The evolution of mechanisms involved in vertebrate endothermy

Lucas J. Legendre[1*] and Donald Davesne[2*]

[1]Jackson School of Geosciences, University of Texas at Austin, Austin, TX, USA
[2]Department of Earth Sciences, University of Oxford, Oxford, UK
[*]Corresponding author: lucasjlegendre@gmail.com; donald.davesne@earth.ox.ac.uk

Abstract

Endothermy, i.e. the endogenous production of metabolic heat, has evolved multiple times among vertebrates, and several strategies of heat production have been studied extensively by physiologists over the course of the 20th century. The independent acquisition of endothermy by mammals and birds has been the subject of many hypotheses regarding their origin and associated evolutionary constraints. Many groups of vertebrates, however, are thought to possess other mechanisms of heat production, and alternate ways to regulate thermogenesis that are not always considered in the palaeontological literature.

Here we perform a review of the mechanisms involved in heat production, with a focus on cellular and molecular mechanisms, in a phylogenetic context encompassing the entire vertebrate diversity. We show that endothermy in mammals and birds is not as well defined as commonly assumed by evolutionary biologists, and consists of a vast array of physiological strategies, many of which currently unknown. We also describe strategies found in other vertebrates, which may not always be considered endothermy, but nonetheless correspond to a process of active thermogenesis. We conclude that endothermy is a highly plastic character in vertebrates, and provide a guideline on terminology and occurrences of the different types of heat production in vertebrate evolution.

Keywords: vertebrates, physiology, endothermy, evolution, metabolic rate, palaeobiology

1. Introduction

Endothermy, i.e. the ability for an organism to generate body heat through a specifically dedicated metabolic activity, is considered to have appeared at least twice in the evolution of vertebrates, in the mammalian and avian lineages [1,2]. Other vertebrate lineages are generally labelled as ectotherms (i.e. organisms relying on their external environment as a main source of heat; see a review with detailed terminology for thermal physiology in [2]). Mammalian and avian types of endothermy have both been characterized in many studies and reviews [3–6], and physiological processes behind metabolic heat production have been extensively studied at the molecular level [7–11]. These processes are energetically very costly, and mammalian and avian basal metabolic rates (BMR, i.e. an estimation of metabolism corresponding to the minimal consumption of oxygen per unit of body mass over time, measured in controlled conditions, i.e. in an adult, post-absorptive, resting, non-reproductive animal [2,12–14]; a closely related estimator, resting metabolic rate (RMR), which is BMR without control for post-absorptive state, is also commonly used, especially for ectotherms [14,15]) are usually higher than that of ectotherms of the same size by at least an order of magnitude [2,16]. However, many particular cases of endothermy diverging from this ‘general model’ have been described among birds and mammals. Other types of thermogenic abilities have also been identified in many other vertebrate groups, some of which being also described as ‘endothermy’ in the literature (see below). Furthermore, the evolutionary origins of such processes are not currently well understood, and many hypotheses have been proposed over the last decades to describe how and why endothermy might have first appeared, with a particular focus on the stem groups of birds (e.g. non-avian dinosaurs [17,18]) and mammals (i.e. non-mammalian synapsids [12,19]). Indeed, since heat is a by-product of all metabolic processes [2], many studies have described members of these lineages as having been subject to various evolutionary constraints that could have favored the selection of endothermy (see below). Such studies have also tried to estimate BMR for fossil species in those groups, using various predictive modeling techniques [20–23]. Most of these studies have focused on non-avian dinosaurs, with numerous and sometimes contradictory results being proposed over the last decade regarding their potential endothermy [24,25].

While dinosaurian endothermy is still a highly controversial subject in the palaeontological community, little attention has been given to the exact physiological strategy that might have been used by non-avian dinosaurs to generate body heat. Many studies have discussed the physiological abilities of several dinosaur clades by comparing them to a few extant endothermic/homeothermic vertebrate species [18,21,22,26–28], suggesting potential analogies between them in terms of heat production strategy and constraints. Other fossil groups among archosauromorphs, as well as many extinct marine reptile clades, have also been studied in this context [22,29–33]. However, none of these studies has ever discussed extensively the physiology of these extant species in the larger context of endothermy in the whole vertebrate clade, and there is no exhaustive review of the different strategies used by endothermic vertebrates that would allow for a clear comparison between them in the context of palaeophysiological inference. Furthermore, it is often difficult to compare two different palaeontological studies describing fossil species as ‘endothermic’, due to the lack of a standard terminology to describe those different strategies for the whole of vertebrates, and most of these studies do not explicitly mention the kind of endothermy they are referring to.

In this paper, we describe as clearly and concisely as possible all major types of vertebrate endothermy identified so far, with a focus on physiological mechanisms at the cellular, tissue and/or organ level, and exceptions to the most common strategy in a given clade (whether ectothermic or endothermic) are also discussed. This description is made in a phylogenetic context (Fig. 1), with each type of endothermy being associated with the lineage(s) it has been described in, and independent acquisitions of a particular type through evolutionary convergence being mentioned explicitly. We also provide a synthetic comparative table (Table 1) for all different types of endothermy, which summarizes all adaptations linked with each of them using a homogeneous nomenclature, and can be used as a quick checklist to navigate through each of them in a straightforward way – which may sometimes prove difficult for non-physiologists. Such a review is thus not as exhaustive as previous works on the subject focusing more specifically on some vertebrate groups (e.g. [2,7,10,34–38]), but rather intended as a framework for palaeontologists and evolutionary biologists to discuss physiological hypotheses regarding the origin of endothermy in a given clade in a more precise way.

2. Mammalian endothermy as a physiological strategy is not as well known as commonly described

Heat production in mammals has been a major field of research in physiology since the early 20th century [39], and the differences in that regard between mammalian clades – monotremes and therians (i.e. marsupials and placentals) – have been extensively studied [9,36,40–42]. For this reason, the physiological strategy of eutherians to produce metabolic heat is probably the best-known type of vertebrate endothermy [43,44]. In placental mammals (i.e. eutherians), metabolic heat is produced in brown adipose tissue (BAT), a structure found only in that clade [11,43] (Table 1), through the activity of UCP1 (Uncoupling Protein 1; Supplementary Note 1). This process is usually labelled mammalian non-shivering thermogenesis [9] (NST, i.e. endogenous heat production outside the thermoneutral zone without contraction of skeletal muscles [43], as opposed to shivering thermogenesis (ST) – see section 5). NST allows most eutherians to maintain their body temperature at a constant level (i.e. homeothermy), usually within a range of 35–40°C [45], and is augmented in animals acclimated to the cold, a phenomenon described as adaptive thermogenesis [43]. This adaptive NST is usually described as the typical mammalian endothermy [9,11]; however, other strategies to produce metabolic heat do exist among mammals, both in eutherians and non-eutherians.

Monotremes (i.e. the platypus *Ornithorhynchus anatinus*, and the four species of echidnas), which do not possess BAT, display lower BMR and body temperatures (T_B) than other mammals [46]. The platypus has the highest metabolic rate of all monotremes, corresponding to about 70-80% of the corresponding eutherian values, whereas echidnas present values of 25-40% of corresponding eutherian BMR, but all monotremes present relatively similar T_B values – around 30–34°C depending on the ambient temperature (T_A) [34,47]. Due to their wide range of T_B depending on T_A, very low BMR values, and ability to enter daily torpor or hibernation (see below) in cold T_A with a drop in T_B of about 10°C compared to its normal range, echidnas have often been characterized as 'protoendotherms', i.e. reflecting a ancestral condition of thermoregulation that might provide insight on the evolution of endothermy in placental mammals [34,35,47]. These low values of BMR and T_B, however, are indeed influenced by their phylogenetic position as monotremes, but also by their burrowing lifestyle [48] and their diet, consisting almost exclusively of small arthropods [34]. Echidnas in particular are considered a very good example of the low metabolic performances found in all myrmecophagous mammals compared to their close relatives with a different diet [49]. Nevertheless, echidnas likely share some characteristics of early adaptations linked with endothermy that might have been present in non-mammalian synapsids, such as moderately high and highly labile BMR with facultative homeothermic endothermy (e.g. in reproductive periods [34,35]), or strong insulation to prevent heat loss [19,35]. The platypus, conversely, does not hibernate nor enter torpor [46], and is able to maintain its body temperature at a constant level even when air or water temperature decreases below 10°C [40]. This might be explained by the presence of local countercurrent exchange systems in its limbs, analogous to that of tunas, lamnids sharks, and leatherback turtles (see description in section 4), ensuring a high internal insulation to complement the integumental one provided by fur and subcutaneous fat at low T_A [34,46]. Therefore, while it is possible that all monotremes share a similar heat production mechanism, the platypus and echidnas have very different thermoregulation strategies, the evolution of which is not yet understood [40].

