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Gene loss, thermogenesis, and the origin of birds

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Compared to related taxa, birds have exceptionally enlarged and diversified skeletal muscles, features that are closely associated with skeletal diversification and are commonly explained by a diversity of avian ecological niches and locomotion types. The thermogenic muscle hypothesis (TMH) for the origin of birds proposes that such muscle hyperplasia and the associated skeletal innovations are instead the consequence of the avian clade originating from an ancestral population that underwent several successive episodes of loss of genes associated with thermogenesis, myogenesis, and skeletogenesis. Direct bird ancestors met this challenge with a combination of behavioral strategies (e.g., brooding of nestlings) and acquisition of a variety of adaptations for enhanced nonshivering thermogenesis in skeletal muscle. The latter include specific biochemical alterations promoting muscle heat generation and dramatic expansion of thigh and breast muscle mass. The TMH proposes that such muscle hyperplasia facilitated bipedality, freeing upper limbs for new functions (e.g., flight, swimming), and, by altering the mechanical environment of embryonic development, generated skeletal novelties, sometimes abruptly, that became distinctive features of the avian body plan.

Keywords: morphological novelty; skeletal muscle; *Glut4*; *UCP1*; myostatin

Introduction

No other vertebrate taxa rival birds in enormity and differentiation of skeletal musculature. The thermogenic muscle hypothesis (TMH) for the origin of birds proposes that such muscle hyperplasia and the associated skeletal innovations are consequences of the avian clade having originated from an ancestral population that underwent several successive episodes of loss of genes associated with thermogenesis, myogenesis, and skeletogenesis. Foremost among these was the loss of the gene specifying uncoupling protein 1 (UCP1).[1] This molecule, also called thermogenin, is a chromosomally encoded mitochondrial protein that is present in the genomes of all vertebrate groups other than reptiles.[2] UCP1 has the function, well described in mammals, of causing proton leakage across the mitochondrial inner membrane in cells of brown adipose tissue (BAT), uncoupling oxidative phosphorylation and thereby enabling the generation of heat within BAT.[3] The resulting nonshivering thermogenesis (NST), which is induced by cold via thyroid hormone and the sympathetic nervous system, is an essential mode of mammalian protection against hypothermia, especially in young and hibernating animals.[4] The bird progenitors (uniquely among the vertebrate classes) also lost the insulin responsive glucose transporter Glut4[5] and several members of the galectin class of multifunctional glycan-binding proteins,[6] with consequences for the development of the musculoskeletal system of their avian descendants.

The essence of the TMH is that the ancestral reptilian group that gave rise to birds remained endothermic, or became increasingly so, by selection for an enhanced capability of skeletal muscles to generate heat. This is proposed to have occurred both by biochemical adaptations of avian muscles and by dramatic increases in skeletal muscle mass.[a] Hyperplastic thigh muscles changed the anatomy

[a]Japanese quail, for example, can have skeletal muscle to total body weight percent ratios of more than 70%,[7] while human values rarely attain 40%.[8]

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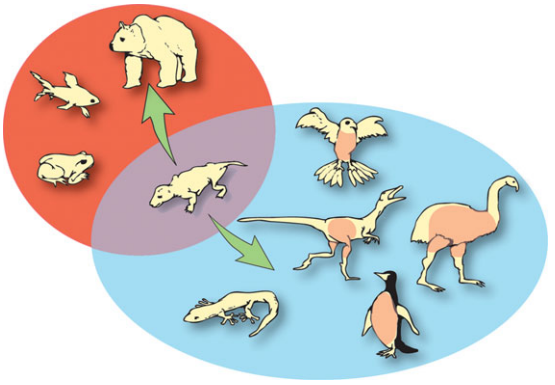

Figure 1. Schematic illustration of thermogenic muscle hypothesis (TMH) for the origin of birds. Animals represented in the red oval—bony fish, amphibians, and mammals—all have the *UCP1* gene, although only mammals have thermogenic brown adipose tissue (BAT). Animals represented in the blue oval—birds and lizards—lack *UCP1* and thus BAT. The common ancestor of lizards and birds, and their descendants, including dinosaurs, also lacked *UCP1*, which is presumed to have been lost in a common ancestor of mammals and saurians, represented by the animal in the purple sector. The TMH asserts that selective pressure for maintenance of elevated body temperature of adults and particularly hatchlings in endothermic egg-laying saurian ancestors of birds led to genetic variants in which nonshivering thermogenesis of skeletal muscle (pink shading) was enhanced and its mass increased. In consequence, descendants exhibited a bipedal stance and sustained tension- and motility-based modifications in their developing skeletons. This led to numerous bird-specific novelties of the legs and thorax, as well as the potential to construct and occupy new ecological niches. Redrawn from Ref. 1.

