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The dinosaurian origin of feathers: perspectives from dolphin (Cetacea) collagen fibers

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Abstract The early origin of birds is a hotly disputed debate and may be broadly framed as a conflict between paleontologists and ornithologists. The paleontological emphasis has shifted from *Archaeopteryx* and its origins to recent finds of Cretaceous birds and “feathered” dinosaurs from China. The identification of alleged feathers has, however, relied principally on the visual image. Some workers have interpreted these integumentary structures as collagen fibers. To test the latter hypothesis, using light microscopy, collagen from the hypodermis (blubber) and subdermal connective tissue sheath was examined from a dolphin that had been buried for a year as part of an experiment. Within the blubber, toward the central thicker parts of the material, the collagen fibers had compacted and the three-dimensional latticework of normal blubber had more or less collapsed. Chromatographic analysis of the blubber revealed pronounced oxidation of the unsaturated lipids, probably accounting for the collapse of the latticework. Fibers normally bound together in bundles became separated into individual fibers or smaller bundles by degradation of the glue-like substance binding them together. These degraded collagen fibers show, in many instances, feather-like patterns, strikingly reminiscent of many of those identified as either “protofeathers” or “modern” feathers in dromaeosaurid dinosaurs. The findings throw serious doubt on the virtually complete reliance on visual image by supporters of the feathered dinosaur thesis and emphasize the need for more rigorous methods of identification using modern feathers as a frame of reference. Since collagen is the main fiber type found in most supporting tissues, the results have wide implications regarding the degradation and fossilization of vertebrate integument, such as that of the ichthyosaurs, dinosaurs and birds.

Introduction

Declarations that any remaining doubts that birds evolved from small, feathered dromaeosaurid dinosaurs should be laid to rest are becoming commonplace. However, a pivotal question of when is a feather a feather, based on fossilized material, is moot, and answers are far from unequivocal. A notable, if controversial, view is that collagen is the biological material preserved in the integument of the famous Chinese dromaeosaurid dinosaurs (Feduccia 1999), not feathers as postulated in a number of recent high-profile articles (Prum 1999; Ji et al. 2001; Norell et al. 2002). This is a plausible interpretation given the wealth of data that has accumulated over the past 25 years on the complex architecture of collagenous fibers in the dermal and subdermal layers of the skin in a wide range of vertebrates, e.g., sharks (Motta 1977; Wainwright et al. 1978), bony fish (Hebrank and Hebrank 1986), dolphins (Pabst 1996), snakes (Jayne 1988), loggerhead turtles (personal observation), urodeles (Frolich and Schmid 1991), and anurans (Greven et al. 1995). Integumental structures in mammoths (Kukhareva and Ileragimov 1981) and ichthyosaurs (Lingham-Soliar 1999, 2001) have also been interpreted as being part of a collagenous fabric.

Type I collagen fibers, the most common type in vertebrates, are normally organized in bundles of fibrils (about 80–90 nm thick), the structural unit of the fibers (Axer et al. 2001). Each fiber is usually 8–10 μm thick. However, what are usually referred to as fibers are in fact bundles or fascicles of fibers (Fig. 1) frequently several hundred microns thick (unpublished data on collagen fiber bundles in the white shark, *Carcharodon carcharias*). There is, thus, considerable potential for post-mortem disorganization of the fibers following degradation, which may be made permanent during fossilization.

Integumentary structures in fossils are difficult to assess because of complex changes following the death of an animal, ranging from taphonomic changes resulting from microbial and chemical degradation (Allison 1988; Allison and Briggs 1991) to compaction and weathering

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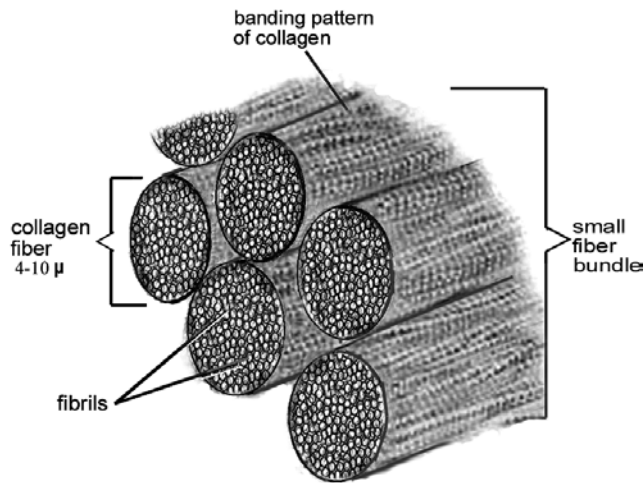


Fig. 1 Schematic illustration of type I collagen fibers. The typical banding of type I collagen fibrils is readily detectable in longitudinal view. The five fibers depicted comprise a bundle and many such bundles may form larger fiber bundles

(Koch et al. 2001). Difficulties include scanty information on postmortem organic changes (humans excluded), in particular on the degradation of integumental collagen.

