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Research article

Preening correlates with lower feather bacteria abundance but not feather coloration in a lek-breeding bird

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Feathers are structures unique to birds that serve important functions such as flight, thermoregulation, and communication. Bacteria that live on the feathers, particularly ones that can break down keratin, have the potential to damage feathers and disrupt their use in communication. We predicted that birds could behaviorally manage their feather bacterial abundances by preening their feathers. We also predicted that individuals with lower feather bacterial abundances would have brighter and more colorful feathers. To test these predictions, we measured the amount of time individuals in a colony of captive Indian peafowl *Pavo cristatus* spent preening their feathers. We also collected feathers to determine bacteria abundance on the feather surface and to measure feather coloration. We found that birds had lower feather bacteria levels when they spent more time preening their own feathers, but only in female birds. We also found that bacteria abundances were not correlated with any feather color variables we measured. These results suggest that birds can manage feather bacterial abundances by preening but feather bacteria may not influence feather coloration in this species.

Key words: color, feather degrading bacteria, feathers, preening

Introduction

Feathers are structures unique to birds and serve important functions in flight (Rayner 1988), thermoregulation (Schwab and Schafer 1972), and communication (McGraw 2008, Weaver et al. 2018). Colorful and elaborate feathers are used as a signal of individual quality across avian taxa (Griggio et al. 2010, Grindstaff et al. 2012, Meadows et al. 2012) and are used to communicate with potential mates and competitors (Hamilton and Zuk 1982, Fitzpatrick 1998). Therefore, the growth and maintenance of feathers are essential for birds' survival and reproduction.

Because feathers are energetically expensive to grow and maintain, they can serve as an honest signal of quality (Lattin et al. 2011, Pap et al. 2013, Møller and Nielsen 2018). Feathers in most species are grown once a year, and condition during feather growth is important for high quality feathers (Pehrsson 1987, Saino et al. 2013).

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However, feathers are not static over time: physical wear, physical aggression, sun bleaching, feather lice, and degradation by bacteria can all damage feathers and alter their appearance (Bonser 1995, Barbosa et al. 2002, Gunderson 2008, Surmacki 2008). Some bacteria that live on feathers can metabolize keratin, which is the main component of feathers (Williams et al. 1990, Bonser 1996). These feather degrading bacteria (FDB) can damage feathers and impact their color (Gunderson et al. 2009, Shawkey et al. 2009, Leclaire et al. 2014).

Birds have evolved in the presence of bacteria, including those on their feathers, and therefore they have developed adaptations to cope with them. Feather maintenance behaviors, such as preening, where birds clean their plumage with their beak, is a way birds can remove dirt, ectoparasites, and bacteria from their feathers (Lenouvel et al. 2009, Waite et al. 2012, Vezzoli et al. 2015). Pigeons with experimentally increased feather bacterial abundance were found to increase preening behavior compared to control birds (Leclaire et al. 2014), suggesting that birds can use preening to control bacterial abundances on their feathers and perhaps preserve their color and quality. When birds preen, they mechanically clean feathers, but they may also use preen oil secreted from the uropygial gland to coat the feathers (but this is not used in every preening bout). This oil can include anti-microbial compounds which could reduce the abundance of FDB (Shawkey et al. 2003). The composition and use of preen oil is highly variable between species and much is still unknown about its functions and impact on fitness (reviewed by Moreno-Rueda 2017). Preening is a costly behavior, as it takes time away from foraging, vigilance to predators, and other behaviors (Redpath 1988). Most species spend a significant amount of time preening, on average 9% of their day (Clayton and Cotgreave 1994). However, preening effort varies based on species ranging from 25% of observation time in common loons *Gavia immer* and less than 1% in ostrich (*Struthio camelus*; Daub 1989, Williams et al. 1993).

Feather bacterial abundance, preening, and feather coloration are interrelated but, to our knowledge, these variables have not been assessed simultaneously. To better understand the relationships among these variables, we examined the natural variation in feather coloration, preening, and both total bacterial abundance and the abundance of FDB in captive Indian peafowl *Pavo cristatus*. Peafowl were chosen for this study due to their colorful plumage that is important for conspecific signaling (Yasmin and Yahya 1996, Loyau et al. 2007, Earl et al. 2022). They also spend a substantial amount of time maintaining this plumage through preening (2.02–14.9%, on average; Walther 2003, Harikrishnan et al. 2010). Peafowl are also generally ground dwelling, which may make them particularly susceptible to FDB, many of which are derived from soil (Burtt and Ichida 1999, Lucas et al. 2003). We predicted that individuals that spent more time preening would have lower abundances of FDB on their feathers and that they would have brighter and more colorful feathers.

