Effects of a FCBP gene polymorphism, location, and sex on Young’s modulus of the tenth primary feather in racing pigeons

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Young’s modulus (E) is a measure for stiffness of a material and a higher E means a higher stiffness. The respective polymorphism of the feather corneous beta-protein gene causes the replacement of glycine by cysteine. We looked for possible effects of the three FCBP genotypes on E in the 10th primaries of racing pigeons. However, we did not find a statistically significant difference of E between the genotypes, even within the sexes and/or within different locations under our test conditions. Our findings do not preclude the possibility that under other conditions (temperature, moisture) an influence of the glycine/cysteine polymorphism on E may exist. Compared to the more proximal locations of the rachis (base and middle) we observed lower values for E in the distal region (tip). The 10th primary constitutes the leading edge of the pigeon wing and this special function may require higher stiffness in the proximal parts of the shaft. We observed significantly higher values of E in females than in males, which result only from statistically significantly higher values in the middle region. The higher stiffness of female primaries may also contribute to the better results of hens compared to cocks in pigeon races.

To carry out their functions during flight, the shafts of avian primaries should be of low weight and tolerate a certain degree of bending without breaking. The primaries consist mainly of the protein β-keratin¹,², which after new findings nowadays should be termed feather corneous beta-protein (FCBP)³,⁴. It is made up by ~ 100 amino acid residues and has a molecular weight near 10 kDa⁵. Like other corneous substances it exhibits a filament/matrix texture²,⁶. The framework of the filament has a helical structure with four repeating units per turn and a pitch length of 9.5 nm. According to EM studies by Filsie and Rogers⁷ the diameter of the filaments (named microfibrils by them) is about 3 nm. They are embedded in the matrix material and the centre-to-centre separation is in the order of 3.5 nm. The two components, filament and matrix, are formed by a single protein⁵.

The rachis of primaries consists of a dense cortex and a foamy medulla⁸–¹¹. These authors agree that the stability of the rachis is mainly based on the geometry of the cortex. The medulla contributes only 16.1% to the dorso-ventral stiffness and 7.8% to the lateral stiffness in pigeon primary shafts¹¹, but it essentially reduces the weight of the rachis. For the calamus cortex, Earland et al.¹² could show that in the interior two thirds the molecules are orientated parallel to the calamus axis whereas the exterior layer lies at right angles to the axis. Astbury and Bell¹³ observed longitudinally directed polypeptide chains for the most part of the rachis and a thin outer layer running at right angles to this. Partial degradation of feathers by microorganisms¹⁴,¹⁵ could visualize fibres of 6–8 µm diameter which are arranged in the dorsal and ventral wall of the rachis cortex in three layers: a thick longitudinal layer adjacent to the medulla, a second layer surrounding the first one circumferentially which is covered by a thin third layer with longitudinally directed fibres. The lateral sides of the cortex, named epicortex, are formed by a cross-fibre architecture, thus enabling rigidity in torsion.

Most authors cited in the last paragraph concurrently state a neglecting role of the chemistry of the corneous material for the mechanical properties of the rachis. In chickens the frizzle locus, which causes curled feather rachis and barbs, is associated with a corneous region enriched with genes coding for FCBPs. Sequence analyses of the keratin gene cluster identified a 69 bp in frame deletion in a conserved region of KRT75, a keratin gene¹⁶.
In domestic pigeons Dybus and Haase\textsuperscript{17} detected a polymorphism in the feather corneous beta-protein gene (feather beta-keratin gene) which causes the replacement of glycine by cysteine and vice versa. Cysteine residues can form disulfide bonds and thereby can play a crucial role for fibrous proteins. Thus, it seems possible that the polymorphism in the \textit{FCBP} gene may affect the mechanical properties of primary feathers. In this paper we are going to study the influence of the \textit{FCBP} gene polymorphism on mechanical characters by comparing rachides from racing pigeons carrying either Cys/Cys or Cys/Gly or Gly/Gly variants.

