

On silver wings: a fragile structural mechanism increases plumage conspicuousness

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We report for the first time the existence of a structural mechanism of feathers different from iridescence that makes plumage conspicuous. By using electron and light microscopy, we show that the mechanism consists of special lengthened and twisted distal barbules that are very susceptible to damage. The dorsal side of these barbules is translucent, which creates a distinctive sheen colouration to feathers that otherwise would be dark. When distal sheen barbules are broken, the black proximal barbules are exposed, thus generating a conspicuous difference between abraded and non-abraded areas. Total and ultraviolet reflectance of sheen (non-abraded) areas are strikingly higher than in abraded areas. We propose that this mechanism represents a case of convergent evolution in species that are limited in developing colourful plumage patches. Future studies should explore the potential of this colour mechanism to act as a signal of individual quality or identity.

Highly reflective silvery structures stand out from their surroundings, as can be seen from the night time shine from the eyes of cats in the beam of a flashlight and the bright silver gleam of baitfish as they twist and turn to avoid a predator. The brightness of a silvery, reflective surface allows for both extreme conspicuousness and visibility from a considerable distance. While we thus might expect this to be exploited by animals for communication or some other function, examples are relatively few. Silvery structures have been described in the exoskeleton of some invertebrates (e.g., silverfish insects *Lepisma saccharina*) and in the scaly integument of some vertebrates (fish; Parker 2005). However, there are few examples of silvery structures in terrestrial vertebrates, with the exception of the reflective eyes of animals with *tapetum lucidum*, a tissue layer in the eye that reflects the light back through the retina. Here we propose that certain modified and sheen coloured feathers present in several avian taxa have properties that are similar to reflective surfaces: when illuminated by sunlight, perceived brightness in these feathers is enhanced compared to more typical feathers (Fig. 1). This is a novel type of structural colouration that appears to have been overlooked even though the production mechanisms of structural colour in the integument of birds have been extensively and intensively investigated and reviewed in the past century (Lucas and Stettenheim 1972, Fox 1976), and many recent advances on their physics and evolution have been published (Prum 2006). Sheen feathers differ from

those with iridescence, defined as the phenomenon of glittering of different colours that change according to the angle with which the object is seen or illuminated (Lucas and Stettenheim 1972, Prum 2006). Iridescent structural colours are produced by coherent scattering from arrays of melanin granules (Prum 2006). The colour of sheen feathers also differs from bright white, which is produced by large air vacuoles in the barbules of feathers, as described, for instance, by Dyck (1976) for the winter plumage of the rock ptarmigan *Lagopus muta*.

Our aim is to describe the structure and optical properties of sheen feathers to stimulate future work on the ecology and evolution of this overlooked type of plumage colouration. We focus on the noticeable example of sheen structure on the dorsal surface of adult bearded vultures *Gypaetus barbatus*, a species whose plumage characteristics some of us have thoroughly studied, as these birds apply cosmetic soils to their feathers (Negro et al. 1999, 2002) and show fine-tuned plumage adaptations (Margalida et al. 2008).

Methods

Juvenile bearded vultures sport a matte dark brown plumage similar to that of other Old World vultures and most other birds of prey (order Falconiformes). The number of sheen feathers increases annually with moult

