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Review

Decoding the Evolution of Melanin in Vertebrates

M.E. McNamara [1,2,*,@], V. Rossi,[1,2] T.S. Slater,[1,2] C.S. Rogers,[1,2] A.-L. Ducrest,[3] S. Dubey,[3,4,5] and A. Roulin[3]

Melanins are widespread pigments in vertebrates, with important roles in visual signaling, UV protection, and homeostasis. Fossil evidence of melanin and melanin-bearing organelles – melanosomes – in ancient vertebrates may illuminate the evolution of melanin and its functions, but macroevolutionary trends are poorly resolved. Here, we integrate fossil data with current understanding of melanin function, biochemistry, and genetics. Mapping key genes onto phenotypic attributes of fossil vertebrates identifies potential genomic controls on melanin evolution. Taxonomic trends in the anatomical location, geometry, and chemistry of vertebrate melanosomes are linked to the evolution of endothermy. These shifts in melanin biology suggest fundamental links between melanization and vertebrate ecology. Tissue-specific and taxonomic trends in melanin chemistry support evidence for evolutionary tradeoffs between function and cytotoxicity.

Highlights

In extant vertebrates melanin fulfils diverse roles including visual communication, photoprotection, antioxidation, and mechanical strengthening of tissues, but the evolution of these functions is debated.

The discovery that melanosomes in fossil and modern vertebrates are associated with tissue-specific suites of trace metals supports hypotheses that melanin has ancient functions in metal homeostasis and antioxidant regulation.

Shifts in melanosome biology across the dinosaur–bird transition reveal intimate links between adaptive and pleiotropic processes relating to the evolution of endothermy, metal homeostasis, photoprotection and the lymphatic system.

Key genes can be mapped onto color pattern phenotypes in fossil vertebrates.

Melanin-based coloration in vertebrates is dominated by melanin forms associated with low cytotoxicity, possibly reflecting selective adaptation against forms linked with greater oxidative stress or co-option of melanin forms with specific metal binding behavior for coloration.

Melanin in Vertebrates

Melanins (see Glossary) are dark to rufous pigments that are widespread in vertebrates and underpin critical functions in physiology and behavior [1]. Fossil evidence of melanin extending to over 300 million years ago has triggered a paradigm shift in paleobiology, prompting remarkable reconstructions of the coloration and behavior of extinct vertebrates [2–6]. New discoveries of internal melanins in vertebrate fossils have broadened our understanding of the functional diversity of ancient melanins [7–9] and invite a re-evaluation of the macroevolutionary history of melanin and its functions. Here, we synthesize trends in the fossil record of melanin and explore fossil evidence for the evolution of melanin function and the genetic basis of melanization. This highlights the value of the fossil record as a resource for tracking melanin evolution through deep time.

Functions of Melanin in Ancient Vertebrates

In extant vertebrates, melanin occurs as micron-sized organelles, **melanosomes,** in the integument, eyes and internal tissues and functions in photoprotection, visual signaling, thermoregulation, immunity, antioxidation, mechanical strengthening, and abrasion resistance [6,10,11] (Figure 1, Box 1). It is unclear which functions evolved first and which selection pressures dominate [11,12]. Fossils preserving evidence of melanin offer a unique temporal perspective.

Melanin has been reported from fossil vertebrates from >25 localities from the **Carboniferous** to the **Pliocene** (Table S1 in the supplemental information online). The fossils include cyclostomes, fish, frogs, lizards, and other squamates, ichthyosaurs, plesiosaurs, turtles, pterosaurs, feathered and nonfeathered dinosaurs, birds, and mammals. This phylogenetically and temporally broad dataset yields evidence for ancient functions of melanin (Figure 2).

[1]School of Biological, Earth and Environmental Sciences, University College Cork, Distillery Fields, North Mall, Cork T23 TK30, Ireland
[2]Environmental Research Institute, University College Cork, Lee Road, Cork T23 XE10, Ireland
[3]Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland
[4]AgroSustain SA, c/o Agroscope, Rte de Duillier 60, 1260 Nyon, Switzerland