In many avian species of various orders striking differences in the plumage between the two sexes can be observed. This sexual dimorphism can concern size, shape, and colour of feathers. In some cases [e.g. mallards (\textit{Anas platyrhynchos}, with curled feathers in the drake's tail) and chickens (\textit{Gallus gallus}, sickle-shaped rectrices in the cock's tail)] gonadal hormones induce the dimorphism, but in other species different genetic mechanisms are involved\textsuperscript{18–20}. To our knowledge no results concerning influences of sex on mechanical properties of primaries have been published. In our material, primaries from both female and male pigeons were investigated. Thus, in the present study, we aimed to detect possible differences affecting mechanical properties in the two sexes. This seems to be of special interest since female pigeons have been reported to show significantly better racing results than males\textsuperscript{21}.

Cross sections of remige shafts look very different depending on the location of the cross section\textsuperscript{8,9}. Several authors observed local differences of mechanical properties in remiges of mute swan\textsuperscript{1}, goose, swan\textsuperscript{22}; pigeon, barn owl\textsuperscript{23} and contour feathers of chicken, turkey, ring-necked phaeanst, herring gull\textsuperscript{24} with increasing values of Young's modulus (E) from the base to the tip. No such differences were found in a primary of the ostrich\textsuperscript{22} and in the tail coverts (train) of the peacock\textsuperscript{24} hinting to the role of flying on local mechanical properties of feather rachides. Experimental recordings of in vivo strains on the shafts of various primaries and a secondary of flying pigeons indicate peak strain values in the 8th primary with a lower value in the 9th and a falling tendency among the more proximal primaries and the secondary\textsuperscript{25}. The 10th primary forms the leading edge of the wing and it differs in morphological and mechanical properties from the 9th and more proximally primaries of pigeons\textsuperscript{9}. In this paper we want to find out whether local differences in Young's modulus of the 10th primary correspond to the findings in other remiges or reflect in some way its special tasks.

**Results**

Three-way ANOVA used to discriminate the effects of location (base, middle, tip), genotype (Cys/Cys, Cys/Gly, Gly/Gly), sex, and interactions between the mentioned factors on the value of Young's modulus showed no statistically significant difference between different \textit{FCBP} genotypes even within different sexes and/or within different locations (p = 0.139).

Therefore, two-way ANOVA (regardless of genetic background) was performed (see Supplementary materials 1). It demonstrated a statistically significant effect of location in Young's modulus (p < 0.001) between tip (5.24 GPa) and base/middle regions (5.77/5.79 GPa) of the feathers (Fig. 1).

The same is true for the effects of sex. We found a statistically significant difference of E between females (5.67 GPa) and males (5.53 GPa). These different values result from the statistically significant difference only in the middle region of the feathers (females 5.94 GPa, males 5.63 GPa), whereas E neither of the feather bases or of the feather tips show no such sexual difference (Fig. 2).

The Young's modulus in the rachis of the 10th primaries of our birds averaged between 5–6 GPa. This agrees well with the findings of Bachmann et al.\textsuperscript{25} on 5th primaries of pigeons and barn owls. These authors, using the nanoindentation technique as well, found no statistically significant differences between the pooled mean values

![Figure 1. Effect of locations on Young's modulus values of feathers. Mean values (bars) and standard error (whiskers) are presented for base, middle, and tip regions of the feather shaft. 32 feathers from 32 animals were used for the comparison. The number of individual measurements in the above-mentioned regions was 572, 537, and 546, correspondingly.](https://doi.org/10.1038/s41598-022-05649-2)
of the two species (pigeon: 5.96 GPa, barn owl: 6.54 GPa). Bonser and Purslow\(^{11}\) performed tensile tests on compact keratin cortex strips from the dorsal side of primaries in 8 avian species belonging to different orders, among them the rock pigeon, and found mean Young's modulus of 2.50 GPa in all species apart from the grey heron (E = 1.78 GPa). Bachmann et al.\(^{23}\) additionally applied bending tests on pieces of primary shafts of their pigeons and barn owls, the measured Young's modulus resembling those of the nanoindenter technique. The relative low values published for pigeons and other species by Bonser and Purslow\(^{11}\) might be due to the use of the tensile tests. In an earlier study, Purslow and Vincent\(^9\) estimated the stiffness of the cortex of pigeon rachis as 7.75–10 GPa by best fit to a bending model (see also Discussion in\(^{23}\)).