Material and methods

This study was conducted in College Station, Brazos County, TX (30°37′40.717″N, 96°20′3.864″W) on a population of captive Indian peafowl (11 males and 19 females) during the summer of 2021 (May–August). The birds were originally captured as adults (exact age unknown) from feral populations in Florida and California between 2009 and 2019. The birds have lived as a single flock in an outdoor enclosure (18.3 × 24.4 × 2.1 m) since capture but have occasionally been separated for other studies (Yorzinski 2019). Individuals had a metal band on one leg and a plastic band on the other leg, both with unique identification codes which allowed us to record individual-level behavioral observations. Individuals were given food and water ad libitum. All methods were approved under the Texas A&M University's Institutional Animal Care and Use Committee (no. 2022-0072).

Preening observations

Each bird was observed for a total of 4 h in 15 min intervals over multiple days between May and August 2021. The order the birds were observed in was determined at random, but a given bird was never observed for more than a single 15 min interval on the same day. All sampling occurred during the hours of 07:00 and 11:00 h and were performed by one individual (KMD). During each sampling period, the focal individual was identified by their leg bands and the observer maintained at least a 3 m distance while still being able to see the focal bird. Humans enter the peafowl enclosure for daily feeding and maintenance and the birds are acclimated to their presence. Therefore, the observer's presence likely did not change the peafowl's behavior. The observer recorded each instance of the bird performing maintenance behaviors towards themselves (when the beak comes into contact with their own feathers; referred to as preening) or towards other bird (the beak of another bird comes into contact with the focal bird's feathers; referred to as allopreening). Once a maintenance behavior began, the observer recorded the start time, the location on the body where preening or allopreening occurred, and the time the behavior ended. We defined the different areas of the body in Fig. 1. If allopreening was observed, the bird that the focal individual was interacting with was recorded as well as if the focal individual gave or received this maintenance behavior. A maintenance behavior event began when the bird non-aggressively brought their beak into contact with their own or another bird's body and ended when the contact ceased. A maintenance behavior event was scored as a single event so long as any pauses in preening did not exceed 10 s. In addition to preening and allopreening, we recorded dustbathing, which is when the bird lays on the ground and tosses dirt onto their wings and back. All times were recorded with a stopwatch. The number of seconds spent preening, allopreening, and dustbathing over all the observation periods were summed for each individual. We ultimately excluded allopreening and dustbathing from our analyses because these events occurred rarely (both

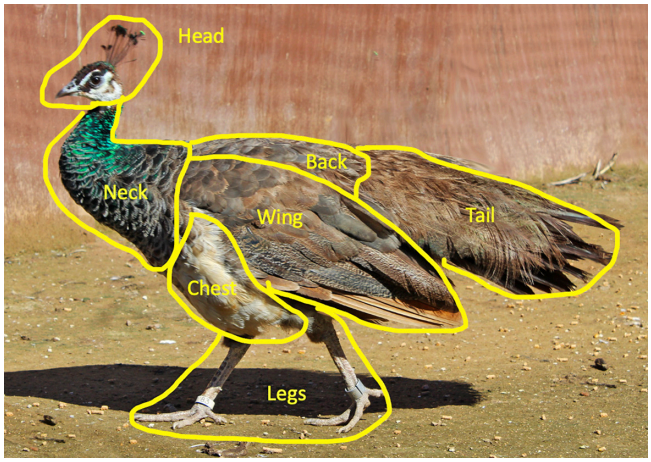

Figure 1. Body regions used to define where preening on a bird occurred.

these activities account for less than 1% of the time birds spent on maintenance behavior). Therefore, the maintenance behavior described in the subsequent analysis and results is preening.

Feather collection

During the final week of peening observations, we collected feathers from each bird to measure color and bacterial abundances on three days in August 2021. We clipped 40 feathers from the neck of each bird, about 10 cm down from the top of the head (Fig. 2): we collected 10 feathers each from the dorsal, ventral, left lateral, and right lateral regions of the neck. The neck feathers were chosen for analysis because the brightness of these feathers positively correlates with dominance in female peafowl (Earl et al. 2022). Alteration by bacteria of these neck feathers may therefore impact intra-specific communication. The feathers were removed by clipping the rachis near the skin below the feather barbs with scissors. We handled the feathers with gloved hands to avoid contamination. In the field, the feathers were stored within opaque envelopes. In the lab, six of these feathers from each side of the neck (24 feathers from each bird total) were mounted on matte black card stock and stored in an opaque envelope in room temperature (20°C) until used for color analysis. The four additional feathers from each side of the neck (16 feathers from each bird total) were immediately stored in a −20°C freezer before microbial analysis (the feathers were stored in the freezer within 6 h of collection). At this time, we also weighed each bird and measured the length of the metatarsus. We used these variables to calculate the scaled mass index, a measure of body condition, using the method described by Peig and Green (2009).

