

# Bacteria as an Agent for Change in Structural Plumage Color: Correlational and Experimental Evidence

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**ABSTRACT:** Recent studies have documented that a diverse assemblage of bacteria is present on the feathers of wild birds and that uropygial oil affects these bacteria in diverse ways. These findings suggest that birds may regulate the microbial flora on their feathers. Birds may directly inhibit the growth of harmful microbes or promote the growth of other harmless microbes that competitively exclude them. If keratinolytic (i.e., feather-degrading) bacteria degrade colored feathers, then plumage coloration could reveal the ability of individual birds to regulate microbial flora. We used field- and lab-based methods to test whether male eastern bluebirds (*Sialia sialis*) with brighter blue structural plumage coloration were better able to regulate their microbial flora than duller males. When we sampled bluebirds in the field, individuals with brighter color had higher bacterial loads than duller individuals. In the lab, we tested whether bacteria could directly alter feather color. We found that keratinolytic bacteria increased the brightness and purity, decreased the ultraviolet chroma, and did not affect the hue of structural color. This change in spectral properties of feathers may occur through degradation of the cortex and spongy layer of structurally colored barbs. These data suggest that bacteria can alter structural plumage color through degradation.

**Keywords:** feather-degrading bacteria, plumage color, structural color, sexual selection, *Sialia sialis*.

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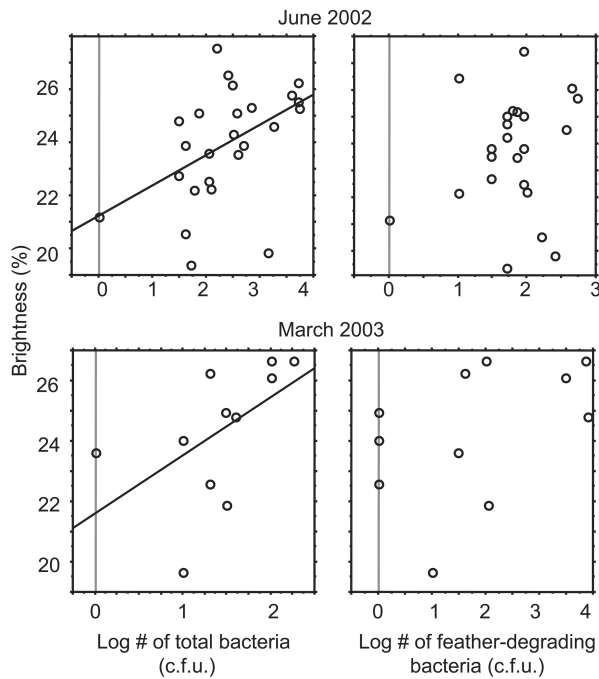
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Indicator models of sexual selection propose that ornamental traits signal aspects of male quality (reviewed in Andersson 1994). One aspect of quality that is important for virtually all animals is the degree of parasitic infection. High-quality males should have fewer parasites than low-quality males, and if ornaments are signals of quality, males with few parasites should be more highly ornamented than males with many parasites. Females should prefer to mate with males with low parasite loads in order to avoid parasite transfer (Freeland 1976; Borgia 1986; Hilgarth 1996), to gain greater parental care (Hamilton 1990; Milinski and Bakker 1990; Møller 1990), or to pass on genes for parasite resistance (Hamilton and Zuk 1982). Numerous studies have tested these hypotheses, most often assessing correlations between individual coloration and numbers of protozoan or metazoan parasites in the blood, gut, or integument (reviewed in Møller et al. 1999).

Despite the ubiquity of bacteria and their frequently deleterious effects on organisms, only a few studies have documented associations between bacterial infection and sexually selected traits (Brawner et al. 2000; Hill et al. 2004), and these studies have focused on how systemic bacterial infections affect the production of ornaments. But bacteria live not just within but also on the surface of birds, in their feathers (reviewed in Burt 1999; Shawkey and Hill 2004). Only a small subset of feather-dwelling bacteria will be detrimental to the bird, and an even smaller subset will cause damage to feathers. Burt and Ichida (1999) isolated the keratinolytic (i.e., able to degrade keratin) bacterium *Bacillus licheniformis* in only 8% of 1,588 wild birds surveyed. Other authors, however, have isolated bacteria with keratinolytic properties in the genera *Vibrio* (Sangali and Brandelli 2000), *Fervidobacterium* (Friedrich and Antranikian 1996), *Pseudomonas* (Shawkey et al. 2003a), *Arthrobacter* (Lucas et al. 2003), and *Streptomyces* (Noval and Nickerson 1959; Mukhopadhyay and Chandra 1990; Böckle et al. 1995; Ichida et al. 2001). Thus, the exclusive focus on *B. licheniformis* by Burt and Ichida



**Figure 1:** Brightness of rump feathers in relation to total and keratinolytic colony forming unit (c.f.u.) abundances among eastern bluebirds in Auburn, Alabama, in June 2002 ( $n = 25$ ) and March 2003 ( $n = 11$ ).

(1999) may have led to an underestimate of the extent to which keratinolytic bacteria are found in feathers.

Many other bacteria isolated from feathers are not able to degrade keratin (Shawkey et al. 2003a, 2005b; Lucas et al. 2005). Some of these bacteria, such as members of the genus *Enterococcus* (Shawkey et al. 2003a), are opportunistic pathogens (Ruoff 1993). Others, such as *Staphylococcus epidermidis*, are part of the normal skin microflora (Tannock 1995). How these bacteria interact with one another and with birds is not clear and needs further exploration. However, these studies clearly indicate that a diverse bacterial assemblage, composed of both keratinolytic and nonkeratinolytic species, is found on feathers. Some recent research suggests that birds can actively regulate the composition of this assemblage.

A recent study by Lucas et al. (2005) demonstrated that birds with experimentally enlarged broods have higher densities of a less diverse bacterial assemblage on their feathers than those with reduced broods. These results suggest a trade-off between energy spent on foraging or reproduction and energy devoted to preening or other sanitation behaviors. By performing sanitation behaviors such as preening, birds may allow harmless or beneficial bacteria to grow and may prevent the growth of pathogenic

bacteria (Bandyopadhyay and Bhattacharyya 1996; Shawkey et al. 2003a).