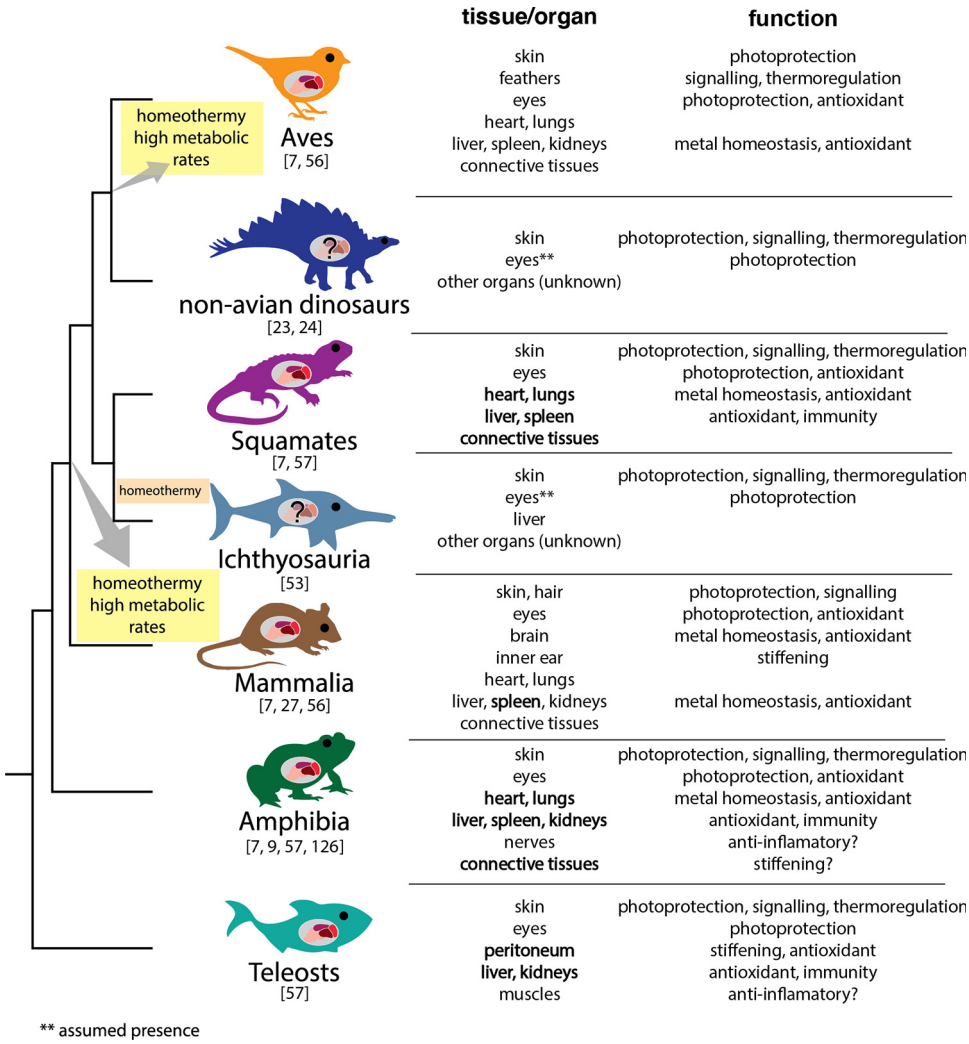

Figure 1. Summary of Current Understanding of the Location of Melanosomes in Different Body Tissues and Organs and Putative Functions in Major Vertebrate Classes. Melanosomes have been reported from diverse tissues across fossil and extant vertebrates (see Table S1 in the supplemental information online) [7,9,23,24,27,28,53,56,57,126,127]. Internal melanosomes are less common in extant birds and mammals than other vertebrate groups. This may reflect the evolution of homeothermy, changes in the immune system, a shift in melanogenesis to the integument and melanosome storage in integumentary appendages such as feathers and hair. Bold text denotes tissues with high content of internal melanin in that taxon. "?" denotes hypothesized function of melanin.

Integumentary Coloration and Visual Signaling

Visual signaling is a major function of melanin [1] and most studies on fossil melanin have focused on integumentary melanins and signaling. Fossil vertebrate melanin has been linked to crypsis [13,14], sexual display [15–18], and aposematism [14]. Integumentary coloration in fossils has been inferred from the visual tone of fossil tissues, melanosome geometry, distributions of melanin-associated metals and/or potential molecular fragments of melanin [3,4]. In the bird *Confuciusornis* [19], clusters of melanosomes of different morphologies (resembling **eumelanosomes** and **pheomelanosomes** of extant birds) and within-feather variations in

[5]HW Romandie, Avenue des Alpes 25, 1820 Montreux, Switzerland

*Correspondence: maria.mcnamara@ucc.ie (M.E. McNamara).
@Twitter: @MariaMcN_palaeo (M.E. McNamara).

Box 1. Melanin Development and Biosynthesis

Melanosome development is a four-stage process [6]. The melanocyte's endosomes produce acidic vesicles (Stage 1) that are filled with a fibrillar matrix derived from PMEL17 glycoproteins (Step 2) [68]. Eumelanin polymerizes as granules (10–30 nm diameter [99]) on this amyloid protein scaffold (Step 3). The resulting mature melanosomes (Stage 4) feature a characteristic rugose surface texture [100]. Pheomelanin-rich melanosomes lack the fibrillar protein matrix (as PMEL17 is downregulated by *ASIP*) and thus have a more spherical, somewhat irregular shape and disordered melanin granule deposition [101]. Fossil melanosomes can exhibit various morphologies and surface texture reminiscent of eu- and pheomelanosomes, but do not preserve evidence of amyloid fibrils.

Biosynthesis of both eumelanin and pheomelanin begins with the oxidation of tyrosine to L-**dopaquinone**, catalyzed by TYR. If L-cysteine concentrations exceed a threshold value [65], and especially if the melanosome internal environment is acidic [102], cysteine combines spontaneously with L-dopaquinone. The resulting cysteinyldopa derivatives are oxidized to cys-dopaquinones and subsequently benzothiazine and its derivatives, some of which are decarboxylated to benzothiazole moieties, yielding pheomelanin polymers [103]. In the absence of L-cysteine, and where pH is neutral [102], dopaquinone undergoes intermolecular cyclization and rearrangement to L-cyclodopa [65], which in turn is oxidized to **5,6-dihydroxyindole-2-carboxylic acid** (DHICA) and **dihydroxylindole** (DHI) in the presence of tyrosinase-related protein 1 (TRP1) or TYR and L-dopachrome tautomerase (TRP2 or L-DCT). DHICA and DHI then undergo unregulated polymerization [5], forming large covalent molecules with complex crosslinks. The diagnostic molecular markers for melanin are therefore benzothiazine and benzothiazole for pheomelanin and DHI and DHICA for eumelanin [66]. The relative abundances of these moieties in fossils has received little attention despite intriguing functional and physiological implications (Box 3).

The rate and nature of melanogenesis are influenced by pH [102] and concentrations of various metal ions [104]. The presence of Cu^{2+} or Zn^{2+} favors DHICA formation [104]. Certain metals preferentially associate with specific functional groups of eumelanin (Ca^{2+} and Zn^{2+}: $COOH^-$; $Fe^{2+/3+}$ and Cu^{2+}: OH^-; $Fe^{2+/3+}$: NH_4^+; these associations can alter with pH [10]). In pheomelanin, Zn^{2+} coordinates with OH^- and with organic sulfur [27] and Cu^{2+}, with $COOH^-$ and quinine–imine (but SH^- and catechol groups at higher pH [105]). The inherent affinity and binding capacity of melanins for different metals underpins proposed functions of melanin in metal regulation and detoxification.

tone indicate complex within-feather patterning [13,20] linked to sexual display and crypsis [13]. Striking dorsoventral patterning (**countershading**) on the torso, and stripes on the tail and face, is inferred for the dinosaur *Sinosauropteryx* [14,20] based on melanosome geometry and spatial distributions of feathers. These features are linked to open habitats and antipredator distraction, respectively [14]. Rufous pheomelanin-based colors on the headcrest of *Anchiornis*, plus high-contrast spangling on the wings, likely functioned in sexual display [16]. Similarly, highly elongate or platelet-like melanosomes and/or ordered melanosome arrays in fossils may have contributed to the generation of weak iridescence [15,17,18,21,22], with potential functions in courtship display, especially where localized to the head, neck and/or chest [17,18].