Feather color measurements

Using UV-Vis spectrometry, we measured the reflectance of the collected feathers. We quantified the color reflectance of feathers using the avian visible spectrum (300–700 nm) using a Maya2000-pro spectrometer and a DH2000-DUV light source (output 190–2500 nm). Each feather was measured individually instead of grouped as recommended by Meadows et al. (2011) to minimize potential error in measurement. We mounted collimating lenses onto the ends of 230 um optical fibers for both illumination and measurement of a spot approximately 2 mm in diameter. The spectrometer illumination probe was placed at 60° to the right of the feather and collection probe at 90° to mimic how the feathers would be viewed by other peafowl (Dakin and Montgomerie 2013, Earl et al. 2022). Reflectance was measured relative

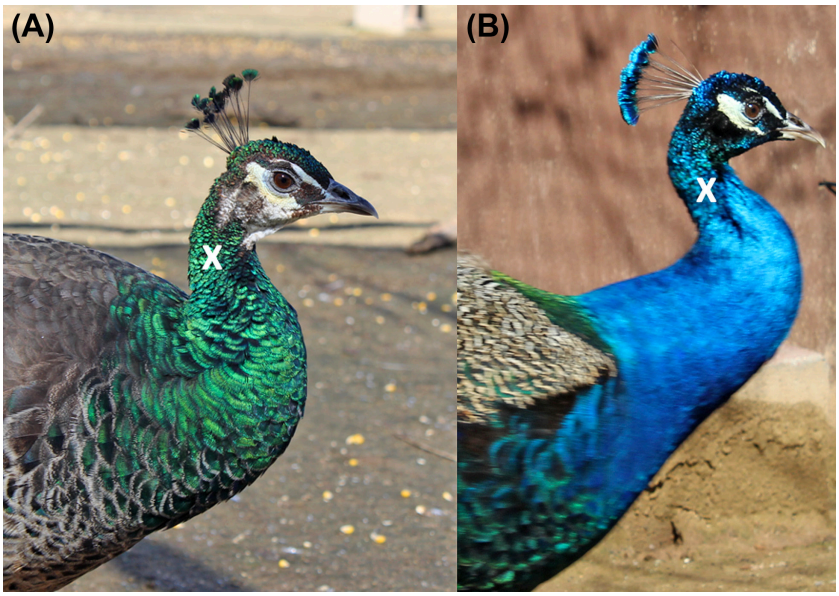

Figure 2. Photograph of a female (A) and male (B) peafowl from our study population demonstrating the species' sexual dimorphism. The white 'X's indicate the locations where the right dorsal feathers were collected.

to a calibrated white (99%) reflectance standard (Spectralon WS-1-SL diffuse reflectance standard; Labsphere, Inc.). The mounted feathers were elevated to be measured at the same distance from the probes as the standard. Dark standards were also taken by removing the collection probe and capping the spectrometer. Dark and white standards were recalibrated approximately every 15 min to minimize drift. We collected reflectance data using the OceanView software (ver. 2.0.8). One experimenter (MRF) measured the feathers without knowledge of their preening behavior and all measurements were taken in a darkroom to minimize ambient light.

We analyzed the reflectance data using the avian visual models for peafowl tetrachromatic vision (R package 'Pavo'; Maia et al. 2013, ver. 2.7.1). Following prior work in this species (Dakin and Montgomerie 2013, Earl et al. 2022), we used a visual model with peafowl chromatic visual sensitivity, the achromatic receptor stimulation for double cone sensitivity of *Gallus gallus* (a close relative of peafowl), and illumination set to 'ideal' which is the homogenous illumination across all bird sensitive wavelengths (Stoddard and Prum 2008). Using this visual model, we quantified four color space variables per feather: brightness, chroma, hue phi (hereafter 'hue UV') and hue theta (hereafter 'hue VIS'; Stoddard and Prum 2008).

