gould 1844) of the Family Dasyuridae (Krajewski et al., 2012) are mouse-sized insectivorous marsupials that inhabit the surface of open habitats, usually in semi-arid and arid regions of Australia. They are active nocturnal predators, catching relatively large, invertebrate prey, such as crickets and beetles (Morton, 1978). Our investigations were in two parts. (1) An analysis of the mitochondrial characteristics of locomotor muscles of *S. crassicaudata*, carried out at the University of New South Wales, Sydney. The animals used in this study were derived from colonies maintained at the University of Adelaide and University of Wollongong. (2) A study of aerobic capacity of *S. crassicaudata* during running, undertaken at the University of Wollongong, using animals from their breeding colony that was established 3 years earlier from free-living animals collected in western Queensland. This study was carried out under approval given by the University of New South Wales and University of Wollongong Animal Care and Ethics Committees (project approval number 00-17 and 04/06, respectively).

During investigation and prior to killing, animals were kept at an air temperature (T_a) of 23±0.4°C, with a 12 h:12 h light:dark cycle (lights on at 06:00 h). They were housed individually in clear plastic containers (55×38×20 cm), which were fitted with wire tops; bedding of straw and shredded paper was provided. A mixture of dried cat food (moistened) and canned dog food was provided *ad libitum*; this was supplemented with live crickets and vitamin drops (Penta-vite infant vitamins, Bayer, Pymble, NSW, Australia). Water was available at all times.

Muscle sample collection and preparation

To assess the muscle mitochondrial parameters of the skeletal muscle of the whole body, we followed a sampling procedure comparable to that developed previously (Hoppeler et al., 1984) and used in other studies of marsupials (Dawson et al., 2004; Webster and Dawson, 2012). The musculature of *S. crassicaudata* was divided into five functional regions, these being head/neck, foreleg, trunk, hindleg and tail. Animals were killed by gassing (carbon dioxide) and weighed to the nearest 0.01 g on an electronic balance (Sartorius AG, Goettingen, Germany) directly before dissection. Four animals were dissected to estimate total skeletal muscle mass and the proportions of muscle in the five body regions. The contributions to M_b of skin, heart and the digestive tract were also determined. In a further five animals the heart plus seven skeletal muscles, including the diaphragm because of its role in ventilation, were then dissected out and small blocks sampled for electron microscopy. The skeletal muscles used were randomly selected, one coming from each region except the hindleg, where two muscles were selected. These were, m. trapezius (head/neck), m. deltoid (forelimb), m. pectoralis minor (trunk), m. multifidi lumborum (trunk), m. gluteus maximus and m. quadriceps (hindleg), and m. multifidi lumborum (tail).

Two small blocks, no greater than 2 mm thick, were randomly cut from each muscle whilst being bathed in a drop of cold

glutaraldehyde fixative solution (2.5% in $0.1\,mol\,l^{-1}$ sodium cacodylate buffer, pH 7.4). Sample blocks were transferred to vials containing the buffered glutaraldehyde fixative solution for proper immersion fixation for a minimum of 4 h. Sample preparation thereafter followed the method of previous studies (Dawson et al., 2004; Webster and Dawson, 2012), with the blocks ultimately being embedded in Spurr's resin (a slow cure, low viscosity epoxy) over a long infiltration period (3–4 days) and cured at 60°C for 48 h. Ultra-thin sections of ~60–80 nm were cut for each muscle sample using glass knives mounted on a Reichert-Jung Ultracut microtome (Leica Microsystems, Vienna, Austria). The sections were placed onto copper grids (200 square mesh) and were immediately stained with uranyl acetate in 50% ethanol for 10 min.

