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How Microbes helped solve a Complex Biomechanical Problem associated with Bird Flight

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Abstract

Bird feathers are made of the toughest natural elastomeric biopolymer, β-keratin. The almost inextricable bond between the fibre and matrix texture of β-keratin has made it virtually impossible to ascertain a fibre hierarchy in the main support structures of the feather, the rachis and barbs, other than filaments nanometres in diameter thick. To circumvent the limits of conventional structure-determination methods microbes were used for the first time to help resolve a biological structural problem. Naturally occurring feather parasites, fungi, were allowed to grow in feathers under laboratory conditions, the hypothesis being that they would preferentially degrade the matrix and release the fibre components. The result was that microbes revealed for the first time the true feather microfiber hierarchy, which included the thickest fibres known in β-keratin by a magnitude of three. These fibres were named syncitial barbules because they showed a system of intermittent nodes as in free down feathers. The side walls of the rachis and barbs were similarly investigated and revealed a crossed-fibre system seen for the first time in feathers. Both discoveries have profound biomechanical significance including a high work of fracture in the feather.

Keywords: Feather; β-keratin; Fibre hierarchy; Syncitial barbule fibres; Crossed-fibre system; Biomechanics

Introduction

Although the role of microorganisms in the transformations of organic matter was not recognized until the middle of the nineteenth century, microbial processes have been used by humans since prehistoric times in the preparation of food drink and textiles. Such traditional microbial processes became perfected to an astonishing degree as used in bread making and production of beer and wine, pickling, making of vinegar, cheese and butter and retting of flax. The rise of microbiology led to great improvements in many of these but also to the development of new industries based on the use of microorganisms [1]. However, the present discussion shows the use of microorganisms in a way that had arguably never been attempted before–in delineating a complex biological structure.

The feather is an extraordinary device and among the most prominent of a series of adaptations that facilitates flight in birds. The main structural support is the rachis from which arise hundreds of side branches, the barbs. The rachis is symmetrically located in contour feathers but nearer the leading edge (asymmetrical) in flight feathers. The outer shell or cortex comprises the bulk of the material of the rachis and has been shown to account for most of its tensile strength [2]. It is constructed of compact β-keratin, the keratin of reptiles and birds (sauropsids), a light, rigid material comprising a fibre-matrix texture [3,4]. Filshie and Rogers' [5] study on the microstructure of the rachis using histological techniques and early use of electron microscopy showed that it comprised fine fibres, nanometers in diameter (Figure 1c). This view has dominated our ideas on feather microstructure through the decades.

A simple construction of longitudinally oriented fibres just nanometers thick posed an important problem for biomechanics: the rachis appeared to lack a high work of fracture mechanism. High work of fracture is a term that refers to a material that has a high ability to resist fracture (Griffith fracture [6]) and if started to resist it spreading throughout the material. Cracks in both natural and man-made materials can be disastrous for structures if they get longer and longer and spread rapidly through the material without some form of crack-stopping mechanism [6]. Without such a mechanism in the feather rachis e.g. it would mean that longitudinal (even transverse) splitting" in the feather rachis would be relatively easy and would severely compromise bird flight. This is clear from the extraordinary demands on the feather connected with flight-requiring qualities that are almost paradoxical, having to be exceedingly light (or the bird would never

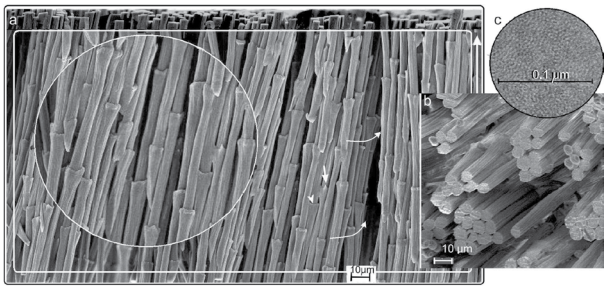

Figure 1: SEM of fibres (syncitial barbules) in the cortex of feather rachis of *Gallus gallus* exposed after fungal biodegradation of matrix (resin embedded and etched). **a** All fibres show regularly spaced syncitial nodes that extend in the proximo-distal direction of the rachis. The syncitial nodes show variations in morphology, terminating in hooks (straight arrow) or a ring (arrowhead), while others are intermediate between the two. Fibres are densely packed through the cortex (curved arrow) and indicate that the nodes are staggered in arrangement on two- and three-dimensional planes. Inset, detail. **b** Cross-section of syncitial barbule fibres of the rachis. **c** Rachis fibre cross-sections revealed by SEM in Filshie and Rogers [5]. About 40 filaments (TS) fit on a 0.1 µm bar. **a** By courtesy of the Royal Society of London and **b** authors unpublished data.