A relationship between feather color and bacterial load on feathers could thus arise in at least two ways. First, color might signal overall quality, and higher-quality birds might be better able to allocate energy to sanitation behaviors that reduce bacterial abundance than poor-quality birds. Feather degradation by bacteria could negatively affect hosts by decreasing the thermoregulation and protection from other bacterial infections provided by feathers (Burt and Ichida 1999; Clayton 1999; Muza et al. 2000), and reduced individual condition could then affect plumage coloration. If this hypothesis is true, then we predict that birds with more elaborate ornamentation would have lower abundances of bacteria. Alternatively, if certain bacteria are beneficial either directly (Lombardo et al. 1999) or indirectly by competitively excluding other harmful bacteria (e.g., Schoeni and Wong 1994; Rabsch et al. 2000), then we predict that birds with brighter plumage color would have higher abundances of total bacteria. Second, bacteria may directly affect plumage color by breaking down the structures involved in the production of color (Shawkey and Hill 2004). Feather degradation could reduce feather coloration as pigments are consumed and chemically modified through microbial action and as microstructures are consumed (Shawkey and Hill 2004). This possibility seems particularly likely in the case of structural plumage color, which is based on the reflection of light from complexly arranged tissues (reviewed in Prum 1999, 2006). Physical change in these structures, such as by alteration of the dimension or the amount of the medullary “spongy layer” (for review, see Prum 2006), may affect the color they produce. Örnborg et al. (2002) recently showed that the structurally based ultraviolet (UV)-blue crown color of blue tits (*Parus caeruleus*) becomes brighter and less UV reflective over the course of the breeding season,

**Table 1:** Spearman rank correlations of general and feather-degrading bacterial loads with color variables of rump feathers of eastern bluebirds

Sampling period	UV-V		Brightness (%)	Spectral saturation (%)
	Hue (nm)	chroma (%)		
General bacterial load:				
June 2002	.05	-.02	.50*	.03
March 2003	.01	.04	.65*	.11
Feather-degrading bacterial load:				
June 2002	-.05	-.13	.16	.10
March 2003	.00	.14	.20	.16

Note: For June 2002,  $n = 25$ , and for March 2003,  $n = 11$ . UV-V = ultraviolet-violet.

\* Significant correlations at  $P < .05$ .

**Table 2:** Comparison of the color of eastern bluebird feathers before and after incubation in humidified chambers with either the feather-degrading bacterium *Bacillus pumilus* or a sham control

	Control before manipulation	Control after manipulation	<i>t</i>	<i>P</i>	Experimental before manipulation	Experimental after manipulation	<i>t</i>	<i>P</i>
Hue (nm)	419.91 ± 3.10	419.61 ± 3.28	.31	.64	419.01 ± 2.52	423.65 ± 3.30	-1.65	.12
Brightness (%)	25.69 ± .85	26.00 ± .95	-.30	.77	24.11 ± .56	28.14 ± 1.42	-2.95	<.01
UV-V chroma (%)	40.00 ± 1.00	40.00 ± 1.00	-.72	.48	39.00 ± .64	36.00 ± .81	3.56	<.01
Spectral saturation (%)	20.31 ± .70	20.56 ± .89	-.26	.80	18.55 ± .60	21.33 ± 1.66	-2.5	.02

Note: Control and experimental values are shown ± 1 SE; *t* and *P* values are from paired *t*-tests comparing the same feathers before and after manipulation.

suggesting that wear on feathers, perhaps partially caused by feather-degrading bacteria, may change their reflective properties. In this case, we would predict a positive relationship between keratinolytic bacteria and the brightness of structural coloration and a negative relationship between keratinolytic bacteria and UV reflectance.

The eastern bluebird (*Sialia sialis*) is an excellent model organism on which to test these hypotheses. Structural UV-blue plumage color of bluebirds appears to be sexually selected; males with brighter blue structural plumage color have greater competitive ability than duller males (Siefferman and Hill 2005), and males with brighter structural and melanin breast coloration have higher reproductive success than duller males (Siefferman and Hill 2003). Furthermore, the anatomical basis of variation in the coloration of bluebird feathers is fairly well understood (Shawkey et al. 2003b, 2005a), allowing us to identify specific mechanisms associated with bacterially mediated changes in plumage color.

We used both correlational and experimental approaches to test whether bacteria cause a change in plumage coloration or are just correlated with such a change. First, we trapped adult male bluebirds, quantified the coloration of their feathers, and measured the abundance of total and keratinolytic bacteria on their feathers. We then correlated measures of bacterial abundance to plumage color. In the lab, we further tested for an effect of bacteria on structural coloration by inoculating groups of feathers with keratinolytic bacteria and examining changes in color and microstructure after a brief incubation period.

## Methods

### Sampling

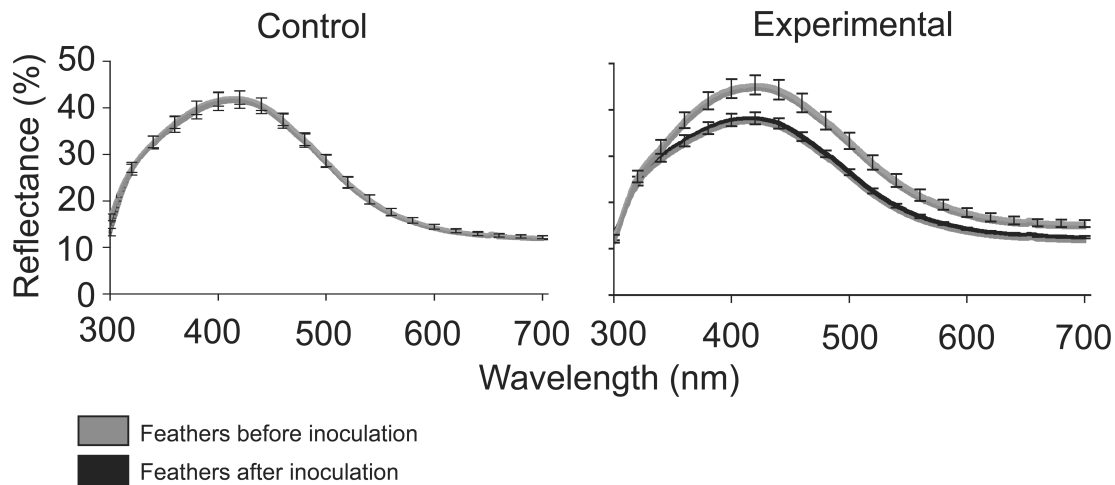
In June 2002 and March 2003, we captured adult male eastern bluebirds in mist nets and box traps on the campus of Auburn University in Lee County, Alabama (32°35'N, 82°28'W). Wearing sterile latex gloves, we gently rubbed a BBL CultureSwab (Becton-Dickinson, Sparks, MD) dipped in sterile phosphate-buffered saline (PBS) on their structurally colored rump feathers. Following bacterial