Coloration has also been inferred for fossil skin. Tonal variation in the dinosaurs *Psittacosaurus* and *Borealopelta* suggests countershading, linked to life in forested habitats with diffuse light for *Psittacosaurus* [23] and strong visual predation pressures for *Borealopelta* [24]. Dorsoventral patterning and vertical striping associated with oblate melanosomes in the cyclostome *Mayomyzon* likely represent melanin-based countershading, disruptive patterning and shallow water ecologies [25]. The relative abundance of preserved **chromatophores**, including **melanophores**, in the skin of a fossil Colubrid snake indicates that the fossil skin was countershaded and originally primarily green, presumably with functions in camouflage [26]; patches of dark brown–black and bright yellow–green skin may have functioned in disruptive coloration [26].

Spatial distributions of Zn, S, and organo-S and organo-Zn species in the fossil mouse *Apodemus* are consistent with **pheomelanin**-based fur [27]. Chemical evidence for **eumelanin** and uniform visible tones in fossil marine reptiles may represent homogeneous dark coloration consistent with background matching, especially in ichthyosaurs (with inferred deep-diving behavior [28]).

Glossary

5,6-dihydroxyindole (DHI) and -2-carboxylic acid (DHICA): principal building blocks of eumelanin.
α-melanocyte-stimulating hormone: arguably the most important of the MSHs in regulating melanogenesis.
Agouti-signaling protein: an antagonist of the MC1R that induces the production of pheomelanin via the downregulation of eumelanin synthesis.
Archosaurs: a group of reptiles that include birds, crocodiles, extinct dinosaurs, and pterosaurs and relatives.
Carboniferous: geological period spanning 358 298 Ma.
Chromatophores: integumentary pigment cells rich in melanin (melanophores), carotenoids (xanthophores), or guanine platelets (iridophores).
Countershading: a form of camouflage where the dorsal surface of an animal is darker than the ventral surface to assist with background adaptation.
Dermal chromatophore unit: a morphologically distinct structure in the dermis of fish, amphibians, and reptiles, comprising three types of pigment cell (melanin-rich melanophore, carotenoid-rich xanthophore, and guanine-rich iridophore).
Dopaquinone: a metabolite of the amino acid L-DOPA and a precursor of all melanins.
Eumelanin: a dark brown to black pigment derived from the polymerization of the indoles DHI and DHICA.
Eumelanosome: a term commonly applied to elongate, eumelanin-rich melanosomes in avian and mammalian feathers.
Melanins: a chemically and functionally disparate family of natural pigments derived from the oxidation of tyrosine. They are characterized by a high refractive index, strong absorption in the UV-visible spectrum, strong affinity for metals and extensive polymerization and crosslinking.
Melanin-concentrating hormone: a cyclic peptide that can act as a neurotransmitter and contributes to melanin regulation in melanophores.
Melanocortin-1-receptor: a seven-transmembrane G-protein-coupled receptor expressed on the surface of melanocytes that regulates the melanin pathway.

Mechanical Properties of Tissues

Melanin substantially increases feather hardness [29]. Densely packed melanosomes [30], dark visible tones [13,16,31,32] and enrichment in Cu [19,32] throughout or in the distal portions of fossil feathers suggest extensive melanization; a common [33] adaptation to high aerodynamic forces [31]. Within-feather melanin gradients may also facilitate buoyancy regulation and/or minimize the physiological costs of melanogenesis [32]. Unusual melanosome morphologies in fossil penguin feathers may have affected the material properties, and thus hydrodynamics, of the feathers underwater [34].

UV Protection

In extant vertebrates, melanin-based coloration often covaries with other phenotypic traits [35,36], including physiological response to UV stress [1]. Such photoprotective functions are inferred for eumelanic dorsal coloration in fossil turtle skin [37]. Melanin is an essential screening pigment in vertebrate eyes [38]. Dark-toned fossil eyespots preserve melanosomes in fossil fish [19,39,40], an unidentified bird [41], the mosasaur *Platecarpus* [42], and in the enigmatic fossil *Tullimonstrum* [43]. Size-specific layering of melanosomes in the eye of a fossil is diagnostic of (a) melanic tissue layer(s); for example, the vertebrate **retinal pigmented epithelium** (RPE) that protects the retina from photooxidative damage. The chemistry of fossil eye melanosomes may carry taxonomic information and has been used to suggest an invertebrate affinity for *Tullimonstrum* [40].

Immunity and Metal Homeostasis

Melanin has important, but relatively understudied, functions in immunity, including antimicrobial and antipathogen functions [44] and metal homeostasis [10]. In extant birds [45] and the seasnake *Emydocephalus* [46], integumentary melanins can bind environmentally derived metal ions (e.g., Ca^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}) that may be excreted via accelerated sloughing [46]. These melanosome-associated metals may represent physiologically important redox-active metals that can be released when needed [47,48], or excess metals derived from metabolic processes (or the external environment) that are isolated from active metabolism, as in hair and feathers [45,46].

Recent discoveries of non-integumentary melanosomes in extant and fossil vertebrates [7–9] offer intriguing insights into regulation of the **metallome**. Melanosomes from different tissues differ in metal content (especially Ca, Fe, Ti, Mn, and Zn) and geometry [7–9]. These signals characterize melanosomes in extant amphibians, reptiles, birds, and mammals, and fossils as old as the **Permian**. Although metal–melanosome associations may be altered during fossilization [8,49], the preservation of tissue-specific signals in fossils strongly suggests that critical functions of melanin in metal regulation, metal-mediated biochemical catalysis, and/or other immune functions (e.g., scavenging **reactive oxygen species** (ROS), originate in deep time.

Intriguingly, spatial distributions of melanin-associated metals can allow the anatomical distribution of internal melanosomes from different tissues to be reconstructed [7]. This offers the potential to interpret the soft tissue anatomy, and resolve the taxonomic affinity, of putative ancient vertebrates [7].