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leave the ground) and at the same time exceedingly tough to cope with the stresses of flight. Such stresses include accelerations that may reach extremely high gravitational (g) forces, in some bird species thought to be as high as 9 g [7], about 3 times that experienced in a jet fighter plane. The notable engineer John Gordon [8] wrote in 1978 "feathers probably do not need to be especially strong, but they do need to be stiff and at the same time resilient and to have a high work of fracture." This intuitively made sense to him despite as he said "the work of fracture mechanism of feathers is something of a mystery; at the time of writing I do not think anybody knows how it works."

The problem remained for a further 30 years. X-ray diffraction analysis while useful with respect to molecular structure and fibre angles [9,10] had produced no data on gross hierarchical structure and morphology of the filaments. Other attempts by workers were made to understand feather ultrastructure by histodifferentiation [11,12]; prematurely they proposed that β-keratogenic tissue of the rachis and barbs was fully characterized i.e. the bulk of the rachis, calamus, and barb rami are comprised of typical, tile-like, stratified squamous epithelial tissues. As we will see not only was this incorrect but it was an architecture that failed to answer the 64-thousand-dollar-question of a crack-stopping mechanism in the feather.

It appeared to me that there had to be a more complex fibre microstructure of the feather that was evading detection by conventional means namely, standard sectioning and histological methods [5] and histodifferentiation [11]. I hypothesized that the bond achieved by the amorphous polymer matrix ('glue') and the polymeric filaments of keratin was so tight that if there was a fibre hierarchy it was lost because of this tightness and efficiency of the bond (analogous to tightly glued matchsticks in which the glue and the matchsticks were of a near similar composition–dissections would simply reveal the inner structure of the matchsticks but not the matchsticks themselves).

It seemed the solution was to find a means to get around the almost inextricable bonding between the filamentous and matrix texture of feather keratin. It is well known that feathers in the living bird harbour naturally occurring keratinophilic fungal genera [13-15]. My hypothesis was that the fungi fed solely on the matrix or glue that held the fibres together rather than the fibres themselves because the latter might be too tough for fungal hydrolyses. This hypothesis was aided by our knowledge on the chemical composition of α-keratin i.e. of two general classes of proteins, a high-sulphur fraction of the amorphous matrix (derived from the sulphur–sulphur cross-links that keep the fibres intact) and a low sulphur fraction of the microfibrillar component. If this was so and similar in β-keratin then by selectively 'feeding' on or hydrolysing the matrix, fungi would release the fibres from their bonds.

The hypothesis may have been complex but the method I used could not have been more basic. I simply allowed the naturally occurring avian fungi to degrade feathers in the disembowelled cadavers (skins with feathers attached, equivalent to mammal pelts) of dead domestic chickens, *Gallus gallus* under laboratory conditions.

Materials and Methods

Details of the experiment were previously published as Supplementary Information (online) in the study [16] and can be seen here in Appendix 1.

