

Evidence that plumage bacteria influence feather coloration and body condition of eastern bluebirds *Sialia sialis*

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Parasites influence the expression of secondary sexual traits and the health of infected individuals. We set out to test the influence of reputed exogenous parasites, plumage bacteria, including feather-degrading bacteria (FDB), on secondary sexual characteristics and body condition of wild adult eastern bluebirds *Sialia sialis*. Previous work has shown that FDB alter the coloration of structurally-colored bluebird feathers *in vitro* (Shawkey et al. 2007). In a correlational study of how bacteria affect birds in the wild, we found that female plumage got duller with increasing FDB intensity. Males tended to get brighter with increasing FDB intensity, but the relationship was not significant. We also found significant associations between plumage bacteria intensity and body condition, but, again, with gender-based differences. Female body condition was negatively associated with plumage bacteria intensity, while male body condition was positively associated with plumage bacteria intensity. Interestingly, plumage bacteria intensity of males and females in nesting pairs was significantly positively correlated. We also report the highest prevalence of FDB measured in a wild bird population, with FDB detected on 67/68 (99%) of individuals. Further work is needed to fully understand the relationships between plumage bacteria and birds, but our data indicate that plumage bacteria may have sex-dependent effects on multiple phenotypic traits.

Sexual selection theory predicts that parasite infection can indirectly influence mating success by altering the expression of secondary sexual characteristics (Andersson 1994). Avian systems have been important models for understanding how parasites influence the expression of these traits. Much of this work has focused on endogenous parasites of the blood (Höglund et al. 1992, Hórák et al. 2001) or gastrointestinal tract (e.g., Hórák et al. 2004, Costa and Macedo 2005, Mougeot et al. 2005), or on exogenous parasites that feed on blood (Fitze and Richner 2002, Doucet and Montgomerie 2003). However, birds also harbor ectoparasites that inhabit the plumage and feed on feathers and skin. Unlike endogenous parasites that can only affect trait expression by influencing health, these integumentary ectoparasites can also directly alter the physical structure of sexually selected traits (e.g. Boyce 1990, Clayton 1990, Kose et al. 1999, Kose and Möller 1999).

Recently, bacteria that can degrade feathers have been isolated from the plumage of wild birds (e.g., Burt and Ichida 1999, Whitaker et al. 2005, reviewed in Gunderson 2008). These bacteria could influence feather condition and individual health, as do other avian ectoparasites (Clayton 1999). Feather-degrading bacteria (hereafter FDB) are a polyphyletic group of microorganisms recognized solely by their ability to hydrolyze the protein β -keratin, which

constitutes over 90% of feather mass. FDB occur on a wide range of avian hosts (Burt and Ichida 1999, Shawkey et al. 2003, Whitaker et al. 2005, Shawkey et al. 2007), but there is no *in vivo* evidence that they influence plumage condition or fitness. All demonstrations of bacterial feather degradation have occurred *in vitro*. However, recent studies have attempted to detect bacterial feather degradation on live birds. Cristol et al. (2005) inoculated captive birds in an outdoor aviary with the FDB *Bacillus licheniformis* (OWU 138B), a strain isolated from the plumage of a willow flycatcher *Empidonax traillii* (Ichida et al. 2001). They found no evidence of increased feather damage on inoculated relative to uninoculated birds. However, aspects of their study may have confounded their ability to detect bacterial feather degradation (Cristol et al. 2005, Gunderson 2008).

Shawkey et al. (2007) attempted to detect bacterial feather degradation in a wild population of eastern bluebirds *Sialia sialis* by looking for a correlation between feather coloration and FDB intensity (the number of individual bacterial cells on an individual bird). First, they demonstrated experimentally that FDB brighten, increase color purity, and decrease UV-chroma of blue, structurally colored male feathers. However, Shawkey et al. (2007) found no relationship between FDB intensity and feather brightness or UV-chroma in a wild bluebird population,

although brightness was positively correlated with total plumage bacteria intensity, including bacteria that cannot degrade feathers.