**Discussion**

**Corneous material composition and biomechanical properties.** During the last 15 years new findings led to a new concept for the classification of corneous materials in vertebrates. The corneous structures in sauropsids like scales, claws, beaks, and feathers are essential formed by small proteins, formerly called beta-keratins (e.g.\(^{1,2,5,6,15,17,26}\)), but nowadays called corneous beta-proteins (CBP). Genes coding for CBPs have evolved within the epidermal differentiation complex (EDC), a locus with no relationship with those of the IF-keratins (reviewed by Alibardi\(^3\) and Holthaus et al.\(^4\)).

Among the various factors that might influence the mechanical properties of feather rachides (see "Introduction") we focused in this paper on the chemical composition of FCBP. After studying the Young’s modulus of primaries in eight avian species, Bonser and Purslow\(^{11}\) concluded that the flexural stiffness of the whole rachis in these species is principally controlled by their cross-sectional morphology rather than by material properties of the FCBP. This view was shared by Bachmann et al.\(^{23}\), who concluded that the flexural stiffness is predominantly influenced by the geometry of the feathers rather than by local material properties. The finding that within a single species, the domestic pigeon, and even within a single breed of it, the homing pigeon, a polymorphism in the feather corneous beta-protein gene was detected\(^{17}\) and offered the chance to further test a contribution of the chemical composition of FCBP on the mechanical properties in an otherwise very homogenous genetic background. This was even more tempting since the described polymorphism in the FCBP gene (\(F\)-KER) resulted in an interchange of cysteine and glycine. Cysteine is known for its ability to form disulfide bonds and its replacement by glycine would prevent the formation of these bridges and could thereby alter the stability of the protein molecule. Additionally, Proskura et al.\(^{21}\) observed a correlation between the racing performances of homing pigeons and their FCBP (\(F\)-KER) genotypes in races from different distances. From distances below 400 km the Gly/Cys birds returned faster than the other 2 genotypes, but this differences was statistically not significant. When released from distances of more than 500 km Cys/Cys pigeons homed with significantly higher speed than Gly/Gly birds.

However, in our measurements an influence of various FCBP genotypes on the Young's modulus of the rachis of the 10th primary could not be detected. Fraser and Parry\(^{26}\) have aligned the amino acid sequences of hard keratins (CBPs) in birds and reptiles. In birds, they found only minor variations in the chain lengths. Feather keratin molecules may be subdivided into three domains: a highly conserved central domain consisting out of 34 residues and the slightly more variable N-terminal and C-terminal domains. The central domain contains a high proportion of \(\beta\)-favoring residues which are thought to be the framework of the filament. The framework of the filament is based on a pair of twisted \(\beta\)-sheets related by a perpendicular diad. The \(\beta\)-sheet consists of three internal strands and two shorter edge strands connected by four turns. Emu (Dromaius novae-hollandiae)
feral pigeons in Vienna and according to30, body mass and lengths of humerus, ulna and carpometacarpus differ it will seem not so surprising that its local Young's modulus does not follow the pattern found in other remiges. metric with the distal one being extremely narrow. Regarding all these peculiarities of the outermost primary observed a slight decrease of E in the distal part of 10th pigeon primaries compared to the middle and the base to the tip. Different from the findings just cited in primaries and contour feathers of birds able to fly, we 50 and 75% of the total length of the rachis, whereas in the goose and in the swan, the values increased from the tail to the tip of the rachis11. In 5th primaries of pigeons and barn owls, Bachmann et al.23 found significant differences of E between the proximal and distal feather parts in the two species, E of the proximal parts being significantly lower than E in the distal parts. In contour feathers taken from the pelvic tract of chicken, turkey, ring-necked pheasant, and herring gull E was found to be higher in distal than in proximal regions of the rachis both in hinging and in tensile tests24. In tail feather coverts of peacock which function in sexual display but not in flight, Weiss and Kirchner observed no significant variation of Young modulus with the position from proximal to distal. In wing feathers from the ostrich, a flightless bird, Cameron et al.21 found similar Young's modulus at 0, 50 and 75% of the total length of the rachis, whereas in the goose and in the swan, the values increased from the base to the tip. Different from the findings just cited in primaries and contour feathers of birds able to fly, we observed a slight decrease of E in the distal part of 10th pigeon primaries compared to the middle and the base region of the rachis. Purslow and Vincent detected differences in the morphology and in mechanical properties between the 10th and the 9th primary of pigeons. The 10th primary was equally stiff laterally as dorso-ventrally, whereas the 9th and other more proximally primaries were much less stiff laterally than dorso-ventrally. Cross-sections of the shafts show that the increase of lateral stiffness of the outermost primary is achieved by a greater width of the rachis compared to feathers proximal to it. These authors also point out that the outermost primary constitutes the leading edge of the wing. The other primaries lie behind the leading edge feather and are thus partly shielded by it. In adaptation to resist the high drag experienced by the 10th primary, it seems possible that an elevated Young's modulus in the basal and middle parts of this feather could be advantageous.