Results and Conclusions

The results of the experiment were quite spectacular [16]. The fungi (identified by rRNA analysis as several species of Alternaria; see Appendix 1), had preferentially degraded the amorphous keratin matrix through the entire depth of the dorsal and ventral walls of the rachidial cortex and left the 'fibres' cleanly exposed as revealed by scanning electron microscopy. The SEMs (Figure 1a,1b) showed for the first time in about 150 years of feather research densely packed, predominantly axially oriented filaments with an average diameter of approximately 6 μm, the thickest fibres by far recorded in the structural elaboration of β keratin by three orders of magnitude (Figure 1c). The fine detail exposed by fungal biodegradation has enabled observation of the most striking and highly unexpected feature of the fibres-nearly periodic nodes at intervals of approximately 70 μm along the entire length of each fibre. Each node terminates in hooks or a ring (Figures 1a, straight arrow and arrowhead resp.). In both features, they resemble structures observed in free, single filamentous, embryonic (chick) and plumulaceous down filaments, the syncytial barbules [17,18] but never before seen comprising a composite structure (the rachidial cortex) of thousands of such filaments. Consequently, these mega-fibrils of the feather rachis microstructure (observed in a number of bird species including e.g. most recently in the pygmy falcon, *Polihierax semitorquatus* (Figure 2) were named syncitial barbule fibres or cells.

With the publication of the paper [6] I sent a copy to the notable expert on feather structure, Peter Stettenheim, co-author of the 2-volume tome on feather structure [18]. He replied immediately stating, "it is a fascinating and very original piece of work, both for its findings and its method of feather preparation… Your finding not only furnishes a good indication of how the rachis originated, but also seems to support the old notion that the earliest feathers were downy, not pennaceous…" He presciently noted that my use of fungi was similar to the use of dermestid beetles (Dermestidae, order Coleoptera) in cleaning furs and skins.

There was more work to be done. The side walls of the rachis are much thinner than the dorsal and ventral but historically also thought to comprise ultra-thin longitudinally oriented fibres. Using microbes again, the findings were just as surprising as the previous study [19]. The major part of the lateral walls of the rachis were not composed of longitudinal fibres at all as previously envisaged. Instead they comprised layers of oppositely oriented fibres forming a trellis- or mesh-like architecture (Figure 3) that had previously been observed in

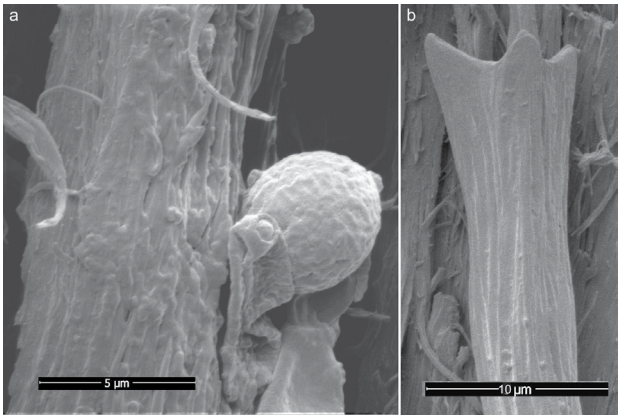

Figure 2: SEM. Mega-fibres (syncitial barbules) in the rachis of a moulted feather of the pygmy falcon, *Polihierax sem*itorquatus (native cortex was peeled longitudinally for direct SEM observation). **a** Shows a fungal species (possibly Alternaria) adjacent to an exposed partially delineated fibre. **b** Shows the nodal end of a delineated fibre complete with hooks.

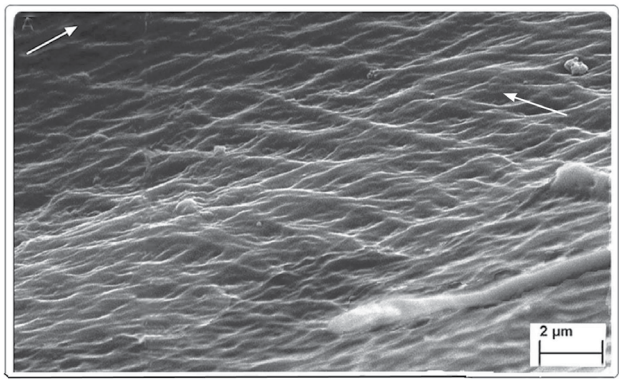

Figure 3: *Gallus gallus*, alternating cross-fibre structure of epicortex or lateral wall of the feather rachis. Arrows show direction of fibres in two adjacent layers. Fungus in bottom right corner shows papulose apical tips of hyphae.