Marsupials, by contrast, also have metabolic rate values lower than those of placentals, but many of them are able to achieve a high level of thermoregulation despite their lack of BAT [41,50]. The ability to display adaptive NST has been identified in several marsupial species, but the mechanism involved is clearly different from that of placentals [41]. A high number of mitochondria and high mitochondrial membrane surface density have long been known to be important contributing factors to adaptive NST in eutherians [51]. It has been shown experimentally, however, that marsupials present a higher mitochondrial proton leak than eutherians of the same size, which is positively correlated with mass-specific BMR in both eutherians and marsupials [50]. Differences in thermogenic abilities between eutherians and marsupials are thus not linked with a difference in mitochondrial proton leak. The UCP1 gene is present in monotremes and marsupials, but has been shown to be more similar in sequence to its ectothermic (e.g. actinopterygians and lissamphibians) rather than eutherian orthologs, and UCP1, if expressed at all, is thus unlikely to present a thermogenic function in those groups [11,52]. Hence, while individual cases of NST in marsupials have been reported (Supplementary Note 2), the exact mechanism underlying the endothermy of non-eutherian mammals is currently unknown (Fig.1).

In fact, even among eutherians, the activation of UCP1 in BAT is not the only available strategy to achieve NST. The *ucp1* coding sequence can be found in many other vertebrate clades, including actinopterygians and amphibians, indicating that the gene likely appeared very early in the evolution of vertebrates – at least in the last common ancestor of osteichthyans [9,52]. The function of UCP1 orthologs in ectothermic vertebrates remains an enigma, and it is widely accepted that UCP1 only acquired thermogenic abilities in the last common ancestor of the eutherians, through its expression in BAT [41,52]. Several recent studies on the evolution of the UCP1 gene in eutherians, however, have shown that inactivating mutations have appeared in many large mammalian clades, which therefore lack the ability to express UCP1 in their mitochondria to achieve NST (Supplementary Note 3). Further research is thus currently needed to understand how alternative mechanisms to achieve NST have evolved independently in placental mammals.

Apart from the regular homeothermic abilities found in most extant mammals and birds, a large number of mammalian and avian species have developed abilities to punctually lower their metabolic rates in response to specific environmental conditions (i.e. heterothermy: being able to produce metabolic heat or not depending on the region of the body, or, like in this case, over a certain period of time [2]). When exposed to cold weather during the night, sometimes associated with food deprivation, several species in both clades will passively enter a state of daily torpor, i.e. a drop in T_B to retain energy and decrease loss of body heat, usually associated with lowering of metabolic rate [43] (Supplementary Note 4). Some species can also display hibernation, i.e. longer, actively controlled periods of torpor for days or weeks, followed by an arousal phase (Supplementary Note 4). Hence both passive and active control of features linked with heat metabolism are ancestral to the mammalian and avian clades, and have been proposed to have strongly influenced the rise of endothermy in non-mammalian synapsids and non-avian dinosaurs [16,19]. Indeed, such a high plasticity in thermogenic metabolism provides the ability to retain energy when needed by lowering BMR and T_B (e.g. during development, or when T_A is high enough to ensure that T_B can be maintained at a relatively stable level through basking behavior [16]), but also to achieve high metabolic performances to regulate incubation temperature (the ‘parental care hypothesis’ [53]), or to forage at night when T_A is low to avoid competition and/or predation from ectothermic vertebrates (the ‘nocturnal bottleneck hypothesis' [54,55]). Departures from homeothermic endothermy in mammals and birds are thus likely to be inherent to the acquisition of those features in the first place, and there is much more than one single strategy to consider in order to understand the whole diversity and evolution of the many physiological processes collectively referred to as mammalian endothermy.

3. Avian endothermy: the full extent of muscular thermogenesis

Birds as a whole have higher metabolic rate than any other vertebrate clade, with BMR values of a typical bird exceeding that of a typical ectotherm by a factor of about 15, compared to about 12 for a typical mammal [2]. For that reason, their ability to achieve NST has been extensively described in the literature [2,10,56]. The mechanism displayed by birds to produce metabolic heat, however, is physiologically distinct from that of mammals: since BAT is a synapomorphy of eutherians, it is absent in birds, and the gene coding for UCP1 was lost by the whole reptile clade, so that birds do not use orthologs of mammalian UCPs to ensure NST [57,58]. Another mitochondrial uncoupling protein, avian UCP (avUCP), has been described in birds, predominantly expressed in skeletal muscles [59]. avUCP has multiple roles in avian mitochondria, such as free-radical production or regulation of lipid use [59], and had originally been hypothesized to have a role in heat production in birds, analogous to that of UCP1 in mammals [60]. However, it has been experimentally shown not to have any role in mitochondrial uncoupling activity nor heat production role in the muscles and liver in birds [10]. Instead, the avian ortholog of the adenine nucleotide translocase (ANT), another protein responsible for the transport of adenine nucleotides (i.e. ADP/ATP) through the inner mitochondrial membrane, found in all eukaryotes [61], has been identified as the main protein responsible for mitochondrial uncoupling in avian skeletal muscles. It has been hypothesized to be directly involved in heat production in skeletal muscle thermogenesis [10,60] (Supplementary Note 5).

However, even if mitochondrial ANT might play an important role in avian NST, heat production in birds is likely to occur mostly through another specialized process, that originally evolved from the muscular thermogenesis already present in all vertebrates: the production of heat through uncoupling of calcium ions in the sarcoplasmic reticulum of muscle cells, activated by sarcolipin (see also section 4 and Supplementary Note 6). In birds, this process most likely became the main way to achieve NST through muscle hyperplasia, particularly that of pectoral muscles, linked to the development of a large keeled sternum allowing muscle-powered flight and a highly compact body plan [19,44,57]. In general, skeletal muscle mass is much more developed in birds than in amniotes of the same size, and avian skeletal muscles involved in flight have a high level of mitochondria and myoglobin, allowing a high aerobic respiration capacity [36]. Avian muscle hyperplasia, likely driven by the ancestral acquisition of bipedality in theropods and subsequent diversification of lifestyles in birds, is thus considered to be a main factor for the emergence of both flapping flight and avian endothermy [2,5,19,36], which could thus be characterized as evolutionary spandrels [62]. The evolution of brooding behaviour and parental care has also been described as a potential factor for muscle hyperplasia and the appearance of endothermy in several bird species [6,53]. Interestingly, the loss of flight in birds also appears to be correlated with low values of BMR and T_B (Supplementary Note 7). The lowest mass-independent BMR values known for birds can be found in the New Zealand kiwis [63], with other flightless palaeognaths (e.g. emus and ostriches) also showing low values compared to other birds [64], and the lowest avian T_B values have been reported in flightless palaeognaths and penguins [45]. High BMR and flight acquisition have also been suggested to be correlated with smaller genome size in birds and mammals, albeit with some controversy [65–68].

Because avian endothermy is considered an exaptation of muscular hyperplasia linked with the acquisition of bipedality in theropods, many evolutionary hypotheses have been proposed over the past five decades regarding the endothermy of non-avian dinosaurs (Fig. 1), especially since other characters previously associated with avian physiology have been identified as present in the whole clade Dinosauria [69–73]. Many studies originally considered dinosaurian metabolic rates to be low, assuming that their large size and inferred high insulation were enough to ensure stable TB without metabolic heat production [17,26,74]. More recently, high BMR values have been predicted by several studies for non-avian dinosaurs, particularly large theropods [18,20–22]. This, however, does not necessarily mean that non-avian dinosaurs achieved such high BMR by using the same physiological strategies as that of modern birds, since similar BMR values can be achieved through many different processes, as shown in this review. For this reason, the commonly inferred endothermy of non-avian dinosaurs cannot currently be linked with any particular physiological strategy. Future studies focusing on estimations of dinosaurian metabolic rates need to consider further palaeobiological features (e.g. linked with pulmonary airflow [75], heart structure [76], lung structure [28], or nasal conchae [77]; Table 1) in order to assess more precisely the actual physiological features that might have given rise to such high metabolic rates in dinosaurs, whether closely related to those of birds or not.

4. Endothermy appeared at least six times in elasmobranchs and teleosts

Mammals and birds are the classical model for endothermic vertebrates, and as such the occurrence of thermogenic strategies in other clades is often overlooked. Indeed, several lineages of elasmobranchs and teleosts independently developed specific forms of muscular endothermy allowing them to elevate their body temperature relative to the ambient water [38,78–81], with two main thermogenic strategies being found. In the first one, heat comes as a byproduct from the contractions of the slow-twitch aerobic red muscles (RM) during constant swimming, a configuration often called "red-muscle endothermy". Specific anatomical specialisations allow the region around RM to retain heat, including insulating them from the outside cold water, and developing networks of counter-current blood vessels – called *retia* – that keep blood warm by reducing convective heat transfer [38,82–85]. In the second one, called "cranial endothermy", extraocular muscles are transformed into a specialised heater organ, in which muscular fibres have lost their contractile activity and perform futile calcium cycling between the cytoplasm and the sarcoplasmic reticulum [7,86–88]. Thus, heater organs produce a form of non-shivering thermogenesis (NST).