of the evolving proto-avians, inclining them toward bipedality. It also freed their upper limbs for adaptive selection for new functions (e.g., flight, swimming), an evolutionary trend that was facilitated by enlargements of pectoral and supracoracoid muscles (also driven by the requirement for heat generation) or vestigiality (Fig. 1).[1]

Experiments that recorded the effects on the chicken skeleton of paralyzing skeletal muscles during development[9–11] and direct examination of musculature compartmentalization effects on skeletogenesis[12] led to the speculation that many of the peculiarities of the bird skeleton—the wishbone or furcula, the fibular crest, the bony bridge between the tibia and fibula (*syndesmosis tibiofibularis*), as well their fusion patterns—were secondary consequences of the increased mechanical stress on the embryonic skeleton associated with the enlarged muscles.[1]

Apart from the novelty of its proposed mechanism for the origins of important aspects of bird anatomy, the TMH deemphasizes the role of flight in the evolution of birds, opening the possibility that swimming and flightless birds may not have passed, phylogenetically, through volant ancestral stages. Although direct evolution of flightless and swimming birds is not a necessary prediction of the hypothesis, it does not depend, as do fight-driven evolutionary scenarios, on the existence of intermediate forms that have not been identified in the fossil record.

Since the TMH was proposed, a number of studies have been published that bear on the plausibility of its assumptions and predictions. In this review, we describe this new work in the context of revisiting the research that motivated and provided the background for the hypothesis. Since much of this new work examines the contributions of apparently independent deletions of genes in several different families to evolutionary innovation, we will discuss the possible role of gene loss and developmental accommodation to such loss in the origin of morphological novelties. We will conclude with a discussion of how the TMH might be refined and tested in the future.

The *UCP1* and *UCP2* genes are deleted in bird genomes

The idea that the origin of birds was tied to the evolutionary loss of *UCP1* emerged from the finding that despite its absence in all sequenced avian genomes, chicken cells have an intact pathway for the expression of the gene.[2] Along with the fact that *UCP1* gene orthologs are present in fish[13] and frog[2] genomes, the strong implication was that the gene was lost in the avian lineage rather than acquired *de novo* in mammals (Fig. 2).[2] Moreover, since the genome of the anole lizard (a squamate) also lacks a *UCP1* gene,[2] the deletion must have occurred no later than the common ancestor of birds and lizards, barring a chromosomal instability that led to multiple independent losses.

Birds are distinguished from lizards by having additionally lost *UCP2* from the tandemly arranged pair *UCP2–UCP3* inherited from a common ancestor with fish (Fig. 3).[14] In contrast, this gene pair was carried through to mammals when they diverged from reptiles. Interestingly, the crocodile, like birds,

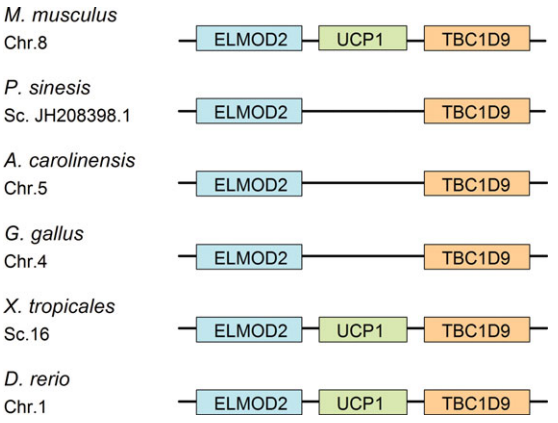

Figure 2. Comparison of vertebrate genomes reveals the absence of the *UCP1* gene in the saurians. Genomic sequences and contexts were analyzed using the Ensembl genome browser http://www.ensembl.org/index.html for all species except lizard and the University of California Santa Cruz genome browser http://genome.ucsc.edu/cgi-bin/hgGateway for the lizard. Lengths and distances between genes not to scale in this and the following figures.

a member of the archosaur clade, nonetheless retains *UCP2*.[15]

Recently, the genome sequence of the Chinese soft-shelled turtle (*Pelodiscus sinensis*) has become available. Turtles belong to the testudine clade, which evidence suggests is the monophyletic sister group of the archosaurs.[16] Like all other nonavian reptiles whose genomes have been sequenced, the turtle retains *UCP2–UCP3*, but like the lizard and birds it lacks *UCP1*. Thus, the evidence of sequenced genomes suggests that reptiles and birds are descendants of an ancestral population in which the *UCP1* gene was deleted, with this lineage plausibly stretching back to the separation of reptiles from mammals. Birds alone have sustained the further loss of *UCP2* (Fig. 3).