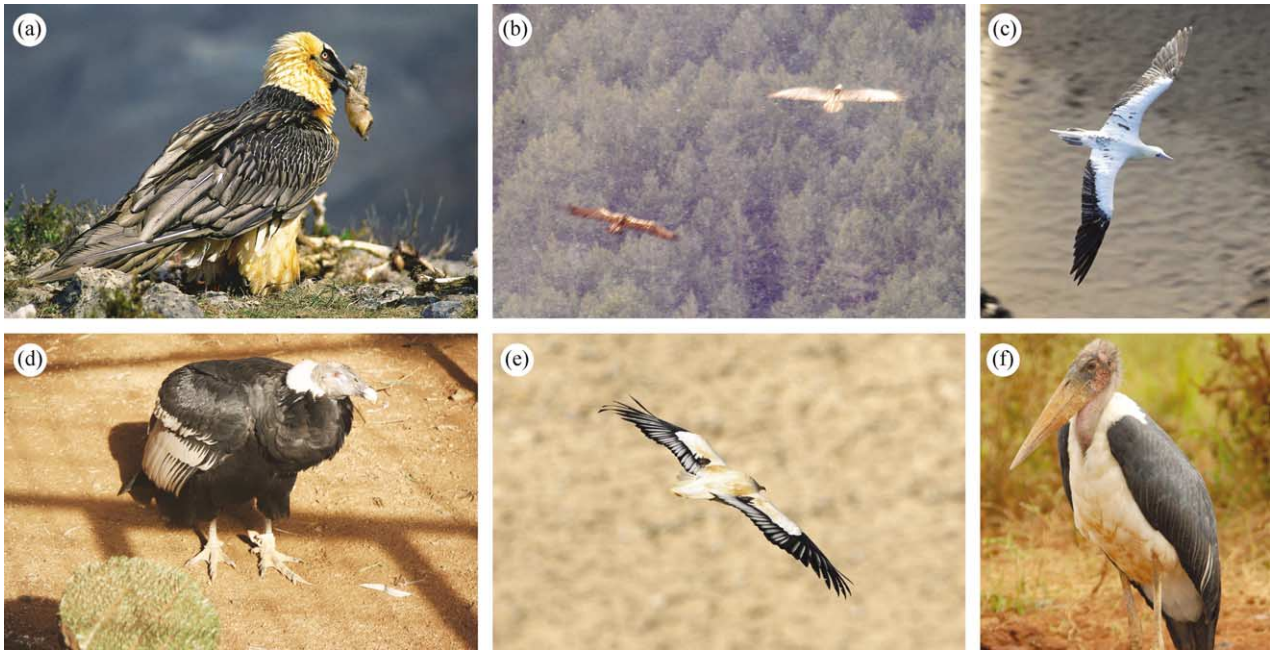


Figure 1. Examples of birds showing feathers with sheen structures. (a) Adult bearded vulture, (b) Immature (left), and adult (right) bearded vultures in flight. Note the bright appearance of the adult bird compared with the immature even on a snowy (i.e. dark) day, (c) Adult, white morph red-footed booby in flight. Note how the sheen structures of flight feathers shine much more in the left wing, which is under sunny light, than in the right wing, which is in the shadow. (d) Adult Andean condor *Vultur gryphus* showing conspicuous whitish feathers on the wing, (e) Adult Egyptian vulture *Neophron percnopterus* showing sheen structures on remiges that give them a whitish appearance. (f) Adult marabou *Leptoptilos crumeniferus*. The white lines on secondary coverts, which acquire that colour because of sheen structures, when fresh are visible all along the wing.

(see photos in Adams and Llopis Dell 2003), such that adult bearded vultures over six years old have a bright sheen appearance on the upperparts (Fig. 1a), from head to tail, and on remiges and rectrices particularly when flying (Fig. 1b).

We used light (LM), and scanning electron microscopes (SEM) to describe the morphological characteristics of the sheen feathers. We also took photographs of these structures with the aim of illustrating the morphological characteristics of feathers that generate the sheen effect. To obtain SEM images of the ultrastructure of feathers, we cut portions of feathers at different points that appeared to the unaided eye as having different levels of abrasion. The samples were analysed with a FEI QUANTA 200 SEM operating in low vacuum mode and 25 kV and using a wavelength dispersive X-ray detector (WDS). The samples were mounted on stainless-steel pegs by using double-sided graphite tape before being transferred to the SEM chamber. The light microscope was an Olympus BX51 with a mounted DP70 camera for image capture.