In elasmobranchs, mackerel sharks (Lamnidae) and the common thresher shark *Alopias vulpinus* (Alopiidae) show elevated temperatures in their axial red muscles and in the brain region [89–91]. In lamnids, the axial RM (part of the muscle blocks that surround the vertebral column and supply most of the propulsive force for swimming) are located in a more medial position than in closely related taxa, allowing them to be better insulated, and are associated with *retia*. The brain region is heated by blood vessels carrying warm blood from the axial RM [81–83]. Cranial and pectoral-fin *retia* have also been detected in several species of devil and manta rays (Mobulidae), but their heat production strategy is so far unknown [92].

In teleosts, a very similar condition to that of lamnid sharks is found in tunas (Scombridae, Thunnini), whose axial RM are internalised, located anteriorly and associated with *retia*. Meanwhile, their ectothermic scombrid relatives such as mackerels (Scombrini) and bonitos (Sardini) have laterally-located axial RM that lack *retia* [38,83,84]. Billfishes, including marlins (Istiophoridae) and the swordfish (Xiphiidae), display brain heaters derived from the superior rectus muscle [86,87], but show no trace of RM endothermy. Their endothermy is therefore limited to the cranial region. The same configuration occurs in the butterfly mackerel *Gasterochisma melampus* (Scombridae), which lacks RM endothermy but has a brain heater derived from the lateral rectus muscle [7,38]. Tunas have elevated brain temperatures and display *retia* in the cranial region: they have both cranial and axial RM endothermy [38,93]. However, the heat-generating mechanism for the cranial region of tunas is unclear: it might pertain to the contraction of the red extraocular muscles [94], or to heat transfer from the axial RM, via special openings in the back of the skull [38]. Only one tuna species, *Allothunnus fallai*, has a true brain heater derived from all four rectus muscles [94]. Finally, the opahs *Lampris* sp. (Lampridiformes) have been recently described to have a unique thermogenic strategy involving both cranial endothermy [95] and a specific whole-body endothermy hypothesized to be NST [96,97] (Supplementary Note 8).

While tunas and billfishes have long been classified together as “scombroids” [98], molecular phylogenies support that they are not closely related within teleosts [99,100]. Within Scombridae, the endothermic “true” tunas form a clade, but *Gasterochisma* is not their immediate outgroup [101,102]. When adding opahs, this implies that endothermy appeared at least four times in teleosts, and two or three times more in elasmobranchs (Fig. 1 – although some analyses support that the common ancestor of lamnid and alopiid sharks was endothermic [103]). Heater organs performing NST appeared three times independently: in billfishes, *Gasterochisma*, and *Allothunnus*. Opahs likely developed another form of NST [97].

Endothermic elasmobranchs and teleosts share high levels of morphological and ecological convergence. They tend to be pelagic, open ocean predators, powerful and fast swimmers that travel over long distances and dive in colder water below the thermocline to pursue their prey [38,104]. Endothermy may also be associated with larger body size [103,105]. Ecological niche expansion, towards broader temperature and geographical ranges is often proposed as the main adaptive advantage of endothermy, and particularly RM endothermy, in these taxa [38,81]. It might be the main driving force behind the convergent appearance of endothermy in at least six different elasmobranch and teleost lineages, as well as in giant extinct marine reptiles such as ichthyosaurs, plesiosaurs, and mosasaurs [33].

5. Punctual thermogenesis in other vertebrates: heat production driven by lifestyle and reproductive behaviour

Shivering thermogenesis (ST) is a very common process in all mammals and birds, occurring during long periods of low T_A or during the arousal phase of a torpor cycle (Supplementary Note 4) to complement heat production achieved through NST [106]. It consists of heat production through asynchronous excitation of muscle fibres, so that often muscles that oppose one another are simultaneously active. This happens without external muscle work and allows for all the ATP accumulated by myofilaments for contraction to be released as heat – a process that also occurs for a fraction of ATP in normal contraction cycles (see a review of ST in [106]). ST has evolved independently in birds and mammals (Table 1) and is considered a mechanism of adjustment of T_B that was likely an evolutionary advantage in the acquisition of endothermy for both groups [106]. However, it is also found in other clades able to achieve homeothermy through specialized muscles. Among squamates, ST has been linked with parental care behaviour in two species of snakes, the Burmese python (*Python bivittatus*) and the diamond python (*Morelia spilota*; Supplementary Note 9). In both species, brooding females are able to display muscle twitching behavior while tightly coiling around their eggs, resulting in ST that can elevate T_B to a constant value of 30-32°C, i.e. warmer than T_A by about 7°C [107], and up to 18°C [108]. This remarkable ability to thermoregulate has led some authors to suggest that ST might not be the only mechanism involved in heat production in these two python species, and that NST may also be possible [109]. Other python species have so far been experimentally shown to be unable to achieve the ST homeothermy of Burmese and diamond pythons [110]. This suggests that this feature might be functionally linked with the acquisition of endothermy, since the use of ST during arousal in small torpid mammals is very similar to that of homeothermic brooding pythons [35].

The ability to temporarily maintain a higher T_B through NST has also been documented in other squamates, i.e. in rattlesnakes during digestion, with a small elevation of T_B (≈ 1°C for a large meal) directly correlated with meal size, that can last for up to 6 days [111], or in some varanids (≈ 0.5°C) [112]. In both examples, despite a strong increase in metabolic rate (3 to 4-fold in varanids [112], 20-fold in some snakes [113]), the increased oxygen intake is dedicated to physiological functions unrelated to heat production, such as elevating visceral blood supply. Thus, increases in T_B always remain very small, also likely because of increased thermal conductance [111], and cannot be used to monitor the rate of lowering T_B at the end of the digestive process [111,112]. More recently, however, seasonal endothermy associated with reproductive behaviour was reported in the black and white tegu (*Salvator merianae*), a large (≈ 2 kg) burrowing lizard endemic to South America [114], providing additional support for an ancestral rise of facultative endothermy linked with lifestyle constraints and parental care (Supplementary Note 10) [11]. The independent acquisition of endothermy by several vertebrate clades is thus likely to have been driven by a handful of key factors, including daily heterothermy and ST, which themselves were subject to very similar environmental and behavioural constraints [35,37].

6. The leatherback sea turtle: homeothermy through large body size

The leatherback sea turtle (*Dermochelys coriacea*), the largest extant turtle species (up to 700 kg [26]), has been described as homeothermic due to its ability to maintain its T_B at a relatively constant level (at least 18°C higher than the ambient temperature of cold water [115]). This thermoregulation is ensured by a very thick insulating layer of subepidermic vascularized adipose tissue, analogous to the blubber in cetaceans and pinnipeds [116]. A blubber layer is also present around the eyes near the retina, and inside the cranial cavity, protecting the neurocranium and salt glands and limiting external heat loss [116]. Additionally, counter-current heat exchangers, analogous to those of tunas and lamnid sharks, can be found in their anterior and posterior flippers [115], and have been shown to provide active thermoregulation through controlled heat dispersion, directly dependent on ambient water temperature (T_W) [117]. These heat exchangers also function as a local heat retainer, maintaining the heat generated through muscular thermogenesis inside the limbs and preventing hyperthermia in the rest of the body [118]. These two adaptations allow the leatherback turtle to maintain its T_B around 25°C, even during active swimming in cold water during deep dives, or in sub-polar waters ($T_W < 5$°C) [26,117].

The presence of endogenous heat production in the leatherback sea turtle is still debated, however, as its physiological correlates of heat metabolism do not differ from those of other reptiles (Supplementary Note 11). For this reason, large body size and high insulation have been proposed as the main drivers behind the homeothermy of leatherback turtles, which would allow heat conservation in the body without requiring a high metabolic rate [26]. This strategy, labelled 'gigantothermy', has subsequently been proposed as a thermoregulatory process in large non-avian dinosaurs [26,119–121]. Indeed, large body size has been described as a potential driver of dinosaurian homeothermy since the late 1970s [74], and the gigantothermy hypothesis has since been discussed in many palaeontological studies and reviews, with contradictory interpretations on the possible influence of phylogeny and ontogeny on this physiological process [15,112–114]. The highly specialized adaptations of the leatherback turtle, however, are heavily linked with its constant swimming associated with an oceanic, pelagic lifestyle. Other possible drivers for countercurrent exchange systems in the limbs of tetrapods might also include a generally aquatic lifestyle, as documented in the platypus [46], and extremely cold ambient temperatures, as observed in penguins [122] or in the Arctic fox

(*Vulpes lagopus*) [123]. The generalization of that strategy to other vertebrate clades on the sole basis of their large body size is thus misleading, especially considering the size difference between a leatherback turtle and large non-avian dinosaurs (e.g. sauropods).

7. Conclusion: palaeophysiological inferences on the evolution of vertebrate endothermy

As described above, vertebrate endothermy consists in multiple strategies that appeared independently through time (Fig. 1). Many of these have only been scarcely investigated, are not necessarily well-defined as endothermy in the literature, and are associated with a number of constraints (parental care, size reduction, nocturnal bottleneck, niche expansion to colder waters, etc.), currently still debated as potential drivers of endothermy among palaeontologists. The various proxys used for palaeophysiological inference (Table 1) in this context have provided many interesting results over the years, and in many cases several of these potential drivers are likely to have jointly constrained the selection for higher BMR in several taxa [19].