sampling, we pulled feathers from the rump of each bird for color analysis. Swabs were immediately refrigerated and then washed in 1 mL sterile PBS within 4 h of collection, and feathers were stored in small manila envelopes in a climate-controlled room (approximately 21°C) until the time of color analysis.

### Media

We used two types of media for quantification of bacteria. Tryptic soy agar (TSA; Difco, Detroit, MI) is a rich medium that supports a wide variety of microorganisms, and its use enabled us to quantify overall bacterial load, including most keratinolytic and other bacterial flora. We sampled for the keratinolytic subset of these bacteria by using feather meal agar (FMA; Sangali and Brandelli 2000), containing 15 g L<sup>-1</sup> feather meal, 0.5 g L<sup>-1</sup> NaCl, 0.30 g L<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, 0.40 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, and 15 g L<sup>-1</sup> agar. FMA was used to specifically estimate keratinolytic bacterial abundance because it contains feather keratin as its sole source of carbon (Sangali and Brandelli 2000). In another study, we found that almost all bacterial isolates taken from this media produced keratinase, an enzyme that catalyzes the hydrolysis of keratin (Shawkey et al. 2003a). Therefore, most bacteria growing on it should be able to digest keratin and could be considered putatively keratinolytic. Both media types contained 100 μg mL<sup>-1</sup> of cycloheximide to inhibit fungal growth (Smit et al. 2001).

We plated 100 μL of our inoculated samples on TSA and FMA and incubated the plates at 37°C. TSA plates were removed after 2 days, while FMA plates were removed after 14 days. The faster growth of microorganisms on TSA than on FMA necessitated this difference in incubation time, as colonies began to merge, and hence became uncountable, after 48 h on TSA. FMA plates were incubated longer because colonies could be clearly distinguished only after 2 weeks. The number of visible colony forming units (CFUs) on each plate was counted for each medium type as an estimate of general and keratinolytic bacterial load (total plate count; see Hambreaus et al. 1990; Bettin et al. 1994; Brock et al. 1994; Miller et al. 1994).



**Figure 2:** Reflectance spectra ( $\pm 1$  SE) of feathers before and after inoculation with either the keratinolytic bacterium *Bacillus pumilus* or a sham control. The dark lines represent groups before treatment, while the light lines represent the same groups after treatment;  $n = 20$  in all cases.

All counts were performed without knowledge of the bird's color scores.

For color analysis, we taped feathers in stacks of five directly on top of one another to gloss-free black construction paper. Blue and gray portions of each feather were aligned with one another so that no mixing of the two colors occurred. This arrangement was similar to that found on the living bird. We recorded spectral data from the blue portion of these feather stacks with an Ocean Optics (Dunedin, FL) S2000 spectrometer (range 250–880 nm). Using a probe holder (RPH-1; Ocean Optics) with matte black interior that excluded ambient light, we held a bifurcated 1- $\mu$  fiber-optic probe at a 90° angle 5 mm from the feather surface, creating a measurement area 2 mm in diameter. The reading area was illuminated by both a UV (D-2000 deuterium bulb; Ocean Optics) and a visible (tungsten-halogen bulb) light source. All data were generated relative to a white standard (WS-1; Ocean Optics). We used OOIbase software (Ocean Optics) to record and average 20 spectra sequentially, and we recorded and averaged measurements from five haphazardly chosen points on each sample.

From these reflectance spectra, we calculated color variables for each sample. We restricted these indexes to wavelengths between 300 and 700 nm because evidence suggests that passerine birds are sensitive to UV wavelengths (300–400 nm; Cuthill et al. 2000) and that 700 nm is the upper limit of the vertebrate visual system (Jacobs 1981). The wavelength of maximum reflectance was used as an index of hue, the principal color reflected by the feathers (e.g., Andersson 1999; Keyser and Hill 1999). Brightness, the mean of reflectances from 300 to 700 nm, is a measure of

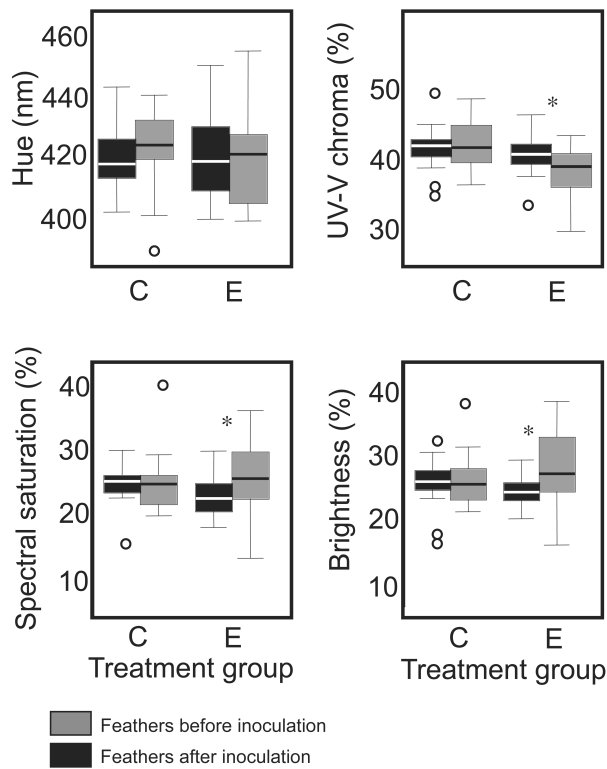
the total amount of light reflected by the feathers (Endler 1990; Andersson 1999). The UV-violet (UV-V) chroma is the percentage of total light reflected in the range of 300–420 nm (Andersson et al. 1998). Spectral saturation, the percentage of total light reflected within a range of 50 nm on either side of the hue value, is an index of color purity (Pryke et al. 2001).