To characterize the optical properties of sheen feathers, we used an Ocean Optics USB2000 spectrophotometer (range 250–800 nm) with ultraviolet (deuterium) and visible (tungsten-halogen) lamps and a bifurcated 400 micrometer fiber-optic probe (Dunedin, Florida). The fibre-optic probe provided both illumination and obtained light reflected from the sample, providing a reading area of $c. 1 \text{ mm}^2$. The measurements were taken at a 90° angle to feather samples. All measurements were relative to a white “Spectralon” tablet (WS-1-SS, Dunedin, Florida). Measurements were taken at different points of feathers differing in the level of abrasion (see Fig. 4), except for

the abrasion experiment (see below) for which three measurements were taken at the same point, removing the probe after each measure. To confirm that the optical properties can be environmentally modified, we experimentally removed the sheen structure in the distal area of a bearded vulture feather by lightly scraping the surface with the blade of a pair of scissors, and then measured the reflectance spectrum at the same point afterwards. As a control, we performed the same experimental manipulation on the distal black area of a primary feather of a European magpie *Pica pica* as this species does not present sheen structures on its feathers.

In addition to the results obtained with the methods described above for the bearded vulture, we also describe the sheen structure observed in other species such as the snow goose *Anser caerulescens* and the red-footed booby *Sula sula*, as this structure can be easily detected without a microscope because at close range they have a unique ‘dusty’ or ‘fuzzy’ appearance that is perceived as greyish or whitish.

Results

Structure of sheen feathers

Observations under LM and SEM (Fig. 3) of sheen feathers of bearded vultures revealed that distal barbules were flattened and twisted, in a similar way as described in some iridescent feathers (Lucas and Stettenheim 1972, Brink and van der Berg 2004), except that distal barbules responsible for iridescence contained large amounts of

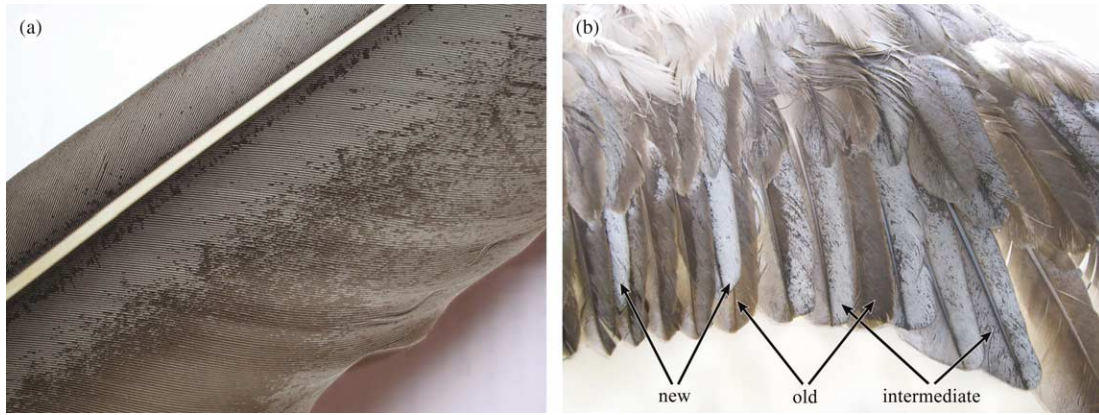


Figure 2. Detailed views of sheen structures in feathers. (a) Primary feather of an adult bearded vulture. (b) Flight and covert feathers of an adult brown morph red-footed booby. In this species, variation in the regularity of the pattern generated by sheen barbules among feathers is due to wear, which allows one to identify feathers of different ages (indicated by arrows).

melanin whereas sheen barbules were translucent. Proximal barbules, however, were flat and dark, as they contain melanin granules.

Sheen feathers of the bearded vulture were speckled with dark spots in an apparently haphazard fashion (Fig. 2a). All individuals examined (i.e., 15 captive and 20 free-ranging individuals) showed these spots, and at least some of the spots were present in growing feathers. The dark spots dotting sheen feathers are places where distal barbules were broken, revealing dark proximal barbules of the barb underneath.

The tip of the distal barbule (i.e. the pennulum) in sheen feathers is lengthened with respect to a 'conventional' barbule, so that its whitish dorsal side is exposed along a greater distance from the point at which it is twisted (Fig. 3; see Shawkey et al. 2005 for additional examples showing the ultrastructure of conventional feathers). Sheen feathers are also different compared to other feather types with lengthened but not twisted barbules (e.g. owls; Bachmann et al. 2007). This lengthening and twisting of distal barbules causes them to break at the point where they twist because they cannot get caught on the barbules of the adjacent barbs from that point on, indicating that this morphology probably increases the likelihood of feather abrasion (Figs. 2 and 3a,b, Fitzpatrick 1998). As for the dark feather spots, their size depends on the number of adjacent distal barbules that are broken (Fig. 3a).