A potential problem with the study of Shawkey et al. (2007) is that they analyzed color characteristics of individuals at one point in time as opposed to associating change in plumage coloration with variation in bacteria load. Coloration at one time may not be indicative of color change over time because feather color can be influenced by many factors, including age (Siefferman et al. 2005) and reproductive output during the previous breeding season (Siefferman and Hill 2005a). Measuring within-individual color change in relation to FDB intensity would increase the probability of detecting an influence of FDB, if it exists.

To address this issue, we tested whether within-individual feather color change during the breeding season was associated with plumage bacteria intensity in a marked population of eastern bluebirds. We quantified FDB and non-FDB intensity within the plumage of nesting pairs of adult eastern bluebirds and objectively measured their feather coloration at two times during the breeding season to look for correlations between bacteria intensity and feather color change. Non-FDB were included because they are hypothesized to influence feather degradation (Shawkey et al. 2007, Gunderson 2008), and because Shawkey et al. (2007) found that FDB and non-FDB intensity combined correlated with bluebird coloration in their population. We hypothesized that FDB can alter the color characteristics of structurally colored feathers, and predicted that FDB would be associated with increased feather brightness and decreased UV-chroma. An association between feather color change and FDB intensity would be the first evidence consistent with FDB degrading the feathers of live birds, in captivity or in natural conditions.

We also looked for evidence consistent with parasite-mediated natural selection via FDB. Under parasite-mediated natural selection, the distribution of parasite intensity among individuals should be aggregated, with most individuals possessing few parasites and a few individuals possessing many parasites, indicated by a high mean/variance ratio in individual parasite intensity within the population (Goater and Holmes 1997). Further, one expects a negative correlation between parasite intensity and host health and fitness metrics (Goater and Holmes 1997). Most studies report only the percentage of individuals carrying FDB in a population (e.g., Burt and Ichida 1999, 2004, Whitaker et al. 2005) and do not address individual variation in FDB intensity (but see Shawkey et al. 2007). No study has reported associations between FDB intensity and any aspect of individual health. This information is essential to determine whether FDB, or plumage bacteria in general, are influencing natural populations of birds (Clayton 1999). Therefore, we looked for an association between FDB intensity and body condition of the adult bluebirds monitored in this study. Under the hypothesis that FDB are parasites, we predicted that FDB intensity would be negatively correlated with body condition. A demonstration of this negative correlation would be the first evidence consistent with FDB influencing the physiological condition of birds.

Materials and methods

Study species

Eastern bluebirds are secondary cavity nesting passerines found across much of the eastern United States. Adults are sexually dichromatic, with males having bright blue wing and dorsal contour feathers, while females are generally blue-grey across the same regions. Male blue coloration is associated with success in competition for nest boxes (Siefferman and Hill 2005b) and, along with the chestnut-colored chest patch, predicts male parental effort and reproductive success (Siefferman and Hill 2003). The chest patch is also sexually dichromatic, being darker in males than in females. Within our population, females typically lay two to three clutches of four to six eggs per breeding season. Nesting pairs are socially monogamous and rarely switch breeding partners between nesting attempts within the same season (J.P. Swaddle unpubl. data). Eastern bluebird plumage has been shown to harbor a diverse array of bacteria (Shawkey et al. 2005).

Field site and first capture of adult bluebirds

Adult male and female bluebirds ($n=68$) were captured using nest box traps (small trap doors set above the entrance holes of breeding boxes) from April 26 to May 31 2007 within York and James City counties, Virginia, USA. All birds were captured during the nestling phase of their first nesting attempt, when eggs had hatched and adults were feeding young. Each bird was banded with a U.S. Geological Survey metal leg band and a unique combination of three plastic colored leg bands. Mass was measured to the nearest 0.1g using an electric balance and unflattened wing chord was measured to the nearest 0.1 mm using dial calipers. We collected feathers from the blue rump patch of each individual for spectroradiometric color analysis. The feather samples were randomly collected from either the right or left half of the rump patch of each individual. We did not take feathers from the entire rump area because we intended to recapture each bird and collect a second feather sample to address color change over the breeding season in relation to FDB intensity. Second feather samples were taken from the side not sampled during the first capture (see below). By leaving one half of the rump undisturbed, and taking our second feather sample from that half, we reduced the chance that feathers collected the second time would be freshly grown as a result of the first sampling. Latex gloves were worn during feather sampling and all further processing of feathers to avoid alteration of feather coloration due to oils from human skin and to minimize contamination of the plumage with bacteria from the researchers' hands.