The TMH proposes that three distinct major lineages arose from the population of amniotes ancestral to the reptiles and mammals.[1] The first lineage, the endotherms that were the direct ancestors of the mammals, retained *UCP1* and thus a mechanism—thermogenesis by BAT—for protecting newborns from hypothermia. The second lineage, leading to the nonavian reptiles, was part of the subpopulation that lost *UCP1*, but since this group consisted of ectotherms, the deletion did not constitute a physiological deficit. The third lineage, the direct ancestors of the theropod dinosaurs and the birds, was also among the group that lost *UCP1*. These animals, insofar as they were endothermic or even heterothermic,[17] must have experienced the loss of the *UCP1* gene as an existential crisis. This lineage was only spared from extinction, according to the TMH hypothesis, by selection for biochemical, physiological, and developmental novelties that facilitated enhanced thermogenesis and expansion of skeletal muscles.

Biochemical and physiological adaptations for thermogenesis in avian skeletal musculature

Like its mammalian orthologs, avian UCP3 (also referred to as avUCP), is expressed mainly in skeletal muscle. Although the *UCP3* gene is not induced in muscle by cold-acclimatization in mammals[18–20] or even the crocodile,[16] it is in birds,[21,22] where it mediates NST.[21] The mechanism of UCP3-dependent NST in avian skeletal muscles is unclear; despite its designation as an uncoupling protein, UCP3 (unlike UCP1) does not exert its metabolic effects via mitochondrial proton leak.[22,23]

Teulier *et al.*[22] discuss two possible bases for skeletal muscle-mediated NST in birds. First, NST could be mediated by decreased efficiency of ATP production in mitochondria through the oxidation of $FADH_2$-linked substrates such as fatty acylcarnitine.[24] This would link avUCP expression to the mobilization of fat reserves and the increased potential of avian skeletal muscle to oxidize fatty acids at low temperatures.[25–27] Second, ATP breakdown could be enhanced through the futile cycling of calcium across the sarcoplasmic reticulum membrane,[28] a mechanism related to malignant hyperthermia.[29] The latter possibility is potentially supported by the recent finding that skeletal muscle NST in mammals (which is less efficient than that in birds[30]) depends on the protein sarcolipin, which promotes heat generation in muscle by uncoupling the sarco/endoplasmic reticulum Ca^{2+}-ATPase (Serca) pump.[31] Sarcolipin genes are present in all sequenced bird genomes (Fig. 4). Although the gene assumed a conserved chromosomal position with the establishment of the tetrapods, it is not present in the Chinese softshell turtle genome (Fig. 4).

A third possible mechanism for avian muscle NST is the induction by cold exposure of the mitochondrial ATP/ADP antiporter (ANT) in

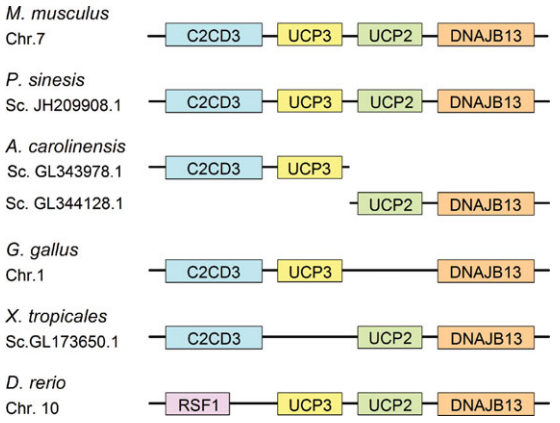

Figure 3. Comparison of vertebrate genomes reveals the absence of the *UCP2* gene in the chicken and the *UCP3* gene in the frog. The other sequenced bird genomes (the zebra finch *Taeniopygia guttata* and the turkey *Meleagris gallopavo*) also lack *UCP2*. Genomic sequences and contexts were analyzed using the Ensembl genome browser.

avian skeletal muscle, where it mediates fatty acid-induced uncoupling of mitochondrial oxidative phosphorylation.[32,33] It is notable, however, that neither ANT nor avUCP is induced by low temperature in neonatal chicks,[33] although response to cold exposure varies widely in relation to developmental stage[34,35] and can differ strongly between the muscles in relation to their functional use and maturation.[36–38]