In recent years, palaeophysiological inference has included a growing number of phylogenetic comparative methods to include phylogenetic information in estimations and reconstructions of metabolic rate [20,22,31,124]. Indeed, multiples “endothermies” have evolved separately in the vertebrate lineage, each of them with its own adaptations to vastly different lifestyles and different pathways to regulate heat production. Discussing the evolution of endothermy in a large vertebrate clade (e.g. Archosauria, Synapsida) requires considering the physiological differences between extant members of the crown group of interest, before even trying to understand how this condition might have appeared in the last common ancestor of this clade. As such, BMR is just another proxy to infer heat metabolism; a high BMR estimate for a given fossil taxon does not provide *per se* evidence for endothermy in this taxon, nor a precise picture of the physiological strategy found in the larger clade to which it belongs. For this reason, we consider that inferences of endothermy in a specific fossil lineage should always include a mention to a specific inferred mechanism, rather than simply defining several taxa as endotherms or ectotherms based on similarity in BMR values, which is imprecise and might be meaningless in a phylogenetic context. Discussions of hypotheses regarding such strategies in such a context have allowed many recent physiological and palaeontological studies to unveil a new, clearer picture of the evolution of vertebrate endothermy, of which this review is only a short summary. Future methodological and conceptual developments will likely lead to important changes in the understanding of such processes in the next few years [125].

Acknowledgements

The authors want to thank Jorge Cubo and Adam Huttenlocker for organizing the Vertebrate Palaeophysiology symposium at the fifth International Palaeontological Conference in 2018, and for guest editing this associated theme issue. Three anonymous reviewers greatly improved the quality of the manuscript.

Funding

This work was supported by a grant to the University of Texas at Austin in support of JA Clarke from the Howard Hughes Medical Institute through the Science Education Program (to LJL), and by a grant of the Leverhulme Trust (RPG-2016-168) in support of RBJ Benson (to DD).

Authors’ Contributions

Both authors conceived and designed this study, prepared figures and tables, authored and reviewed drafts of the paper, and approved the final draft.

Competing Interests

We have no competing interests.

References

1. Hulbert AJ, Else PL. 2000 Mechanisms underlying the cost of living in animals. *Annu. Rev. Physiol.* **62**, 207–235. (doi:10.1146/annurev.physiol.62.1.207)
2. Clarke A, Pörtner H-O. 2010 Temperature, metabolic power and the evolution of endothermy. *Biol. Rev.* **85**, 703–727. (doi:10.1111/j.1469-185X.2010.00122.x)
3. Seebacher F, Schwartz TS, Thompson MB. 2006 Transition from ectothermy to endothermy: the development of metabolic capacity in a bird (*Gallus gallus*). *Proc. R. Soc. B Biol. Sci.* **273**, 565–570. (doi:10.1098/rspb.2005.3333)
4. Pörtner HO. 2004 Climate variability and the energetic pathways of evolution: the origin of endothermy in mammals and birds. *Physiol. Biochem. Zool.* **77**, 959–981. (doi:10.1086/423742)
5. Newman SA, Mezentseva NV, Badyaev AV. 2013 Gene loss, thermogenesis, and the origin of birds. *Ann. N. Y. Acad. Sci.* **1289**, 36–47. (doi:10.1111/nyas.12090)
6. Angilletta MJ, Cooper BS, Schuler MS, Boyles JG. 2010 The evolution of thermal physiology in endotherms. *Front. Biosci. Elite Ed.* **2**, 861–881.
7. Block BA. 1994 Thermogenesis in Muscle. *Annu. Rev. Physiol.* **56**, 535–577.
8. Else PL, Turner N, Hulbert AJ. 2004 The evolution of endothermy: role for membranes and molecular activity. *Physiol. Biochem. Zool.* **77**, 950–958. (doi:10.1086/422767)
9. Hughes DA, Jastroch M, Stoneking M, Klingenspor M. 2009 Molecular evolution of UCP1 and the evolutionary history of mammalian non-shivering thermogenesis. *BMC Evol. Biol.* **9**, 4. (doi:10.1186/1471-2148-9-4)
10. Walter I, Seebacher F. 2009 Endothermy in birds: underlying molecular mechanisms. *J. Exp. Biol.* **212**, 2328–2336. (doi:10.1242/jeb.029009)
11. Oelkrug R, Polymeropoulos ET, Jastroch M. 2015 Brown adipose tissue: physiological function and evolutionary significance. *J. Comp. Physiol. B* **185**, 587–606. (doi:10.1007/s00360-015-0907-7)
12. McNab BK. 1978 The evolution of endothermy in the phylogeny of mammals. *Am. Nat.* **112**, 1–21. (doi:10.1086/283249)
13. Bennett A, Ruben J. 1979 Endothermy and activity in vertebrates. *Science* **206**, 649–654. (doi:10.1126/science.493968)
14. IUPS Thermal Commission. 2001 Glossary of terms for thermal physiology. Third Edition. *Jpn. J. Physiol.* **51**, 245–280.
15. Burton T, Killen SS, Armstrong JD, Metcalfe NB. 2011 What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc. R. Soc. B Biol. Sci.* **278**, 3465–3473. (doi:10.1098/rspb.2011.1778)
16. Geiser F, Stawski C, Wacker CB, Nowack J. 2017 Phoenix from the ashes: fire, torpor, and the evolution of mammalian endothermy. *Front. Physiol.* **8**, 842. (doi:10.3389/fphys.2017.00842)
17. Seebacher F. 2003 Dinosaur body temperatures: the occurrence of endothermy and ectothermy. *Paleobiology* **29**, 105–122.
18. Pontzer H, Allen V, Hutchinson JR. 2009 Biomechanics of running indicates endothermy in bipedal dinosaurs. *PLOS ONE* **4**, e7783. (doi:10.1371/journal.pone.0007783)
19. Lovegrove BG. 2017 A phenology of the evolution of endothermy in birds and mammals. *Biol. Rev.* **92**, 1213–1240. (doi:10.1111/brv.12280)
20. Werner J, Griebeler EM. 2014 Allometries of maximum growth rate versus body mass at maximum growth indicate that non-avian dinosaurs had growth rates typical of fast

growing ectothermic sauropsids. *PLOS ONE* **9**, e88834. (doi:10.1371/journal.pone.0088834)

21. Grady JM, Enquist BJ, Dettweiler-Robinson E, Wright NA, Smith FA. 2014 Evidence for mesothermy in dinosaurs. *Science* **344**, 1268–1272. (doi:10.1126/science.1253143)

22. Legendre LJ, Guénard G, Botha-Brink J, Cubo J. 2016 Palaeohistological evidence for ancestral high metabolic rate in archosaurs. *Syst. Biol.* **65**, 989–996. (doi:10.1093/sysbio/syw033)

23. Rey K *et al.* 2017 Oxygen isotopes suggest elevated thermometabolism within multiple Permo-Triassic therapsid clades. *eLife* **6**, e28589. (doi:10.7554/eLife.28589)

24. Myhrvold NP. 2013 Revisiting the estimation of dinosaur growth rates. *PLoS ONE* **8**, e81917. (doi:10.1371/journal.pone.0081917)

25. Myhrvold NP. 2016 Dinosaur metabolism and the allometry of maximum growth rate. *PLOS ONE* **11**, e0163205. (doi:10.1371/journal.pone.0163205)

26. Paladino FV, O'Connor MP, Spotila JR. 1990 Metabolism of leatherback turtles, gigantothermy and thermoregulation of dinosaurs. *Nature* **344**, 858–860. (doi:10.1038/344858a0)

27. Köhler M, Marín-Moratalla N, Jordana X, Aanes R. 2012 Seasonal bone growth and physiology in endotherms shed light on dinosaur physiology. *Nature* **487**, 358–361. (doi:10.1038/nature11264)

28. Brocklehurst RJ, Schachner ER, Sellers WI. 2018 Vertebral morphometrics and lung structure in non-avian dinosaurs. *Open Sci.* **5**, 180983. (doi:10.1098/rsos.180983)

29. Seymour RS, Bennett-Stamper CL, Johnston SD, Carrier DR, Grigg GC. 2004 Evidence for endothermic ancestors of crocodiles at the stem of archosaur evolution. *Physiol. Biochem. Zool.* **77**, 1051–1067.