Optical properties of sheen feathers

When we measured the reflectance of a bearded vulture feather at a point where no broken barbules were observed (i.e. the sheen colour was uniform), the resulting reflectance spectrum showed a defined peak in the ultraviolet (UV) range, followed by a decrease and a subsequent increase in reflectance at longer wavelengths (Fig. 4a). The spectrum obtained for the sample taken at a point where some broken barbules were observed showed a similar shape but a slightly lower UV peak. However, the spectrum for a point at the darkest part of the feather, with a higher number of broken barbules, also showed a lower UV peak but with considerably higher and increasing reflectance values at longer wavelengths, similar to the typical reflectance spectrum

of feathers with melanin-based colours (McGraw 2006). Similar results were obtained when a bearded vulture feather was experimentally abraded (Fig. 4c). The fact that differences in UV reflectance between abraded and non-abraded parts of bearded vulture feathers is more marked after an experimental abrasion than in a naturally abraded feather, indicates that differences between curves obtained (from abraded and non-abraded points) could be observed only after the experimental abrasion of feathers (Fig. 4c).

Wear-induced damage was also evident in the plumage of the other species with sheen feathers that were examined (Fig. 2b). Indeed, differences in shape of spectra between abraded and non-abraded parts of feathers were even more marked in the snow goose feather (Fig. 4b) than in the bearded vulture feather (Fig. 4a). Therefore, it appears that for some species, physical abrasions have to be very severe before UV reflectance changes with the disappearance of the silvery sheen, or, alternatively, the silvery sheen is associated with but not fully coupled to the UV reflectance of these feathers. In contrast, reflectance curves for abraded and non-abraded areas of a European magpie feather overlapped along a large proportion of the spectral range, and the total reflectance after the experimental abrasion had the opposite effect and even increased after manipulation (Fig. 4d).

Species that have evolved sheen feathers

In addition to the species used in this study, we have observed sheen structures in several other species. We confirmed that these structures were the same as those in the case of the bearded vulture by using light microscopy. In particular, we have noted the presence of this type of colouration in pelicans (Pelecaniformes), ducks and geese (Anseriformes), storks and ibises (Ciconiiformes), New World vultures, kites, harriers, Egyptian and bearded vultures (Falconiformes), cranes (Gruiformes) and sand-grouse (Pteroclidiformes; see Fig. 1, videoclips 1 and 2, and Fig. S1–S6 in suppl. materials). Even though we examined numerous birds in the order Passeriformes, we failed to find sheen feathers.