While nestlings obtain some protection against hypothermia from brooding by fully endothermic adults, it is significant that they also exhibit metabolic adaptations of their own that do not depend on mature functions. In addition to accumulation of muscle mass, acquisition of homeothermy is associated with developmental changes in the proportion of the muscle fiber types from fast- to slow-twitch,[39] with a concomitant increase in the abundance of mitochondria[40] and rapid elevation of citrate synthase and 3-hydroxyacyl-CoA dehydrogenase, two enzymes involved in mitochondrial substrate oxidation, in their breast and leg muscles just before hatching.[36,41] The shift in oxidative capacity during the 24 h before hatching is common in precocial birds[36,42]—for which brooding by adults sharply decreases after incubation ends. In cold-tolerant precocial species, such as the common eider (*Somateria mollissima*), the activity of cytochrome c oxidase in leg muscle increases more than three- to fourfold during the 24 h before hatching.[42–44]

Whereas a substantial proportion of thermogenic needs in adult birds can be met with shivering metabolism that requires muscle contractions, the acquisition of thermogenesis in nestlings, especially in altricial species (i.e., having prolonged dependence on adult care), commonly precedes onset of muscle contractive abilities and functional differentiation. It is clear then that the skeletal muscles of adult and even newly hatched birds have acquired adaptations over the course of evolution that enable efficient NST in response to cold. In combination with shivering thermogenesis, such changes enable survival of a warm-blooded clade emerging from the population of amniote ancestors that lost *UCP1*.

Developmental and maturational adaptations in birds promote hyperplasia of skeletal muscle

In addition to features that enable NST in skeletal muscle of birds, an increase in the mass of this tissue is another important determinant of its capacity to promote homeothermy. Indeed, the enormity, relative to body size, of avian upper leg or breast muscles, compared to all other vertebrate classes, is the hallmark of the distinctive anatomy of birds that the TMH was devised to explain.[1] In theory, muscle biochemical adaptations alone might have been sufficient to carry forward an endothermic lineage of vertebrates lacking BAT, but robust survival plausibly required the synergistic effects in skeletal muscle of thermogeny and increased mass. According to the TMH, it was the latter that produced the unique anatomy of birds. By virtue of the ecospace opened up as a consequence of bipedality, a powerful upper torso and skeletal alterations developmentally secondary to the morphogenesis of a massive musculature, the new clade came to inhabit, with remarkable success, novel niches on the ground, water, and air. Secondarily, a significant accumulation of muscle mass, and its thermogenic ability, might have enabled diversification of other aspects of avian life history, from prolonged incubation without feeding under subzero ambient temperatures to extraordinarily long nonstop transoceanic migrations. Several alternative choices to the ones taken in the evolution of mammalian development underlie the striking hyperplastic expansion of avian skeletal muscles during development, the maturation of juveniles, and the plasticity in response to incidental and seasonal changes in

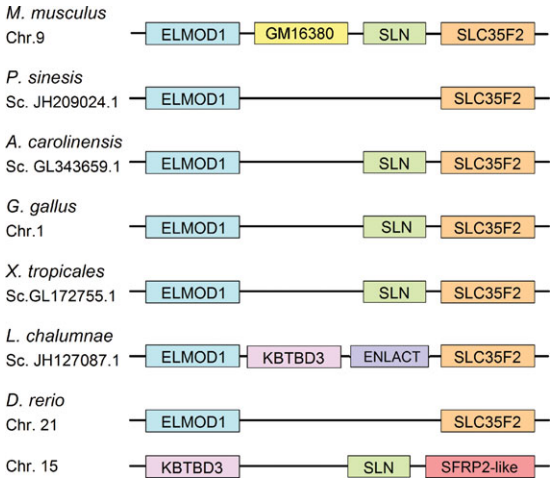

Figure 4. Comparison of vertebrate genomes reveals the absence of the sarcolipin (*SLN*) gene in the turtle and coelacanth (*L. chalumnae*). The National Center for Biotechnology Information protein databases were searched using the lizard sarcolipin protein sequence (Accession XP_003228550.1) for homologs in the target species. In the case of chicken, the mRNA sequence in the NCBI database was conceptually translated to obtain the protein sequence. The species-specific sarcolipin sequences were then used to locate the corresponding genes using the Ensembl genome browser. The chromosomal position of the sarcolipin gene in the zebrafish, a ray-finned fish, is different from that in the tetrapods. The tetrapod arrangement was apparently brought about by a chromosomal rearrangement that also occurred in the coelacanth, a lobe-finned fish, but with the deletion of *SLN* in the latter case.

temperature. As with the precipitating deletion of *UCP1*, some of these choices appear to have involved the jettisoning of genes.