Mitochondrial volume and inner mitochondrial membrane surface area

Grids were viewed at 10,000× magnification with either a Hitachi 7000 (Tokyo, Japan) or JEOL 1400 (Tokyo, Japan) transmission electron microscope (TEM). Ten grid squares were selected per sample block using a systematic random sampling method (Howard and Reed, 1998). Digital micrographs were taken in the top left corner of the grid squares using an Olympus SQ (Tokyo, Japan) digital camera and software package AnalySIS (attached to the Hitachi 7000 TEM) or a Gatan (Pleasanton, CA, USA) digital camera and software package Gatan Digital Micrograph (attached to the JEOL 1400). For each animal, a total of 160 micrographs were taken (10 micrographs per block × two blocks per muscle × eight muscles).

Mitochondrial volumes were determined using the methods of previous studies (Dawson et al., 2004; Webster and Dawson, 2012). Briefly, mitochondria were identified and selected in digital images by a human operator. The total percentage area covered by the mitochondria (mitochondrial area fraction) in each micrograph was estimated using either an image processing plug-in to Adobe Photoshop (Adobe Systems Inc., San Jose, CA, USA) or the software ImageJ (US National Institutes of Health, Bethesda, MD, USA). According to the Delesse principle, the mitochondrial volume fraction $Vv(mt,f)$, often referred to as mitochondrial volume density, is equivalent to the mitochondrial area fraction (Weibel, 1980; Howard and Reed, 1998). The total mitochondrial volume $V(mt,m)$ for each muscle region (in ml) was calculated from Eqn 1:

$$V(mt,m) = M_m \times Vv(mt,f) \times Vv(f,m) \times d^{-1}, \tag{1}$$

where M_m is regional muscle mass, $Vv(mt,f)$ is the volume fraction of mitochondria, $Vv(f,m)$ is the volume fraction of muscle occupied by muscle fibres, and d is the density of the muscle. For this study, it was assumed that $Vv(f,m)$ was equal to 1 (Hoppeler et al., 1987) and that d was equal to $1.06\,g\,ml^{-3}$ (Mendez and Keys, 1960) as the myofibril fraction and density are considered constant in all muscles (Mendez and Keys, 1960; Barth et al., 1992).

The surface density of the inner mitochondrial membranes was estimated in four muscles (m. gluteus maximus, m. deltoid, heart and diaphragm). For each animal, a total of 40 mitochondria (five mitochondria per block × two blocks per muscle × four muscles) were examined and micrographs taken at up to 40,000× magnification (using the Hitachi 7000 TEM with attached Olympus digital camera). The surface density of inner mitochondrial membranes per unit volume of mitochondria, $Sv(im,mt)$, was estimated using the same method as in previous studies (Dawson et al., 2004; Webster and Dawson, 2012). An overall estimation of the total surface area of inner membranes in each muscle is given by Eqn 2:

$$S(im,m) = V(mt,m) \times Sv(im,mt). \tag{2}$$

Aerobic capacity

To ensure that maximum aerobic capacity (MMR) was achieved, we followed procedures published elsewhere (Seeherman et al., 1981); such procedures were used in comparable studies on placental mammals (see Weibel et al., 2004). The essence of these procedures was extensive treadmill training (running) that ensured an accurate and reproducible MMR. Seeherman and colleagues found that at least 2–6 weeks of training were needed for this to be achieved for most of the species that they investigated (Seeherman et al., 1981). We trained *S. crassicaudata* for treadmill running for 6–8 weeks by exercising them at speeds of up to $1.5\,m\,s^{-1}$, generally on alternate days. The highest training speed at which an animal could maintain 5 min of constant running, following an initial speed adjustment period of 30 s, was used during the measurement of MMR; such speeds ranged between 1 and $1.5\,m\,s^{-1}$. The MMR obtained was the highest 2 min period of instantaneous oxygen consumption when an animal ran for at least 5 min. The method for obtaining instantaneous oxygen consumption (Bartholomew et al., 1981) involved initially determining the washout characteristics of the chamber, at the flow rate used, by tracking the dynamics of a sudden pulse of O_2-depleted air followed by an immediate return to room air.