Thermoregulation

Melanin can convert electronic energy into heat (photon–phonon transformation); for example, in the pecten in the eyes of migratory birds and lizard skin [50]. Internal melanins can minimize oxidative tissue damage during freezing and thawing, especially in species with **supercooling** capacity [51,52]. Fossil evidence for such functions is lacking, although dark eumelanin-based coloration in marine reptiles, especially in dorsal body regions, is implicated in thermoregulation,

Melanocytes: melanin-producing pigment cells, widespread in vertebrates.
Melanocyte-stimulating hormones: peptides derived from the *POMC* gene produced mainly in the pituitary gland and also in the hypothalamus and skin. α-MSH stimulates the production of melanin by melanocytes.
Melanomacrophages: melanin-rich phagocytes in ectothermic vertebrates.
Melanophores: melanin-containing pigment cells that form part of the dermal chromatophore unit in fish, amphibians, and reptiles.
Melanosomes: micron-sized lysosome-like organelles, produced by melanocytes and melanophores, where melanin synthesis and storage as supermolecular aggregates take place.
Metallome: the complement of metal species and metalloids in a cell, tissue, or organism.
Ornithodira: the clade that includes pterosaurs and dinosaurs and their last common ancestor.
Permian: a period of geological time spanning 298.9–251.9 Ma.
Pheomelanin: a sulfur-rich pigment derived from the reaction of cysteine with dopaquinone and associated with the production of yellowish to orange–brown colors in skin, hair, and feathers.
Pheomelanosome: a term commonly applied to spheroidal, pheomelanin-rich melanosomes in extant avian feathers.
Pliocene: epoch of geological time spanning 5.333–2.58 Ma.
***PMEL* genes:** these encode transmembrane glycoproteins essential for the structural organization of pre-eumelanosomes.
Reactive oxygen species: unstable, highly reactive molecules or molecular species (free radicals) derived from molecular oxygen, generated during oxidative metabolism.
Retinal pigment epithelium: a monolayer of cells in the vertebrate eye between the choroid and the retinal photoreceptors.
Sox10: a transcription factor critical for development of the neural crest and that is expressed in melanocyte nuclei.
Supercooling: the ability of an organism to cool its internal fluids below their freezing temperatures without solidification of the fluids.
Taphonomy: the suite of processes occurring from the moment of death to recovery of a fossil in the field, including key steps such as transport, deposition, decay, thermal maturation, diagenetic fluid interactions, and uplift.

accelerated growth [37] and occupation of cold-water habitats [28,53]. Comparative analysis of the distributions of internal melanin in extant ectothermic and endothermic [54] reptiles and fossils could shed light on dinosaur metabolism.

Triassic: the period of geological time spanning 251.9–201.3 Ma.
Tyrosinase: a Cu-containing enzyme that catalyzes melanogenesis via the oxidation of the amino acid tyrosine.
***WNT*:** a gene family involved in regulation of cell fate and patterning during development.

Taxonomic and Tissue-Specific Trends in Melanin Evolution

Melanosome Abundance and Anatomical Distribution

In extant amphibians, nonintegumentary melanosomes outnumber those from the skin [9]. This also applies to fossil amphibians, where layers of nonintegumentary melanosomes are thicker (10–200 μm) than those from the skin (2–5 μm) [9]. In reptiles, both melanin sources contribute equally to the melanin complement, whereas integumentary melanosomes dominate in birds and mammals [7]. This reveals a shift in the location of melanogenesis during bird and mammal evolution, presumably linked to endothermy. The evolution of full-body coverings of feathers or hair for thermoregulation would require melanogenesis to shift to the integumentary appendages to maintain functions in UV absorption. This spatial shift may also be adaptive, reducing physiological costs of generating and/or storing melanin due to the potential for accumulating ROS [55] and excess quantities of heavy metals [56]; another adaptive consequence is mechanical strengthening of integumentary appendages [29]. Why, then, do birds and mammals retain internal melanin at all? The physiological benefits of metal homeostasis and/or antioxidant response presumably outweigh the physiological costs of ROS production during melanogenesis.

An additional factor is the evolution of the lymphatic system. In birds and mammals the primary immune response derives from macrophages of the splenic germinal center (GC) and, in mammals, lymph nodes [57]. Fish, amphibians, and reptiles lack lymph nodes and GCs; instead, immunity derives primarily from **melanomacrophage** centers (MMCs) of the spleen. These MMCs contain abundant **melanocytes** and are considered precursors of the mammalian GCs [57,58]. Lower abundances of internal melanosomes in birds and mammals relative to amphibians and reptiles may therefore reflect, at least in part, an adaptive shift of immune response from MMCs of internal organs to GCs and, in mammals, the lymph system. This shift may have initiated in **archosaurs** prior to the origins of feathers and may have facilitated the shift of melanogenesis to integumentary appendages in birds and, especially, mammals.

New analyses of melanosome-associated metals highlight melanin functions in metal regulation [7]. Vertebrate integumentary melanosomes are usually enriched in Ca and Zn (plus Fe and Cu in feathers [59]). Melanosomes from the heart, lungs, spleen, liver, and kidneys are usually enriched in Fe (plus Cu in the liver). Melanosomes from connective tissues are usually enriched in Ca; eye melanosomes contain Zn and, to a lesser extent, Ti and Fe. These consistent patterns of metal enrichment across vertebrates suggest strong controls on melanin–metal chemistry. Whether these are traits under active selection, (in)direct effects of physiology, controlled by the melanin form (monomer) present (Box 2) or represent pleiotropic effects [60] is, however, uncertain.

Melanosome Geometry

Melanosomes show intriguing taxonomic trends in geometry [36]. Oblate/ovoid integumentary melanosomes are typical of turtles, lizards, crocodiles, and nonfeathered dinosaurs. Mammalian integumentary melanosomes show a greater range of rod-shaped geometries; melanosomes in feathered dinosaurs and birds include ovoids, spheres, elongate rods, and flattened platelets. This diversification of melanosome geometries has been linked to pleiotropic shifts in the melanocortin system that controls melanogenesis and metabolic rate [61] across the dinosaur–bird transition, and in particular, the physiological transition to endothermy [36]. These changes may ultimately derive from the entry of a new player in the control of melanogenesis; for example, **Agouti signaling protein** (ASIP).