Corning and Biewener recorded in vivo strains on the shafts of the 9th, 8th, 6th, 5th, and 4th primary and the 2nd secondary of slowly flying pigeons using strain gauges attached to the dorsal side of the rachides approximately 2 cm distal to the calamus. Compressive strains during the downstroke exceeded tensile strains during the upstroke. The peak values were found in the downstroke of the 8th primary (− 0.0034) and in the 6th − 0.0036 with a falling tendency to the 2nd secondary (− 0.0021). These finding indicate that the different remiges experience different strains and consequently may vary in stiffness. Dorso-ventral deflexions of the shaft under static load applied at distances of 50% to 60% of the length resulted in higher values for the 10th than for the 9th primary (− 0.0053, Fig. 6). Moreover, the 10th primary like the others is covered dorsally by the plumage. In several species of the order Columbiformes, both sexes look rather similar appearance of male and female pigeons, slight size differences have been described between the two sexes. Thus, Glutz von Blotzheim reports average body weights of 238.1 g for male and 231.5 g for female feral pigeons in Vienna and according to26, body mass and lengths of humerus, ulna and carpometacarpus differ in C. livia with males being bigger. In our material the tenth primaries in males (185.3 mm) were longer than those of females (180.5 mm). It might be that these size differences are related to the sex differences in Purslow and Vincent studying primaries from five pigeons with body weights between 265 and 460 g reported that shape and size of the cortex, as measured by its second moment of area, have relations the body weight of the birds. On the other hand, Bonser and Purslow11 comparing primaries from seven avian species ranging in size from the common starling (60 g) to the mute swan (10 kg) found similar Young's modulus in these species.

Young's modulus differs between the sexes. So far we found no sources describing biomechanical differences related to sexual feather dimorphisms. In several species of the order Columbiformes, both sexes look alike (monomorphic or monochromatic) and this also holds in the genus Columba. Looking more closely, the hens plumages sometimes seem to be duller and poorer in contrast compared to the males, but often it is almost impossible to identify the sex of a rock pigeon, a feral or a domestic pigeon merely on the plumage. In spite of this, Purslow and Vincent studying primaries from five pigeons with body weights between 265 and 460 g reported that shape and size of the cortex, as measured by its second moment of area, have relations the body weight of the birds. On the other hand, Bonser and Purslow comparing primaries from seven avian species ranging in size from the common starling (60 g) to the mute swan (10 kg) found similar Young's modulus in these species.