For actual measurement, one *S. crassicaudata* was contained within an inverted 1.2 l rectangular plastic container on a stationary treadmill belt. The treadmill speed was then adjusted to that required, ie. the highest training speed for that individual. A constant airflow of $2.0\,l\,min^{-1}$ was aspirated through the container at all treadmill speeds. Air entered through two small holes in the front of the chamber and also through the bottom edges of the chamber in contact with the belt. Flow rate was monitored with a Sierra Top-Trak mass-flow meter (Sierra, Monterey, CA, USA). Oxygen content of inlet and outlet air was measured using a Sable Systems FC-1 oxygen analyser (Las Vegas, NV, USA), with a detection sensitivity of 0.0005%. Water and CO_2 were removed from sampled air prior to gas analysis using Drierite and soda lime, respectively. The O_2 throughout this exercise period was determined using appropriate corrections for the system configuration (Hill, 1972). Values were adjusted for variations in chamber air leakage at different treadmill speeds. Air leakage was determined by delivering a gas mix into the chamber *via* a mass flow controller with a percentage O_2 similar to that while a *S. crassicaudata* was running. Readings were first taken while the treadmill belt was stationary and then recorded at each belt speed used in the MMR determinations. Corrections ranged from 7% at the lowest running speed to 14% at the highest.

Statistical analysis

Comparisons between muscles were analysed using one-way analysis of variance (ANOVA). A Student–Newman–Keuls (SNK) multiple-range test was applied when significant differences were indicated by the ANOVA (using Statistica for Mac, StatSoft, Tulsa,

Table 1. Body mass of the fat-tailed dunnart, including contributions of muscle and other body components

M_b (g)	15.0±1.28
Total skeletal muscle (g)	4.83±0.223
Total skeletal muscle (% M_b)	32.3±1.96
Gut + liver (% M_b)	14.2±1.23
Heart (% M_b)	0.79±0.068
Skin (% M_b)	17.5±0.986

M_b, body mass.
Values are means ± s.d., $N=5$.

Table 4. Relationship between mitochondrial content of the skeletal muscle and aerobic capacity in the fat-tailed dunnart compared with that of the 'athletic' placental wood mouse and two small marsupials

Parameter	Dunnart	Wood mouse	Rat-kangaroo	Opossum
Mitochondrial content				
M_b (g)	15.0±1.28	20.3	1000	89.4
M_m/M_b (%)	32.3±1.96	42.5	43.5	32
$Vv(mt,f)$ (%)	14.0±1.33	11.0	8.7	8.4
$V(mt)/M_b$ (ml kg^{-1})	45.0±4.26	43.5	36.0	30.1
Aerobic capacity				
$\dot{V}_{O_2,max}/M_b$ (ml O$_2$ min^{-1} kg^{-1})	272±30.9	264	177	129
BMR (ml O$_2$ min^{-1} kg^{-1})	21.3±1.77	28.0	7.8	9.53
BMR (ml O$_2$ min^{-1} kg$^{-0.72}$)	6.6	9.4	7.8	4.9
fAS	12.8±1.45	9.4	23	13.6
$\dot{V}_{O_2,max}/Vv(mt)$ (ml O$_2$ min^{-1} ml^{-1})	6.1±0.56	5.0	4.9	4.3

$Vv(mt,f)$, volume fraction of mitochondria; $V(mt,m)/M_b$, mass-specific mitochondrial volume; $\dot{V}_{O_2,max}$, maximum aerobic metabolic rate.
For the dunnart, values are means ± s.d.
Data sources other than the current study: fat-tailed dunnart (*Sminthopsis crassicaudata*), BMR from Dawson and Hulbert (1970); wood mouse (*Apodemus sylvaticus*), BMR from Haim et al. (1995), other data from Hoppeler et al. (1984); rat-kangaroo (*Bettongia penicillata*) data from Webster and Dawson (2003, 2012); short-tailed opossum (*Monodelphis domestica*), BMR from Dawson and Olson (1988), other data from Schaeffer et al. (2003).

red kangaroo (*M. rufus*) has an M_m/M_b value of 47% (Dawson et al., 2004). The lower M_m/M_b of *S. crassicaudata* compared with *A. sylvaticus*, however, is offset by its relatively higher $Vv(mt,f)$, which is 14% *versus* 11% in *A. sylvaticus* (Table 4). The very similar total heart mitochondrial volumes in the two species reflect this balance. This trait is a reliable predictor of the MMR of equivalent sized species among marsupials (Dawson et al., 2003) and placentals (Karas et al., 1987). The heart masses were 0.79% and 0.78% of M_b, respectively, for *S. crassicaudata* and *A. sylvaticus*, while $Vv(mt,f)$ in the hearts of both species was approximately 34%.

