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Colourful parrot feathers resist bacterial degradation

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The brilliant red, orange and yellow colours of parrot feathers are the product of psittacofulvins, which are synthetic pigments known only from parrots. Recent evidence suggests that some pigments in bird feathers function not just as colour generators, but also preserve plumage integrity by increasing the resistance of feather keratin to bacterial degradation. We exposed a variety of colourful parrot feathers to feather-degrading *Bacillus licheniformis* and found that feathers with red psittacofulvins degraded at about the same rate as those with melanin and more slowly than white feathers, which lack pigments. Blue feathers, in which colour is based on the microstructural arrangement of keratin, air and melanin granules, and green feathers, which combine structural blue with yellow psittacofulvins, degraded at a rate similar to that of red and black feathers. These differences in resistance to bacterial degradation of differently coloured feathers suggest that colour patterns within the Psittaciformes may have evolved to resist bacterial degradation, in addition to their role in communication and camouflage.

Keywords: *Bacillus licheniformis*; plumage coloration; Psittaciformes; psittacofulvins

1. INTRODUCTION

The colourful plumage of birds has long attracted the attention of behavioural ecologists, who have emphasized the importance of bright colours in visual signalling [1,2]. However, pigment molecules deposited in tissues, even non-living structures like feathers, may also serve non-visual functions, such as abrasion resistance [1] and metal binding [3]. Another well-supported function is the importance of melanin for resisting bacterial degradation by keratinolytic micro-organisms, such as *Bacillus licheniformis*, *Bacillus pumilus* and other *Bacillus* species [4–6]. Keratinolytic microbes are ubiquitous among birds [4,7,8] especially in humid or salty habitats where feather-degrading bacilli are abundant and increased melanization of the plumage may be selected to minimize microbial damage [9,10]. The selection of melanin for its resistance to bacterial degradation raises the possibility that other feather pigments may serve a similar protective function.

Parrots (order Psittaciformes) are a group of largely tropical species that display a remarkable variety of plumage colours. Their red, orange and yellow colours are unique, in that they are not derived from carotenoids, the common pigment source of such colours in birds, but from a class of endogenously synthesized pigments—psittacofulvins—found in no other organisms [11–13]. Given the humid, tropical habitat of many parrot species, their sexual monochromatism, at least to the human eye, and the lack of colour variation with age or diet ([14], but see [15] for an exception), we hypothesized that psittacofulvin pigments protect feathers from bacterial degradation. Our hypothesis is based, in part, on the preliminary data of Grande *et al.* [16], who found that green feathers from blue-crowned parakeets (*Aratinga aduticaudata*) were unusually resistant to bacterial degradation. We tested our hypothesis by exposing parrot feathers of several colours and species to *B. licheniformis* under controlled laboratory conditions and evaluating spectrophotometric signatures of feather break-down products [5] daily for 5 days after inoculation of the feathers with feather-degrading bacilli.

2. MATERIAL AND METHODS

(a) *Samples*

Colourful rectrix and remige feathers of 13 parrot species (see the electronic supplementary material, table S1 for species and scientific names) were acquired from owners of pet parrots in 2002 [12]. Feathers were categorized into six broad colour categories: blue, green, red, yellow, black and white (see electronic supplementary material, materials and methods for additional information).

(b) *Degradation of differently coloured parrot feathers*

Feather degradation tests and analyses were performed using a medium described by Williams *et al.* [17] and methods adapted from those of Goldstein *et al.* [5]. (see electronic supplementary material, materials and methods for additional information).

(c) *Dimensions of barbs from differently coloured parrot feathers*

Bacterial degradation of feathers is an enzymatic process that depends on surface area. Therefore, we matched differently coloured barbs as closely as possible for size (see the electronic supplementary material for additional information). In addition, the cortex of melanic barbs is thicker than that of non-melanic barbs [18]. A thicker cortex could increase resistance to bacterial degradation, independent of surface area or pigment presence/concentration. Since the relationship between feather colour and cortical thickness of barbs is unknown in parrots, we measured the cortex of differently coloured barbs at their thickest point to account for this potential confound (see electronic supplementary material for additional information). To control for differences in size of the barbs, we divided cortical thickness by the length of the cross section and compared degradation rates as a function of adjusted cortical thickness.