#### Experimental Methods

We performed experimental inoculation of bluebird feathers. We arbitrarily picked five rump feathers from each of 40 different males from a large collection of feathers in our lab and measured them using a spectrometer as above. We then divided these feathers into experimental and control groups of 20 individuals each. We sterilized feathers with 10 kGy of  $\gamma$ -radiation at the Auburn University Space Research Center. This sterilization method effectively kills almost all (>99.9%) microbes (including fungi) on any surface (Roberts 1985; Silverman 1991).

To simulate a humid environment, we constructed simple humidified chambers in petri dishes by using layers of sterile wet and dry blotter paper and plastic. Sterile circular pieces of blotter paper were saturated with sterile water and placed on the outermost position of the top and bottom portions of the dishes. Between these wet layers, we placed two pieces of dry sterile blotter paper sandwiching two pieces of sterile plastic.

In a previous study, we had identified a large number of bacterial isolates from bluebird feathers (Shawkey et al. 2005a). We tested a subset of these bacteria for keratinolytic activity following the methods of Shawkey et al.



**Figure 3:** Box plots of color variables measured before and after inoculation with either the keratinolytic bacterium *Bacillus pumilus* or a sham control. The dark boxes represent groups before treatment, while the light boxes represent the same groups after treatment. The line within each box represents the median color variable, the upper and lower borders of each box are the twenty-fifth and seventy-fifth percentiles, and the lower and upper bars are the tenth and ninetieth percentiles. Asterisks indicate significant ( $P < .05$ ) difference;  $n = 20$  in all cases.

(2003a) and chose for our experimental treatment a highly keratinolytic bacterium, identified as closely related to *Bacillus pumilus* by 16S rDNA sequence analysis (Shawkey et al. 2005a). Colonies of this bacterium were grown for 24 h on TSA and were added to sterile PBS until a turbidity comparable to McFarland standard 1.0 (equivalent to about  $3 \times 10^8$  bacterial cells  $\text{mL}^{-1}$ ) was reached. We then inoculated feathers with 500  $\mu\text{L}$  of either this bacterial solution (experimental group) or sterile PBS (control group), patted them with sterile tissues to absorb excess moisture, and placed them between the two plastic layers of the humidified chambers.

We incubated these chambers at 37°C for 72 h and then removed them and placed the feathers in new sterile petri dishes. We washed feathers in ethanol and allowed them to air-dry in a sterile hood. After drying for 24 h, all feathers were taped to black construction paper and measured with the spectrometer as above.

### Microscopy

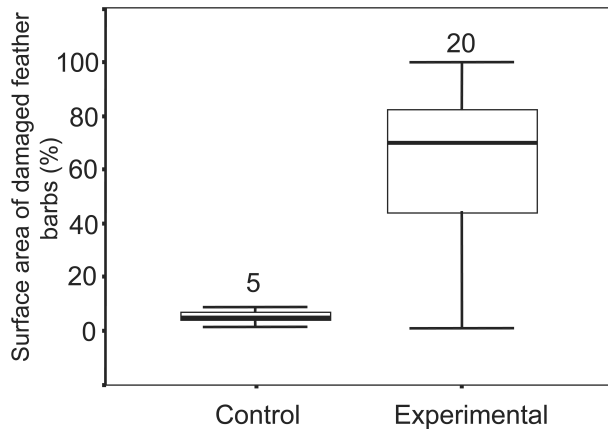
To determine how bacteria damaged feathers and whether this damage caused change in feather color, we examined feathers from our inoculation experiment with a scanning electron microscope (SEM). We mounted one feather from each experimental sample and one feather from five control samples on stubs (Ted Pella, Redding, CA) using carbon tape (Ted Pella), sputter-coated them with gold on a Electron Microscopy Sciences sputter coater (Hatfield, PA) and viewed them on a Zeiss DSM 930 SEM (Oberkochen, Germany). We took five consecutive photos of barbs at  $\times 500$  magnification, moving clockwise from the distal end of the central rachis of each feather. We defined damaged surface area as any disruption in the integrity of the barb's surface (see fig. 4). We then measured total surface area and damaged surface area in NIH Image, version 1.62 (available for download at <http://rsb.info.nih.gov/nih-image>). We divided damaged surface area by total surface area as a relative index of feather damage.

### Analyses

All analyses were performed on SPSS, version 10, for Macintosh (SPSS 2002). Percentage data were arcsine transformed, and bacterial count data were log transformed. We correlated TSA and FMA plate counts with color variables using Spearman's rank correlation test because our data were not normally distributed. We used paired  $t$ -tests to compare the color of experimental and control groups before and after manipulation and then created a proportional "color change" variable for each color measurement by subtracting each postexperiment color measurement from the corresponding preexperiment measurement and dividing by this original measurement. Finally, we correlated these color change variables with the amount of feather damage using Pearson's correlation test. All tests were two tailed.

### Results

In June 2002, overall CFU abundance and brightness were significantly positively correlated (Spearman rank correlation,  $r_s = 0.504$ ,  $P = .010$ ,  $n = 25$ ; fig. 1; table 1), but keratinolytic CFU abundance and brightness were not significantly correlated ( $r_s = 0.164$ ,  $P = .456$ ,  $n = 25$ ; fig. 1; table 1). No other variables were significantly correlated (all  $P > .4$ ; table 1). When we replicated this experiment in March 2003, we found similar patterns. Again, overall CFU abundance and brightness were significantly positively correlated (Spearman rank correlation,  $r_s = 0.654$ ,  $P = .029$ ,  $n = 11$ ; fig. 1; table 1), but keratinolytic CFU abundance and brightness were not significantly correlated



**Figure 4:** Box plot of percentage of feather barb surface area that was damaged following inoculation with either the keratinolytic bacterium *Bacillus pumilus* or a sham control. The line within each box represents the median percentage of damaged area, the upper and lower borders of each box are the twenty-fifth and seventy-fifth percentiles, and the lower and upper bars are the tenth and ninetieth percentiles.