The surface area of the inner mitochondrial membranes, $S(im,m)$, has been consistently correlated with the activity of the terminal respiratory chain enzymes in vertebrate groups (Else and Hulbert, 1981), and appears to be functionally linked with aerobic metabolic capacity. The surface density of inner mitochondrial membranes per unit volume of mitochondria, $Sv(im,mt)$, in the muscles of *S. crassicaudata* is ~35 m^2 cm^{-3}, which is similar to that of other marsupials (Dawson et al., 2004; Webster and Dawson, 2012) and placentals including *A. sylvaticus* (Hoppeler et al., 1981; Hoppeler et al., 1984; Schwerzmann et al., 1989). As $S(im,m)$ equals $V(mt,m)$ multiplied by $Sv(im,mt)$ (Eqn 2), mitochondrial volume in skeletal muscle can be used as a proxy for $S(im,m)$. The high overall $Vv(mt,f)$ of *S. crassicaudata* relative to that of *A. sylvaticus* and those of other placentals compiled by Weibel and colleagues (Weibel et al., 2004) results from high mitochondrial volume densities in all muscles across the body (Tables 2, 3). The $Vv(mt,f)$ of individual muscles, except for the diaphragm, did not vary through the body (Table 2); this would reflect *S. crassicaudata*'s active quadrupedal lifestyle. The pattern differs in the more specialised kangaroos, whereby muscle $Vv(mt,f)$ is markedly higher in the region of the pelvis and lower back where the bulk of the skeletal muscle is also found (Dawson et al., 2004).

These data from *S. crassicaudata* considerably extend our understanding of the overall aerobic capacities of marsupials relative to those of placentals. Initially, an investigation of the cardio-respiratory allometry in marsupials (Dawson and Needham, 1981) identified them as having the capability for a considerable aerobic capacity. Dawson and Dawson further challenged the notion that marsupials, with their low BMRs, were 'low energy' mammals (Dawson and Dawson, 1982). Two small marsupials species that they exposed to cold had generally larger fAS values, 8–9 as against 4–6 for similar-sized placental species, and aerobic capabilities equivalent to those of the placentals. Data from Hinds and colleagues

further highlighted relatively high fAS values in a range of marsupials (Hinds et al., 1993). In response to cold, marsupials and placentals were able to increase aerobic metabolism above BMR by 8.3 and 5.1 times, respectively; values during locomotion were almost twice those observed in the cold (Hinds et al., 1993) and fAS values were again higher in marsupials (17) than in placentals (13.5). However, subsequent locomotor investigations indicate that these fAS values were underestimated in marsupials (see below).