 Watching their behaviour is a better criterium to distinguish between sexes, but here, again, problems arise, since the behavioural differences between the sexes are rather qualitatively than quantitatively. However, one behaviour trait, wing clapping, is mainly performed by the males. After copulation the male takes off loudly
clapping his wings over his back for 3–5 wingbeats\textsuperscript{30–32}. This makes a clapping sound\textsuperscript{33}. This behaviour is part of the ‘display flight and it can also be observed in other situations as well, e.g. when a male notices another pigeon on the wing, especially if it is above him; or “when he sees his mate or another pigeon in display flight nearby” or “when about to alight at or near his home after having been away foraging” and also “when flying in company with his mate” (\textsuperscript{31}, p. 296). When he heavily courts a female, he may fly up for a few flaps clapping his wings or when the hen runs or flies away from him while she is courted he may follow her loudly clapping his wings (personal observation E.H. and A.D.). Slow motion videos from pigeons during take-off show that clapping during normal take-off results from primaries and secondaries that beat together over the birds back at the end of the upstroke\textsuperscript{33,34}. During the upstroke the primaries are bent ventrally but at the end of the upstroke they become bent dorsally and meet the contralateral primaries over the bird's back. This bending may be facilitated by the lower stiffness (E) in the middle of the males shafts compared to females. Clapping related to courtship looks like an intensified version of off-take clapping and is primarily performed by the males.

In short (< 400 km) races as well as in long (< 500 km) races hens performed significantly better than cocks\textsuperscript{21}. In conclusion our biomechanical findings are consistent with the idea that the higher stiffness of the females primaries contributes to the difference in the speed of homing.

Methods and materials
Study approval. This study was carried out in strict accordance with the recommendations of the National Ethics Committee on Animal Experimentation. The protocol was approved by Local Ethics Committee for Animal Testing of the West Pomeranian University of Technology in Szczecin (Protocol Number: 36/2012).

Homing pigeons from the lofts of A.D. and friends in Poland and E.H. in Kiel were used in this study. The birds had previously been genotyped for their \textit{FCBP} alleles by A.D. using the method described in\textsuperscript{17} on DNA extracted from trunk feathers collected during the annual molt or from blood samples. Tenth (most distal) primaries of 32 adult (> 1.5 years old) birds (16 males, 16 females) were collected at the time of shedding in late fall and early winter. These feathers had grown about 12 months ago during the molting cycle of the previous year. 12 of these birds carried the \textit{TT} or Cys/Cys \textit{FCBP} genotype, 11 were heterozygous (\textit{TG} or Cys/Gly) and the remaining 9 were homozygous (\textit{GG} or Gly/Gly). In 27 birds the basic colour was black (wild type) showing different patterns: blue barred (+), checker (C), dark checker (C\textsuperscript{T}), and uniformly black (S), in 5 pigeons the basic colour was red (ash red \textit{BA}) with the patterns barred, check and dark check (nomenclature and genetic symbols after Sell\textsuperscript{35}). In the first group the dominating pigment was eumelanin, whereas in the second group phaeomelanin predominated\textsuperscript{36}. Mean feather length was 185.3 mm in the males and 180.5 mm in the females.

Samples of the dorsal cortex of each rachis (2–3-mm long and 0.5–1.5 mm wide were cut from three regions: base (at the boundary between rachis and calamus), middle and tip (15% of feather length from the feather tip). These samples (2–3 mm long and 0.5–1.5 mm wide) were gently cut with a very sharp scalpel to avoid stress deformation of the material. For nanoindentation they were mounted to aluminum cylinders at room temperature with cyanoacrylate instant glue (ergo 5925 elastomer, Kislung AG, Wetzikon, Switzerland), which produces a very thin glue layer. The fixed samples were checked using a New View 4 k white light interferometer (Zygo, Middlefield, CT, USA) to determine the surface topography. Only areas with average surface roughness (R\textsubscript{s} < 60 nm) were used for further nanoindentation measurements.

