A Diverse Assemblage of Late Cretaceous Dinosaur and Bird Feathers from Canadian Amber

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The fossil record of early feathers has relied on carbonized compressions that lack fine structural detail. Specimens in amber are preserved in greater detail, but they are rare. Late Cretaceous coal-rich strata from western Canada provide the richest and most diverse Mesozoic feather assemblage yet reported from amber. The fossils include primitive structures closely matching the protofeathers of nonavian dinosaurs, offering new insights into their structure and function. Additional derived morphologies confirm that plumage specialized for flight and underwater diving had evolved in Late Cretaceous birds. Because amber preserves feather structure and pigmentation in unmatched detail, these fossils provide novel insights regarding feather evolution.

Although amber offers unparalleled preservation of feathers (*1–4*), only isolated specimens of uncertain affinity have been reported from the Late Cretaceous (*5*). This contrasts with the rich Early Cretaceous compression assemblage from northeastern China (*6–8*), leaving a substantial temporal gap in our understanding of feather evolution. Late Cretaceous amber from Grassy Lake, Alberta (late Campanian), is derived from lowland cupressaceous conifer forests that occupied the margin of the Western Interior Seaway and is best known for its diverse insect inclusions (*9*). Eleven feather or protofeather specimens (*10*) were recovered by screening over 4000 Grassy Lake amber inclusions predominantly within the Royal Tyrrell Museum of Palaeontology (TMP) and University of Alberta (UALVP) collections. These fossils have disparate morphologies that span four evolutionary stages for feathers (*11, 12*). Specimens include filamentous structures similar to the protofeathers of nonavian dinosaurs that are unknown in modern birds (*13–15*), as well as derived bird feathers displaying pigmentation and adaptations for flight and diving.
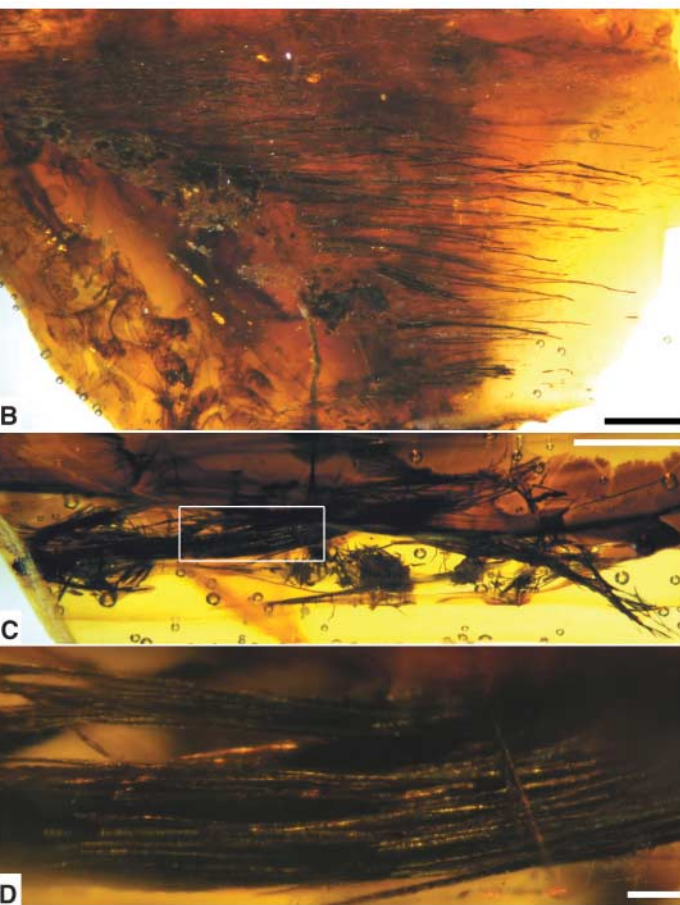
The currently accepted (*11, 12*) evolutionary-developmental model for feathers (Fig. 1A) consists of a stage I morphology characterized by a single filament: This unfurls into a tuft of filaments (barbs) in stage II. In stage III, either some tufted barbs coalesce to form a rachis (central shaft) (IIIa), or barbules (segmented secondary