Two samples of plumage bacteria were collected from each bird concurrently with feather collections. One sample was collected from rump feathers ("rump bacteria") and another was taken from feathers over the rest of the body ("body bacteria"). Rump bacteria were sampled from only one side of the rump patch, the same side from which rump feathers were collected (before removing feathers for color analysis). Bacterial samples were collected by dipping a sterile cotton-tipped applicator in 2 ml sterile phosphate

buffered saline (PBS) with 0.01% Tween-80 (pH = 7.25) contained in a sterile 15 ml falcon tube, and running the applicator, while rotating it, through the plumage. The applicator was then placed back in the PBS until bacterial plating. A.R. Gunderson collected all bacteria samples, and all birds were sampled in an identical manner. A new pair of latex gloves was worn while processing each bird, and the gloves were put on immediately before handling to ensure that all bacteria collected were derived from the plumage. All birds were released immediately following this sampling and none of them abandoned their nests.

One to three hours after collection, bacteria were removed from the cotton applicator by vortexing, and were grown in 15 ml culture plates on two different growth media; trypticase soy agar, a general microbial growth medium, and feather meal agar, a medium upon which only microbes that can hydrolyze keratin can grow (Sangali and Brandelli 2000). Bacteria grown on trypticase soy agar are referred to as “non-FDB”, while bacteria grown on feather meal agar are referred to as “FDB”. 100 μ l of sample was inoculated onto each medium from both the rump and body samples of each bird (thus, four samples were grown per bird). Plates were incubated for 5 d at 35° C, and colony-forming units (CFU) present on each plate were counted. Of the 272 plates (68 birds \times 4 plates/bird), 9 yielded bacterial colonies that were too numerous to count and an accurate count could not be made. For these plates, the colony count was recorded as 1,000 CFU, which is likely an underestimate. The highest accurate count made on a plate was one with 903 colonies. A.R. Gunderson completed all bacterial plating and counting without knowledge of the color or body condition of the birds.

Second capture of adult birds

A minimum of 30 d after initial capture (range: 40–69 d), adult bluebirds were recaptured and a second rump feather sample was collected for color analysis (resamples collected between June 20 and July 28 2007). The second feather sample was taken from the opposite side of the rump from which the initial sample was taken (see above). All body measurements were taken again, as they were during the first capture. Due to an uncharacteristically low rate of second nesting attempts in our population in 2007, we were able to recapture only 32 adult bluebirds (Males $n = 15$, females $n = 17$) of the 68 originally sampled.

Color analysis

Nine feathers from each bird were stacked on top of each other to mimic their placement on the bird, and taped to a standard matte black cardboard surface. Color analyses were conducted with an Ocean Optics USB2000 uv-vis spectroradiometer (range 300–700 nm) with a PX-2 pulsed xenon light source. The probe was held at a 90° angle to the feathers inside a metal sheath to exclude external light, with the distance from the feathers adjusted to sample an area with a diameter of 3 mm. One sample with the spectroradiometer consisted of the average of 20 reflectance spectra taken sequentially. We sampled the feathers from each bird three times in this way, and averaged the three outputs to

obtain the reflectance spectra for the rump patch of each bird used in analyses.

From the reflectance spectra, we calculated hue (wavelength of maximum reflectance), brightness (total area under the reflectance curve from 300–700 nm), and UV-chroma (proportion of total reflectance from 300–400 nm; birds can see UV light (Hart and Hunt 2007)).

Body condition

Body condition, here defined as mass corrected for body size, was calculated as the residuals of body mass regressed over wing length. Because of sexual size dimorphism, regressions were conducted on male and female body measurements separately.