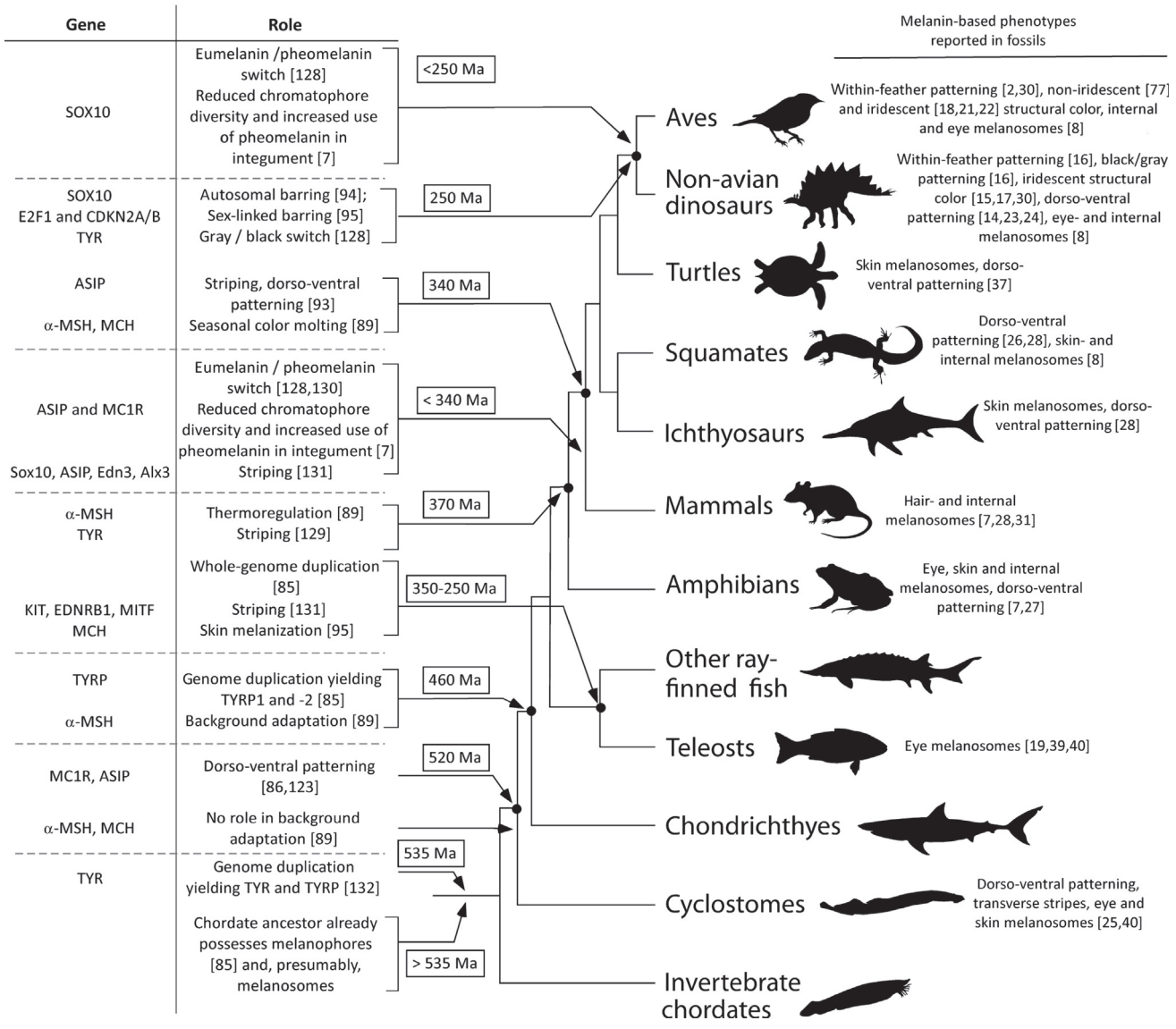

Figure 2. Melanin-Based Phenotypes Reported in Fossil Vertebrates and Possible Genomic Events Underlying the Functional Evolution of Melanin in Vertebrates. Phylogenetic bracketing of genomic data from the literature [85,86,89,93–95,123,128–132] suggests that the emergence of certain regulatory genes can be linked tentatively to key nodes in the simplified phylogenetic tree. Dates (in millions of years, Ma) for key nodes in the phylogeny indicate when the capacity for certain functions may have emerged. Melanin regulation was already established in the earliest chordates 535 Ma, with early genome-duplication events yielding TYR, TYRP1, and TYRP2 prior to the evolution of vertebrates. Following the emergence of regulatory roles for background adaptation in the earliest vertebrates, roles for α-MSH in thermoregulation and for MC1R in the eumelanin/pheomelanin switch emerged 390 Ma in early tetrapods. Other key components for melanin-based patterning emerged at the origin of mammals and birds 340 Ma, and within-feather patterning at the base of dinosauromorphs 250 Ma. Abbreviations: α-MSH, α-melanocyte-stimulating hormone; ASIP, Agouti-signaling protein; MC1R, melanocortin-1 receptor; MCH, melanin-concentrating hormone; MITF, microphthalmia-associated transcription factor; TYR, tyrosinase.

Alternatively, these trends could reflect the loss of the **dermal chromatophore unit** (DCU) during modification of the skin in tandem with the origins of feathers and hair [2]. In extant amphibians and reptiles, the DCU generates diverse hues plus iridescence using melanophores and other chromatophores [26]. Bird and mammal skin lacks iridophores and xanthophores and thus has a reduced color gamut. The diversification of melanosome geometry in birds from plesiomorphic

Box 2. Pheomelanin and Evolutionary Tradeoffs

In physiological terms, pheomelanin production is more costly than that of eumelanin as it is associated with higher levels of ROS in tissues [50]: its biosynthesis generates oxidative free radicals and it is readily photolyzed by UV. Pheomelanin is more abundant in all tissues of birds and mammals, especially feathers and hair, relative to those in amphibians and reptiles [7]. The selective advantages of pheomelanin are unclear, but in adult birds may relate to camouflage or to the generation of plumage patches with striking hues as quality indicators [5]. The cost of incorporating pheomelanin in inert feathers or hair may be low compared to the cost for skin or other tissues. In juvenile birds, pheomelanin may function in cysteine homeostasis, especially in carnivorous species that may consume excessive dietary cysteine [106]. Evidence of pheomelanin has been reported from diverse fossils and tissues, including several taxa where it is considered to dominate integumentary melanin, producing ginger to brown colors [14,20,23,24,27]. This is somewhat surprising as pheomelanin is more soluble, and presumably less resistant to degradation, than eumelanin; some pheomelanin may have been protected by a eumelanin casing, but this remains to be tested. Whether these fossil data represent a real biological signal, or a taphonomic artifact, is a key consideration for further research.