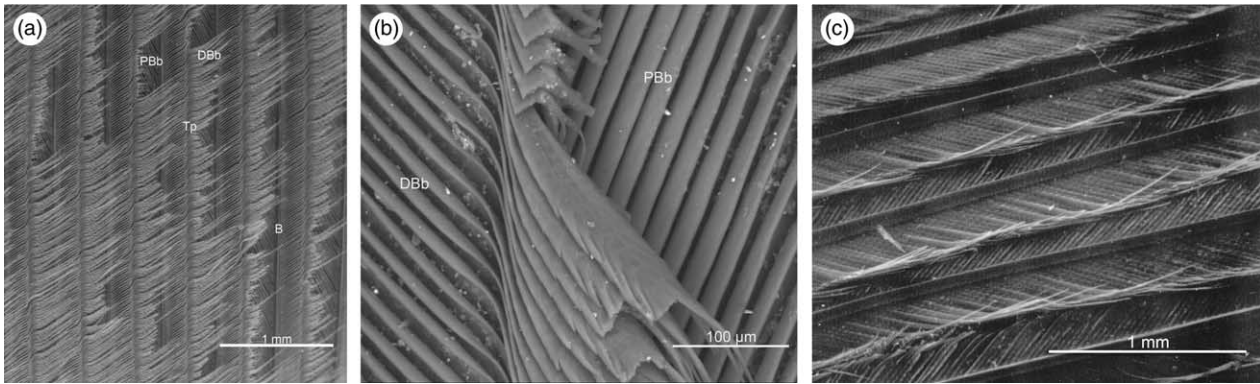


Figure 3. Ultrastructure of feathers containing sheen barbules. (a) SEM micrograph of a primary feather of an adult bearded vulture with sheen barbules with different levels of wear. Distal barbules (DBb), which cover the proximal barbules (PBb) of the adjacent barbs (b), twist at the middle of their length (Tp) following an apparently regular pattern. After twisting, DBb show a translucent area that creates the sheen colour that contrasts against dark PBb. Note that DBb always break at the Tp point, not at the point of insertion in the barb, hence not losing their capacity of binding adjacent barbs. (b) SEM image showing the detail of distal barbules as in: (a) at Tp. (c) SEM image of an adult magpie primary feather. The picture was taken at the junction of the black and white areas of the feather, but there are no morphological difference causing the different hues as there is in sheen feathers. In this type of ‘conventional’ feather, barbules are almost completely straight.

Discussion

Structural characteristics

We consider sheen colouration unique in being fragile, as it is highly susceptible to damage. As we showed (Fig. 4b), one can easily take a blade or abrasive medium (even a fingernail) and remove the barbules to reveal the dark feather underneath. The loss of feather elements has

sometimes been reported as a mechanism to create ornaments in birds, such as the impressive racketed tail of the turquoise-browed motmot *Eumomota superciliosa*, due to the selective loss of barbs in the rectrices (Murphy 2007), and the black bib of male sparrows (e.g., Bogliani and Brangi 1990).

In some species where the density of barbules is high, the appearance of sheen can be pure white, as in the flight feathers of the Andean condor *Vultur gryphus* (Fig. 1d and

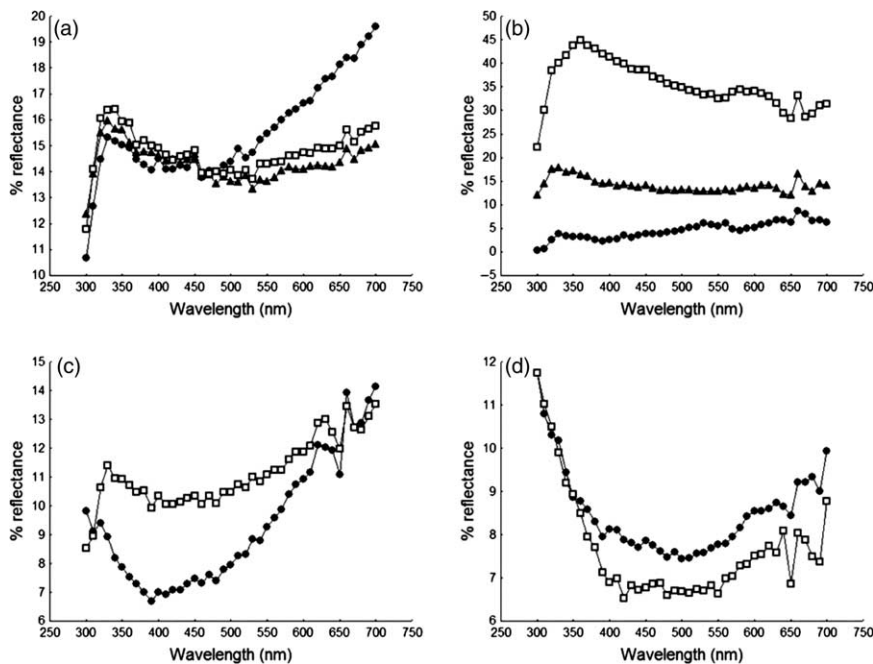


Figure 4. Reflectance spectra obtained at an area without broken barbules (squares), a totally abraded (i.e. black/brown) area (circles) and an area with an intermediate degree of broken barbules (triangles) in a bearded vulture (a) and a snow goose (b) feather. A similar result (c) was obtained when a bearded vulture feather was experimentally abraded (circles: reflectance spectrum from an area after barbules were experimentally abraded; squares: reflectance spectrum at the same area before barbules were abraded). (d) The result of the same experimental abrasion on a European magpie feather without sheen structure. Note how reflectance curves for abraded (circles) and non-abraded (squares) areas largely overlap, and, contrary to the effect in (b), the reflectance for the abraded area slightly increased compared to the non-abraded area.