Repurposing of insulin as a skeletal muscle growth factor

In the mouse, insulin acts on skeletal muscle as a metabolic regulator and as an inducer of myogenic differentiation.[45] In the latter developmental function, the insulin signaling pathway relieves the inhibitory effect of p38–MAPK on the myoblast-specific master transcription factor MyoD.[46] In developing skeletal muscle, MyoD induces not only the suite of contractile proteins necessary for the tissue's biomechanical function, but also the insulin-responsive glucose transporter Glut4.[47] Glut4, in turn, mediates the myogenesis-promoting effect of insulin.[48] Birds, however, lack Glut4,[5] a loss that abrogated the insulin-dependent myogenic circuit and enabled the repurposing of the insulin pathway in the avian clade. Indeed, insulin is an inhibitor of MyoD in newly hatched chicks, but insulin administration at these early stages increased the mass of the differentiation-suppressed skeletal muscles.[49] The proliferation-promoting effect was associated with an increase in the levels of Pax7, a transcription factor that promotes the survival and proliferation of muscle stem cells.[50,51] These effects of insulin on muscle stem cells have not been described in mammals.

The absence of Glut4 in birds (resulting from a clade-specific gene deletion in the avian lineage; Fig. 5) limits the uptake of glucose by skeletal muscle. Birds are thus considered insulin-resistant, and chickens, for example, have blood glucose levels twice as high as most mammals. In mammals, high glucose levels lead to high plasma insulin, but this is not the case in birds, where circulating insulin levels are comparable to those in mammals.[52] Birds appear to have novel mechanisms for keeping insulin secretion in check. In mammals, UCP2 acts in pancreatic islet cells to negatively regulate insulin secretion in response to glucose,[53] but as noted above, birds, among all vertebrate classes, are unique in having lost the *UCP2* gene.

Environmental plasticity of myogenin expression

The TGF-β family member myostatin is an autocrine/paracrine inhibitor of skeletal muscle growth during embryogenesis across all the vertebrate clades. Plasticity in the expression of myostatin is seen in postnatal chickens, with the protein declining in abundance with concomitant increase of skeletal muscle mass in response to cold exposure for the first week posthatching, but not beyond.[54]

In other bird species, myostatin expression remains plastic in adults, closely associated with seasonal muscle mass increase to enable greater thermogenesis in overwintering birds[55,56] and otherwise mediate seasonal phenotypic flexibility in cold tolerance. In house sparrows (*Passer domesticus*), gene expression of myostatin decreased significantly in winter, with summer values exceeding winter values by half.[57] Although myostatin expression is seasonally downregulated in a hibernating mammal, the ground squirrel (*Spermophilus lateralis*), this is not in response to cold exposure, nor is it mass increasing, but rather atrophy sparing.[58] The adaptive regulation of myostatin to enhance

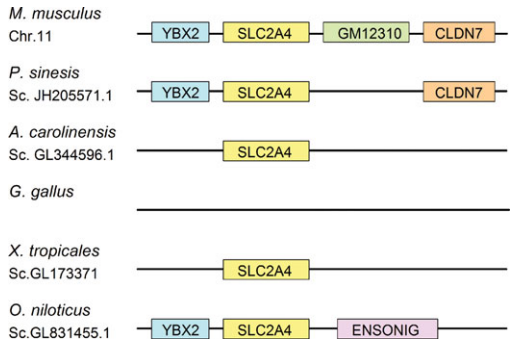

Figure 5. Comparison of vertebrate genomes reveals the absence of the glucose transporter 4 gene (*SLC2A4*) in birds. The chicken result is shown, but the other sequenced bird genomes also lack *SLC2A4*. Genomic sequences and contexts were analyzed using the Ensembl genome browser.

the mass of avian skeletal muscles requires further study.

Thus, as predicted by the TMH, birds have adapted to being warm-blooded species without BAT by increasing thermogenic capacity and mass of their skeletal musculature. Interestingly, the muscle modifications that arose in the avian lineage in association with the loss of the *UCP1* gene (it is unclear in what temporal relation) involved the loss of additional genes. The most important of these was *Glut4*, since it enabled the repurposing of insulin as a skeletal growth factor, but the additional absence in birds of the *UCP2* gene points to avian-specific modes of glucose homeostasis.