Selection on the synthesis of certain melanin monomers is also important. Eumelanogenesis via the DHI pathway (Box 1) yields melanin with enhanced photoprotective properties but causes higher oxidative stress than the DHICA pathway [107]. In extant vertebrates, eumelanin comprises primarily DHICA, which, at least in birds and mammals, is the primary determinant of eumelanin-based plumage color [107]. Similarly, benzothiazole is associated with less production of ROS than benzothiazine during pheomelanogenesis and is the primary contributor to pheomelanic color [107]. This suggests adaptive selection of color phenotypes dominated by more carboxylated melanin monomers, potentially linked to reduced oxidative stress during or after melanogenesis. This is supported by the inferred DHICA content of fossil melanosomes in a pterosaur [62]. Selection for different forms has implications for metal–melanosome associations, as DHI has a higher capacity for binding certain elements, such as Cu and Fe, at OH groups, relative to DHICA; conversely Ti has a higher capacity for binding to DHICA (at COOH groups). Whether this is actively selected for, and whether the previously-mentioned trends also apply to more basal vertebrates, and to nonintegumentary melanosomes, is an important knowledge gap in our understanding of melanin evolution.

[62] oblate geometries may reflect selective pressures to expand melanin-based color space [2,62] via selection for more extreme melanosome morphologies [2,62]. The speed of this transition is unknown and the comparative color gamut of these groups has not been investigated quantitatively (but see [63]). Melanosome geometries in mammals are intermediate in diversity relative to those in reptiles and birds; this may reflect reduced capacity for color vision, potentially due to nocturnal ancestry.

These trends relate to the chemistry of melanin in melanosomes of different geometries. Traditionally, feather melanosomes are characterized as elongate, eumelanin-rich eumelanosomes and spheroidal, pheomelanin-rich pheomelanosomes. There is increasing evidence, however, that most melanosomes have a mixed composition [64–66]. The casing model of melanosome structure [64] posits initial synthesis of pheomelanin followed by eumelanin, yielding mixed melanosomes [65] with a pheomelanin core surrounded by a eumelanin shell of variable thickness [64,65]. The switch from pheo- to eumelanogenesis occurs when L-cysteine is depleted below threshold levels [65]. This is regulated by **α-melanin-stimulating hormone** (α-MSH) binding to **melanocortin 1-receptor** (MC1R), which upregulates eumelanogenesis by preventing binding of the antagonist ASIP [67].

Given known constraints on melanosome geometry (Box 1), diversification of melanosomes from an ovoid precursor, and expansion of color space, may thus reflect stronger partitioning of eumelanin and pheomelanin into melanosomes of different geometries. Delay and/or suppression of the switch from ASIP- to α-MSH regulation [67] during melanogenesis (Box 2) would generate spheroidal pheomelanosomes. Earlier activation of the switch, and/or enhanced production of PMEL glycoproteins [68] (regulated by ASIP- and/or α-MSH [69]; Box 1) would yield elongate eumelanosomes (although other genes may also play a role [70–72]). In pterosaurs, oblate melanosome geometries similar to the plesiomorphic state [73,74] suggest that such genetic shifts in melanogenesis and their phenotypic expression occurred after the origins of feathers in **Ornithodira** [74] but prior to the evolution of dinosaurs.

Nonintegumentary melanosomes also show expanded geometries in birds and mammals [7]. This may reflect pleiotropic effects during melanogenesis that are independent of tissue location, but the selective forces involved are unknown.

These hypotheses could be tested as follows. Where feathers are colored primarily by melanin, melanosome geometry is presumably diverse and (with melanosome chemistry) under strong selection to yield particular colors. In feathers where other pigments, such as carotenoids, dominate, melanosomes function as a backing pigment and are predicted to show less diverse, plesiomorphic geometries and mixed chemistries (although other selective forces may apply). Similarly, comparative analysis of these parameters for melanosomes from skin of different colors, and nonintegumentary tissues, of amphibians and reptiles will test whether melanosomes in these tissues show partitioning of melanosome geometry and chemistry. Until then, melanin-based color in fossil amphibians and basal archosaurs may be difficult to determine, especially where preserved melanosomes show indiscriminate oblate geometries.

Structural Coloration

Iridescent structural coloration in feathers has multiple independent origins in birds and nonavian theropods [75] and is often associated with modified melanosome geometries, organized melanosome arrays, high melanosome density and flattened barbule geometries [75]. Platelet-like melanosomes in the fossil bird *Caihong* resemble iridescence-generating melanosomes in extant birds in geometry but not internal structure, which suggests decoupling of the two features [6,17,76]. Similarly, solid melanosomes in feathers of the trogon *Primotrogon* predicted as iridescent contrast with the hollow melanosomes of iridescent feathers in extant relatives [75]. Melanosome arrays in iridescent feathers may reflect self-assembly [6,77,78]; narrower melanosomes may migrate to the outer cortex during feather development [78,79]. Evolution of noniridescent structural coloration from gray coloration may reflect selection for large melanosomes that concentrate in the feather core during keratinization, generating a thick cortex that can house quasiordered photonic structures.

Evolution of Melanin Functions in Early Vertebrates

Eumelanin is often considered the ancestral form of melanin in vertebrates [6]. This reflects, in part, an incomplete understanding of the phylogenetic distribution of pheomelanin. Our recent research shows that pheomelanin is not restricted to mammals, birds, and reptiles [6] but also occurs in amphibians and fish (and cephalopods) [7,40,80]. It is thus unlikely that pheomelanin has multiple independent origins in vertebrates [81]; a single deep origin (for vertebrates and potentially for Metazoa) is more parsimonious. This hypothesis is supported by evidence that pheomelanogenesis is the default melanin pathway [65] and is less complex (involving fewer regulatory proteins) than eumelanogenesis [82]. An evolutionary transition from pheomelanogenesis to eumelanogenesis could reflect selection for pathways associated with lower production of cytotoxic ROS and with enhanced capacity for broadband absorption across the UV-visible spectrum (Box 2).