Statistical analyses

The effects of bacteria on rump plumage color were tested using regression analyses. Color change was converted to a rate (color change/d) by subtracting the color score at the time of the first color sampling from the color score at the time of the second sampling and dividing by the number of d between samples. We used bacteria intensity from only the rump feathers for these analyses. We hypothesized that plumage brightness and UV-chroma would be affected by FDB, and thus these two color variables were treated as dependent variables in separate analyses (brightness and UV-chroma were uncorrelated in our feather samples). Associations between plumage bacteria intensity and body condition were tested using mixed-model analysis of variance (ANOVA). The model included body condition as the dependent variable, with bacteria intensity as an independent variable with covariates that may influence body condition (sex, number of chicks in the nest, date of sampling). All variables and interactions were included in the initial model, with interaction terms removed until the most parsimonious model was found. FDB and non-FDB intensity were highly correlated and, thus, were included as independent variables in separate analyses to avoid problems of colinearity. Bacteria count data were log-transformed to adhere to the assumption of normality. All statistical analyses and computation of color characteristics were performed with the R statistical programming package (v. 2.4.1).

Results

Distribution of plumage bacteria within the population

We detected FDB on 99% (67/68) of the adult bluebirds sampled. To determine the total FDB intensity on each individual, we summed the number of FDB detected on the rump and body. The distribution of FDB intensity among birds was highly skewed to the right (mean/variance ratio = 886.88), with most individuals having relatively few FDB, and some individuals having many FDB (Fig. 1). A similar pattern was seen for non-FDB (mean/variance ratio = 781.983; data not shown).

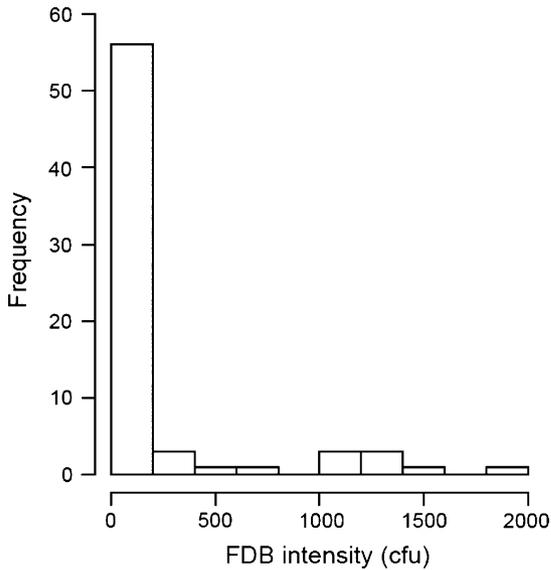


Figure 1. Distribution of total FDB intensity (“rump” and “body” FDB intensities summed for each individual), measured in colony-forming units (cfu), among 68 adult bluebirds (males and females included).

Log-transformed rump and body bacteria intensities from the same individual bird were highly correlated for both FDB (Pearson product-moment correlation, $r = 0.688$, $df = 67$, $P < 0.001$) and non-FDB ($r = 0.557$, $df = 76$, $P < 0.001$). Thus, within individuals, bacteria intensity in one area of the plumage is indicative of bacteria intensity in other areas. The significant correlation between independent samples from the same individual also indicates that our method of bacteria quantification is repeatable.

Plumage bacteria intensities of males and females in nesting pairs were significantly correlated (FDB: $r = 0.385$, $df = 25$, $P = 0.047$; non-FDB: $r = 0.470$, $df = 25$, $P = 0.013$, Fig. 2).