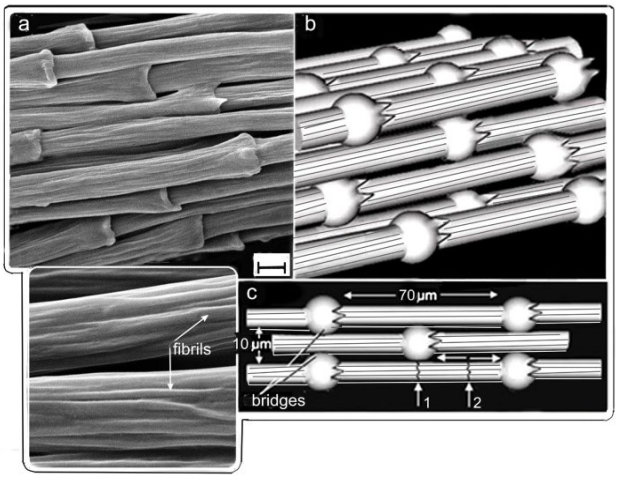

Figure 4: Mechanical structure of syncitial barbule cells (mega-fibres). **a** Syncitial barbule cells in the cortex of the feather rachis showing nodes (inset below shows detail of the syncitial barbule cells, comprised of smaller fibrils and they in turn of smaller fibrils and so on). **b** Diagrammatic representation of fibre bundling (syncitial barbules) in three-dimensions. **c.** Diagrammatic brick-bridge mortar structure between syncitial barbules and polymer matrix demonstrating crack-stopping mechanisms (see text). Scale bar 5 µm. Permission of Journal of Ornithology.

a range of invertebrate animals [20] and subsequently in high-speed swimmers such as tuna [21], dolphins [22] and the white shark [23,24], with profound biomechanical implications. However, this was the first time it was seen in birds and in keratin per se.

All the discoveries above were exactly the same for the feather barbs (the hundreds of side branches on either side of the rachis that make the feather venation.

Finally, this still left the critical question, did the new understanding of feather microstructure, revealed so beautifully by microbes, advance our understanding of feather microstructural biomechanics and principally of a crack-stopping mechanism or high work of fracture in bird feathers? We look at this briefly here although more details can be found elsewhere [6,25].

With respect to crack-stopping the most significant features were the nodes seen at the highest thickness level (hierarchy) of the fibres of the rachis and barbs i.e. the characteristic structure of the syncitial barbule fibres. The node-to-node regions of the syncitial barbule cell (~60–70 µm long) effectively create a repeated dogbone shape along the fibre length. Also, the nodes of one fibre are invariably staggered with those of adjacent fibres in both 2- and 3-dimensional planes (Figure 1a and 4a). Rather than the traditional brick and mortar arrangement [16], the periodic nodes suggest an architecture perhaps even more comparable with the "brick-bridge mortar" structure proposed for nacre [26,27]. The syncitial barbule nodes provide connectivity for the entire fibre system by bridging the space occupied by the matrix. As in nacre [27], such bridges are considered here to influence the strength and toughness at the interfaces and resistance to axial fracture by the pattern of crack extension. Simply put, because there is no longer an unbroken continuum of the matrix along the feather rachis, i.e. it is broken up between nodes, the crack consequently is trapped or stopped between nodes (Figure 4b,4c). Transversely, too, the staggering of the nodes would act to block or deflect the crack (Figure 4b,4c).

We will look at a few other problems that the unique fibre structure of the feather helps solve. In engineering systems, there are a number of major problems associated with materials involving polymer fibres, e.g. axial fibre fracture, fibre pull-out and, delamination of fibres as a consequence of debonding of the matrix [28]. Goodfellow [28] found through fracture mechanics that interfacial fracture might be reduced by increasing the fiber diameter or by coating the exposed fibre with silicone or a similar material. Recently, Naraghi et al. [29] working with carbon nanotubules, which are known to have among the highest individual toughness in synthetic materials, found that when they were bundled together they lose strength because of lateral slippage. They found that, by adding a polymer between the nanotubules, it resulted in very high ductility and a very high toughness (reported to be higher than Kevlar), with the ability to absorb and dissipate large amounts of energy before failure. Amazingly, Naraghi et al.'s [29] findings of bundling of fine fibres into thicker fibres and bonding the latter together with a glue to create a highly efficient structure had been 'invented' by birds 150 million years ago [6,25], a powerful testament in support of biomimetics, i.e. the lessons engineers can learn from biological structures.