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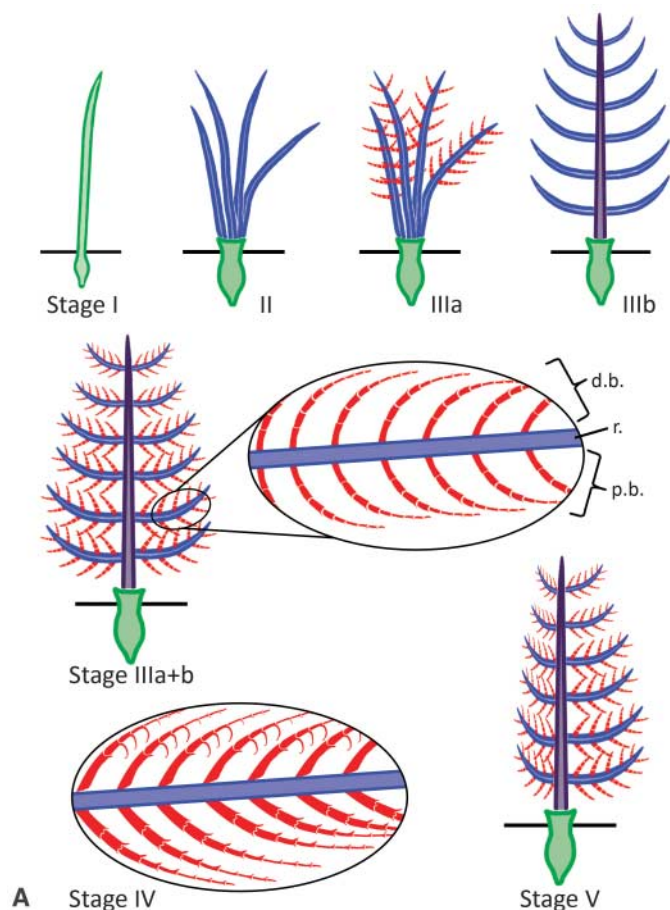
Fig. 1. Feather evolutionary-developmental model (*11*), terminology (*17*), and stage I and II specimens from Canadian amber. (**A**) Feather stages outlined within text. Green, calamus or equivalent; blue, barbs; purple, rachis; red, barbule internodes; d.b., distal barbules; r., ramus; p.b., proximal barbules. (**B**) Field of filaments cut obliquely (stage I), UALVP 52821. (**C**) Filament clusters variably oriented (stage II), UALVP 52822. (**D**) Close-up of (C), showing filaments that comprise clusters. Pigmentation coupled with comparatively thick outer walls produces darker color than in isolated filaments. Scale bars, (B) and (C) 1 mm, (D) 0.1 mm (*10*).

branches) stem from the barbs (IIIb); then, these features combine to produce tertiary branching (IIIa+b). Barbules later differentiate along the length of each barb, producing distal barbules with hooklets at each node to interlock adjacent barbs and form a closed pennaceous (vaned) feather (stage IV). Stage V encompasses a wide range of additional vane and subcomponent specializations. Most modern birds possess stage IV or V feathers or secondary reductions from these stages (11, 16). Modern feathers exhibit a range of morphologies that are associated with their various functions and remain discernible in some of their finest subunits, the barbules (17). This is particularly important in the study of amber-entombed feathers because preservation is biased toward feather subcomponents, which provide the basis for our morphological comparisons.

Stage I is represented by UALVP 52821, which contains a dense forest of regularly spaced, flexible filaments with a mean diameter of 16.4 ± 4.2 μm (Fig. 1B and figs. S1 to S4). Filaments are hollow with the internal cavity comprising ~60% of total diameter, have no obvious internal pith, and taper apically. Where surface texture is observable, filaments bear a faint cross-hatching pattern but lack surface topography. The filaments are not plant or fungal remains because they lack cell walls and are relatively large. Comparatively small diameters and a lack of cuticular scales imply that they are not mammalian hairs, as does direct comparison to a hair from this amber deposit. Their closest morphological match is the filamentous covering found of nonavian dinosaurs such as the compsognathid *Sinosauropteryx prima* (18). The amber-entombed specimens are slightly finer than those of *Sinosauropteryx*, which may have been distorted by compression and permineralization. The amber filaments display a wide range of pigmentation, ranging from nearly transparent to dark (fig. S2). No larger-scale color patterns are apparent. [Additional specimen details are provided in supporting online material (SOM) text.]