The origin of melanogenesis has been linked to defense against ROS [11], with melanin co-opted later for photoprotection [5]; these were probably important functions for melanin in early vertebrates. The evolution of melanogenesis may even be a secondary effect of metal homeostasis and metal-mediated catalysis of biochemical reactions [11], which have deep evolutionary origins [83] and are evidenced by tissue-specific metal-melanosome associations in diverse vertebrate fossils [7,8]. Integration of melanin into the immune responses of protostomes and deuterostomes [84] suggests additional immune functions for melanin in early metazoans, including early vertebrates. Current fossil evidence indicates that the gamut of functions outlined

in the preceding text (antioxidant, photoprotection, metal regulation, and signaling) had evolved by the Carboniferous period. Phylogenetic bracketing, however, suggests that these functions were present in basal vertebrates and are probably ancestral to the group. Collectively, these data suggest that the plesiomorphic functions of melanin in vertebrates were homeostasis and photoprotection, with greater emphasis on signaling during the evolution of endotherms in the early **Triassic**, a period of major ecosystem reorganization. This, plus potential links between antioxidant functions and rising oxygen levels in the Early Cambrian period, suggests indirect links between melanin evolution and global-scale environmental change.

Fossil Insights into the Genetic Basis of Melanization

The genetic architecture for melanin production is highly conserved across vertebrates [85], featuring several whole-genome duplication events [86] and with important roles for **melanocyte-stimulating hormones** (MSHs), **melanocyte-concentrating hormones** (MCHs), MC1R, **tyrosinase** (TYR), and ASIP [87] (Box 3 and Figure 2). The biosynthetic pathways of integumentary and nonintegumentary melanins, however, may differ and the genetic basis of nonintegumentary melanins and potential homeostatic functions is unclear [56]. The following discussion therefore focusses on fossil insights into the genetic basis of melanin-based color patterning: the genetic mechanisms involved have been characterized for extant vertebrates and integumentary coloration is a major focus of previous studies of fossil melanin (Table S1 in the supplemental information online).

Color patterns in fossils may provide unique minimum temporal constraints on the expression of specific coloration phenotypes through deep time (Figure 2). For instance, patterning in fossil

Box 3. Genetic Basis of Melanogenesis

The melanin pathway and its regulation are conserved in most vertebrates: 605 genes with melanin-related phenotypes have orthologs in human, mouse (*Mus musculus*) and zebrafish (*Danio rerio*) [108]. Processes such as the migration of melanocytes and melanophores from the neural crest [109] and their survival and proliferation [110] in other organs are spatially and temporally regulated by paracrine factors from adjacent keratinocytes and fibroblasts and by neuroendocrine factors [111]. Collectively, these factors belong to the ***WNT*** pathway and *EDN3*, *BMP*, *HOX*, *KIT*, *MITF*, *PAX*, and *SOX* genes [112,113].

Melanin pigmentation is also tightly controlled by, and coordinated with, melanosome maturation. The main key regulators of melanin type and quantity and of melanosome biogenesis are α-MSH and ACTH, ASIP, and MC1R [114]. Binding of α-MSH to MC1R triggers adenylate cyclase to induce cAMP-dependent and -independent pathways. This subsequently enhances the expression of transcription factors such as microphthalmia-associated transcription factor (MITF) [115], which in turn stimulates transcription of enzymes responsible for melanin synthesis (TYR, TRP1, and TRP2), transport proteins (SLC45A2/OCA4, OCA2/pink-eyed dilution [116]) and melanosome maturation (PMEL17 [117] and MART1/MELAN, GPR143/OA1 [118]).

Melanosome biogenesis also involves proteins such as (BLOC)-1–3, Rab38, Rab32, Cargo, and PYKfyve and adaptor protein complexes that function in melanin trafficking (AP1–3) [119]. The small GTPase Rab27a with melanophilin and myosin Va anchor melanosomes to actin-based motors to deliver melanosomes to the plasma membrane [120] and, subsequently, to keratinocytes.

In fish, amphibians, and reptiles, integumentary melanin is produced in melanosomes of melanophores [121]. Key neuroendocrine and paracrine regulators of melanophore activity are α-MSH for melanin synthesis, melanophore proliferation and differentiation (as in mammals and birds), and α-MSH, MCH, noradrenaline, and melatonin for melanosome aggregation and dispersion [122]. In all vertebrates, α-MSH induces melanosome dispersion, while MCH may either be a dispersant in teleost fish or an aggregant similar to melatonin in other taxa, facilitating substrate matching (background adaptation) [89]. Long-term substrate matching may also induce melanophore apoptosis or proliferation [123]. Melanosome movement and melanin synthesis appear more complex in chromatophores with multiple receptors and antagonists; for example, for the melanocortins expressed in the skin of sea bass (*Dicentrachus labrax*) [123,124]. Moreover, melanophore regulation in fish, amphibians, and reptiles is influenced by the presence of other chromatophores [125].

cyclostomes [25] constitutes the oldest evidence for differential melanogenesis across the body. In extant birds this is regulated, at least in part, by **Sox10** [88], suggesting that α-MSH and/or MCH regulation of substrate matching [89] emerged early during vertebrate evolution.

The development of melanosome amyloid fibrils and thus melanosome geometry is controlled by ***PMEL*** genes (Box 1); expanded melanosome diversity in birds and mammals may thus reflect shifts in the evolution of these genes in the archosaurian ancestor, or convergent evolution. The evolution of more-specialized melanosome geometries in birds and mammals, including those dominated by pheomelanin or eumelanin, has implications for the timing of ASIP–MC1R binding and, in turn, intracellular cysteine levels [65]. Assuming that the widespread ovoid melanosome geometries in amphibians and reptiles are plesiomorphic and represent mixed compositions, the selective pressures to generate striking coloration via more specialized melanosome morphologies, especially elongate eumelanosomes in feathers and hair, may override the plesiomorphic and adaptive advantage of mixed melanogenesis by decoupling cysteine consumption from melanosome morphology.