30. Wintrich T, Hayashi S, Houssaye A, Nakajima Y, Sander PM. 2017 A Triassic plesiosaurian skeleton and bone histology inform on evolution of a unique body plan. *Sci. Adv.* **3**, e1701144. (doi:10.1126/sciadv.1701144)

31. Fleischle CV, Wintrich T, Sander PM. 2018 Quantitative histological models suggest endothermy in plesiosaurs. *PeerJ* **6**, e4955. (doi:10.7717/peerj.4955)

32. Harrell TL, Pérez-Huerta A, Suarez CA. 2016 Endothermic mosasaurs? Possible thermoregulation of Late Cretaceous mosasaurs (Reptilia, Squamata) indicated by stable oxygen isotopes in fossil bioapatite in comparison with coeval marine fish and pelagic seabirds. *Palaeontology* **59**, 351–363. (doi:10.1111/pala.12240)

33. Bernard A *et al.* 2010 Regulation of body temperature by some mesozoic marine reptiles. *Science* **328**, 1379–1382. (doi:10.1126/science.1187443)

34. Nicol SC. 2017 Energy homeostasis in monotremes. *Front. Neurosci.* **11**. (doi:10.3389/fnins.2017.00195)

35. Grigg GC, Beard LA, Augee ML. 2004 The evolution of endothermy and its diversity in mammals and birds. *Physiol. Biochem. Zool.* **77**, 982–997. (doi:10.1086/425188)

36. Rowland LA, Bal NC, Periasamy M. 2015 The role of skeletal-muscle-based thermogenic mechanisms in vertebrate endothermy: Non-shivering thermogenic mechanisms in evolution. *Biol. Rev.* **90**, 1279–1297. (doi:10.1111/brv.12157)

37. Ruf T, Geiser F. 2015 Daily torpor and hibernation in birds and mammals. *Biol. Rev.* **90**, 891–926. (doi:10.1111/brv.12137)

38. Dickson KA, Graham JB. 2004 Evolution and consequences of endothermy in fishes. *Physiol. Biochem. Zool.* **77**, 998–1018.

39. Martin CJ. 1903 Thermal adjustment and respiratory exchange in monotremes and marsupials. A study in the development of homaeothermism. *Philos. Trans. R. Soc. Lond. Ser. B Contain. Pap. Biol. Character* **19**, 1–37.

40. Brice PH. 2009 Thermoregulation in monotremes: riddles in a mosaic. *Aust. J. Zool.* **57**, 255–263. (doi:10.1071/ZO09039)

41. Jastroch M *et al.* 2008 Marsupial uncoupling protein 1 sheds light on the evolution of mammalian nonshivering thermogenesis. *Physiol. Genomics* **32**, 161–169. (doi:10.1152/physiolgenomics.00183.2007)

42. Gaudry MJ *et al.* 2017 Inactivation of thermogenic UCP1 as a historical contingency in multiple placental mammal clades. *Sci. Adv.* **3**, e1602878.

43. Cannon B, Nedergaard J. 2004 Brown adipose tissue: function and physiological significance. *Physiol. Rev.* **84**, 277–359. (doi:10.1152/physrev.00015.2003)

44. Periasamy M, Herrera JL, Reis FCG. 2017 Skeletal muscle thermogenesis and its role in whole bbody energy metabolism. *Diabetes Metab. J.* **41**, 327–336. (doi:10.4093/dmj.2017.41.5.327)

45. Clarke A, Rothery P. 2008 Scaling of body temperature in mammals and birds. *Funct. Ecol.* **22**, 58–67. (doi:10.1111/j.1365-2435.2007.01341.x)

46. Grant TR, Dawson TJ. 1978 Temperature regulation in the platypus, *Ornithorhynchus anatinus*: production and loss of metabolic heat in air and water. *Physiol. Zool.* **51**, 315–332.

47. Schmidt-Nielsen K, Dawson TJ, Crawford EC. 1966 Temperature regulation in the echidna (*Tachyglossus aculeatus*). *J. Cell. Physiol.* **67**, 63–71. (doi:10.1002/jcp.1040670108)

48. McNab BK. 1979 The influence of body size on the energetics and distribution of fossorial and burrowing mammals. *Ecology* **60**, 1010–1021. (doi:10.2307/1936869)

49. McNab BK. 1984 Physiological convergence amongst ant-eating and termite-eating mammals. *J. Zool.* **203**, 485–510. (doi:10.1111/j.1469-7998.1984.tb02345.x)

50. Polymeropoulos ET, Heldmaier G, Frappell PB, McAllan BM, Withers KW, Klingenspor M, White CR, Jastroch M. 2012 Phylogenetic differences of mammalian basal metabolic rate are not explained by mitochondrial basal proton leak. *Proc R Soc B* **279**, 185–193.

51. Else PL, Hulbert AJ. 1985 An allometric comparison of the mitochondria of mammalian and reptilian tissues: The implications for the evolution of endothermy. *J. Comp. Physiol. B* **156**, 3–11. (doi:10.1007/BF00692920)

52. Klingenspor M, Fromme T, Hughes DA, Manzke L, Polymeropoulos E, Riemann T, Trzcionka M, Hirschberg V, Jastroch M. 2008 An ancient look at UCP1. *Biochim. Biophys. Acta BBA - Bioenerg.* **1777**, 637–641. (doi:10.1016/j.bbabio.2008.03.006)

53. Farmer CG. 2000 Parental care: The key to understanding endothermy and other convergent features in birds and mammals. *Am. Nat.* **155**, 326–334. (doi:10.1086/303323)

54. Crompton AW, Taylor CR, Jagger JA. 1978 Evolution of homeothermy in mammals. *Nature* **272**, 333–336. (doi:10.1038/272333a0)

55. Gerkema MP, Davies WIL, Foster RG, Menaker M, Hut RA. 2013 The nocturnal bottleneck and the evolution of activity patterns in mammals. *Proc. R. Soc. B Biol. Sci.* **280**, 20130508–20130508. (doi:10.1098/rspb.2013.0508)

56. Ruben J. 1995 The evolution of endothermy in mammals and birds: from physiology to fossils. *Annu. Rev. Physiol.* **57**, 69–95.

57. Newman SA. 2011 Thermogenesis, muscle hyperplasia, and the origin of birds. *BioEssays* **33**, 653–656. (doi:10.1002/bies.201100061)

58. Mezentseva NV, Kumaratilake JS, Newman SA. 2008 The brown adipocyte differentiation pathway in birds: An evolutionary road not taken. *BMC Biol.* **6**, 17. (doi:10.1186/1741-7007-6-17)

59. Emre Y, Hurtaud C, Ricquier D, Bouillaud F, Hughes J, Criscuolo F. 2007 Avian UCP: the killjoy in the evolution of the mitochondrial uncoupling proteins. *J. Mol. Evol.* **65**, 392–402. (doi:10.1007/s00239-007-9020-1)

60. Toyomizu M, Ueda M, Sato S, Seki Y, Sato K, Akiba Y. 2002 Cold-induced mitochondrial uncoupling and expression of chicken UCP and ANT mRNA in chicken skeletal muscle. *FEBS Lett.* **529**, 313–318. (doi:10.1016/S0014-5793(02)03395-1)

61. Santamaria M, Lanave C, Saccone C. 2004 The evolution of the adenine nucleotide translocase family. *Gene* **333**, 51–59. (doi:10.1016/j.gene.2004.02.013)

62. Gould SJ. 1997 The exaptive excellence of spandrels as a term and prototype. *Proc. Natl. Acad. Sci.* **94**, 10750–10755. (doi:10.1073/pnas.94.20.10750)

63. Calder WA, Dawson TJ. 1978 Resting metabolic rates of ratite birds: The kiwis and the emu. *Comp. Biochem. Physiol. A Physiol.* **60**, 479–481. (doi:10.1016/0300-9629(78)90020-8)

64. Maloney SK, Dawson TJ. 1993 Sexual dimorphism in basal metabolism and body temperature of a large bird, the emu. *The Condor* **95**, 1034–1037. (doi:10.2307/1369441)

65. Organ CL, Shedlock AM, Meade A, Pagel M, Edwards SV. 2007 Origin of avian genome size and structure in non-avian dinosaurs. *Nature* **446**, 180–184. (doi:10.1038/nature05621)

66. Waltari E, Edwards SV. 2002 Evolutionary dynamics of intron size, genome size, and physiological correlates in archosaurs. *Am. Nat.* **160**, 539–552. (doi:10.1086/342079)

67. D'Emic MD, Benson RBJ. 2013 Measurement, variation, and scaling of osteocyte lacunae: a case study in birds. *Bone* **57**, 300–310. (doi:10.1016/j.bone.2013.08.010)

68. Kapusta A, Suh A, Feschotte C. 2017 Dynamics of genome size evolution in birds and mammals. *Proc. Natl. Acad. Sci.* **114**, E1460–E1469. (doi:10.1073/pnas.1616702114)

69. Farmer CG. 2015 The evolution of unidirectional pulmonary airflow. *Physiology* **30**, 260–272. (doi:10.1152/physiol.00056.2014)

70. O'Connor PM, Claessens LPAM. 2005 Basic avian pulmonary design and flow-through ventilation in non-avian theropod dinosaurs. *Nature* **436**, 253–256. (doi:10.1038/nature03716)

71. Claessens LPAM, O'Connor PM, Unwin DM. 2009 Respiratory evolution facilitated the origin of pterosaur flight and aerial gigantism. *PLoS ONE* **4**, e4497. (doi:10.1371/journal.pone.0004497)

72. Norell MA, Xu X. 2005 Feathered dinosaurs. *Annu. Rev. Earth Planet. Sci.* **33**, 277–299. (doi:10.1146/annurev.earth.33.092203.122511)

73. Xu X, Zheng X, You H. 2010 Exceptional dinosaur fossils show ontogenetic development of early feathers. *Nature* **464**, 1338–1341. (doi:10.1038/nature08965)