The aerobic factorial scope of *M. rufus* is of the order of 54 (Dawson et al., 2004). How could this be so much greater than the value of 17 given by Hinds and colleagues (Hinds et al., 1993) for the fAS of marsupials during locomotion? The answer comes from investigations on placentals (see Weibel et al., 2004; Weibel and Hoppeler, 2005). Allometric equations from these studies show that MMR is more loosely associated with BMR than was previously considered. When MMR is plotted against M_b for placentals, two distinct patterns occur (Weibel et al., 2004). One group of species has a relatively high MMR while most other species tend to have a distinctly lower MMR; the former was designated 'athletic' and the latter 'sedentary' (Fig. 1). Furthermore, these MMR patterns vary with M_b in a different manner from that of BMR. While the allometric equations usually used for BMR have an exponent of 0.75, the exponents found for MMR of 'athletic' and 'sedentary' placentals were much steeper, 0.942 and 0.849, respectively (Fig. 1). Weibel and co-workers have shown that MMR is largely set by the energy needs of active cells, primarily those in muscle, during maximal work and that total skeletal muscle mitochondrial volume, $V(mt)$, is a superior proxy for this (Weibel et al., 2004). 'Athletic' species had greater $V(mt)$ than 'sedentary' species, which was due to either greater M_m/M_b and/or higher $Vv(mt,f)$. Overall, as initially proposed (Hoppeler and Lindstedt, 1985), there was a strong and consistent correlation between MMR and $V(mt)$ (Fig. 2). Our previous studies (Dawson et al., 2004; Webster and Dawson, 2012) have shown that both the hopping marsupials have MMRs that fall within the 'athletic' grouping in relation to M_b (Fig. 1), and that the relationship between MMR and $V(mt)$ is indistinguishable from that of placentals (Fig. 2). Given the large evolutionary distance and the disparity in body form between modern placentals and the kangaroos and rat-kangaroos, we were somewhat surprised to find comparable relationships. The volume of muscle, its total mitochondrial content and its overall vascular supply were essentially identical in the Macropodiformes to values seen in 'athletic' placental mammals.
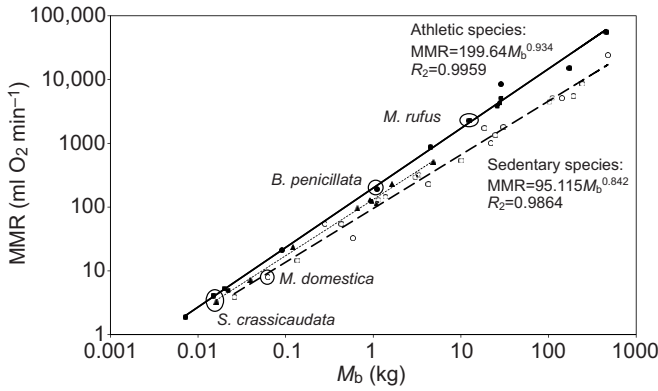
Fig. 1. Maximum metabolic rate (MMR) as a function of body mass (M_b) in mammals. 'Athletic' mammals (filled circles and solid line) have a different relationship between MMR and M_b from that of more 'sedentary' mammals (open circles and dashed line). Marsupial species values may fall either on the 'athletic' line (*Sminthopsis crassicaudata*, *Bettongia penicillata*, *Macropus rufus*) or on the 'sedentary' line (*Monodelphis domestica*). *Sminthopsis crassicaudata* data are from the present study; *B. penicillata*, *M. rufus* and *M. domestica* data are from previous studies (Seeherman et al., 1981; Kram and Dawson, 1998; Schaeffer et al., 2003). Placental data are from Weibel et al. (Weibel et al., 2004). Allometric equations shown on the graph include all species (placentals and marsupials) but slopes and elevations are not significantly different from previously published placental-only equations (Weibel et al., 2004). Also shown are data for several marsupial species (including *S. crassicaudata*) from previous work (Hinds et al., 1993); the allometric equation for this data set is MMR=$131.76 M_b^{0.882}$, with r^2=0.9949 (triangles and dotted line). This line falls between the 'sedentary' and 'athletic' lines, and may indicate incomplete treadmill training of the individuals used in the study; see Discussion for details.

Fig. 2. MMR as a function of total mitochondrial volume [V(mt)] in mammals. Marsupial species data (open circles) were obtained as follows: *S. crassicaudata*, present study; *M. domestica*, *B. penicillata* and *M. rufus*, previous studies (Schaeffer et al., 2003; Webster and Dawson, 2012; Dawson et al., 2004). Numbers on the right identify the placental mammal species (filled circles) (Weibel et al., 2004): 1, wood mouse; 2, mole rat; 3, white rat; 4, guinea pig; 5, agouti; 6, fox; 7, goat; 8, dog; 9, pronghorn; 10, horse; 11, steer.