Nanoindentation involves the application of a controlled load to the surface to induce local surface deformation using a sharp indenter tip with high elastic modulus, Fig. 3A. Smooth surface and well-defined geometry are required for the tip as well. The most used material for tips is diamond with an elastic modulus \textit{E} = 1140 GPa. A three-sided sharply pointed Berkovich tip (the total included angle is 142.3°, a tip radius in our experiments was 150 nm, Fig. 3B) is more efficient over spherical, conical or pyramidal indenters, especially for a wide range of materials, including biomaterials.

With a 1 μN to 20 mN force range and 1 nm to 20 μm displacement range, nanoindentation bridges the gap between atomic force microscopy and macroscale mechanical testing. Because of its small probe size, nanoindentation can be used to measure local material properties in small, thin, and heterogeneous samples. Nanoindentation is also useful for measuring mechanical properties of microstructure within bulk samples, characterizing the properties of individual component within heterogeneous samples, mapping mechanical properties across a sample surface. This allows testing of samples unsuitable for other mechanical testing techniques and makes the nanoindentation indispensable for mechanical testing of biomaterials. So, nanoindentation has been used to investigate the mechanical properties of radula teeth in gastropods\textsuperscript{37}, skin by different snake species\textsuperscript{38}, caries lesions in dentin\textsuperscript{39}, etc.

Displacement in our nanoindenter (MTS nanoindenter II, MTS Systems Incorporation, Oak Ridge, USA) was monitored by capacitance gauge, while force actuation was provided through magnetic coils. A schematic of a nanoindenter system is shown in Fig. 3A. Such properties as Young's modulus and hardness are calculated from the load–displacement curves using well-established equations based on elastic contact theory\textsuperscript{40}. \textit{E} can be calculated by the formula:

\[ E = \frac{S \sqrt{\pi}}{2 \sqrt{A}}, \]

where \textit{S} is the contact stiffness, and \textit{A} is the contact area, which can be found from the dependence of contact area from the contact depth after tip shape calibration procedure using a standard material with well-known properties (e.g. fused quartz with \textit{E} = 69.6 GPa)\textsuperscript{41}. Finally, the Young's modulus was averaged at penetratration depth exceeding 400 nm to exclude the effect of the surface roughness on the measurements, Fig. 3C.

Dynamical Young's modulus was determined using continous stiffness measurements method\textsuperscript{42,43} with nanoindenter controlling software Test Work 4 (MTS Systems Inc.).
**Statistical analysis.** Two-and three-way ANOVA was performed with SigmaPlot 12.5 (Systat Software, Inc. Erkrath, Germany). Data normality distribution and variance equality were checked before post-hoc analysis. Kolmogorov-Smirnov test was used for distribution normality check. Holm-Sidak method was used for post-hoc all pairwise multiple comparison procedure.

**Statement.** Our study is reported in accordance with ARRIVE guidelines46.

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**References**

1. Fraser, R. D., MacRae, T. P., Parry, D. A. & Suzuki, E. The structure of feather keratin. *Polymer* 12, 35–56 (1971).
2. Weiss, I. M. & Kirchner, H. O. K. The peacock’s train (*Pavo cristatus* and *Pavo cristatus* mut. Alba). I. Structure, mechanisms, and chemistry of the tail feather coverts. *J. Exp. Zool.* 313, 690–703 (2010).
3. Alibardi, L. Review: Cornification, morphogenesis and evolution of feathers. *Protoplasma* 254, 1259–1281 (2017).
4. Holthaus, K. B., Eckhart, L., Dalla Valle, L. & Alibardi, L. Review: Evolution and diversification of corneous beta-proteins, the characteristic epidermal proteins of reptiles and birds. *J. Exp. Zool. B Mol. Dev. Evol.* 330, 438–453 (2018).