Feather color change and plumage bacteria

Plumage brightness decreased over time, on average, in males (-1831.79 ± 3140.86) and females (-347.46 ± 3959.63), although brightness did increase in some individuals. Rump FDB intensity showed a positive but non-significant association with rate of brightness change in males ($y = -61.75 + 34.625x$, $r^2 = 0.196$, $P = 0.123$, Fig. 3), but a significant negative relationship with rate of brightness change in females ($y = 24.516 + (-17.872)x$, $r^2 = 0.223$, $P = 0.032$, Fig. 3). A test for difference of slopes showed a significant sex-by-FDB intensity interaction ($t = 2.265$, $P = 0.031$), indicating rate of color change in relation to FDB intensity is different in males and females. A similar pattern was found between brightness change and non-FDB intensity (males: $y = -39.430 + 6.386x$, $r^2 = 0.007$, $P = 0.767$; females: $y = 42.047 + (-19.545)x$, $r^2 = 0.274$, $P = 0.021$), although there was no significant difference in slope between males and females ($t = 1.137$, $P = 0.265$).

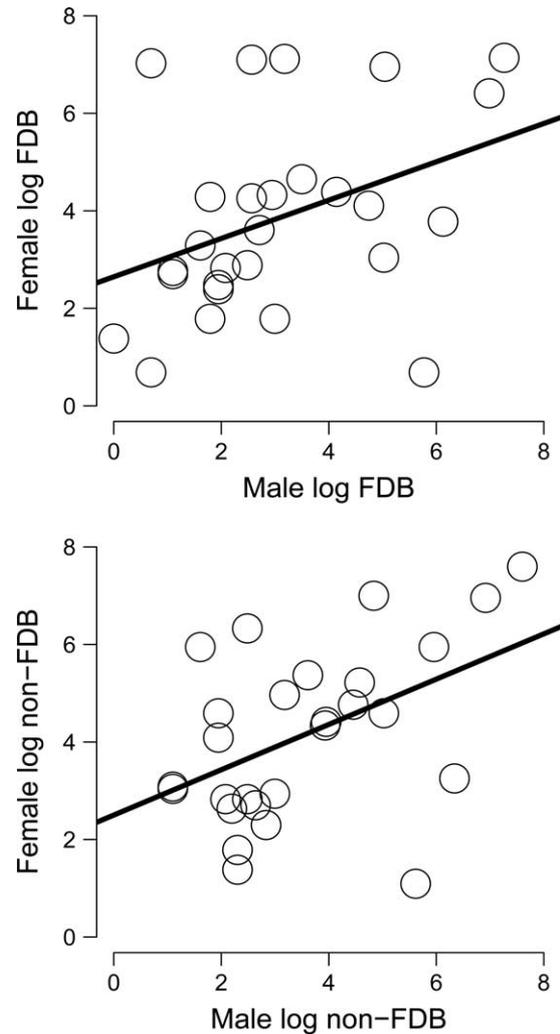


Figure 2. Associations between total plumage bacteria intensity (“rump” and “body” bacteria intensities summed for each individual) of adult male and female bluebirds in nesting pairs. Top: FDB. Bottom: non-FDB.

UV-chroma decreased on average in both males (-0.006 ± 0.012) and females (-0.009 ± 0.020), although it did increase in some individuals. There was no association between change in UV-chroma and FDB intensity in either sex (males: $y = 0.000 + 0.000x$, $r^2 = 0.089$, $P = 0.397$; females: $y = 0.000 + 0.000x$, $r^2 = 0.003$, $P = 0.816$; data not shown). Results were similar with non-FDB (males: $y = 0.000 + 0.000x$, $r^2 = 0.078$, $P = 0.430$; females: $y = 0.000 + 0.000x$, $r^2 = 0.003$, $P = 0.812$; data not shown).

Associations of plumage bacteria with body condition

None of the independent variables significantly predicted body condition alone, whether FDB or non-FDB was used as the independent variable for bacteria intensity (Table 1). There was however, a significant sex-by-bacteria intensity interaction (FDB: $F_{1,59} = 6.746$, $P = 0.012$; Non-FDB: $F_{1,59} = 13.020$, $P = 0.001$; non-FDB). This interaction is