( $r_s = 0.422$ ,  $P = .196$ ,  $n = 11$ ; fig. 1; table 1). No other variables were significantly correlated (all  $P > .4$ ; table 1).

In the humidified chamber experiment, color measurements of the control group did not change significantly following sham manipulation (all  $P > .4$ ; table 2; figs. 2, 3). The experimental group had significantly higher brightness ( $t = -2.95$ ,  $P = .008$ ; table 2; figs. 2, 3) and spectral saturation ( $t = -2.50$ ,  $P = .02$ ; table 2; figs. 2, 3) and significantly lower UV-V chroma ( $t = 3.56$ ,  $P = .002$ ; table 2; figs. 2, 3) following incubation with keratinolytic bacteria. Hue did not change significantly ( $P > .12$ ; table 2; figs. 2, 3).

Bacteria appeared to damage feathers primarily by thinning the cortex and breaking barbs and barbules (fig. 4). As expected, feathers in the experimental group were significantly more damaged than those in the control groups (Mann-Whitney  $U = 5.00$ ,  $Z = -3.06$ ,  $P = .001$ ; figs. 4, 5). Within our experimental group, change in UV-V chroma during the experiment was positively correlated with feather damage such that feathers that were heavily damaged during the experiment lost more UV-V reflectance than those that were lightly damaged ( $r = 0.52$ ,  $P = .02$ ; fig. 6). Feather damage was not significantly correlated with change in any other color variable (brightness:  $r = 0.12$ ,  $P = .62$ ; spectral saturation:  $r = 0.20$ ,  $P = .41$ ; hue:  $r = -0.33$ ,  $P = .16$ ; fig. 6).

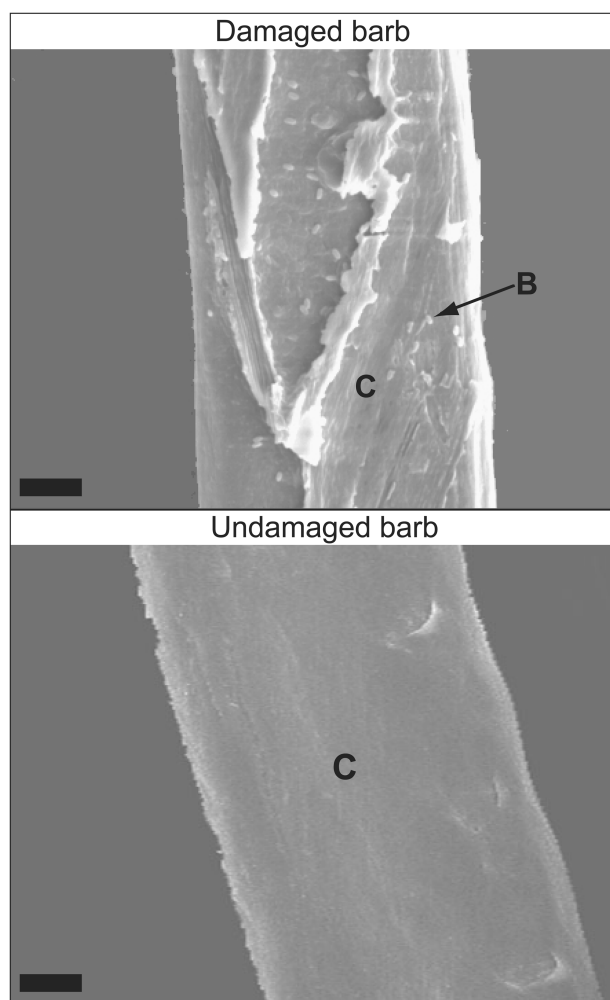
### Discussion

We found that male bluebirds with brighter structural color had a higher overall CFU abundance on their feathers

than males with duller plumage. These patterns did not support the hypothesis that bright coloration indicates overall ability to inhibit microbial growth. This positive relationship between bacterial load and color could have arisen in a number of ways. First, bright, dominant male eastern bluebirds may allocate more time to display and territorial defense than to sanitation behaviors, leading to an increase in bacterial numbers on feathers. This possibility could be tested by comparing the time spent preening by dull versus bright birds. Other studies have shown a negative link between social dominance and condition (e.g., Poiani et al. 2000), suggesting that dominance may entail health costs. Similarly, European starlings *Sturnus vulgaris* with experimentally increased reproductive workloads had more numerically abundant bacterial assemblages on their feathers than starlings with reduced workloads (Lucas et al. 2005), perhaps because they devoted less time to sanitation behavior. Brighter bluebirds may also raise a larger number of young than duller birds and thus invest more energy in reproduction, leaving less for self-maintenance (Siefferman and Hill 2003). However, we found a similar relationship between brightness and bacterial load both before egg laying and after the completion of the last clutch, so this explanation seems unlikely.

Second, some of these bacteria may be beneficial (Lombardo et al. 1999), and brighter birds may be better able to acquire or enhance the growth of these bacteria than duller birds. In particular, some bacteria may competitively exclude the growth of pathogenic bacteria, as the normal microflora does on human skin (Tannock 1995). Testing these hypotheses will first require research into the effects that different species of bacteria have on birds both alone and in assemblages. At present, our knowledge of these effects is largely limited to a small number of pathogenic bacteria (reviewed in Charlton 2000). Such studies would greatly enhance our understanding of the complex interactions between birds and microbes but are beyond the scope of this article.