We calculated the mean of each color space variable (brightness, chroma, hue UV, and hue VIS) for each of the four sides of each bird's neck. These color variables are commonly used to describe feather color in the avian visual system (Stoddard and Prum 2008). Male and female birds have different feather colors and therefore were analyzed separately.

Bacteria culture

To collect bacteria on the feathers, we removed the feathers from the freezer and placed four feathers from each individual (one from each side of the neck) in a falcon tube with 1.3 ml of sterile water. The tube was then shaken and vortexed for 15 min to dislodge bacteria from the feathers' surface into solution. Then, 200 ul of this solution was plated onto four agar plates: two plates of generalist media, tryptic soy agar (TSA), and two plates of selective media, feather meal agar (FMA; 15 g l^{-1} feather meal, 0.5g l^{-1} NaCl, 0.3 g l^{-1} K_2HPO_4, 0.4 g l^{-1} KH_2PO_4, 15 g l^{-1}, agar; Sangali and Brandelli 2000). Feather meal agar is made with feather meal, or ground up feathers, which selects specifically for bacteria that can use feathers as a nutrient source. TSA plates were then incubated for 24 h at 35°C. Growth is slower on FMA plates (Shawkey et al. 2009), so they were included for 48 h at 35°C. Positive or negative control plates were not included in the incubation. However, plates were prepared using standard aseptic lab techniques, following methodology from previous studies on this topic (Møller et al. 2009, Shawkey et al. 2009). Once the plates were finished incubating, they were photographed against a standard black background with a digital camera. All plates were incubated in October 2021. The photos were opened with ImageJ (ver. 1.53) and the point tool was used to count the individual colonies, which are distinct visible clusters of bacteria cells. We did not attempt to identify colonies or distinguish them morphologically, so it is possible some of the colonies were fungal rather than bacteria. The effect of fungi on feathers is not as well characterized but there is some evidence that certain strains may also degrade feathers (Kaul and Sumbali 1999, Robicheau et al. 2019). One experimenter (MRF) counted all plates without knowledge of preening or feather coloration. We then averaged the number of colonies on the duplicate TSA and FMA plates to get one average colony count per individual per plate type. The values of the duplicate plates were generally similar (Pearson correlation $p < 0.0001$).

Statistical analysis

All statistical analyses were performed using R (ver. 4.1.2, www.r-project.org). Due to the ecological and physical differences between the sexes of this species, we analyzed male and female data separately. Because we measured bacteria from the neck region, we used the time birds spent preening the neck in our analyses, which was highly correlated with the time birds spent preening overall in both sexes (Pearson correlation: females $p < 0.01$; males $p < 0.001$). First, to determine the relationship between preening and total bacteria and FDB abundances, we fit linear models with colony counts from TSA or FMA plates as the response variable. The explanatory variables were scaled mass and time spent preening the neck region. And second, to determine which factors predict feather color score, we fit linear models with brightness, chroma, hue UV, or hue VIS as the response variables. The explanatory variables were the total bacteria or FDB abundances, amount of time spent preening the neck, and scaled mass index. A previous study on peafowl neck ornamentation found the color variables from all regions of the neck to be similar and used the average of colors from all neck regions in their analysis (Earl et al. 2022). However, we did not find a similar correlation so we included the region of the neck feathers, as well as individual ID, as random effects in the models.

Results

We found that males and females had similar bacterial colony counts when grown on TSA (mean colonies: female: 328.6, male: 384.9; 1; $p = 0.25$) and FMA plates (mean colonies: female: 163.6, male mean: 194.1; $p = 0.45$). There were marginally significant differences between the sexes in the amount of time spent preening with females spending more of their time preening (female: 2441 s, male: 1715 s; $p = 0.05$). Females also spent significantly more of their time preening the neck region than males (female mean: 313.2 s, male mean: 185.1 s; $p < 0.01$). The neck feathers of male and female birds are distinct in color space (Fig. 3).

We found that preening was correlated with the number of bacterial colonies grown from feathers in females, but not males. We found that female birds that spent more of