Fossilized soft tissue preservations are both rare and valuable (in information content) and consequently warrant rigorous tests to enable reliable interpretations. The aim of the present investigation was to test how collagen might be preserved in fossils by burying a dolphin for a year so as to obtain data on the important stages of decomposition that immediately precede potential fossilization following the death of an animal.

Materials and methods

The experiment involved burial of an entire juvenile bottlenose dolphin, *Tursiops truncatus* (1.2 m long), in semiporous river sand for 1 year (December 2001–December 2002) at a depth of approximately 0.75 m (recovery of skin tissue was first made after 6 months but fiber degradation was not significant). Thin fragments of the blubber and subdermal connective tissue sheath (SDS; Pabst 1996) were recovered from the decomposing dolphin (December 2002) and slide-mounted along tangential planes (TP; parallel to skin surface). For comparison, TP sections (about 25 μm thick) of fresh dolphin blubber (adult female 2.46 m long) and of the dermis of the white shark, *Carcharodon carcharias* (adult female 4.7 m long) and tiger shark *Galeocerdo cuvier* (cover), were prepared by cryostat-sectioning. Materials were examined and photographed using a Zeiss Axiophot light microscope with differential interference contrast (DIC) (Fig. 2). Fiber measurements were made using a Panasonic digital biomed analyzer.

Results

Light microscopy revealed that the SDS comprised collagen fiber bundles 30–40 μm in diameter in several compressed layers. Fibers were eroded, although occasional patches of fairly well preserved fibers, oppositely oriented in two discernible layers (Fig. 2a), were evident.

In other areas of the SDS, the fibers had a clearly wavy structure that produced a downy appearance (Fig. 2b). Within the blubber, toward the central thicker parts of the section, the collagen fibers had compacted and the three-dimensional latticework, observable in fresh dolphin blubber, had more or less collapsed. Chromatographic analysis of this layer revealed pronounced oxidation of the unsaturated lipids, which probably accounts for the collapse of the latticework (only saturated fatty acids with 14, 16, and 18 carbon atoms remained) (O. Grahl-Nielsen, personal communication 2003; Fig. 3). Nearer the edges of the blubber sections (Fig. 2c–e) there was wholesale breakdown and disruption of fiber bundles of various size classes (Fig. 2f, g). The process of “peeling” apart of the collagen bundles was evident in many places (Fig. 2h).