Finally, bacteria may directly alter plumage color (Shawkey and Hill 2004). If brighter males have more bacteria because bacteria affect feathers in a way that increases brightness, we would expect to see a stronger correlation between keratinolytic CFU abundance and color than between overall CFU abundance and color. We observed the opposite: there was a strong relationship between feather color and overall bacterial load but not feather color and keratinolytic CFU abundance. These results remain puzzling, but bacteria live in complex communities, and even bacteria that are not able to break down keratin themselves may play a role in feather degradation (Lucas et al. 2005). Thus, entire assemblages of microbes may be needed for feather breakdown in the wild. Additionally, certain keratinolytic bacteria may be more ef-



**Figure 5:** Scanning electron micrograph ( $\times 500$ ) of the outer surface of a feather barb experimentally damaged by the keratinolytic bacterium *Bacillus pumilus* and an undamaged feather barb. C = cortex, B = bacterial cells.

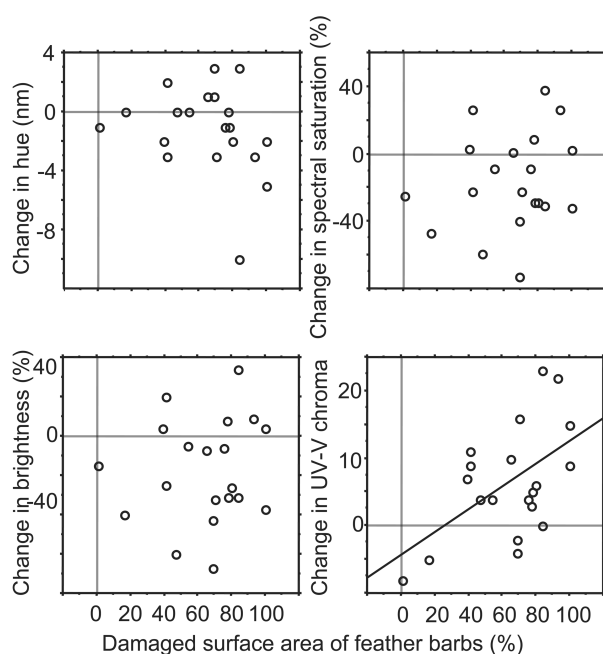
fective at breaking down feathers than others but may not be numerically dominant. The relationship between these important keratinolytic bacteria and plumage color may have been swamped in our analysis.

We tested the hypothesis that bacteria can directly change feather color in a lab experiment. This experiment provided direct evidence that keratinolytic bacteria are able to alter structural plumage coloration. It is worth noting, however, that our inoculation of feathers with a single bacterial strain was a highly simplified test that did not account for the synergistic or antagonistic effects of other bacteria on feathers. Indeed, Cristol et al. (2005) did not find any effect of inoculation with a single strain of keratinolytic bacteria on feathers of captive birds. Degradation

of feathers may occur quite differently under natural conditions with the complex bacterial communities that appear to exist on feathers (Lucas et al. 2005; Shawkey et al. 2005a). Future studies should inoculate entire assemblages of bacteria on feathers.

Bacterial degradation appears to strongly increase overall brightness of feathers. As figure 2 illustrates, the change in reflectance over most wavelengths (excluding from 300 to 320 nm) of the experimental feathers following inoculation is higher than that of the controls. This increase, equivalent to multiplying the reflectance values of the experimental feathers before inoculation by a constant  $>1$ , probably explains the changes in other color variables. Multiplication of the large reflectance values around the peak wavelength results in a greater increase than at other wavelengths and thus heightened saturation. Because these peak wavelengths are outside of the UV, reflectance values within the UV increase proportionally less, causing decreased UV chroma. This change does not affect hue because it is independent of brightness.

The anatomical explanation for these differences probably involves changes in the thickness of the cortex and subsequent exposure of the spongy layer. As described previously (Shawkey et al. 2003b), the color-producing spongy layer of eastern bluebird feather barbs lies beneath a keratin cortex and above a layer of melanin granules surrounding large central vacuoles. In this study, we found that bacteria tended to degrade the cortex and expose the spongy layer. The loss of light-absorbing cortex (Finger 1995) may cause overall reflectance to increase through greater exposure of light to the spongy layer (Shawkey et al. 2005a). This increase could cause greater reflection of light from the spongy layer and hence higher brightness. Furthermore, such damage to the cortex would not affect the dimensions of the spongy layer itself, and this pattern is consistent with the observed constancy of hue values of the experimental group following treatment. Why UV chroma decreased in the experimental group is less clear. Reflection at very short UV wavelengths (300–320 nm) appears not to be affected by the experimental treatment (fig. 2), suggesting either that the elements of barb morphology responsible for reflection at these wavelengths were not damaged by the bacteria or that their reflective properties were not altered by damage. The lack of change in these wavelengths coupled with the overall increase in brightness of the experimental feathers may have caused a decrease in UV-V chroma. Weak or absent correlations between these color variables and one simple measure of barb damage suggests that other unmeasured types of damage, such as loss of barbules and depth of damage to the barb, may be critical to color change. Future studies should examine these and other damage variables in relation to color in wild populations of birds.



**Figure 6:** Scatter plots of change in color variables versus relative surface area of damaged feather barbs following inoculation with the keratinolytic bacterium *Bacillus pumilus*;  $n = 20$  in all cases.

It is clear from our results that keratinolytic bacteria can directly alter structural plumage color, and this result is novel and potentially important for sexual signaling. A number of studies have shown evidence that females prefer males with more UV-reflective feathers in species with both noniridescent (Andersson et al. 1998; Hunt et al. 1999; Sheldon et al. 1999) and iridescent (Bennett et al. 1997) colors. Other studies have found evidence that the brightness and UV-V chroma of structural coloration signals the resource-holding potential of males (Keyser and Hill 1999; Siefferman and Hill 2005). Thus, if bacteria change the color of feathers in wild birds, they may play an important role in the evolution of the signaling function of structural color. Before we confer an important role to feather-degrading bacteria in the signal content of blue feathers, we will first have to show that the type of damage shown in our experiment occurs in wild populations of birds and that it is correlated with measures of color. Bacteria may not degrade feathers so extensively in the wild and may have beneficial or commensal roles.