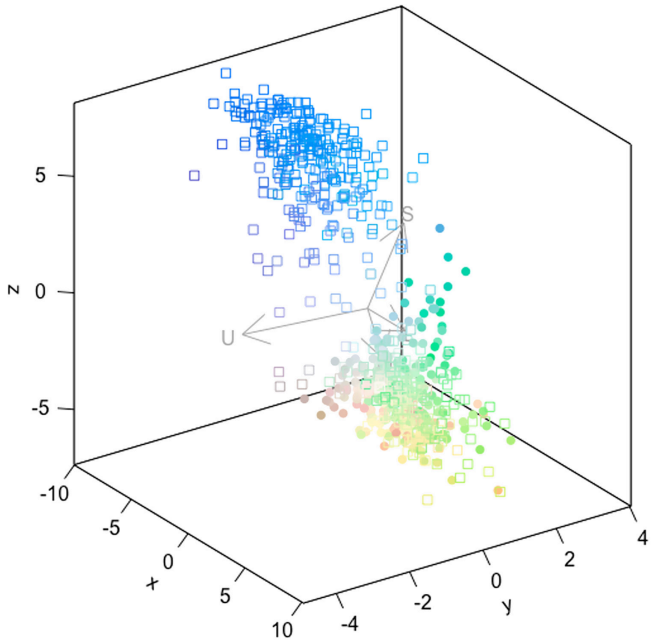

Figure 3. Distribution of male and female neck feather color plotted in tetrahedral color space. Male feathers are represented by open square points and females as filled circular points.

their time preening their neck had statistically significant lower bacteria colony counts when measuring total bacteria and marginally statistically significant lower bacteria colony counts when measuring FDB (TSA: F value = 5.96, p-value = 0.02, Pearson's r = −0.50; FMA: F-value = 3.64, p-value = 0.07, Pearson's r = −0.41; Table 1; Fig. 4). However, there was no relationship between preening and colony counts in males (TSA: F-value = 0.21, p-value = 0.65; FMA: F-value = 0.01, p-value = 0.96; Table 1; Fig 4). Total bacteria or FDB abundances did not significantly predict any of the feather color variables in males or females (Table 2). We did find that scaled mass was correlated with Hue Vis in females, such that smaller birds had greener feathers (F-value = 5.01, p-value = 0.04; Table 2).

Discussion

We found that the time spent preening correlated with feather bacterial abundances in female but not male peafowl. Despite this, we did not find relationships between feather color scores and feather bacterial abundances nor feather color scores and preening. These results suggest that preening may be able to control the abundances of bacteria on female birds' feathers, but this may have little influence on the color of their feathers and the signals that they communicate.

We predicted that preening would be negatively correlated with bacteria abundance on the feathers as preening behavior can remove bacteria (Leclaire et al. 2015). We found support for this prediction in female but not male birds. The region of the body we measured bacteria abundance from, the neck feathers, is likely used for communication in females (Earl et al. 2022) but it is not known if this region has a similar function in the males of this species. Therefore, female birds may have more incentive to remove potentially harmful bacteria from these feathers than males do. Female birds spent more time preening (both overall and the neck region specifically) than males. It would be interesting to test if preening correlates with bacterial abundance in the tail feathers of this species, which are important signals in mate choice in males (Yasmin and Yahya 1996, Yorzinski et al. 2013). In females, we found that the correlation between preening and abundance in total bacteria is strongly significant but the correlation between preening and the abundance of FDB was only marginally significant. This suggests that preening is removing bacteria indiscriminately and not specifically targeting the potentially damaging feather degrading bacteria.

Despite the relationship between bacterial abundances and preening effort, we did not find relationships between bacterial abundances and feather color variables, suggesting that removing FDB from feathers does not preserve feather color signals in this species. Several studies have found FDB are related to feather color (Gunderson et al. 2009, Shawkey et al. 2009, Kilgas et al. 2012, Leclaire et al. 2014), however, other studies have found no effect (Cristol et al. 2005, Jacob et al. 2014). Blue and iridescent feathers colors, like those in peafowl, are produced by complex structures in the feather barbules composed of keratin, melanin, and air pockets (Prum 2006) that may be disrupted by FDB. Female bluebirds *Sialia sialis* with higher abundance of FDB on their feathers had duller plumage, as well as lower body condition (Gunderson et al. 2009). Iridescent color in pigeon feathers *Columba livia* with higher abundances of (FDB) was duller (Leclaire et al. 2014). The complex and varied structures of feathers may be why not all studies have found the same results. A study in male bluebirds found that birds with higher abundances of FDB had brighter plumage (Shawkey et al. 2007) and in great tits *Parus major* experimentally increasing FDB had no effect on feather color (Jacob et al. 2014). FDB may not have degraded the color of the iridescent feathers in this study due to the melanin in their feather matrix. Melanin may prevent FDB degradation by binding to keratinases that hydrolyze keratins (Gunderson et al. 2008). Understanding

Table 1. Results from a model investigating the relationship between total bacteria and FDB abundance and time preening the neck region and scaled mass index in peafowl. F-values and their associated p-values (in parentheses) are reported.