Discussion

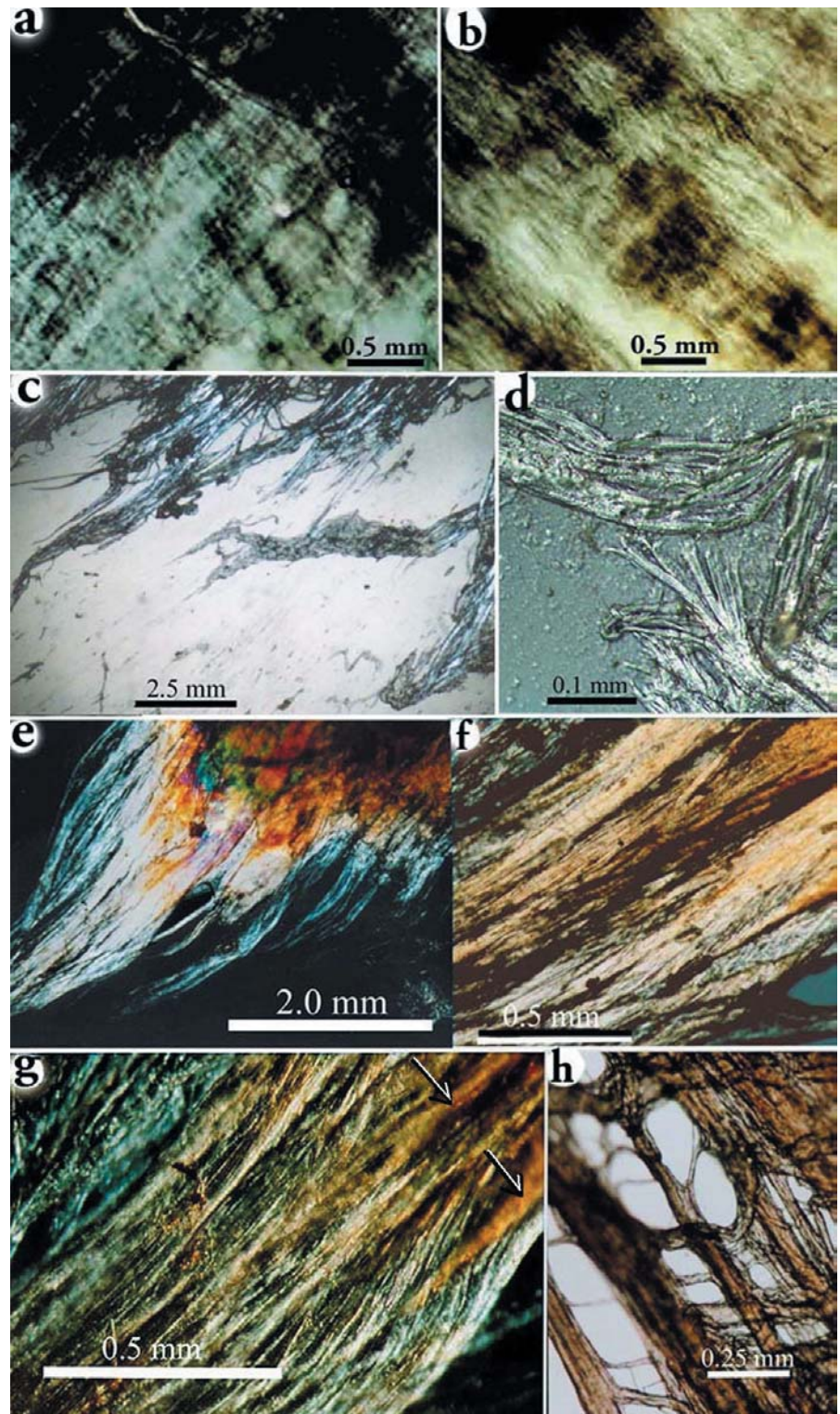
Lewis and Johnson (2001) noted that during the failure process of cartilage, peeling of collagen fibrils occurs, apparently as a consequence of the breakdown of a “glue” that is considered to hold them together. I interpret the peeling, in this case of the fibers and fiber bundles (Fig. 3h), to be caused by the degradation of a similar “glue”, presumably as a consequence of bacterial activity. The “glue” was less evident along the edges of the sections where more of the fibers had been released from their bundles.

A feature widely noted in the preserved decomposing tissue concerns the bead-like structure of the collagen fibers. A similar condition was noted in fossilized integumental fibers in ichthyosaurs. Under polarized light at different azimuths, TP sections of dolphin blubber and shark skin showed peaks and troughs of the fibers, a consequence of regular, short waves (at about 50 μm intervals), which coincided with the observed bead-like structure (cover) and confirmed that they were not breaks in the fibers. The feature was an artifact of preparation and dehydration. All sections were air-dried to increase transparency and birefringence during transmission microscopy, which resulted in slight contraction of the fibers.

Given that degrading collagen fibers demonstrate a striking resemblance to feathers, it is clearly very difficult to assign objective meaning to interpretations of dinosaur feathers, and more so to “protofeathers”, purely from visual images. This highlights the need for additional studies in which measurements are made and strict comparisons with the structure and proportions of modern feathers are presented.

The new “filament-to-feather” model for feather origin (Prum 1999) has served as a challenge to the classic “scale-to-feather” model (Maderson 1972; Regal 1975; Maderson and Alibardi 2000). The new model, allegedly supported by “branched” structures, based on scanty fossil evidence, was recently shown to be highly speculative (Lingham-Soliar 2003). I demonstrate here how difficult it would be to distinguish between collagen fibers of the integument, with an almost limitless potential for pattern