Thus, our data for *S. crassicaudata* provide a wide size range over which marsupials have aerobic capabilities that are essentially similar to those of 'athletic' placentals (Fig. 1), despite the significantly lower BMR of these marsupials (Table 4). Notably, while V(mt) is high in *S. crassicaudata*, the relationship between MMR and V(mt) follows the general mammalian pattern (Fig. 2). Does a pattern of 'athleticism' pertain for most other marsupials or do marsupials also have variable aerobic potentials, as do placentals (Weibel et al., 2004; Weibel and Hoppeler, 2005)? Available information is equivocal in regard to this question. The only marsupial for which comparable information is also available on MMR and on muscle and muscle mitochondria volumes (Table 4) is the grey short-tailed opossum (*Monodelphis domestica*, Family: Didelphidae) (Schaeffer et al., 2003). The MMR of *M. domestica* is relatively low (Table 4, Fig. 1) and falls in line with those of the 'sedentary' placentals, not with 'athletic' small mammals such as the placental *A. sylvaticus* and the marsupials, *S. crassicaudata* and *B. penicillata*. This is somewhat surprising given its fAS at 13.6 is relatively large, but this mostly reflects it having a BMR that is low, even for a marsupial (Table 4). The BMR of *M. domestica* is approximately 70% of the value predicted for a marsupial of its mass (including other didelphids) from allometric equations (Dawson and Hulbert, 1970; Withers et al., 2006). However, the relationship between MMR and V(mt) in *M. domestica* is similar to that of mammals generally (Fig. 2) and its Vv(mt,f) is also relatively low (Table 4).

Apart from the results for *M. domestica*, other data suggest that high aerobic capacity may be a general characteristic of marsupials. For example, marsupials tend to have larger hearts than placentals (Dawson et al., 2003), a trait that benefits attaining high MMR. Also, the data collected by Hinds and colleagues (Hinds et al., 1993)
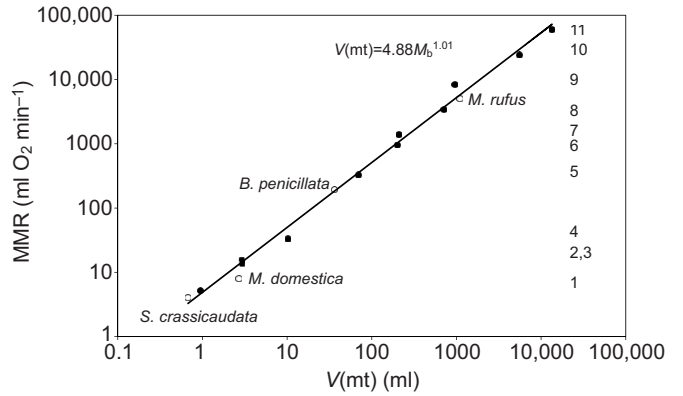
corroborates the greater aerobic potential of marsupials when it is examined in detail. The MMR of marsupials during locomotion that they report are from untrained animals, but still, with their expanded fAS values, they mostly exceed those of trained 'sedentary' placentals (Fig. 1). Full treadmill training presumably would increase the MMR of many of these species to 'athletic' levels, as we found for *S. crassicaudata* (Fig. 1). A clear supporting framework for these abilities is apparent in other species so far examined. As in 'athletic' placentals (Weibel et al., 2004; Weibel and Hoppeler, 2005), the peak aerobic demands associated with maximum energy output by muscle are met *via* the commensurate, matched oxygen supply system from the lungs to the muscle mitochondria *via* the expanded
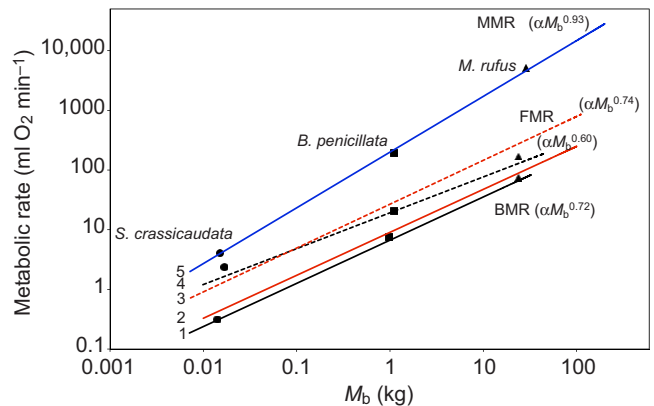
Fig. 3. Different levels of metabolic rate (basal metabolic rate, BMR; field metabolic rate, FMR; and MMR) as a function of M_b in mammals. Numbers on the left: 1, marsupial BMR; 2, placental BMR; 3, placental FMR; 4, marsupial FMR; 5, 'athletic' mammal MMR. Allometric equations for the relationships of these levels of metabolic rate to M_b are shown in Table 5. The measured values of BMR, FMR and MMR for three species of marsupial are also shown: *S. crassicaudata* (circles) – BMR (Dawson and Hulbert, 1970), FMR (Nagy et al., 1988) and MMR data (present study); *B. penicillata* (squares) – BMR (Webster and Dawson, 2003), FMR (Nagy, 1994) and MMR data (Seeherman et al., 1981); and *M. rufus* (triangles) – BMR (Dawson et al., 2000a), FMR (Munn et al., 2008) and MMR data (Dawson et al., 2004).