(d) *Chemical analysis of differently coloured parrot feathers*

Beyond the broad colour categories mentioned above, we conducted a more refined test of the relationship between psittacofulvin coloration/pigmentation and feather degradation. Since we previously showed [12] that the hue of colourful parrot feathers is significantly correlated with pigment content (and confirmed that relationship in our sample here; $r = -0.76$, $n = 11$, $p = 0.027$), we evaluated whether or not pigment concentration of yellow and red feathers was correlated with rates of bacterial degradation of the feathers. We determined the psittacofulvin concentration of each sample using a heated, acidified pyridine extraction and high-performance liquid chromatography, as previously described [12].

(e) *Statistical analyses*

We used an Analysis of Covariance and Tukey test to compare the degradation rate of the differently coloured feathers (see electronic

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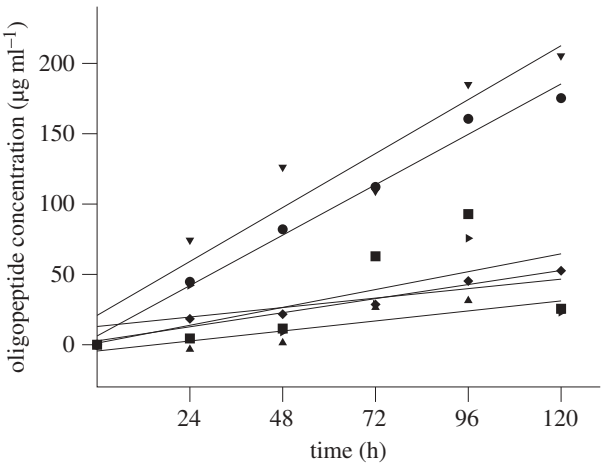

Figure 1. Bacterial degradation of feathers releases oligopeptides into the feather medium. Comparison of the rate of increase in the mean concentration of these by-products (microgram oligopeptides per millilitre) indicates that bacterial degradation is faster for white and yellow parrot feathers than for black, blue, green or red feathers. Pre-inoculation concentrations of oligopeptides were subtracted from all subsequent measurements for all colours. Inverted triangles, white; circles, yellow; diamonds, red; upright triangles, green; right-pointing triangles, blue; squares, black.

Table 1. Pairwise comparisons of the degradation rates of differently coloured parrot feathers. Statistically significant differences are in bold.

colour comparison	mean difference	critical difference	p
black–green	0.068	0.309	0.657
black–blue	0.153	0.346	0.374
black–red	0.111	0.261	0.393
black–yellow	−0.178	0.309	0.251
black–white	−0.363	0.324	**0.029**
green–blue	−0.085	0.346	0.620
green–red	0.043	0.261	0.739
green–yellow	−0.246	0.309	0.115
green–white	−0.432	0.324	**0.011**
blue–red	−0.042	0.304	0.781
blue–yellow	−0.331	0.346	0.060
blue–white	−0.517	0.359	**0.006**
red–yellow	−0.289	0.261	**0.031**
red–white	−0.475	0.279	**0.002**
yellow–white	−0.186	0.324	0.254

supplementary material for additional information), We used an Analysis of Variance to compare the cross-sectional width and length of barbs (electronic supplementary material, table S2) and a Pearson's correlation test to evaluate the effect of cortical thickness on degradation rate (electronic supplementary material, figure S1) and the relationship between concentration of red and yellow feather psittacofulvins and bacterial degradation rate.

3. RESULTS

(a) *Degradation of differently coloured parrot feathers*

Feather colour significantly affected bacterial degradation rate (figure 1). White feathers degraded more rapidly than black, blue, green and red feathers (table 1). Red, blue and green feathers degraded at rates comparable to black (table 1). It is worth noting that green feathers have both psittacofulvins and melanin [19], but the combination of pigments did not slow the rate of bacterial degradation relative to feathers with only melanin (blue, black) or only psittacofulvin (red). Yellow feathers degraded significantly more rapidly than red feathers (table 1).