74. Benton MJ. 1979 Ectothermy and the success of dinosaurs. *Evolution* **33**, 983–997. (doi:10.2307/2407661)

75. Cieri RL, Farmer CG. 2016 Unidirectional pulmonary airflow in vertebrates: a review of structure, function, and evolution. *J. Comp. Physiol. B* **186**, 541–552. (doi:10.1007/s00360-016-0983-3)

76. Seymour RS. 2016 Cardiovascular physiology of dinosaurs. *Physiology* **31**, 430–441. (doi:10.1152/physiol.00016.2016)

77. Bourke JM, Ruger Porter Wm, Ridgely RC, Lyson TR, Schachner ER, Bell PR, Witmer LM. 2014 Breathing life into dinosaurs: tackling challenges of soft-tissue restoration and nasal airflow in extinct species. *Anat. Rec.* **297**, 2148–2186. (doi:10.1002/ar.23046)

78. Carey FG, Teal JM, Kanwisher JW, Lawson KD, Beckett JS. 1971 Warm-bodied fish. *Integr. Comp. Biol.* **11**, 137–143. (doi:10.1093/icb/11.1.137)

79. Block BA. 1991 Endothermy in fish: thermogenesis, ecology and evolution. In *Biochemistry and Molecular Biology of Fishes*, pp. 269–311. Elsevier. (doi:10.1016/B978-0-444-89124-2.50015-4)

80. Block BA, Finnerty JR. 1994 Endothermy in fishes: a phylogenetic analysis of constraints, predispositions, and selection pressures. *Environ. Biol. Fishes* **40**, 283–302. (doi:10.1007/BF00002518)

81. Watanabe YY, Goldman KJ, Caselle JE, Chapman DD, Papastamatiou YP. 2015 Comparative analyses of animal-tracking data reveal ecological significance of endothermy in

fishes. *Proc. Natl. Acad. Sci.* **112**, 6104–6109. (doi:10.1073/pnas.1500316112)

82. Graham JB, Koehrn FJ, Dickson KA. 1983 Distribution and relative proportions of red muscle in scombrid fishes: consequences of body size and relationships to locomotion and endothermy. *Can. J. Zool.* **61**, 2087–2096. (doi:10.1139/z83-274)

83. Graham JB, Dickson KA. 2001 Anatomical and physiological specialization for endothermy. In *Fish Physiology. Volume 19: Tuna: Physiology, Ecology, and Evolution*, p. 45. San Diego: Academic Press.

84. Graham JB, Dickson KA. 2000 The evolution of thunniform locomotion and heat conservation in scombrid fishes: New insights based on the morphology of Allothunnus fallai. *Zool. J. Linn. Soc.* **129**, 419–466. (doi:10.1111/j.1096-3642.2000.tb00612.x)

85. Katz SL. 2002 Design of heterothermic muscle in fish. *J. Exp. Biol.* **205**, 2251–2266.

86. Carey FG. 1982 A brain heater in the swordfish. *Science* **216**, 1327–1329.

87. Block BA. 1986 Structure of the brain and eye heater tissue in marlins, sailfish, and spearfishes. *J. Morphol.* **190**, 169–189. (doi:10.1002/jmor.1051900203)

88. Morrissette JM, Franck JPG, Block BA. 2003 Characterization of ryanodine receptor and Ca2+-ATPase isoforms in the thermogenic heater organ of blue marlin (*Makaira nigricans*). *J. Exp. Biol.* **206**, 805–812. (doi:10.1242/jeb.00158)

89. Block BA, Carey FG. 1985 Warm brain and eye temperatures in sharks. *J. Comp. Physiol. B* **156**, 229–236. (doi:10.1007/BF00695777)

90. Wolf NG, Swift PR, Carey FG. 1988 Swimming muscle helps warm the brain of lamnid sharks. *J. Comp. Physiol. B* **157**, 709–715. (doi:10.1007/BF00691001)

91. Bernal D, Sepulveda CA. 2005 Evidence for temperature elevation in the aerobic swimming musculature of the common thresher shark, *Alopias vulpinu*s. *Copeia* **2005**, 146–151. (doi:10.1643/CP-04-180R1)

92. Alexander RL. 1996 Evidence of brain-warming in the mobulid rays, *Mobula tarapacana* and *Manta birostris* (Chondrichthyes: Elasmobranchii: Batoidea: Myliobatiformes). *Zool. J. Linn. Soc.* **118**, 151–164. (doi:10.1111/j.1096-3642.1996.tb00224.x)

93. Linthicum DS, Carey FG. 1972 Regulation of brain and eye temperatures by the bluefin tuna. *Comp. Biochem. Physiol. A Physiol.* **43**, 425–433. (doi:10.1016/0300-9629(72)90201-0)

94. Sepulveda CA, Dickson KA, Bernal D, Graham JB. 2008 Elevated red myotomal muscle temperatures in the most basal tuna species, *Allothunnus fallai*. *J. Fish Biol.* **73**, 241–249. (doi:10.1111/j.1095-8649.2008.01931.x)

95. Runcie RM, Dewar H, Hawn DR, Frank LR, Dickson KA. 2009 Evidence for cranial endothermy in the opah (*Lampris guttatus*). *J. Exp. Biol.* **212**, 461–470. (doi:10.1242/jeb.022814)

96. Wegner NC, Snodgrass OE, Dewar H, Hyde JR. 2015 Whole-body endothermy in a mesopelagic fish, the opah, *Lampris guttatus*. *Science* **348**, 786–789.

97. Franck JPC, Slight-Simcoe E, Wegner NC. 2019 Endothermy in the smalleye opah (*Lampris incognitus*): A potential role for the uncoupling protein sarcolipin. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **233**, 48–52. (doi:10.1016/j.cbpa.2019.03.024)

98. Carpenter KE, Collette BB, Russo JL. 1995 Unstable and stable classifications of scombroid fishes. *Bull. Mar. Sci.* **56**, 379–405.

99. Orrell TM, Collette BB, Johnson GD. 2006 Molecular data support separate scombroid and xiphioid clades. *Bull. Mar. Sci.* **79**, 16.

100. Alfaro ME, Faircloth BC, Harrington RC, Sorenson L, Friedman M, Thacker CE, Oliveros CH, Černý D, Near TJ. 2018 Explosive diversification of marine fishes at the Cretaceous–Palaeogene boundary. *Nat. Ecol. Evol.* **2**, 688. (doi:10.1038/s41559-018-0494-6)

101. Block BA, Finnerty JR, Stewart AFR, Kidd J. 1993 Evolution of Endothermy in Fish:

Mapping Physiological Traits on a Molecular Phylogeny. *Sci. New Ser.* **260**, 210–214.

102. Miya M *et al.* 2013 Evolutionary origin of the scombridae (tunas and mackerels): members of a Paleogene adaptive radiation with 14 other pelagic fish families. *PLOS ONE* **8**, e73535. (doi:10.1371/journal.pone.0073535)

103. Pimiento C, Cantalapiedra JL, Shimada K, Field DJ, Smaers JB. 2019 Evolutionary pathways toward gigantism in sharks and rays. *Evolution* **73**, 588–599. (doi:10.1111/evo.13680)

104. Bernal D, Dickson KA, Shadwick RE, Graham JB. 2001 Review: Analysis of the evolutionary convergence for high performance swimming in lamnid sharks and tunas. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **129**, 695–726. (doi:10.1016/S1095-6433(01)00333-6)

105. Ferrón HG. 2017 Regional endothermy as a trigger for gigantism in some extinct macropredatory sharks. *PLOS ONE* **12**, e0185185. (doi:10.1371/journal.pone.0185185)

106. Hohtola E. 2004 Shivering thermogenesis in birds and mammals. In *Life in the cold: evolution, mechanisms, adaptation, and application. 12th International Hibernation Symposium*, pp. 241–252. B. M. Barnes, H. V. Carey.