I will mention a last point with respect to a further key function of the dogbone shape of the syncitial barbules in the feather rachis and barbs namely, to prevent or minimise 'pull out' of the fibres from the surrounding matrix and improve the transmission of forces (Figure 4a,b). In engineering, this is analogous in structure and function to steel rebars used as concrete reinforcement in composite materials in high-rise building construction [6,30].

Lastly, we will consider the biomechanics of the cross-fibre system in the lateral walls (epicortex) of the barbs and rachis. The cross-fibre architecture shows an anisotropic structure far more complex than previously thought. This importance of the cross-fibre architecture of the epicortex is emphasized by the fact that it occupies a surface area at least equal to that of the cortex [19]. The mechanical consequences, which can only be considered briefly here, are significant. The crossfibre system involves a specialist bioengineering design principle [6,20] that enables rigidity in torsion in cylindrical structures (high contraction or Poisson ratio). The system is widely found in nature and may be comprised of a variety of structural fibres in different organisms, including collagen, chitin, cellulose [20,31] and keratin [25]. Wainwright et al. [32] describe in shark skin the mechanical principles involved: "Since twisted cylinders of a homogeneous material fail by splitting

at 45° to their long axis the best design for torsional stiffness in the shark's caudal peduncle would be collagen fibers in the skin wrapped at 45° to the body's long axis." In the feather, the cross-fibre architecture may provide a key mechanism for preventing damage to the rachis and barbs. However, a rigid system risks being loaded with dangerously high forces during flight. In this context, it is noteworthy that the longitudinal fibre system of the cortex not only provides stiffness but, in contrast to the cross-fibre system of the epicortex, importantly, allows torsion, which would help to lower the critical bending moment needed to cause local buckling failure [33]. At the core of this understanding is the presence of two distinctive fibre systems, that of the epicortex and of the corte in feather microstructurex, which in given circumstances will inevitably function in synergy to promote ideal feather aerodynamics. Astonishingly, we owe these recent finding to my most diligent 'lab assistants', as one journalist referred to them, the microbes.

Appendix 1

Materials and methods

Feather biodegradation and SEM preparation: At the time the project was commenced in 2004-2005 there was little to go on with respect to feather keratin selective biodegradation as an experimental possibility. We proceeded in the knowledge that naturally occurring keratinophilic fungal genera occur in birds with evidence of maximum occurrence (47%) on domestic chickens (*Gallus gallus*, our principle test animal. We also knew that certain fungi i.e., white-rot fungi are known to preferentially degrade lignin from other components such as cellulose in woody plant material. We gambled on selective biodegradation occurring as a consequence of the different molecular composition of the matrix and fibers as mentioned in the introduction. Because we had little to model our biodegradation experiment on we allowed decomposition to proceed as close to nature as we thought possible-without any interference e.g. inoculation of fungi, or raising or lowering temperature and humidity (some studies on mass feather degradation were beginning to appear in the literature for industrial applications rather than as an investigative tool).

Feathers were allowed to biodegrade on 5 domestic chickens, *Gallus gallus*, in the laboratory at normal room temperature (22-30°C) and humidity (50-70%) after removal of flesh underlying the skin as well as the internal organs (similar to taxidermy preparation). For the first few weeks the specimens were placed in a fume cupboard provided with an intermittent extractor fan after which the fan was turned off.