Table 5. Allometric equations relating BMR, FMR and MMR to M_b in mammals, corresponding to the lines in Fig. 3

Metabolic rate	Mammalian group	a	b	Line no. in Fig. 3	Source
BMR	Marsupials	6.68	0.72	1	Capellini et al., 2010[1]
	Placentals (all)	7.57	0.72	n.a.	Capellini et al., 2010[1]
	Placentals (four Orders only)[2]	9.04	0.72	2	Data from Capellini et al., 2010; Heyssen and Lacy, 1985[2]
FMR[3]	Marsupials	19.21	0.60	4	Capellini et al., 2010[1]
	Placentals	26.76	0.74	3	Capellini et al., 2010[1]
MMR	All 'athletic' mammals	199.64	0.93	5	Fig. 1 (present study)

BMR, basal metabolic rate; FMR, field metabolic rate; MMR, maximum metabolic rate.

In each case, equations are of the form MR=$aM_b{}^b$, with MR in ml O_2 min^{-1} and M_b in kg.

[1]Values for the intercept 'a' were provided by I. Capellini (personal communication).

[2]For comparison with the four placental Orders for which MMR data are available (Weibel et al., 2004), we calculated an equation relating BMR to M_b for just these four Orders, using data for the Orders Artiodactyla, Carnivora and Rodentia from Capellini et al. (2010) and data for the horse, *Equus caballus* (Order Perissodactyla), from Hayssen and Lacy (1985).

[3]Conversion of FMR from units of kJ day^{-1} (Capellini et al., 2010) to units of ml O_2 min^{-1} assumed that 1000 ml of O_2 provides 20.1 kJ of energy.

supply of erythrocytes. This is the concept of symmorphosis (Weibel, 2000) and it also pertains to *M. rufus* and *B. penicillata* (Dawson et al., 2004: Webster and Dawson, 2012). Following this concept, there are numerous other studies that lend support for a generally high MMR in marsupials. These examined lung structure and function of the respiratory system (Dawson and Needham, 1981; Hallam et al., 1989; Chappell and Dawson, 1994; Dawson et al., 2000b), heart structure and capacities (Dawson and Needham, 1981; Dawson et al., 2003), blood oxygen affinities (Hallam et al., 1995) and relative haematocrit levels (Agar et al., 2000).