The stage II morphotype (Figs. 1, C and D, and fig. S5) consists of tightly adpressed clusters approximately 0.2 mm in width and composed of filaments that are otherwise similar to those already discussed (10). Five clusters are preserved together in UALVP 52822. As in stage II primitive feathers (11), filaments in each cluster appear to diverge from a common basal region without branching, but no rachis is visible where the clusters exit the amber. These filaments bear some resemblance to fibrils that compose pycnofibers (tufted filaments) in pterosaur compression fossils (19), except the amber specimens lack the secondary organization observed in pycnofiber bundles. The most morphologically comparable compression fossils are protofeathers associated with the dromaeosaurid *Sinornithosaurus millenii* (10, 20). These clusters exhibit generally comparable sizes and shapes to the amber specimens and even have the more loosely bundled appearance distally where individual filaments have more variable lengths.

In contrast to stages I and II, additional specimens from Canadian amber have barbules specialized for discrete functions. In TMP 96.9.334 (Figs 2, A to C, and figs. S6 and S7) (10), a thick-

Fig. 2. Specialized barbules in Canadian amber. (**A**) Coiled barbules surrounding thickened rachis (arrow), cut obliquely, TMP 96.9.334. (**B**) Close-up of coils in isolated barbule. (**C**) Semi-flattened internodes and weakly expanded node of (A). Diffuse, variable barbule pigmentation produces pale overall color. (**D**) Isolated barb with differentiated barbules and thickened ramus, in spider's web, UALVP 52820. (**E**) Barbules