Feather biodegradation as revealed by light microscopic examination (x1000) was slow (I (T.L-S) now realize that it was possibly light microscopy failure to reveal the degradation rather than the slowness of the degradation). After 18 months feathers were examined by SEM. The feathers examined were from the wing (primaries) and breast. Native and decomposing feathers from corresponding areas in the species examined (tangential sections of the rachis taken at ca. the mid-rachis length) were cut into 1 cm segments and split manually to expose longitudinal views of the cortex of the rachis (tangential sections i.e. parallel to the rachis surface), gold sputter-coated and examined by SEM . Alternatively, intact segments were dehydrated in a graded acetone series and gradually infiltrated and embedded in epoxy resin as done conventionally during the preparation of biological samples for transmission electron microscopy (a 42 year-old museum specimen of the Lanner falcon was also investigated for potentially degraded feather rachis, resin embedded and etched, (Figure 3A). Samples within resin blocks were sectioned both transversely and longitudinally to expose structures in these planes. The block faces were subsequently immersed for 15 min in a solution of potassium methoxide to dissolve the resin and expose the sectioned faces. Etched blocks were then rinsed thoroughly in methanol and air dried, mounted on stubs using double-sided carbon tape, rendered conductive by sputter coating lightly with gold and viewed at 5 kV using a LEO 1450 scanning electron microscope. Images were digitized and measurements of features in the samples were performed on calibrated images using Image Pro-Plus software (MediaCybernetics, USA). Several of the prepared samples showed little signs of degradation of the matrix. Native feathers were also treated to the same resin embedding and etching process; they showed similar tight bonding by the matrix as in direct SEM analysis (above) (Figure 5). Further, to ensure that the fibers and their nodes were not affected by the degradation and the resin embedding process, native rachi of domestic chicken and a number of wild birds were examined without resin embedding and etching. Although by normal histological techniques delineating of the fibres (FBRs) was extremely difficult because of the tight bond between the matrix and fibres we simply persevered by making numerous tangential sections of the rachis and hoping that forearmed with the wisdom of hindsight of what we were looking for we would eventually find regions of intact FBRs, even if not as cleanly exposed from the matrix as those from the biodegraded specimens (see ESM Figures 2 and 3).

However, we believe as far as the biodegradation part of the study is concerned it is a beginning and at the time we started there was very little to model our investigation on-we considered it best to let nature take its course. With better understanding of the fungi involved and their selectivity (certainly unrecorded before) in future biodegradation experiments on feather keratin (perhaps other keratins too) it may be more simple to inoculate feathers with concentrations of the most appropriate fungi and subject them to ideal temperatures. Significantly, one of the principle fungal species that we isolated and identified, *Alternaria tenuissima*, we subsequently discovered was also

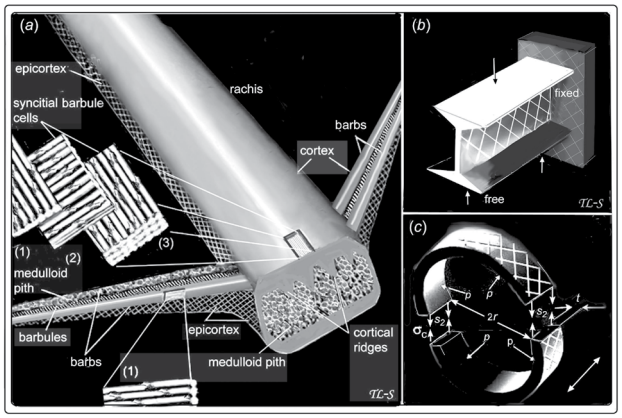

Figure 5: A new microstructural fibre model of feather rachis and barbs and classic engineering analogues. **a** An exploded view of three fibre divisions of the rachidial cortex and one of the barb cortex (both in dorsal and ventral walls). The cortex is identified by the thick syncitial barbules cells (6–8 μm in diameter). The lateral walls of the rachis and barbs, the epicortex, are characterized by a crossed-fibre structure and absence of syncitial barbules cells. One barb shows cortex removed to expose the medullary pith cells. **b** Diagrammatic view of rachis and barb as an I-beam (here, a cantilever) in which most material is concentrated in the upper (tension) and lower (compression) surfaces to resist maximum stresses–with the "web" in the middle to resist shearing forces at ~45°C. Diagrammatic view of barb as a thin-walled pressure cylinder. Slice in latter shows circumferential stress is twice the longitudinal stress. Double-headed arrow long axis of cylinder [19].