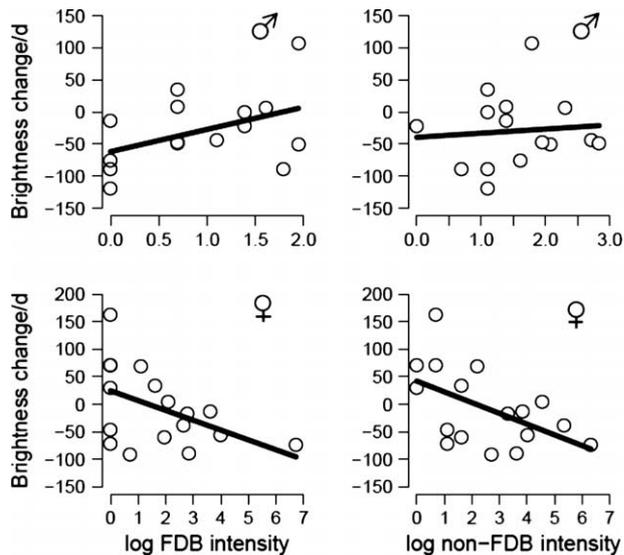


Figure 3. Associations between rump plumage bacteria intensity and rate of brightness change (change/d) among adult male and female bluebirds. Top left: males, FDB. Top right: males, non-FDB. Bottom left: females, FDB. Bottom right: females, non-FDB.

driven by male and female body condition being associated with bacteria intensity in opposite directions. Body condition (corrected for date of sampling) correlates negatively with body FDB intensity in females ($y = 1.053 + (-0.275)x$, $r^2 = 0.118$, $P = 0.041$, Fig. 4), but positively with body FDB intensity in males ($y = -0.682 + 0.220x$, $r^2 = 0.129$, $P = 0.044$, Fig. 4). This is true with non-FDB intensity as well (females: $y = 1.522 + (-0.366)x$, $r^2 = 0.167$, $P = 0.013$; males: $y = -0.949 + 0.266x$, $r^2 = 0.150$, $P = 0.029$, Fig. 4).

Because of the relationship between female condition and plumage bacteria intensity, we looked for an association between female reproductive success and female body condition to determine if plumage bacteria could have fitness consequences for females. Data for this analysis came from a much larger sample of females from three years (2005–2007) of ongoing field studies. Morphological data in all years were collected as described in the Methods. We found no association between female body condition and number of chicks fledged from a nest ($y = 3.882 + 0.015x$, $r^2 = 0.0007$, $n = 143$, $P = 0.751$). However, we did find a positive association between female body condition and

mean body condition of nestlings in the nest ($y = 0.055 + 0.208x$, $r^2 = 0.040$, $n = 167$, $P = 0.010$), although the amount of variance explained by female body condition was very low. Nestling condition and mean condition of chicks in a nest were calculated following Kight and Swaddle (2007). We did not address male reproductive success in relation to body condition because we have relatively few body measurements on males in our population and did not test for paternity using molecular methods.

Discussion

We found the highest prevalence (% individuals infected) of FDB yet reported in a wild bird population (99%). Previous multispecies surveys of FDB prevalence on wild birds by Burt and Ichida (1999) and Whitaker et al. (2005) found maximum prevalences of 29% and 59%, respectively, while Shawkey et al. (2007) found FDB on 89% of male eastern bluebirds in a population in Alabama. Our data indicate that FDB can be a ubiquitous feature of plumage, at least in some species and/or populations.

Furthermore, we found that FDB have an aggregated distribution within our bluebird population (mean/variance ratio = 886.88; see Goater and Holmes 1997 for examples of intensity mean/variance ratios for several other avian parasites). Most bluebirds have relatively few FDB in their plumage, while a minority of bluebirds carry many FDB (Fig. 1). A highly aggregated parasite distribution on hosts is expected if parasites are mediating natural selection because the influence of parasites should select for individuals with high parasite resistance (Goater and Holmes 1997), shifting the distribution of individual parasite loads toward low levels within the population.

For parasites to mediate natural selection, they must also influence host fitness. We found evidence that plumage bacteria influence body condition, a trait ultimately thought to affect reproductive success (Jensen et al. 2004, Blums et al. 2005, Dyrce et al. 2005, O'Dwyer et al. 2006). Consistent with our prediction, female body condition correlates negatively with plumage bacteria intensity. However, contrary to our prediction, male plumage bacteria intensity shows a positive correlation with male body condition. Sex differences in parasite infection are common, with prevalence of infection and susceptibility to parasites often greater in one sex relative to the other (Zuk and McKean 1996, Schalk and Forbes 1997, McCurdy et al.