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Figure 3. Nanoindentation experiments. (A) A scheme of the nanoindenter (adopted from Klein et al.44). (B) Berkovich tip imprints on a feather sample after nanoindentation. (C) CSM measurement curve of Young’s modulus (E). The material parameters were averaged over the hatched area. CMA is a coil/magnet assembly, springs are supporting springs, CG is a capacitance gauge, tip is a Berkovich indentation tip, ST is a 2D moving/positioning stage with a sample tray.
5. Fraser, R. D. & Parry, D. A. Molecular packing in the feather keratin filament. *J. Struct. Biol.* **162**, 1–13 (2008).
6. Pabisch, S., Puchegger, S., Kirchner, H. O., Weiss, I. M. & Peterlik, H. Keratin homogeneity in the tail feathers of *Pavo cristatus* and *Pavo mut. alba*. *J. Struct. Biol.* **172**, 270–275 (2010).
7. Filshie, B. K. & Rogers, G. E. An electron microscope study of the fine structure of feather keratin. *J. Cell Biol.* **13**, 1–12 (1962).
8. Rutschke, E. Die submikroskopische Struktur schillernder Federn von Entenvögeln [Submicroscopic structure of duck feathers]. *Z. Zellforsch. Mikrosk. Anat.* **73**, 432–443 (1966).
9. Purslow, P. P. & Vincent, J. F. V. Mechanical properties of the primary feathers from the pigeon. *J. Exp. Biol.* **72**, 251–260 (1978).
10. Crenshaw, D. G. Design and materials of feather shafts: Very light, rigid structures. *Symp. Soc. Exp. Biol.* **34**, 485–486 (1980).
11. Bonser, R. & Purslow, P. The Young's modulus of feather keratin. *J. Exp. Biol.* **198**, 1029–1033 (1995).
12. Earland, C., Blakey, P. R. & Stell, J. G. Studies on the structure of keratin IV. The molecular structure of some morphological components of keratins. *Biochim. Biophys. Acta* **6**, 268–274 (1962).
13. Astbury, W. T. & Bell, F. X-ray data on the structure of natural fibres and other bodies of high molecular weight. *Tabulæ Biol.* **17**, 90–112 (1939).
14. Lingham-Soliar, T. Feather structure, biomechanics and biomimetics: Its incredible lightness of being. *J. Ornithol.* **155**, 323–336 (2014).
15. Lingham-Soliar, T. & Murugan, N. A new helical-crossed fibre structure of β-keratin in flight feathers and its biomechanical implications. *PLoS ONE* **8**, e68549. [https://doi.org/10.1371/journal.pone.0068549] (2013).
16. Ng, C. S. et al. The chicken frizzle feather is due to an α-keratin (KRT75) mutation that causes a defective rachis. *PLoS Genet.* **8**, e1002748. [https://doi.org/10.1371/journal.pgen.1002748] (2012).
17. Dybus, A. & Haase, E. Feather keratin gene polymorphism (F-KER) in domestic pigeons. *Br. Poultry Sci.* **52**, 173–176 (2011).
18. Witschi, E. Sex and secondary sexual characters. In *Biological and Comparative Physiology of Birds II* (ed. Marshall, A. J.) (Academic Press, 1961).
19. Haase, E., Ito, S. & Wakamatsu, K. Influences of sex, castration, and androgens on the eumelanin and pheomelanin contents of different feathers in wild mallards. *Pigment Cell Res.* **8**, 16–170 (1995).
20. Kimball, R. T. & Ligon, J. D. Evolution of avian plumage dichromatism from a proximate perspective. *Am. Nat.* **154**, 182–193 (1999).
21. Proskura, W. S. et al. The Cys83Sly amino acid substitution in feather keratin is associated with pigeon performance in long-distance races. *Vert. Med.* **62**, 221–225 (2017).
22. Cameron, G. J., Wess, T. J. & Bonser, R. H. Young's modulus varies with differential orientation of keratin in feathers. *J. Struct. Biol.* **143**, 118–123 (2003).
23. Bachmann, T., Emmerlich, J., Baumgartner, W., Schneider, J. M. & Wagner, H. Flexural stiffness of feather shafts: Geometry rules over material properties. *J. Exp. Biol.* **215**, 405–515 (2012).