Characteristic	Isolate 1	Isolate 2
Texture	cottony	powdery
Pigmentation	Mustard-Brown at the front and orange at the back	Cream at the front, light brown at the back
Spore shape	Club-shaped	Round with a flat base
Spore and hyphal septation	Spores are divided into several cells by transverse and vertical walls. Hyphae are septate	Single celled spores. Hyphae are septate
Spore pigmentation	Brown	Colourless
Suggested identity	Alternaria sp.	Scopulariopsis sp.

Table 1: Fungal identification in degraded feather rachis.

an important species used in industrial biodegradation of feathers and showing high keratinolytic activity. We believe the biodegradation under ideal conditions (artificially created) may be substantially quicker and that it may also be aided by the absence of inhibitory toxins that may e.g. have been present in the whole chicken experiments. However, we emphasize that for the purposes of our investigation the end result i.e. separating the keratin filaments from the matrix was achieved, and we were able to robustly compare our results with those from native feathers in a range of bird species.

As a footnote, I (TL-S) believe that the above findings point the way to the use of hydrolytic enzymes to selectively delineate the fibre-matrix texture of β-keratin.

[ESM data above is available online [16].

Microbiology

This part of the analysis i.e. identification of the fungi was performed by Evodia Setati (then UKZN, now at Institute for Wine Biotechnology, Stellenbosch University)

Fungal Identification. Feathers were cut into 1 cm pieces and placed directly onto malt extract agar (MEA) and Rose Bengal chloramphenicol agar. The plates were incubated at 28°C and observed daily under the stereo microscope for formation of mycelia. Growing mycelia were sub-cultured twice onto fresh MEA plates to obtain pure cultures.

Pure isolates were inoculated onto MEA and incubated at 28°C for 5 days. Macroscopic characteristics were noted and slide preparations were made for microscopic analysis. Samples were collected from the edge of the growing mycelia and inoculated into 50 ml of medium containing (KH2PO4, 7 g/l; K2HPO4, 2 g/l; $MgSO_4.7H_2O$, 0.5 g/l; $(NH4)2SO_4$, 1 g/l; yeast extract, 0.6 g/l). The cultures were incubated with shaking at 120 rpm for 5 days. Mycelia were harvested and ground under liquid nitrogen. Genomic DNA was isolated. The fungal primer pair D1 and D2 were used to amplify the D1-D2 region of the large ribosomal subunit, using the PCR programme: 94°C (2 min); 94°C (30s), 53°C (45s), 72°C (45s); 72°C 7 min. The amplicons were then sequenced by Inqaba Biotec.

Two isolates were obtained from the feathers inoculated on MEA. The macroscopic and microscopic characteristics are summarized in Table 1. The sequence analysis of the D1D2 region was inconclusive for isolate 2 as it showed 96% similarity to *Massarina igniaria*, 93% *to Byssothecium circinans* and 94% to *Sporidesmium tengii*, and only displayed 87% similarity to *Scopulariopsis brevicaulis*. Therefore, the ITS1-5.8S-ITS2 region in the small ribosomal subunit was also sequenced, using the primer pair ITS5 and ITS4. This attempt was also not conclusive as the highest sequence similarity (95%) was with uncultured fungi.