Table 1. Summary of ANOVA models of factors influencing body condition. Summary statistics from analysis with total FDB as the "bacteria intensity" variable are given first. Values in parentheses are those from the same analysis with total non-FDB representing "bacteria intensity".

	df	SS	MS	F	P
Bacteria Intensity	1	0.803 (1.101)	0.803 (1.101)	0.433 (0.661)	0.513 (0.419)
Sex	1	2.430 (2.376)	2.430 (2.376)	1.311 (1.426)	0.257 (0.237)
Date	1	0.068 (0.124)	0.068 (0.124)	0.036 (0.075)	0.849 (0.786)
No. of chicks	1	0.058 (0.070)	0.058 (0.070)	0.032 (0.042)	0.860 (0.839)
Bacteria intensity × Sex	1	12.506 (21.691)	12.506 (21.691)	6.75 (13.020)	0.012 (0.001)
Bacteria intensity × date	1	0.571 (2.879)	0.571 (2.879)	0.308 (1.728)	0.581 (0.194)
Sex × date	1	10.382 (8.350)	10.382 (8.350)	5.60 (5.012)	0.021 (0.029)
Bacteria intensity × sex × date	1	5.132 (6.448)	5.132 (6.448)	2.768 (3.870)	0.101 (0.054)
Residuals	59	109.387 (98.297)	1.854 (1.666)		

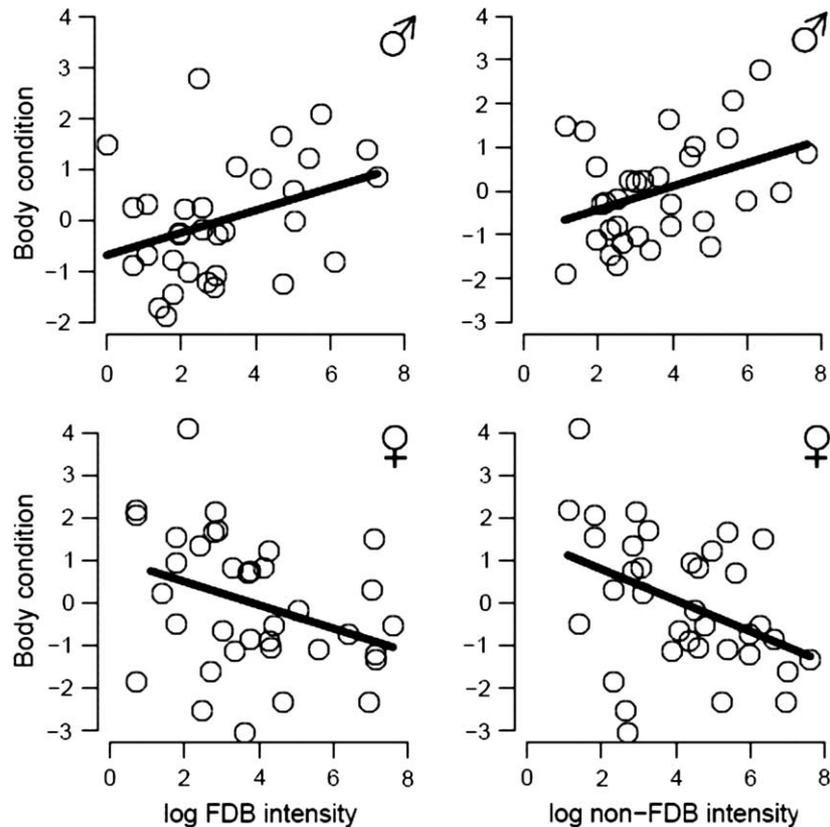


Figure 4. Associations between total plumage bacteria intensity (“rump” and “body” bacteria intensities summed for each individual) and body condition of adult male and female bluebirds. The effect of measurement date has been removed from the body condition scores. Top left: males, FDB. Top right: males, non-FDB. Bottom left: females, FDB. Bottom right: females, non-FDB.