Perhaps most important, these data also suggest that, contrary to the dominant paradigm (reviewed in Hill and McGraw 2006), brightness may not always be a reliable signal of male quality. More damaged feathers are brighter, and birds may thus be naturally bright or they may become bright by feather damage. If the system is open to “cheat-

ers,” then the signal content of the color display will be eroded, and both males and females should lose interest in it over evolutionary time (Hill 1994). Perhaps achromatic variables such as brightness that can be heavily altered by environmental damage (Örnberg et al. 2002; this study) are not as reliable as those that are not so susceptible, such as hue. Alternatively, the aerodynamic or insulation properties of damaged feathers may be superior to those of undamaged feathers. All of these hypotheses will require further research.

This study shows that bacteria on the surface of feathers may play an important, hitherto unexamined, role in the maintenance and evolution of structural plumage. The potential for interactions between microbes and feathers to affect the signal content of structural color display is enormous and should be explored through correlative and experimental studies.

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### Literature Cited

- Andersson, M. A. 1994. *Sexual selection*. Princeton University Press, Princeton, NJ.
- Andersson, S. 1999. Morphology of UV reflectance in a whistling-thrush: implications for sexual selection. *Journal of Avian Biology* 30:193–204.
- Andersson, S., J. Örnberg, and M. Andersson. 1998. Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proceedings of the Royal Society B: Biological Sciences* 265:445–450.
- Bandyopadhyay, A., and S. P. Bhattacharyya. 1996. Influence of fowl uropygial gland and its secretory lipid components on growth of skin surface bacteria of fowl. *Indian Journal of Experimental Biology* 34:48–52.
- Bennett, A. T. D., I. C. Cuthill, J. C. Partridge, and K. Lunau. 1997. Ultraviolet plumage colors predict mate preferences in starlings. *Proceedings of the National Academy of Sciences of the USA* 94: 8618–8621.
- Bettin, K., C. Clabots, P. Mathie, K. Willard, and D. N. Gerding. 1994. Effectiveness of liquid soap vs. chlorhexidine gluconate for the removal of *Clostridium difficile* from bare hands and gloved hands. *Infection Control and Hospital Epidemiology* 15:697–702.
- Böckle, B., B. Galunsky, and R. Müller. 1995. Characterization of a



- keratinolytic serine proteinase from *Streptomyces pactum* DSM 40530. *Applied and Environmental Microbiology* 61:3705–3710.
- Borgia, G. 1986. Satin bowerbird parasites: a test of the bright male hypothesis. *Behavioral Ecology and Sociobiology* 19:355–358.
- Brawner, W. R. I., G. E. Hill, and C. A. Sundermann. 2000. Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. *Auk* 117:952–963.
- Brock, T. D., M. T. Madigan, J. M. Martinko, and J. Parker. 1994. *Biology of microorganisms*. Prentice Hall, Englewood Cliffs, NJ.
- Burt, E. H., Jr. 1999. Think small. *Auk* 116:878–881.
- Burt, E. H., Jr., and J. M. Ichida. 1999. Occurrence of feather-degrading bacilli in the plumage of birds. *Auk* 116:364–372.
- Charlton, B. R. 2000. *Avian disease manual*. 5th ed. American Association of Avian Pathologists, Athens, GA.
- Clayton, D. H. 1999. Feather-busting bacteria. *Auk* 116:302–304.
- Cristol, D. A., J. L. Armstrong, J. M. Whitaker, and M. H. Forsyth. 2005. Feather-degrading bacteria do not affect feathers on captive birds. *Auk* 123:222–230.
- Cuthill, I. C., J. C. Partridge, A. T. D. Bennett, S. C. Church, N. S. Hart, and S. Hunt. 2000. Ultraviolet vision in birds. *Advances in the Study of Behavior* 29:159–215.
- Endler, J. A. 1990. On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society* 41:315–352.
- Finger, E. 1995. Visible and UV coloration in birds: mie scattering as the basis of color production in many bird feathers. *Naturwissenschaften* 82:570–573.
- Freeland, W. J. 1976. Pathogens and the evolution of primate sociality. *Biotropica* 8:12–24.
- Friedrich, A. B., and G. Antranikian. 1996. Keratin degradation by *Fervidobacterium pennavorans*, a novel thermophilic anaerobic species of the order Thermotogales. *Applied and Environmental Microbiology* 62:2875–2882.
- Hambreaux, A., J. Hoborn, and W. Whyte. 1990. Skin-sampling validation of a pad method and comparison with commonly used methods. *Journal of Hospital Infection* 16:19–27.
- Hamilton, W. D. 1990. Mate choice near and far. *American Zoologist* 30:341–352.
- Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites. *Science* 218:384–387.
- Hilgarth, N. 1996. Parasites and female choice in the ring-necked pheasant. *American Zoologist* 40:227–233.
- Hill, G. E. 1994. Trait elaboration via adaptive mate choice: sexual conflict in the evolution of signals of male quality. *Ethology Ecology and Evolution* 6:351–370.
- Hill, G. E., and K. J. McGraw. 2006. *Bird coloration*. Vol. 1. Mechanisms and measurements. Harvard University Press, Cambridge, MA.
- Hill, G. E., K. L. Farmer, and M. L. Beck. 2004. The effect of mycoplasmosis on carotenoid plumage coloration in male house finches. *Journal of Experimental Biology* 207:2095–2099.
- Hunt, S., I. C. Cuthill, A. T. D. Bennett, and R. Griffiths. 1999. Preferences for ultraviolet partners in the blue tit. *Animal Behaviour* 58:809–815.
- Ichida, J. M., L. Krizova, C. A. LeFevre, H. M. Keener, D. L. Elwell, and E. H. Burt Jr. 2001. Bacterial inoculum enhances keratin degradation and biofilm formation in poultry compost. *Journal of Microbiology Methods* 47:199–208.
- Jacobs, G. H. 1981. *Comparative color vision*. Academic Press, New York.
- Keyser, A. J., and G. E. Hill. 1999. Condition-dependent variation in the blue-ultraviolet colouration of a structurally based plumage ornament. *Proceedings of the Royal Society B: Biological Sciences* 266:771–777.
- Lombardo, M. P., P. A. Thorpe, and H. W. Power. 1999. The beneficial sexually transmitted microbe (STM) hypothesis of avian copulation. *Behavioral Ecology* 10:333–337.
- Lucas, F. S., O. Broennimann, I. Febbraro, and P. Heeb. 2003. High diversity among feather-degrading bacteria from a dry meadow soil. *Microbial Ecology* 45:282–290.
- Lucas, F. S., B. Moureau, V. Jourdie, and P. Heeb. 2005. Brood size modifications affect plumage bacterial assemblages of European starlings. *Molecular Ecology* 14:639–646.
- Milinski, M., and T. C. M. Bakker. 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitised males. *Nature* 344:330–333.
- Miller, A. J., F. J. Schultz, A. Oser, J. L. Hallman, and S. A. Palumbo. 1994. Bacteriological safety of swine carcasses treated with reconditioned water. *Journal of Food Science* 59:739–741.
- Møller, A. P. 1990. Effects of parasitism by the haematophagous mite *Ornithonyssus bursa* on reproduction in the barn swallow *Hirundo rustica*. *Ecology* 71:2345–2357.
- Møller, A. P., P. Christe, and E. Lux. 1999. Parasitism, host immune function, and sexual selection. *Quarterly Review of Biology* 74:3–20.
- Mukhopadhyay, R. P., and A. L. Chandra. 1990. Keratinase of a streptomycete. *Indian Journal of Experimental Biology* 28:575–577.
- Muza, M. M., E. H. Burt Jr., and J. M. Ichida. 2000. Distribution of bacteria on feathers of some eastern North American birds. *Wilson Bulletin* 112:432–435.
- Noval, J. J., and W. J. Nickerson. 1959. Decomposition of native keratin by *Streptomyces fradiae*. *Journal of Bacteriology* 77:251–263.
- Örnborg, J., S. Andersson, S. C. Griffith, and B. C. Sheldon. 2002. Seasonal changes in a ultraviolet colour signal in blue tits, *Parus caeruleus*. *Biological Journal of the Linnean Society* 76:237–246.
- Poiani, A., A. R. Goldsmith, and M. R. Evans. 2000. Ectoparasites of house sparrows (*Passer domesticus*): an experimental test of the immunocompetence handicap hypothesis and a new model. *Behavioral Ecology and Sociobiology* 47:230–242.
- Prum, R. O. 1999. The anatomy and physics of avian structural colors. Pages 1635–1653 in N. J. Adams and R. H. Slotow, eds. *Proceedings of the 22nd International Ornithological Congress*. BirdLife South Africa, Johannesburg.
- . 2006. Anatomy, physics and evolution of avian structural colors. Pages 295–353 in G. E. Hill and K. J. McGraw, eds. *Bird coloration: mechanisms, function, and evolution*. Harvard University Press, Cambridge, MA.
- Pryke, S. R., S. Andersson, and M. J. Lawes. 2001. Sexual selection of multiple handicaps in the red-collared widowbird: female choice of tail length but not carotenoid display. *Evolution* 55:1452–1463.
- Rabsch, W., B. M. Hargis, R. M. Tsohis, R. A. Kingsley, K. Hinz, H. Tschäpe, and A. J. Bäuml. 2000. Competitive exclusion of *Salmonella* enteritidis by *Salmonella* gallinarum in poultry. *Emerging Infectious Diseases* 6:443–448.
- Roberts, T. 1985. Microbial pathogens in raw pork, chicken and beef: benefit estimates for control using irradiation. *American Journal of Agricultural Economics* 67:957–965.
- Ruoff, K. L. 1993. The genus *Streptococcus*: medical. Pages 1450–1464