Skeletal muscle hyperplasia and developmental mode

Birds have exceptional diversification in developmental modes and juvenile traits with even closely related species often differing along the altricial–precocial spectrum.[59,60] Remarkably, the entire altricial–precocial spectrum of developmental events varies along a single defining dimension: accumulation and maturation of muscle mass,[59,61] contributed mostly by pectoral and leg muscles.[60] Accumulation of muscle mass is strongly proportional to the onset of homeothermy and independent of developmental modes or the onset of functional use; across all examined species there is a remarkably constant relationship between the timing of the first endothermic response to cold stress and the accumulation of muscle mass.[34,62–64]

Importantly, whereas shivering thermogenesis typically requires acquisition of normal contractive abilities of muscle[65] (experimental limitation of contraction and function, for example, wing flapping, precludes shivering thermogenesis[66]), NST does not, and thus can precede the functional maturation of muscles.[10] Further, by uncoupling mitochondria, NST can impair the energetic efficiency of the contractive processes,[10] such that functional maturation of muscles (for locomotion and tension generation) might be sacrificed for this type of thermogenesis in nestlings. Indeed, in a number of species, muscle use for thermogenesis precedes muscle functional maturation, differentiation, and use.[10,34] For example, in procellariiformes (e.g., albatrosses and petrels), large pectoral muscles of neonates are a major source of thermogenesis months before they begin to differentiate and mature for actual flight.[67] In other species, there is an ontogenetic switch between thermogenic muscles. Precocial galliformes, which start to fly at an early age, rely on leg muscles for early thermogenesis with pectoral muscle thermogenesis taking over by late ontogeny.[36,68] In some altricial species, the direction is reversed with smaller (and not yet functional) pectoral muscles contributing more to thermogenesis than much larger leg muscles.[69,70] The difference can be due to active use of leg muscles for standing in the nest in these nestlings and with pectoral muscles being closer to the core of the body and thus better able to regulate core body temperature. Overall, across species, pectoral and leg muscles together account for 73% of variation in nestling thermogenesis.[34,38,64,71]

Developmental accumulation of muscle mass is also closely associated with nestling cooling time. Although hatchlings of all species depend on brooding to some degree, time spent brooding is lost for feeding, either during off-nest bouts by parents in altricial species or during independent feeding forays in precocial hatchlings. Thus, in addition to selection for early acquisition of skeletal muscle thermogenesis, there is a strong selection for tolerance of low body temperature in nestlings. Both acquisition of homeothermy and cooling time are most strongly linked to accumulation of muscle mass,[10,34,37,64,72] regardless of the onset of muscle functionality or developmental stage. Before acquisition of full capacity for thermogenesis, nestlings of all species are remarkably tolerant of low body temperatures. In most species, nestlings routinely tolerate body temperatures of 30 °C and lower,[35,72,73] but many

avian neonates routinely remain active and tolerate body temperatures below 10 °C for hours without any detrimental effects.[44,74] In some species, nestlings can become hypothermic for several days during inclement weather, when adults are forced to temporarily abandon nests.[75,76]

The validity of the TMH, however, hinges on evidence that the accumulated evolutionary effects of extraordinarily hyperplastic muscles on the development of the musculoskeletal system led to the generation of the avian body plan.

Developmental plasticity and morphological novelties in avian skeletal development

Muscle activity is required for normal skeletogenesis in birds,[9,11,77,78] with the elements of the skeleton that most distinguish birds from other tetrapods being most dependent on muscular activity.[78,79] The molecular–developmental bases of avian skeletal plasticity are unknown. It is notable, however, that the spatiotemporal arrangement of the primary cartilages that form the templates of the limb skeletal elements in the chicken embryo is mediated by a regulatory network consisting of two members (CG-1A and CG-8) of the galectin family of multifunctional carbohydrate-binding proteins.[80] The galectins comprise several structural subcategories whose members have partly overlapping functions. In addition to their role in limb skeletogenesis, one or more of them has highly specific patterns of expression during early avian embryogenesis, including in developing skeletal muscle.[81] The avian clade, with only five galectin family members, sustained a substantial loss of galectin genes during phylogeny, the zebrafish having 8 such genes, the frog 12, and the lizard 7. In contrast, mammals underwent an expansion of the family relative to their presumed common ancestors with the birds, the mouse having 8 or 9 galectin genes, depending on strain, the rat 9, the chimpanzee 10, and the human 13.[6] Under the assumption that these proteins participate in skeletal development in all vertebrate classes, it is possible that the relative plasticity of skeletogenesis in birds in response to external stress is tied to the lack of functional redundancy in the pared-down galectin family of this clade.

While most avian developmental sequences are remarkably constant,[82] one aspect that shows great variability among even closely related species is the extent of ossification by the time of hatching, a variability that is unrelated to either developmental mode or incubation duration.[83,84] Instead, the rate of ossification is closely linked to the onset of muscle compartmentalization and functionality.[12] The strong effect of muscle placement and activity on skeletogenesis is mediated by the secondary cartilage,[85] an avascular tissue whose development closely depends on muscle activity[86] and whose abundance is closely associated with bone growth rate across bird species.[84] Secondary cartilages, which appear at the margins of developing membrane bones, are responsive to mechanical stresses exerted by muscles and determine the shape and growth of their associated bones;[87] experimental removal of musculature leads to significant cartilage expansion.[86,87] Bird neonates have large cartilage volumes at hatching—50% in precocial species and up to 90% in altricial species[84]—and both developmental modes undergo substantial postnatal growth and diversification of their bony skeletons guided by musculature attachment and strength.