Fig. 2 Fine fibers from the SDS (subdermal connective tissue sheath) of the decomposing dolphin observed by polarized light microscopy: **a** layers of fine collagen fibers undergoing degradation but still retaining tension; **b** fine collagen fibers losing their tension and taking on a wavy appearance. Collagen fibers in decomposing dolphin blubber observed under polarized light: **c** peripheral collagen fiber bundles extending about 10–15 mm from the edge of the small sample (they may be traced a similar distance into the material); **d** detail of single fibers in **c** showing component fibers during degradation, including filamentous branching patterns; **e** plume-like patterns of some of the fibers; **f** two thick fiber bundles; **g** fiber bundles in **f** showing disorganization of the finer fibers and bundles to produce overlapping feather-like patterns (note that **f** and **g** indicate how different levels of magnification and preservation capture feather-like patterns of different size classes; *arrows* show small fiber bundles); **h** peeling of fibers and coalescing with others, indicating a glue-like substance binding fibers together

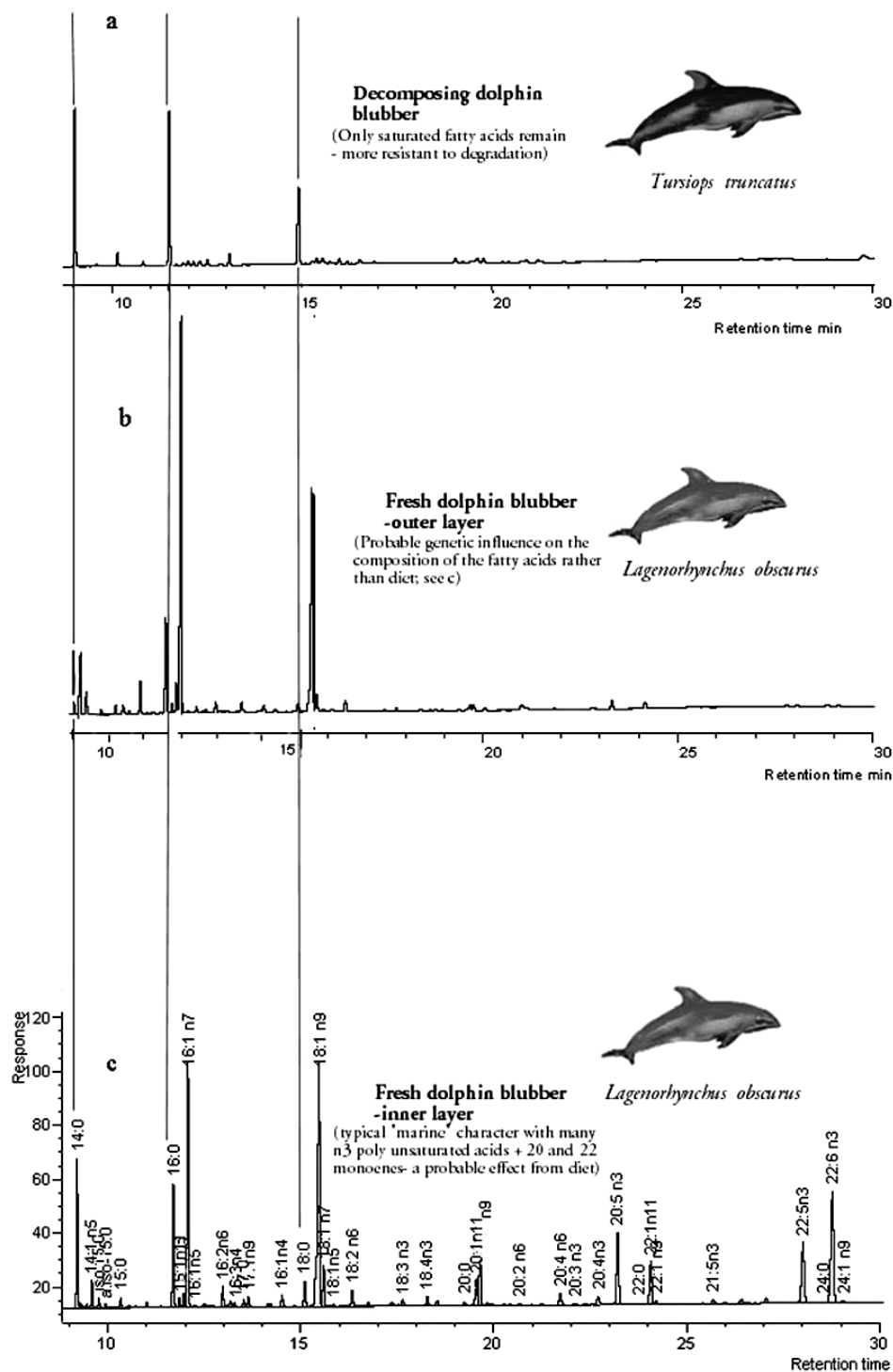


permutations, and the alleged external “appendages” in *Sinornithosaurus millenii* (Xu et al. 2001, their figure 2).