Broadly then, clades of mammals, both placental and marsupial, have evolved elevated aerobic capacities that can be sustained by small species for at least several minutes and for relatively longer periods in larger species. The evolutionary forces behind such elevated capabilities are likely to be diverse, but the predator–prey 'arms race' (Vermeij, 1987) initially comes to mind. The MMR that a mammal can attain is clearly determined by the functional characteristics of muscle mitochondria, for which V(mt) is an appropriate proxy (Fig. 2). V(mt) results from various mixes of M_m/M_b ratios and V(mt,m) levels of individual muscles, which can also vary markedly. In regard to the link between MMR and BMR, it seems to be much more loose than previously accepted. The patterns differ considerably between marsupials and placentals, as indicated by their differing fAS values. Although fAS shows much plasticity (consider the value of 54 for *M. rufus*), there is an apparent upper limit to MMR based at the 'athletic' level (see Weibel et al., 2004; Weibel and Hoppeler, 2005), which we have shown is also reached by marsupials. Some mammalian groups may have evolutionarily varied their energetic profile by varying their basic energetic structure, i.e. their BMR. For example, a relatively low BMR in *M. domestica* is reflected in a low MMR (Table 4), which is seen in the converse in red-toothed shrews of the subfamily Soricinae such as *Blarina brevicauda* and *Sorex araneus* (Dawson and Olson, 1987; Poppitt et al., 1993). However, underlying patterns are common to marsupials and placentals, and indicate that the basic structure–function framework for mammalian aerobic capabilities is ancient. It must at least predate the divergence of the therians.

The high MMR of *S. crassicaudata* does not completely explain the unusual patterns in the allometry of FMR in marsupials and placentals, whereby small marsupials have higher FMRs than placentals (Koteja, 1991; Nagy et al., 1999; Cooper et al., 2003; Capellini et al., 2010). The BMR of *S. crassicaudata* is 75% of that predicted for a similar sized placental, yet its FMR at ~7 times BMR (Nagy, 1988) is almost double the predicted FMR for a placental

(Fig. 3). In the context of its high MMR, *via* a fAS of 13, *S. crassicaudata* has ample aerobic capacity for such a FMR (Fig. 3). The reasons behind the high FMRs of small marsupials are conjectural, but the fact that most small marsupials are insectivores/carnivores could be an underlying feature. Note, that while *A. sylvaticus* has a high MMR, this omnivorous rodent has a FMR of only ~3.2 times BMR (Speakman, 1997). Nagy and co-authors highlight the plasticity of FMR in mammals and point to numerous causes (Nagy et al., 1999; Nagy, 2005).

The fact that FMR and MMR, with its clear connection to V(mt), may not be closely linked *via* BMR in mammals is highlighted by energetic profiles displayed among marsupials. As with placentals, these show marked impacts associated with M_b (Fig. 3, Table 5) and it is instructive to compare the overall data for *S. crassicaudata* with that for the large kangaroo, *M. rufus* (Fig. 3). While the BMR of *M. rufus* is ~75% of that of a placental, its FMR is low, 50% of that predicted for a placental (Munn et al., 2008). That *M. rufus*, with a fAS of 54, has one of the highest mammalian MMRs highlights the looseness in connections between the energy 'levels' of mammals. The patterns in the levels of energy use among mammals that we have clarified also robustly contest the proposal that design features of the O_2 transport system lock in an allometric exponent of 0.75 for the relationship between M_b and BMR (West et al., 1997; West et al., 1999) that extends mechanistically to MMR and FMR, as in the 'metabolic theory of ecology' (Brown et al., 2004).

LIST OF SYMBOLS AND ABBREVIATIONS

d	density of muscle
fAS	factorial aerobic scope
M_b	body mass
M_m	muscle mass
S(im,m)	total surface area of inner mitochondrial membranes
Sv(im,mt)	surface density of inner mitochondrial membranes per unit volume of mitochondria
V(mt,m)	mitochondrial volume of individual muscles (or muscle regions)
V(mt)	total mitochondrial volume of skeletal muscle
$\dot{V}_{O_2,max}$	maximal aerobic oxygen consumption
Vv(f,m)	volume fraction of muscle occupied by muscle fibres
Vv(mt,f)	volume fraction of mitochondria

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