1998, Klein 2000). We are unaware of any cases, however, in which reputed parasites show a positive association with health or condition metrics in one sex while showing a negative association with the same variables in the other.

We found evidence that plumage bacteria influence female but not male plumage coloration. Females with more FDB tend to get duller than females with few FDB. In males the relationship between FDB and brightness was positive, as we predicted, but not significant. Thus, FDB may not alter male coloration in the wild. These data should be interpreted cautiously, however, because of our small sample size and the relatively low bacteria loads of the males we recaptured (note the range of bacteria intensities for males vs. females in Fig. 3). We predicted UV-chroma would be negatively affected by FDB, but there was no evidence for this in either sex. Overall, these results are inconsistent with our original predictions that FDB intensity would positively correlate with change in brightness and negatively correlate with change in UV-chroma. However, they are consistent with FDB reducing female feather brightness. It should be noted that the results of Shawkey et al. (2007), from which we derived our predictions, are based on bacterial degradation of male bluebird feathers only. Male and female rump feathers have different structural organizations (Shawkey et al. 2005), and thus predictions derived from male color change data may not be applicable to females. If FDB influence structural feather coloration, they could influence sexually and non-sexually selected plumage color signals. This could apply

to carotenoid-based signals as well, as structural coloration contributes to the expression of feathers colored with carotenoids (Shawkey and Hill 2005).

Sex differences in the relationship between plumage bacteria intensity and feather coloration and body condition require explanation. Two non-mutually exclusive mechanisms could create this pattern (*sensu* Zuk and McKean 1996). 1) Behavioral and/or physiological differences between males and females may lead to sex differences in the composition of plumage bacterial communities. For example, differences in nesting and feeding behavior could lead males and females to encounter different substrates with different bacterial communities. Sex differences in preen oil composition (Jacob et al. 1979, Kolattukudy 1987, Reneerkens et al. 2002) could lead to sex differences in plumage bacteria composition because preen oil can influence bacterial growth (Bandyopadhyay and Bhattacharyya 1996, Jacob et al. 1997, Shawkey et al. 2003, Reneerkens et al. 2008). This mechanism requires that different bacteria affect birds differently, and that these effects can be positive or negative, depending on the bacterium. This has been demonstrated for endogenous bacteria (Potti et al. 2002, Moreno et al. 2003). 2) Behavioral and/or physiological differences between males and females cause them to respond differently to bacteria. In this scenario, the composition of the bacterial community in the plumage does not differ between the sexes, but the effect of the bacteria on each sex does differ. The mechanisms responsible for driving the patterns in our data

may differ depending on the metric (i.e., body condition or feather coloration) being considered, and determining which of the above alternatives might be at work in our population is difficult with our limited data set. However, the correlated bacteria intensities of nesting males and females in our population (Fig. 2) suggests males and females are experiencing similar environments with respect to bacteria.

Our results are the first consistent with FDB damaging the feathers of live birds in a wild population. This has broad implications for other avian systems. FDB appear to be cosmopolitan, occurring on a wide range of bird species (Burtt and Ichida 1999, Whitaker et al. 2005), and feather damage influences a range of traits that impact fitness (Booth et al. 1993, Swaddle and Witter 1997, Ferns and Lang 2003, Williams and Swaddle 2003). Our results also suggest that FDB may influence feather coloration, an important component of communication and crypsis in many birds. This result is an interesting compliment to previous results demonstrating that feathers colored with melanins resist bacterial degradation (Goldstein et al. 2004, Gunderson et al. 2008). Thus, interactions between birds and FDB may be important in driving the evolution of avian coloration. Finally, we have shown data consistent with plumage bacteria influencing the condition of birds, although with opposite effects for males and females. Manipulative experimental work *in vivo* is now necessary to determine whether plumage bacteria can act as a causal agent generating variation among individuals in body condition and feather coloration.

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