near distal tip of (D), with clearly defined distal and proximal barbule series (left and right sides of ramus, respectively). (**F**) Close-up of distal barbule in (E), showing nodal prongs and ventral tooth on basal plate (arrow) adjacent to abrupt transition into pennulum. Banded pattern of dark pigmentation within basal plate, and diffuse dark pigmentation within pennulum, suggest a gray or black feather (24). Scale bars, (A) 0.4 mm; (B), (D), and (E) 0.2 mm; (C) and (F) 0.05 mm (10).
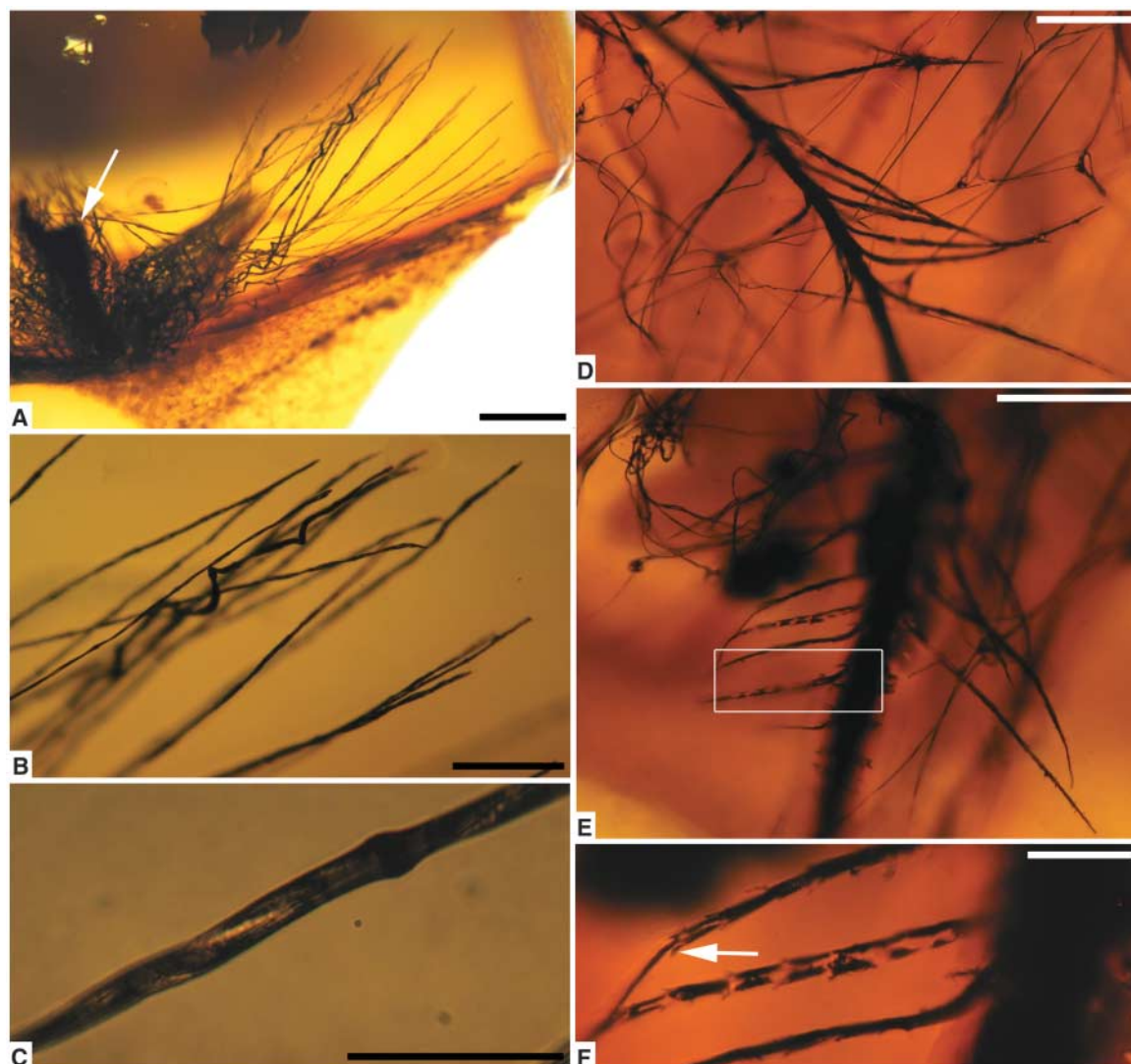
ened rachis is surrounded by numerous barbules with tightly coiled bases. The barbules undergo three or more complete whorls and are composed of semi-flattened internodes (~120 μm long, 9 μm wide) separated by weakly expanded nodes (~12 μm wide). This coiling cannot be attributed to interaction between barbules and resin during amber polymerization because it only occurs at the base of each barbule. Mod-ern seedsnipes and sandgrouse (21, 22) possess belly feathers with similar basal barbule coil-ing, which allows water to be retained for trans-port to the nest for distribution to nestlings or for cooling incubating eggs. Grebes also have coiled barbules that absorb water into plumage, facili-tating diving by modifying buoyancy, reducing hydrodynamic turbulence, and improving insu-lation (23). In all of these instances, the keratin of coiled barbules interacts with water to uncoil and absorb water through capillary action (22). The high number of coils in TMP 96.9.334 is most similar to that reported from grebes (23, 24), implying that the Cretaceous barbules are related to diving behavior.