References

1. Stanier RY, Ingraham JL, Wheelis ML, Painter PL (1989) General microbiology. 2nd Edition, Macmillan Education Ltd, Basingstoke.
2. Purslow PP, Vincent JFV (1978) Mechanical properties of primary feathers from the pigeon. J Exp Biol 72: 251-260.
3. Fraser RD, Parry DA (2008) Molecular packing in the feather keratin filament. J Struct Biol 162: 1-13.
4. Fraser RDB, Parry DAD (2011) The structural basis of the filament matrix texture in the avian/reptilian group of hard b-keratins. J Struct Biol 173: 391-405.
5. FILSHIE BK, ROGERS GE (1962) An electron microscope study of the fine structure of feather keratin. J Cell Biol 13: 1-12.
6. Lingham-Soliar T (2015) The Vertebrate Integument. Structure, Design and Function. Springer, Heidleberg.
7. Clark CJ (2009) Courtship dives of Anna's hummingbird offer insights into flight performance limits. Proc Biol Sci 276: 3047-3052.
8. Gordon JE (1978) Structures. Penguin, Harmondsworth.
9. Rudall KM (1968) Comprehensive biochemistry. Elsevier 23: 559-594.
10. Fraser RDB, MacRae TP, Parry DAD, Suzuki E (1971) The structure of feather keratin. Polymer 12: 35-56.
11. Alibardi L, Toni M (2008) Cytochemical and molecular characteristics of the process of cornification during feather morphogenesis. Prog Histochem Cytochem 43: 1-69.
12. Maderson PF, Hillenius WJ, Hiller U, Dove CC (2009) Towards a comprehensive model of feather regeneration. J Morphol 270: 1166-1208.
13. Dixit AK, Kushwaha RK (1991) Occurrence of keratinophilic fungi on Indian birds. Folia Microbiol (Praha) 36: 383-386.
14. Deshmukh SK (2004) Keratinophilic fungi on feathers of pigeon in Maharashtra, India. Mycoses 47: 213-215.
15. Kushwaha RKS, Gupta M (2004) Diversity of keratinophilic fungi in soil and on birds, in Microbiology and biotechnology for sustainable development. Microbiology 21: 59-70.
16. Lingham-Soliar T, Bonser RH, Wesley-Smith J (2010) Selective biodegradation of keratin matrix in feather rachis reveals classic bioengineering. Proc Biol Sci 277: 1161-1168.
17. Chandler AC (1916) A study of the structure of feathers with reference to their taxonomic significance. Univ Calif Publ Zool 13: 243-446.
18. Lucas AM, Stettenheim PR (1972) Avian anatomy-the integument. U.S. Government Printing Office, Washington DC.
19. Lingham-Soliar T, Murugan N (2013) A new helical crossed-fibre structure of b-Keratin in flight feathers and its biomechanical implications. Plos ONE 8: 1-12.
20. Wainwright SA, Biggs WD, Currey JD, Gosline JM (1976) Mechanical design in organisms. Edward Arnold, London.
21. Hebrank MR, Hebrank JH (1986) The mechanics of fish skin: lack of an 'external tendon' role in two teleosts. Biol Bull 171: 236-247.
22. Pabst DA (1996) Morphology of the subdermal connective sheath of dolphins: a new fiberwound, thin-walled, pressurized cylinder model for swimming vertebrates. J Zool Lond 238: 35-52.

23. Lingham-Soliar T (2005) Dorsal fin in the white shark, Carcharodon carcharias: a dynamic stabilizer for fast swimming. J Morphol 263: 1-11.

24. Lingham-Soliar T (2005) Caudal fin in the white shark, Carcharodon carcharias (Lamnidae): a dynamic propeller for fast, efficient swimming. J Morphol 264: 233-252.

25. Lingham-Soliar T (2014) Feather structure, biomechanics and biomimetics: the incredible lightness of being. J Ornithol 155: 323-336.

26. Song F, Bai Y (2001) Analysis of the strengthening and toughening of a biomaterial interface. Sci China Ser A 44: 1596-1601.

27. Katti KS, Katti DR (2006) Why is nacre so tough and strong? Mater Sci Eng C 26: 1317-1324.

28. Goodfellow B (2004) Design and application of a fiber pullout test for examining controlled interfaces in fiber reinforced polymers. NNIN REU Research Accomplishments 1: 62-63.

29. Naraghi M, Filleter T, Moravsky A, Locascio M, Loutfy RO, et al. (2010) A multiscale study of high performance double-walled nanotube-polymer fibers. ACS Nano 4: 6463-6476.

30. Santos PMD, Julio ENBS, Silva VD (2007) Correlation between concrete-to-concrete bond strength and the roughness of the substrate surface. Constr Build Mater 21: 1688-1695.

31. Gibson LJ (2012) The hierarchical structure and mechanics of plant materials. J R Soc Interface 9: 2749-2766.

32. Wainwright SA, Vosburgh F, Hebrank JH (1978) Shark skin: function in locomotion. Science 202: 747-749.

33. Corning WR, Biewener AA (1998) In vivo strains in pigeon flight feather shafts: implications for structural design. J Exp Biol 201: 3057-3065.