- in A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer, eds. The prokaryotes. 2nd ed. Springer, New York.
- Sangali, S., and A. Brandelli. 2000. Feather keratin hydrolysis by a *Vibrio* sp. strain kr2. *Journal of Applied Microbiology* 89:735–743.
- Schoeni, J. L., and A. C. Wong. 1994. Inhibition of *Campylobacter jejuni* colonization in chicks by defined competitive exclusion bacteria. *Applied and Environmental Microbiology* 60:1191–1197.
- Shawkey, M. D., and G. E. Hill. 2004. Feathers at a fine scale. *Auk* 141:652–655.
- Shawkey, M. D., S. R. Pillai, and G. E. Hill. 2003a. Chemical warfare? effects of uropygial oil on feather-degrading bacteria. *Journal of Avian Biology* 34:345–349.
- Shawkey, M. D., A. M. Estes, L. M. Siefferman, and G. E. Hill. 2003b. Nanostructure predicts intraspecific variation in ultraviolet-blue plumage colour. *Proceedings of the Royal Society B: Biological Sciences* 270:1455–1460.
- Shawkey, M. D., A. M. Estes, L. M. Siefferman, and G. E. Hill. 2005a. The anatomical basis of sexual dichromatism in non-iridescent ultraviolet-blue structural colouration of feathers. *Biological Journal of the Linnean Society* 85:259–271.
- Shawkey, M. D., K. L. Mills, C. Dale, and G. E. Hill. 2005b. Microbial diversity of wild bird feathers revealed through culture-based and culture-independent techniques. *Microbial Ecology* 50:40–47.
- Sheldon, B. C., S. Andersson, S. C. Griffith, J. Örnborg, and J. Sen-decka. 1999. Ultraviolet colour variation influences blue tit sex ratios. *Nature* 402:874–877.
- Siefferman, L., and G. E. Hill. 2003. Structural and melanin coloration indicate parental effort and reproductive success in male eastern bluebirds (*Sialia sialis*). *Behavioral Ecology* 14:855–861.
- . 2005. UV-blue structural coloration and competition for nest boxes in male eastern bluebirds (*Sialia sialis*). *Animal Behaviour* 69:67–72.
- Silverman, G. J. 1991. Sterilization and preservation by ionizing radiation. Pages 566–579 in S. S. Block, ed. *Disinfection, sterilization, and preservation*. 4th ed. Lea & Febiger, New York.
- Smit, E., P. Leeflang, S. Gommans, J. van den Broek, S. van Mil, and K. Wernars. 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Applied and Environmental Microbiology* 67:2284–2291.
- SPSS. 2002. SPSS 10.0 for Macintosh. SPSS, Chicago.
- Tannock, G. W. 1995. *Normal microflora*. Chapman & Hall, New York.

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