see videoclip 1 in suppl. materials). This kind of white, however, can be easily distinguished from 'conventional' white like that described by Dyck (1976), as these feathers have a matte white and a 'dusty' appearance. The particularly striking example of the Andean condor illustrates how this mechanism of special barbules can generate brightness in feathers that are in fact dark. This suggests that originally black feathers, may have evolved as such because of selective pressures acting on melanization (e.g. environmental wear or need for crypsis; Lucas and Stettenheim 1972, Bortolotti 2006, Margalida et al. 2008 and references therein), and then a mechanism developed to change colour by increasing the brightness and thus the general conspicuousness of individuals.

When distal barbules were intact, the UV peak in the reflectance spectrum of feathers also stood out against the remaining portion of the spectrum. This type of spectral shape for sheen-white feathers, with an initial defined peak at the UV range followed by a decrease and stabilization in reflectance values, has also been observed for the white plumage of other species with 'conventional' feathers without the sheen structure discussed here (Penteriani et al. 2006, McGlothlin et al. 2007). At this stage we cannot determine whether sheen feathers have the effect of increasing overall plumage brightness or only reflectance at UV wavelengths, but it is noticeable that signalling mechanisms to stand out against dark environments often involve the reflectance of UV light. This has been shown for nestling skin, mouth and carotenoid-based plumage colour (Heeb et al. 2003, Hunt et al. 2003, Jourdie et al. 2004, Galván et al. 2008), and for the colour of blue-green eggs in cavity nesting birds (Avilés et al. 2006). A similar signalling device involving the production of startling flashes of UV light has been recently discovered in fish living in habitats with limited environmental light (Novales Flamarique et al. 2007). Therefore, it is likely that sheen feathers increase the conspicuousness of birds by increasing their UV reflectance, as UV light strongly contrasts against certain dark backgrounds (see references above). Of course, this would not necessarily exclude the possibility that sheen feathers increase conspicuousness by increasing brightness at all spectral regions. Further studies should search for associations between prevalence of sheen feathers and detailed habitat characteristics.

Possible functions of sheen structures

A first possibility is that the breakage susceptibility of the pennulum of sheen barbules make them a signalling device without losing their mechanical function of binding adjacent barbs, as the dark bases of the distal barbules are not easily destroyed by abrasion. Alternatively, inter-individual differences in the pattern of abrasion of these barbules is caused by behavioural attributes of birds and thus signals, for example, dominance status if that pattern is modified by agonistic interactions with conspecifics. Lastly, it is possible that sheen structures serve as signals for individual recognition, if they do not confer significant and differential costs with respect to quality to bearers and show high (i.e. multimodal) variation (Tibbetts and Dale 2007). Future studies should address these possibilities.

Given the occurrence of sheen feathers in different avian orders, the structure creating this type of plumage has surprisingly been overlooked. Selection pressures are strong in feather types that are critical for flight and for protecting certain parts of the body from abrasion, and the increased resistance to wear may frequently have been an important factor favouring melanization of this type of feather (Lucas and Stettenheim 1972, Bortolotti 2006, McGraw 2006, Schreiber et al. 2006). The plumages of all species in which we have observed sheen feather structures is mostly composed of black, brown, grey and white colours, suggesting a lack of other pigments responsible for bright colours, such as carotenoids. Thus, sheen structures may represent an alternative, and perhaps inexpensive, way to become more brightly coloured, or at least to increase 'achromatic conspicuousness' by increasing total plumage brightness, in those species that are limited in generating bright colours through the presence of other pigments.

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Appendix