The importance of muscle activity on ossification is demonstrated by experimental paralysis of bird embryos that induce severe changes across the entire developing skeleton.[77,88] These effects are particularly strong in bones with abundant secondary cartilage, such as the sternal plates and the clavicles, which require movements of the attached pectoral muscles for fusion in the midline (and to form the furcula). Importantly, the fusion of the sternum seems proportional to the extent of muscle paralysis: complete paralysis arrests sternum fusion whereas intermediate muscle forces induce only partial fusion.[85,89] It is probable that the typical nonavian tetrapod clavicle would form in birds in which the developing primordia were subject to a balance of forces intermediate between those exerted by the bird musculature and the paralyzed state, (i.e., the typical tetrapod configuration). In addition, paralyzed chick embryos fail to develop the fibular crest,[78,88,90] which is a morphological novelty in theropod dinosaurs and a necessary component in the development of the avian-specific syndesmosis tibiofibularis.[88,91]

In birds, which are oviparous, embryos are isolated from maternal movements during development (unlike in mammals), and thus the embryo's own muscle activity must play a greater role in skeletogenesis. Spontaneous muscle activity

in avian embryos occurs as early as day three of incubation[92] and even young embryos are known to respond to egg cooling by increasing muscle electric or mechanical activity.[72] In chickens, developing leg muscles undergo periodic bouts of 20–30 s every few hours throughout development, although coordinated movements of limbs do not emerge until shortly before hatching.[9,92] Along with positional self-adjustment of embryos in response to periodic egg turning by incubating females, such bouts of undirected activity are thought to provide important positional feedback for skeletal development[86] and in particular to enable formation of joints.[11,89] Once the movements and muscle contractions become more coordinated and associated with greater load (e.g., after hatching in precocial species), their growth-inducing and diversification effects on ossification are gradually replaced with greater effects on osteogenesis that influence bone density and diameter.[10] In altricial hatchlings, which accumulate a significant amount of musculature that does not play a strong supportive role until later in development, bones continue to undergo shaping and extensive growth at the expense of bone strength.[60,84] In precocial hatchlings and at later developmental states in altricial species, onset of locomotion and associated load closes cartilaginous gaps in bones, with bones becoming histologically reorganized and gaining greater diameter and mechanical strength.[10] Across species, increasing locomotive requirements are associated with slower bone growth and greater strength.[10]

The bipedal condition, a side effect of expanded thigh and breast musculature, has developmental consequences of its own during the postnatal period. Although this has not been systematically explored, it is strikingly illustrated in the famous case described by Slijper of a goat born without fore legs, which adapted to this condition by hopping on its hind legs.[93] The goat's musculoskeletal system, examined upon its accidental death at the age of one year, exhibited a number of novel accommodations to the bipedal stance, including a flattened and extended pelvic ischium, also a distinguishing character of the bird skeleton.

Hyperplasia of skeletal muscle and diversification of avian life histories

An extraordinary accumulation of muscle mass and its thermogenic ability might have secondarily enabled diversification of other aspects of avian life histories, including prolonged incubation, parental care, altricial development, and long-distance migration. First, birds incubate eggs throughout the entire embryonic period and this requires significant thermogenesis at near motionless conditions for long periods of time (up to four months in some species).[94,95] For example, skeletal musculature enables prolonged incubation periods in emperor penguins (*Aptenodytes forsteri*),[96] where exaggerated pectoral muscles provide both thermogenesis and nutrient supply during a 150-day incubation without adult feeding (or much movement) under sub-freezing temperatures (the muscle mass being depleted by as much as 40% during this time).[97]