107. Snow RW, Wolf AJ, Greeves BW, Cherkiss MS, Hill R, Mazzotti FJ. 2010 Thermoregulation by a brooding burmese python (*Python molurus bivittatus*) in Florida. *Southeast. Nat.* **9**, 403–405. (doi:10.1656/058.009.0215)

108. Sup DJ, Shine R. 1988 Reptilian endothermy: a field study of thermoregulation by brooding diamond pythons. *J. Zool.* **216**, 367–378. (doi:10.1111/j.1469-7998.1988.tb02435.x)

109. Brashears JA, DeNardo DF. 2013 Revisiting Python Thermogenesis: Brooding Burmese Pythons (*Python bivittatus*) Cue on Body, not Clutch, Temperature. *J. Herpetol.* **47**, 440–444. (doi:10.1670/12-050)

110. Stahlschmidt ZR, DeNardo DF. 2009 Effect of nest temperature on egg-brooding dynamics in Children’s pythons. *Physiol. Behav.* **98**, 302–306. (doi:10.1016/j.physbeh.2009.06.004)

111. Tattersall GJ, Milsom WK, Abe AS, Brito SP, Andrade DV. 2004 The thermogenesis of digestion in rattlesnakes. *J. Exp. Biol.* **207**, 579–585. (doi:10.1242/jeb.00790)

112. Bennett AF, Hicks JW, Cullum AJ. 2000 An experimental test of the thermoregulatory hypothesis for the evolution of endothermy. *Evolution* **54**, 1768–1773. (doi:10.1111/j.0014-3820.2000.tb00720.x)

113. Secor SM, Diamond JM. 2000 Evolution of regulatory responses to feeding in snakes. *Physiol. Biochem. Zool.* **73**, 123–141. (doi:10.1086/316734)

114. Tattersall GJ, Leite CAC, Sanders CE, Cadena V, Andrade DV, Abe AS, Milsom WK. 2016 Seasonal reproductive endothermy in tegu lizards. *Sci. Adv.* **2**, e1500951–e1500951. (doi:10.1126/sciadv.1500951)

115. Greer AE, Lazell JD, Wright RM. 1973 Anatomical evidence for a counter-current heat exchanger in the leatherback turtle (*Dermochelys coriacea*). *Nature* **244**, 181. (doi:10.1038/244181a0)

116. Davenport J, Fraher J, Fitzgerald E, McLaughlin P, Doyle T, Harman L, Cuffe T. 2009 Fat head: an analysis of head and neck insulation in the leatherback turtle (*Dermochelys coriacea*). *J. Exp. Biol.* **212**, 2753–2759. (doi:10.1242/jeb.026500)

117. Bostrom BL, Jones TT, Hastings M, Jones DR. 2010 Behaviour and physiology: the thermal strategy of leatherback turtles. *PLOS ONE* **5**, e13925. (doi:10.1371/journal.pone.0013925)

118. Davenport J, Jones TT, Work TM, Balazs GH. 2015 Topsy-turvy: turning the counter-current heat exchange of leatherback turtles upside down. *Biol. Lett.* **11**. (doi:10.1098/rsbl.2015.0592)

119. Chinsamy-Turan A. 2005 *The Microstructure of Dinosaur Bone - Deciphering*

Biology with Fine-Scale Techniques. Baltimore: Johns Hopkins University Press.

120. Sander PM *et al.* 2011 Biology of the sauropod dinosaurs: the evolution of gigantism. *Biol. Rev.* **86**, 117–155. (doi:10.1111/j.1469-185X.2010.00137.x)

121. Seymour RS. 2013 Maximal aerobic and anaerobic power generation in large crocodiles versus mammals: implications for dinosaur gigantothermy. *PLoS ONE* **8**, e69361. (doi:10.1371/journal.pone.0069361)

122. McCafferty DJ, Gilbert C, Thierry A-M, Currie J, Le Maho Y, Ancel A. 2013 Emperor penguin body surfaces cool below air temperature. *Biol. Lett.* **9**, 20121192–20121192. (doi:10.1098/rsbl.2012.1192)

123. Prestrud P. 1991 Adaptation by the arctic fox (*Alopex lagopus*) to the polar winter. *ARCTIC* **44**, 132–138. (doi:10.14430/arctic1529)

124. Huttenlocker AK, Farmer CG. 2017 Bone microvasculature tracks red blood cell size diminution in Triassic mammal and dinosaur forerunners. *Curr. Biol.* **27**, 48–54. (doi:10.1016/j.cub.2016.10.012)

125. Polymeropoulos ET, Oelkrug R, Jastroch M. 2018 Editorial: the evolution of endothermy–from patterns to mechanisms. *Front. Physiol.* **9**. (doi:10.3389/fphys.2018.00891)

126. Naylor G, Caira J, Jensen K, Rosana K, Straube N, Lakner C. 2012 Elasmobranch phylogeny: a mitochondrial estimate based on 595 species. In *Biology of Sharks and Their Relatives, Second Edition* (eds J Carrier, J Musick, M Heithaus), pp. 31–56. CRC Press. (doi:10.1201/b11867-4)

127. Betancur-R R, Wiley EO, Arratia G, Acero A, Bailly N, Miya M, Lecointre G, Ortí G. 2017 Phylogenetic classification of bony fishes. *BMC Evol. Biol.* **17**, 162. (doi:10.1186/s12862-017-0958-3)

128. Harrington RC, Faircloth BC, Eytan RI, Smith WL, Near TJ, Alfaro ME, Friedman M. 2016 Phylogenomic analysis of carangimorph fishes reveals flatfish asymmetry arose in a blink of the evolutionary eye. *BMC Evol. Biol.* **16**, 224. (doi:10.1186/s12862-016-0786-x)

129. Simões TR, Caldwell MW, Tałanda M, Bernardi M, Palci A, Vernygora O, Bernardini F, Mancini L, Nydam RL. 2018 The origin of squamates revealed by a Middle Triassic lizard from the Italian Alps. *Nature* **557**, 706–709. (doi:10.1038/s41586-018-0093-3)

130. Tarver JE *et al.* 2016 The Interrelationships of placental mammals and the limits of phylogenetic inference. *Genome Biol. Evol.* **8**, 330–344. (doi:10.1093/gbe/evv261)

131. Prum RO, Berv JS, Dornburg A, Field DJ, Townsend JP, Lemmon EM, Lemmon AR. 2015 A comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing. *Nature* **526**, 529–573. (doi:10.1038/nature15697)

132. Davesne D, Meunier FJ, Friedman M, Benson RBJ, Otero O. 2018 Histology of the endothermic opah (*Lampris* sp.) suggests a new structure–function relationship in teleost fish bone. *Biol. Lett.* **14**, 20180270. (doi:10.1098/rsbl.2018.0270)

133. Davesne D, Meunier FJ, Schmitt AD, Friedman M, Otero O, Benson RBJ. 2019 The phylogenetic origin and evolution of acellular bone in teleost fishes: insights into osteocyte function in bone metabolism. *Biol. Rev.* **94**, 1338–1363. (doi:10.1111/brv.12505)

134. Atkins A *et al.* 2014 Remodeling in bone without osteocytes: Billfish challenge bone structure–function paradigms. *Proc. Natl. Acad. Sci.* **111**, 16047–16052. (doi:10.1073/pnas.1412372111)

135. Hillenius WJ. 1992 The evolution of nasal turbinates and mammalian endothermy. *Paleobiology* **18**, 17–29. (doi:10.1017/S0094837300012197)

136. Chinsamy A, Hurum JH. 2006 Bone microstructure and growth patterns of early mammals. *Acta Palaeontol. Pol.* **51**, 325–338.

137. Hayward JS, Lisson PA. 1992 Evolution of brown fat: its absence in marsupials and monotremes. *Can. J. Zool.* **70**, 171–179. (doi:10.1139/z92-025)

138. Kabat AP, Rose RW, Harris J, West AK. 2003 Molecular identification of uncoupling

proteins (UCP2 and UCP3) and absence of UCP1 in the marsupial Tasmanian bettong, *Bettongia gaimardi*. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **134**, 71–77. (doi:10.1016/S1096-4959(02)00189-6)

139. Kolb C, Scheyer TM, Veitschegger K, Forasiepi AM, Amson E, Van der Geer AAE, Van den Hoek Ostende LW, Hayashi S, Sánchez-Villagra MR. 2015 Mammalian bone palaeohistology: a survey and new data with emphasis on island forms. *PeerJ* **3**, e1358. (doi:10.7717/peerj.1358)

140. Bal NC *et al.* 2012 Sarcolipin is a newly identified regulator of muscle-based thermogenesis in mammals. *Nat. Med.* **18**, 1575–1579. (doi:10.1038/nm.2897)

141. Wallace BP, Jones TT. 2008 What makes marine turtles go: A review of metabolic rates and their consequences. *J. Exp. Mar. Biol. Ecol.* **356**, 8–24. (doi:10.1016/j.jembe.2007.12.023)

142. Lindgren J *et al.* 2018 Soft-tissue evidence for homeothermy and crypsis in a Jurassic ichthyosaur. *Nature* **564**, 359–365. (doi:10.1038/s41586-018-0775-x)

143. de Ricqlès A, Meunier FJ, Castanet J, Francillon-Vieillot, H. 1991 Comparative microstructure of bone. In *Bone, Volume 3: Bone matrix and bone specific products*, pp. 1–78. United States: Brian K. Hall.

Figure captions

FIGURE 1. Phylogeny of vertebrates showing independent appearances of heat production strategies described as endothermy in the literature, with taxa where endothermy has appeared at least once being labelled in bold. A distinction is made between non-shivering thermogenesis (NST), i.e. the strategy most commonly referred to as endothermy (see text), and other strategies; some taxa, such as mammals and birds, are able to achieve NST, but can also occasionally rely on other mechanisms to generate body heat (see text). Fossil taxa that have been inferred as potential endotherms are also labelled. The leatherback sea turtle is labelled with a question mark, since NST has not been convincingly demonstrated in that species. Reference phylogeny compiled from several sources [102,126–131]; silhouettes of terminal taxa obtained from PhyloPic (http://phylopic.org/).