Evolution of patterns from homogeneous colors could be explained by the appearance of point mutations [90] rather than supergenes [91], or by expression of genes with major phenotypic effects. Changes in pattern elements could reflect selection bias towards genetic hot spots [92] or highly modular genetic control on color phenotypes [93]. Dorsoventral patterns and stripes depend on ASIP expression [93]. Within-feather patterning, as in the feathered dinosaur *Anchiornis*, may arise from one of two processes. For example, in the domestic chicken *Gallus*, autosomal (i.e., irregular) barring and spangling is linked to expression of Sox10 at the *DB* genetic locus [94]. Sex-linked (regular) barring is associated with expression of E2F1 controlled by the *CDKN2A/B* gene [95]. Complex spot formation in feathers may also arise from expression of both ASIP and MC1R [96]. There is increasing evidence, however, that periodic within-feather patterns arise from a combination of genetic signaling (linked to the orientation of pattern elements) and Turing-like self-assembly [97] controlled by physical interactions between melanocytes [98]. Detailed mapping of color patterns in fossil plumage may inform on the evolution of different pattern formation mechanisms, especially given that the regulatory networks associated with periodic patterning are highly conserved in vertebrates [98].

Color patterns are often much more complex in in extant birds relative to mammals [96]. This may relate to enhanced temporospatial regulatory freedom afforded by the availability of both mediolateral (from the rachis to the margins of the feather vane) and anterior–posterior (opposite sides of the vane) regulatory domains in addition to the proximal–distal domain (the only such domain in hair) [96]. Fossils with feathers representing early developmental stages, for example, lacking barbules or a rachis, may shed light on whether pattern complexity increased in tandem with structural evolution of the feather.

Limitations of the Fossil Record

Preservational biases relating to stratigraphic gaps in the rock record, nonpreservation of soft tissues and taxa and the potential for chemical and morphological alteration of melanosomes, yield a record of melanin that is far from complete. Limited fossils preserving soft tissues and restrictions on destructive sampling result in small datasets that are not always amenable to statistical testing. To maximize the palaeobiological potential of fossils, the spatial distribution, morphology and chemistry of preserved melanosomes should be fully characterized using relevant imaging and chemical techniques. More comprehensive characterization of these melanosome attributes in extant vertebrates (and how these vary with ontogeny, gender, and environment), will reveal how representative are data from individual fossils. This, plus a deeper

understanding of melanin and melanosome **taphonomy**, will enhance the usefulness of the melanin fossil record.

Concluding Remarks and Future Perspectives

Recent fossil discoveries underpinned by an improved understanding of melanin biology and preservation provide new opportunities to test models for the functional evolution of melanin and raise important questions (see Outstanding Questions). New advances emphasize the importance of melanin in regulating the metallome and unexpected evolutionary conservatism in metal–melanin associations. Shifts in melanosome location, geometry, and chemistry across the dinosaur–bird transition highlight intimate links between adaptive and pleiotropic processes relating to the evolution of endothermy, metal homeostasis, photoprotection, and the lymphatic system. Color patterns in individual fossils can allow genomic events underlying the functional evolution of melanin to be mapped onto phylogenies. Better integration of genetic and fossil data may inform on the underlying genomic and developmental controls on melanization and may provide unique temporal and ecological constraints on the origins of specific genotypic and phenotypic features.

Characterizing variation within populations, and within a lineage over time, will provide further genetic insights.

The most exciting future developments relate to macroevolutionary perspectives on the role of melanin in enabling key transitions in vertebrate evolution; that is, the origins of the clade, of the tetrapod transition to life on land and of endothermy. Future studies focusing on basal vertebrates and their close relatives will shed light on the original functions of melanin in vertebrates.

The transition from aquatic to terrestrial lifestyles is associated with fundamental physiological transformation. Similar to the evolution of endothermy, this may be accompanied by changes in melanin biology. Testing this hypothesis will require full characterization of melanin biology in extant and fossil fish and comparative analysis of these data with data from fish, amphibians and basal reptiles.

Evolutionary tradeoffs associated with the production of certain forms of melanin and integumentary coloration are particularly intriguing in the context of selection against pro-oxidant melanin forms. Does the evolution of specialized melanosome morphologies imply selection for an expanded color gamut for signaling despite potential oxidation damage or toxic levels of cellular cysteine during melanin synthesis? Analysis of the relative abundance of different melanin forms in fossil and extant vertebrates will yield new insights into evolutionary processes and drivers.

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Declaration of Interests

No interests are declared.

Supplemental Information

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Outstanding Questions

How have the functions of melanin evolved in vertebrates through time? Was melanin co-opted for integumentary coloration only after other functions were already established?

Given the cytotoxicity of pheomelanin, why did it evolve? Were early functions in cysteine metabolism expanded to include new functions in communication, and when?

What is the significance of changes in the location, morphology, and chemistry of internal melanosomes during vertebrate evolution?

Why are melanosomes in different tissues associated with different metals?

Why do mammals and birds have less internal melanin than amphibians and reptiles? Is this a crown group feature or apparent in early representatives of both groups?

What is the genetic basis for the evolution of specific melanosome geometries associated with iridescent and noniridescent structural colors in the feathers of birds and nonavian dinosaurs?

Are shifts in the geometry, chemistry and spatial distribution of melanosomes across the evolutionary transition from reptiles to birds mirrored, or controlled by, associated transformations in melanogenic genetic regulatory networks?

What is the anatomical distribution, morphology and chemistry of melanosomes in basal vertebrates, and what are the implications of these for the functional evolution of melanin, vertebrate origins and the transition to life on land?

Do melanosomes in the skin of amphibians show selection for extreme morphologies as in many extant bird feathers? Conversely, do melanosomes in bird feathers show less derived morphologies when not under selection for coloration; for example, where the melanosomes serve as a backing pigment for feathers colored by carotenoids or other pigments?

How representative are data from individual fossils? Can we gauge this using fossilization experiments and comprehensive testing of statistically robust datasets on extant vertebrates?

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