Second, thermogenic abilities of nestlings and their cooling rates—both determined by their muscle mass[34,64]—determine parental presence on the nest after hatching and corresponding partitioning of parental provisioning between males and females, which in turn can drive diversification in avian mating systems.[98,99] Third, the altricial mode of development, which is a derived trait in birds,[59] might itself have been enabled by significant muscle expansion by hatchling birds and corresponding evolution of remarkable cold tolerance in avian neonates. In turn, altriciality is associated with reduced allocation to eggs and shortened incubation periods and related changes in life histories.[59] Fourth, in altricial nestlings, thermogenesis and cooling time can exert selection pressure on the evolution of brood size, because collective mass of several nestlings huddled together essentially becomes homeothermic (thus enabling longer foraging trips by parents) earlier than acquisition of homeothermy by individual nestlings. Finally, exaggerated musculature is a chief source of fuel and, through amino acids from protein serving as gluconeogenic precursors, of glucose itself (the latter being particularly important for the central nervous system) during uniquely long-range trans-oceanic migrations in birds.[100–103] For example, bar-headed godwits (*Limosa lapponica*) cross the entire length of the Pacific Ocean from Alaska to New Zealand—a distance of 11,000 km—in a single nonstop flight that lasts for 10 or more days.[104,105] Muscle mass (mostly pectoralis) sustains nine times its tissue-specific basal metabolic rate during the entire duration of the flight.[104,106]

Conclusions

The thermogenic muscle hypothesis for the origin of the avian clade is well supported by anatomical, physiological, genetic, ecological, and behavioral evidence. For a population of amniote ancestors adapted to niches requiring endothermy (the legacy of which is marked in the bird physiological genetic repertoire by the presence of an essentially intact BAT pathway[2]), loss of thermogenic brown fat would have been detrimental without compensatory changes. The ancestral lineage of birds could have survived the loss of *UCP1* by accumulating modifications that turned its skeletal muscle into a tissue that is more thermogenic than that of mammals, its *UCP1*-retaining warm-blooded sister clade. In addition to losing *UCP1*, birds lost *Glut4*,[5] separating them from the nonavian reptiles. This suggests that the repurposing of insulin as a skeletal muscle growth factor consequent to the loss of that gene was an important part of the avian survival adaptations. Genetic change that brought the myogenesis inhibitory factor myostatin under negative regulation by cold[54,57] was another important adaptation.

It is striking that several of the key evolutionary transitions leading to birds involved gene loss (Table 1). Apart from *UCP1* and *Glut4*, birds also lack *UCP2*, at least one of whose functions in mammals is insulin/glucose homeostasis.[53] In the absence of the Glut4-mediated uptake of glucose by skeletal muscle found in other vertebrates, avian ancestors recruited other physiological modules to this purpose, since insulin and glucose are both stringently regulated in birds.[52] In addition, a major loss of genes specifying members of the galectin family of glycan-binding proteins occurred during the evolution of the birds, causing this clade to have the smallest complement of this family's proteins among the vertebrates.[6] As discussed above, galectins are involved in the development of the embryonic muscle and skeletal systems in chickens.[80,81] They also function, at least in mammals, in postnatal and adult muscle differentiation and regeneration.[107] Loss of galectin genes, which compromised functional redundancy, potentially rendered avian musculoskeletal development less canalized and thus more plastic.[108] Thus, developmental plasticity enabled mechanical stress to become a morphogenetic determinant of the bird skeleton, resulting in several bird-specific skeletal novelties.[9,77]

Table 1. Gene loss in birds

	UCP1	UCP2	Glut4	Galectins[a]
Fish	Yes	Yes	Yes	8
Frog	Yes	Yes	Yes	12
Mammal	Yes	Yes	Yes	8–13
Lizard	No	Yes	Yes	7
Bird	No	No	No	5

[a]Galectin data from Ref. 6, except for the Anole lizard, which was searched using the UCSC Genome Browser: http://genome.ucsc.edu/cgi-bin/hgGateway.

The suggestion that some macroevolutionary changes (e.g., the origin of the reptiles, and within them, the origin of the birds) were tied to one or a few gene losses represents a challenge to conventional evolutionary narratives, which favor gradualist scenarios. The "trees down" and "ground up" theories of bird evolution, which focus on flight as the defining character of birds, are two such scenarios.[109] The standard argument is that a subpopulation of organisms with a macromutation (such as the losses of *UCP1* and *Glut4* discussed above) will be incapable of surviving in the ecological niche in which they arose.[110] The post-Darwinian rejoinder is that organisms do not simply occupy premade niches, but construct new ones, inventing ways of life that suit their biological endowments.[111] If there are preexisting characters (e.g., feathers) and behavioral modes (e.g., brooding of hatchlings) that can ameliorate the disability, some individuals will get through the crisis. But there will also be enormous unidirectional selective pressures on variable traits (e.g., skeletal muscle thermogenicity and mass) to compensate for the deficit. All of these effects are amply illustrated by the birds, who met the ancestral loss of brown fat by evolving a range of novel and versatile anatomies that enabled them to populate a vast range of habitats on the land, in the waters, and in the air.

Conflicts of interest

The authors declare no conflicts of interest.

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