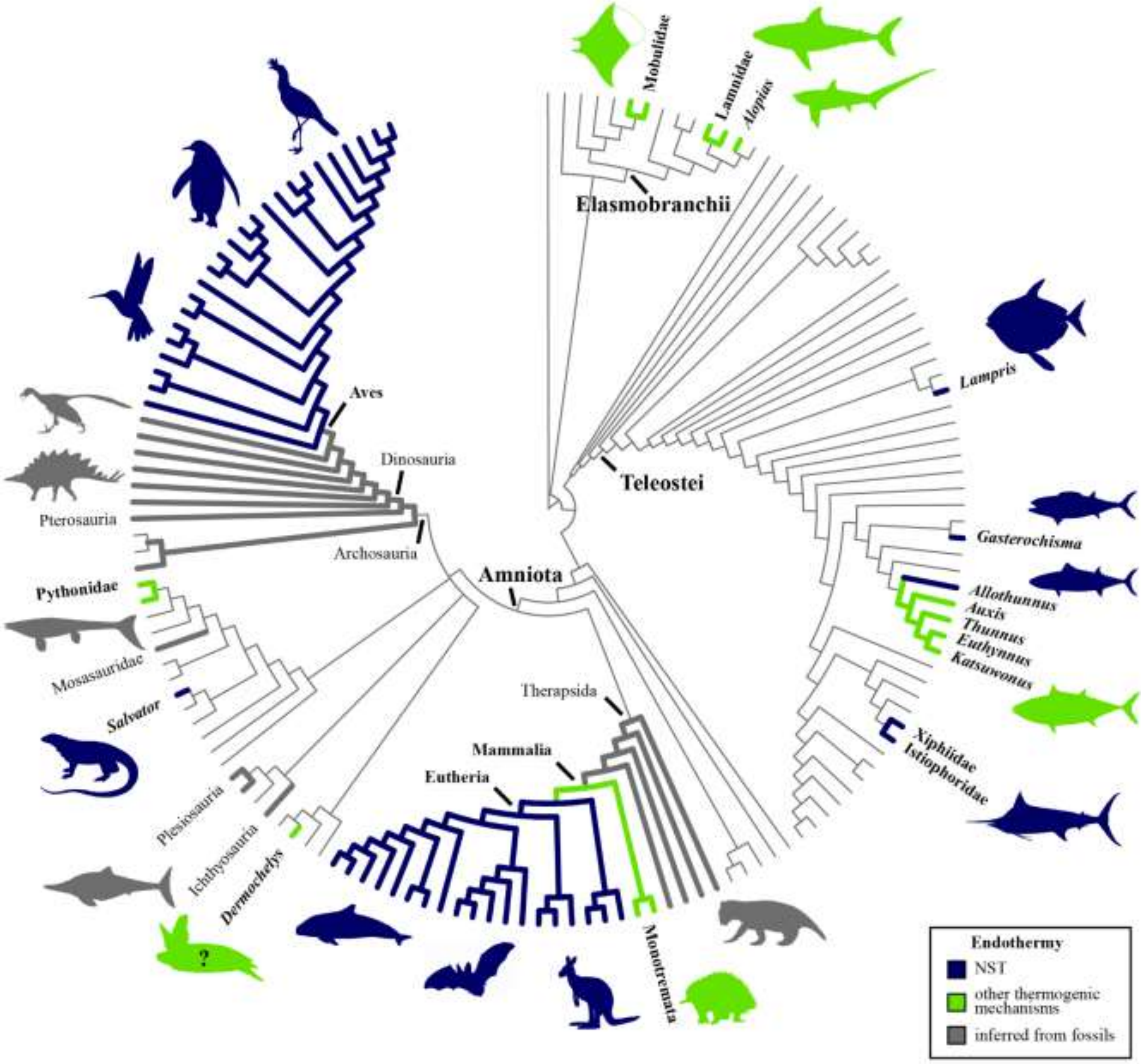
Mobulidae
Lamnidae
Alopias
Elasmobranchii
Lampris
Teleostei
Gasterochisma
Allothunnus
Auxis
Thunnus
Euthynnus
Katsuwonus
Xiphiidae
Istiophoridae
Aves
Dinosauria
Pterosauria
Archosauria
Amniota
Pythonidae
Mosasauridae
Salvator
Plesiosauria
Ichthyosauria
Dermochelys
?
Therapsida
Mammalia
Eutheria
Monotremata
Endothermy
NST
other thermogenic mechanisms
inferred from fossils

TABLE 1. Summary of physiological strategies described as endothermy in the literature for each vertebrate taxon discussed in the text, with emphasis on phylogeny, cellular/molecular mechanisms (if known), and palaeophysiological proxys for associated fossil stem-groups.

Taxonomy	**Endothermic lineage**	**Type of endothermy**	**Anatomical specialisation**	**Thermogenic mechanism**	**Possible palaeophysiological proxys**
Elasmobranchii, Lamniformes	Lamniidae (mackerel sharks) [89]	Red muscle (RM) endothermy, cranial endothermy [89]	Internalised axial RM, axial *retia*, vascular connection to braincase [90]	Byproduct of RM contraction [89,90]	None known, maybe body size and palaeobiogeography [105]
Elasmobranchii, Lamniformes	*Alopias vulpinus* (common thresher shark) [91]	RM endothermy [91]	Internalised axial RM, axial *retia* [91,104]	Byproduct of RM contraction [91,104]	None known
Elasmobranchii, Myliobatiformes	Mobulidae (manta rays) [92]	Cranial endothermy (suggested) [92]	Cranial *retia* [92]	Unknown	None known
Teleostei, Lampridiformes	*Lampris* sp. (opahs) [96]	Whole-body endothermy [96], cranial endothermy [95]	Specialised pectoral RM, insulating adipose tissue, gill arches *retia* [96]	Byproduct of RM contraction [96], proposed NST in the sarcoplasmic reticulum (sarcolipin) [97]	Osteocytic bone [132,133]
Teleostei, Istiophoriformes	Istiophoridae, Xiphiidae [86]	Cranial endothermy [86]	Heater organ (modified ocular muscles), cranial *retia* [86,87,101]	NST in the sarcoplasmic reticulum (ryanodine receptor) [88]	High remodelling in bone of the rostrum [134]
Teleostei, Scombriformes, Scombridae	*Gasterochisma melampus* (butterfly mackerel) [7]	Cranial endothermy [7]	Heater organ (modified ocular muscles), cranial *retia* [7]	NST in the sarcoplasmic reticulum [88]	None known
Teleostei, Scombriformes, Scombridae	Thunnini (“true” tunas) [84]	RM endothermy, cranial endothermy [83,84,93]	Internalised axial RM, axial and cranial *retia* [38,83,84], heater organ (only in *Allothunnus fallai* [94])	Byproduct of RM contraction [38], NST in the sarcoplasmic reticulum (*A. fallai* [94])	Osteocytic bone [133]
Amniota, Mammalia	Monotremata (monotremes) [40]	Facultative endothermy, shivering [35,40]	Insulating fur and fat, four-chambered heart, countercurrent vessels in limbs (platypus) [40,46]	Shivering [106]	Fur, respiratory turbinals [135], specific bone histological features [136]
Amniota, Mammalia	Metatheria (marsupials) [137]	Whole-body endothermy, shivering [41,137]	Insulating fur, respiratory turbinals, four-chambered heart [2,11]	Shivering, NST (mechanism unknown) [50,138]	Fur, respiratory turbinals [135], specific bone histological features [139]
Amniota, Mammalia	Eutheria (placental mammals) [2]	Whole-body endothermy, shivering [2]	BAT, insulating fur or blubber, respiratory turbinals, four-chambered heart [2,11]	Shivering, NST in BAT (UCP1)[43,106] – albeit with	Fur, respiratory turbinals [135], specific bone histological features [139]

				exceptions [42,52], NST in skeletal muscles (sarcolipin) [140]	
Amniota, Squamata, Pythonidae	*Python bivittatus* (Burmese python), *Morelia spilota* (diamond python) [109]	Muscle thermogenesis [109]	Specialised muscles [108]	Byproduct of muscle contraction, possible NST [109]	None known
Amniota, Squamata, Teiidae	*Salvator merianae* (black and white tegu) [114]	Seasonal reproductive endothermy [114]	Unknown	Unknown (proposed NST) [114]	None known
Amniota, Chelonia	*Dermochelys coriacea* (leatherback sea turtle) [26]	Gigantothermy [26]	Insulating adipose tissue, counter-current vessels in flippers [116,141]	Probably byproduct of muscle and metabolic activity [141]	None known (traces of blubber detected in extinct marine amniotes) [142]
Amniota, Archosauria	Aves (birds) [10]	Whole-body endothermy, shivering [4,10]	Insulating feathers, respiratory turbinals, unidirectional airflow in lungs, muscle hyperplasia, four-chambered heart, small genome size (debated) [2,57,58,68]	Shivering, NST in skeletal muscles (ANT, sarcolipin) [57,60,106]	Feathers, respiratory turbinates, specific bone histological features, bone pneumaticity (respiratory air sacs), [28,29,68,72,77,143]