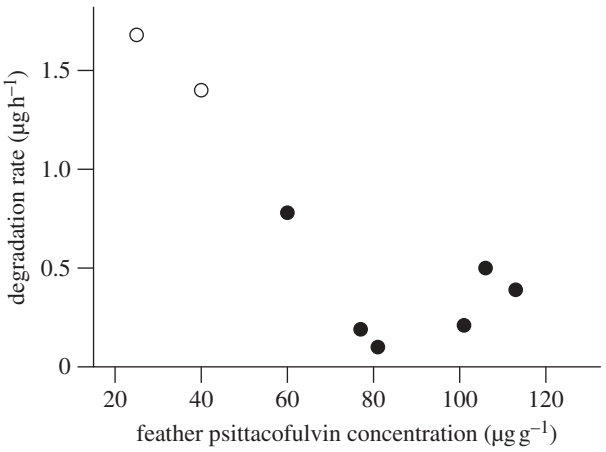

Figure 2. Bacterial degradation of feathers slows as the concentration of yellow (open circles) and red (filled circles) psittacofulvins increases.

(b) *Measurement of cortical thickness from differently coloured barbs*

Cross-sectional length ($F_{1,14} = 59.09$, $p < 0.001$) and width ($F_{1,14} = 53.55$, $p < 0.001$) differed significantly among differently coloured barbs (electronic supplementary material, table S2). Cortical thickness, even when controlled for barb size, was significantly and negatively correlated with degradation rate (electronic supplementary material, table S2 and figure S1; $r = -0.607$, $p < 0.02$).

(c) *Relationship between feather psittacofulvin concentration and resistance to bacterial degradation*

Psittacofulvin concentration of red and yellow feathers was significantly and negatively correlated with the rate of bacterial degradation (figure 2; $r = -0.83$, $p = 0.008$).

4. DISCUSSION

Our findings are consistent with the hypothesis that colourful pigments reduce microbial damage to parrot feathers. First, feathers containing red psittacofulvins degraded more slowly than white feathers. Second, red feathers degraded more slowly than yellow feathers, which contain only short-chain psittacofulvins that have fewer double-bonds than red forms [12]. Long-chain psittacofulvins may bind the layers of the pleated sheet structure of the β-keratin molecules more tightly than the short-chain psittacofulvins, making it difficult for the β-keratinase to position itself within the pleated sheet. Psittacofulvins, like melanin [18], thicken the cortex of the barb, which may make it more difficult for feather-degrading bacilli to break through the structure of the barb. Third, pigment concentration of yellow and red feathers was

negatively correlated with bacterial degradation rate. Fourth, green feathers, like black and red feathers, resisted bacterial degradation. Green parrot feathers contain both yellow psittacofulvin and melanin, which increase the resistance of the feather to abrasion [1,20]. For many species of parrots, green also provides camouflage against foliage. These several advantages of green may be why it is so widespread among parrots compared with shades of brown and grey, which are also cryptic and common among birds [1], but uncommon among parrots.

Feather-degrading bacilli occur in the plumage of all avian species sampled to date [4,21]. Furthermore, in the only field study, Gunderson *et al*. [22] showed that feather-degrading bacilli were active in the plumage of eastern bluebirds (*Sialia sialis*) and altered feather colour, reduced body condition and lowered reproductive success. We assume that feather-degrading bacilli are present in the plumage of parrots and that their presence has potential consequences.

Our study does not rule out alternative visual and non-visual functions of the several pigments found in parrot feathers. Psittacofulvin-based colours appear to serve only rarely as variable sexual signals [15], but they may still work as contrasting colours that help females (as in *Eclectus roratus*) or all members of a species contrast against green leaf backgrounds to indicate nest occupancy [23] or maintain social cohesiveness in large flocks. The fact that the cortex of barbs with melanin or psittacofulvin was significantly thicker than the cortex of barbs lacking these pigments suggests that psittacofulvins may increase the abrasion resistance of parrot feathers, though this possibility is unstudied. Our data suggest that the effects of melanin and psittacofulvins are not additive, at least in green feathers, but this needs to be tested with larger sample sizes and perhaps experimentally (e.g. by removing pigments chemically). Most importantly, our results emphasize that, in addition to their optical properties, biologists must consider the physical and chemical effects of pigments in animal integuments [1–3], even those as visually striking as the brilliant red of the scarlet macaw (*Ara macao*).

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