In the light of the present findings, the nature of feather-like impressions in dromaeosaurs (Ji et al. 2001) is difficult to resolve, given that integumental fibers in ichthyosaurs are known to simulate similar patterns (Lingham-Soliar 2003). Nevertheless, some reports of “modern” feathers in

an alleged dromaeosaur (Norell et al. 2002) are compelling, although not beyond dispute. However, it is important to emphasize that there is no evidence that relates possible “true feathers” in non-avian dinosaurs (Norell et al. 2002) with filamentous “protofeathers” (Xu et al. 2001). For instance there is similarly no implicit evolutionary connection in the occurrences of hair and

Fig. 3 Chromatogram of lipids found in dolphins: **a** the decomposing dolphin blubber in which only three saturated fatty acids predominate (14, 16, and 18 carbon atoms); in the outer layer (**b**) and inner layer (**c**) of fresh dolphin hypodermis, the unsaturated fatty acids predominate. Note, degradation of the bulk of the unsaturated fatty acids evidently accounts for the collapse of the fiber matrix in the decomposing blubber (modified after O. Grahl-Nielsen, personal communication, 2003, Bergen University, Norway)



collagen fibers found in close association in mammoths (Kukhareva and Illegimov 1981).

The “wavy” feature of the fibers shown in Fig. 2b is consistent with the behavior of collagen. When tension is lost via loss of muscular tone, collagen fibers may bend or fold sharply (Gordon 1978). Dehydration, as observed in preparations, may on the other hand produce shorter, regularly spaced creases or waves in the fibers. Thus, during fossilization, collagen fibers may only appear

straight if they are rapidly mineralized (Allison and Briggs 1991) before loss of muscular tone, or dehydration. Disregard for such conditions has led to several narrowly focused views in assessments of the Chinese “feathered” dinosaurs. For instance, “wavy” fossilized fibers described in the theropod dinosaur, *Sinosauropteryx* (Currie and Chen 2001), as “soft and pliable”, conform in nature and behavior to collagen fibers. Waviness suggests that mineralization may have been relatively

slow. Slower mineralization may also account for darker edges and lighter centers of integumentary structures (figure 1f in Lingham-Soliar 2003), in contrast to the interpretations of similar features in a dromaeosaur as reflecting hollow integumental structures resembling the hollow rachis of feathers (Currie and Chen 2001).

We may obtain a better idea now of how some rare preservations of fine integumental fibers occurred in the ichthyosaur, *Ichthyosaurus* (see figure 2a in Lingham-Soliar 1999), from strikingly similar patterns of fibers of the decomposing dolphin SDS (Fig. 2a). For example in both cases erosion of fibers, providing “windows” into the underlying fibers, suggests that microbial degradation may have occurred during burial in mud on the sea floor, and the beaded form of some fibers suggests possible dehydration. The fine preservation of layers of fibers (Lingham-Soliar 1999) was evidently aided by encasement in a mudstone nodule, perhaps comparable with burial of the dolphin. Thus, some features of the preservation suggest that geological erosion and compaction may play a significantly smaller role in the loss of information, in certain circumstances, than, for example, pre-fossilization processes such as the rate of decomposition of the tissue and the role of authigenic minerals in “freezing” the material (Briggs and Wilby 1996) before the loss of vital information. Under laboratory conditions, experimentally induced mineralization was found to be initiated within 2 weeks (Briggs and Kear 1993). However, certain specific conditions must prevail in order for mineralization of soft tissue to occur. The present documentation of the survival of collagenous fibers (and muscle tissue) after a year’s burial show that minimally two conditions for preservation – rapid burial and inhibition of bacterial decomposition (Allison 1988) – increase the chances of subsequent mineralization in, for instance, phosphate-rich sediment pore waters (Allison and Briggs 1991).

Skepticism about the theory of the dinosaurian origin of feathers is supported by other areas of study too. For instance, recently Sawyer et al. (2003) showed that feather β -keratins are also expressed in embryonic scutate scales. Moreover, relevant to the present findings, it must be emphasized that that none of the papers that allege the presence of feathers in dinosaurs allude to an available technique for distinguishing between β -keratin and collagen (Davis and Briggs 1995).

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