Barbules displaying all characteristics neces-sary for forming vaned feathers are also present in Canadian amber (Fig. 2, D to F, and fig. S8)
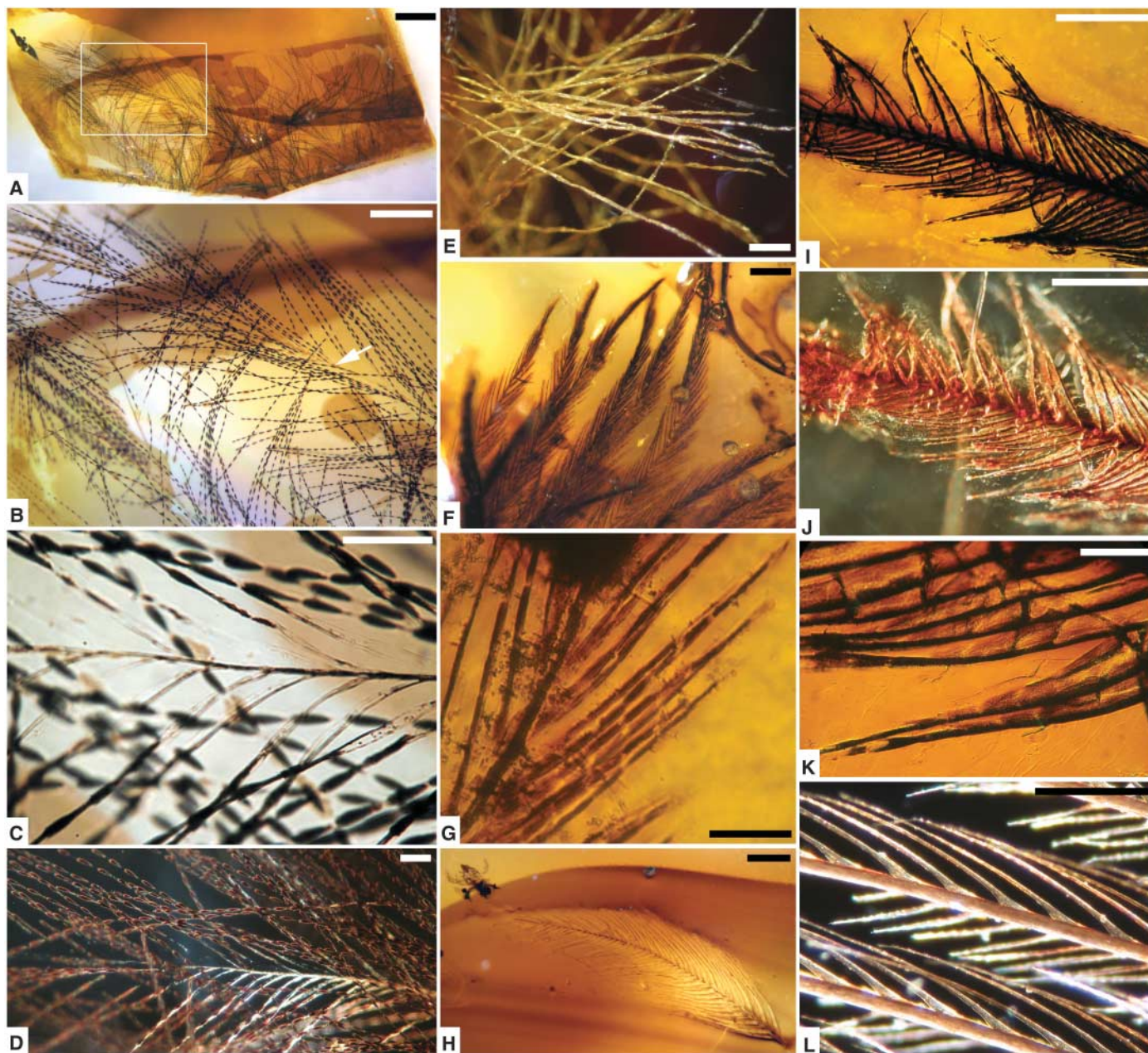
Fig. 3. Pigmentation in Canadian amber feathers. (**A** to **D**) Semi-pennaceous feathers, TMP 96.9.997: (A) six barbs; (B) close-up of box in (A), arrow indicates unpigmented ramus; (C) detail of ramus and barbule bases; (D) dark-field micro-photograph of (C), showing brown coloration with ramus and basal internodes minimally pigmented. Density and distribution of pigments (24, 25) are con-sistent with medium- to dark-brown modern feathers. (**E**) Unpigmented downy barbules, TMP 79.16.12. (**F** to **K**) Poorly differentiated, flattened barbules: (F) partial overview of 16 pennaceous barbs, TMP 96.9.553; (G) close-up of (F), showing variable, diffuse pigmentation within barbule bases (ventral plates translucent, dorsal flanges pigmented); (H) unpigmented, isolated barb with juvenile mite, TMP 96.9.546; (I) central portion of isolated barb, TMP 94.666.15; (J) dark-field microphotograph of (I), showing overall color; (K) banded pig-mentation within basal plate of proximal barbule in (I), indicating 5 to 6 com-ponent internodes. (**L**) Reduced pennaceous barbs from non-interlocking region of dark brown and white mottled chicken contour feather for comparison. Scale bars, (A) 0.5 mm; (B), (E), (F), (H) to (J), and (L) 0.2 mm; (C), (D), (G), and (K) 0.04 mm.