24. MacLeod, G. D. Mechanical properties of contour feathers. *J. Exp. Biol.* **87**, 65–71 (1980).
25. Cornning, W. R. & Biewener, A. A. In vivo strains in pigeon flight feather shafts: Implications for structural design. *J. Exp. Biol.* **201**, 3057–3065 (1998).
26. Fraser, R. D. & Parry, D. A. Amino acid sequence homologies in the hard keratins of birds and reptiles, and their implications for molecular structure and physical properties. *J. Struct. Biol.* **188**, 213–224 (2014).
27. Taylor, A. M., Bonser, R. H. C. & Farrent, J. W. The influence of hydration on the tensile and compressive properties of avian keratinous tissues. *J. Mater. Sci.* **39**, 939–942 (2004).
28. Goodwin, D. *Pigeons and Doves of the World* (Cornell University Press, 1977).
29. Glutz von Blotzheim, U. N. & Bauer, K. M. *Handbuch der Vögel Mitteleuropas. vol. 9 Columbiformes-Piciformes* (Akademische Verlagsgesellschaft, 1980).
30. Goodwin, D. Behaviour. In *Physiology and Behaviour of the Pigeon* (ed. Abs, M.) (Academic Press, 1983).
31. Heinroth, O. & Heinroth, K. Verhaltensweisen der Felsentaube (Haustaube) *Columba livia livia* L. Z. Tierpsychol. **6**, 153–201 (1949).
32. BBC Earth Unplugged [https://www.youtube.com/watch?v=UfYSHFb4qM].
33. Wing-clapping [https://www.birdnote.org/listen/shows/wing-clapping].
34. Sell, A. *Pigeon Genetics—Applied Genetics in the Domestic Pigeon* (Verlag Sell, 2012).
35. Haase, E., Ito, S., Sell, A. & Wakamatsu, K. Melanin concentrations in feathers from wild and domestic pigeons. *J. Heredity* **83**, 64–67 (1992).
36. Krings, W., Kovalev, A., Glaubrecht, M. & Gorb, S. Differences in the Young modulus and hardness reflect different functions of teeth within the taeniolabis radula of gastropods. *Zoology* **137**, 125713 (2019).
37. Klein, M.-C.G., Deuschle, J. K. & Gorb, S. N. Material properties of the skin of the Kenyan sand boa *Gonyosoma colubrinus* (Squamata, Boidae). *J. Comp. Physiol. A* **196**, 659–668 (2010).
38. SigmaPlot (Systat Software).
39. Schwendicke, F. et al. In vitro induction of residual caries lesions in dentin: Comparative mineral loss and nano-hardness analysis. *Caries Res.* **49**, 259–265 (2015).
40. Fischer-Cripps, A. C. *Nanoindentation* (Springer, 2002).
41. Oliver, W. C. & Pharr, G. M. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. *J. Mater. Res.* **7**, 1564–1583 (1992).
42. Meiss, R. A. Stiffness of active smooth muscle during forced elongation. *Am. J. Physiol.* **253**, C484–493 (1987).
43. Hochstetter, G., Jimenez, A. & Loubert, J. L. Strain-rate effects on hardness of glassy polymers in the nanoscale range. Comparison between quasi-static and continuous stiffness measurements. *J. Macromol. Sci. B* **38**, 681–692 (1999).
44. Klein, M.-C.G., Deuschle, J. K. & Gorb, S. N. Material properties of the skin of the Kenyan sand boa *Gonyosoma colubrinus* (Squamata, Boidae). *J. Comp. Physiol. A* **196**, 659–668 (2010).
45. SigmaPlot (Systat Software).
46. Pericé du Sert, N. et al. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol.* **18**, e3000411 (2020).

**Author contributions**

E.H., A.D., S.G., An.K., and Al.K. planned and designed the research; A.D. and E.H. collected feathers for the nanoindentation analysis; A.D. carried out DNA analyses; An.K, S.G. and AL.K. carried out the nanoindentation analysis. Al.K. and S.G. carried out the statistical analysis; Al.K. and E.H. designed figures for the paper; E.H. and A.D. wrote the paper; All authors read and approved the final manuscript.

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