(10). These were probably borne by an animal capable of flight. Within UALVP 52820, barbules of unequal lengths arise from either side of the barb, producing a differentiated series of longer proximal (~0.42 mm) and shorter distal (~0.24 mm) elements, all having spinose nodal projections. Barbules are widely spaced along a thick ramus (barb shaft) adapted for rigidity and are strongly differentiated to interlock with adjacent barbs to form a vane (10).

On the basis of the presence of a rachis in TMP 96.9.334 and differentiated barbules in UALVP 52820, these specimens can be assigned conservatively to stages IV and V and are attributed to Late Cretaceous birds. The remaining six feathers are fragmentary downy and contour feathers (Fig. 3). Although they offer limited insight concerning the identity or behavior of their bearer, their structure and pigmentation bear directly on feather evolutionary stages. Four of the six feather fragments in TMP 96.9.997 (Fig. 3, A to D, and fig. S9) are aligned. Superficially, these exhibit an intermediate morphology (stage IIb) proposed for an Early Cretaceous (late Albian) French amber specimen (4). In the Canadian specimens, as in the French material, the main axis preserved is short (3.7 mm) and weakly defined, dorsoventrally flattened, and composed of fused secondary branches in an opposite arrangement. However, in the Canadian specimens intense pigmentation in each internode produces a beaded appearance, highlighting segmentation that is otherwise difficult to discern based on barbule diameter variation (Fig. 3C). Segmentation identifies the finest branches as barbules attached to narrow rami, and not barb equivalents attached to a rachis. This interpretation identifies these small specimens as subcomponents of a larger feather, such as basal barbs on a contour feather (17), and not a distinct stage in feather evolution lacking barbules (4). This interpretation probably extends to the French material as well. Pigmentation is preserved with fidelity in all additional specimens. Although downy feathers are consistently transparent, and would have been white in life, pennaceous feathers are more variable, with diffuse, transparent, and mottled patterns of pigmentation (Fig. 3, E to L) that match those observed in modern birds (10, 24, 25).

Although neither avian nor dinosaurian skeletal material has been found in direct association with amber at the Grassy Lake locality, fossils of both groups are present in adjacent stratigraphic units. Hadrosaur footprints are found in close association with the amber, and younger (late Campanian and Maastrichtian) strata of western Canada contain diverse nonavian dinosaur (26) and avian (27, 28) remains. There is currently no way to refer the feathers in amber with certainty to either birds or the rare small theropods from the area (26). However, the discovery of end-members of the evolutionary-developmental spectrum in this time interval, and the overlap with structures found only in nonavian dinosaur compression fossils, strongly suggests that the proto-feathers described here are from dinosaurs and not birds. Given that stage I filaments were present in densities relevant for thermoregulation and protection, and that comparable structures are preserved as coronae surrounding compression fossils, it becomes apparent that protofeathers had important nonornamental functions. Specialized barbule morphologies, including basal coiling, suggest that Campanian feather-bearers had already evolved highly specialized structures similar to those of modern grebes to enhance diving efficiency.

Canadian amber provides examples of stages I through V of Prum's (11) evolutionary-developmental model for feathers. None of the additional morphotypes observed in compression fossils of nonavian dinosaurs (8, 15) or amber (4) were found here, suggesting that some morphotypes may not represent distinct evolutionary stages, or may not have persisted into the Late Cretaceous. The snapshot of Campanian feather diversity from Canadian amber is biased toward smaller feathers, subcomponents of feathers, feathers that are molted frequently, and feathers in body positions that increase their likelihood of contacting resin on tree trunks. Despite these limitations, the assemblage demonstrates that numerous evolutionary stages were present in the Late Cretaceous, and that plumage already served a range of functions in both dinosaurs and birds.

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Trace Metals as Biomarkers for Eumelanin Pigment in the Fossil Record

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Well-preserved fossils of pivotal early bird and nonavian theropod species have provided unequivocal evidence for feathers and/or downlike integuments. Recent studies have reconstructed color on the basis of melanosome structure; however, the chemistry of these proposed melanosomes has remained unknown. We applied synchrotron x-ray techniques to several fossil and extant organisms, including *Confuciusornis sanctus*, in order to map and characterize possible chemical residues of melanin pigments. Results show that trace metals, such as copper, are present in fossils as organometallic compounds most likely derived from original eumelanin. The distribution of these compounds provides a long-lived biomarker of melanin presence and density within a range of fossilized organisms. Metal zoning patterns may be preserved long after melanosome structures have been destroyed.

Feather color in birds stems mostly from chemical pigments, of which the most widely used are melanins (1). Resolving color patterns in extinct species may hold the key to understanding selection processes that acted during crucial evolutionary periods and