		Female		Male	
	df	Total bacteria	FDB	Total bacteria	FDB
Time preening neck	1	5.96 (0.02*)	3.64 (0.07)	0.21 (0.65)	0.01 (0.96)
Scaled mass index	1	0.66 (0.42)	0.27 (0.60)	1.01 (0.34)	0.21 (0.65)

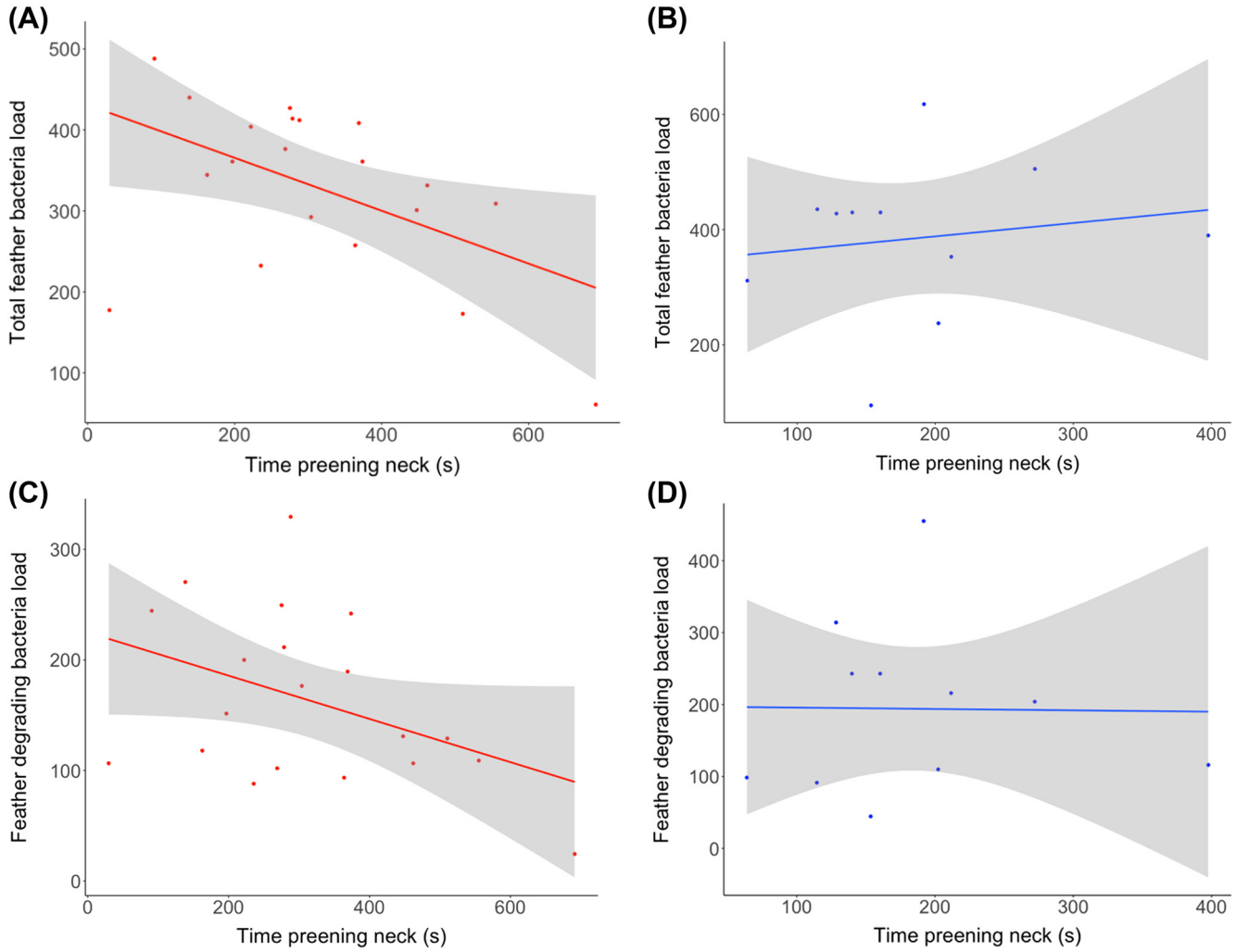

Figure 4. Relationship between time spent preening the neck and total bacteria abundances (A: females; B: males) and FDB abundances (C: females; D: males).

Table 2. Results from a model investigating the relationship between the color scores and feather bacteria abundance (A: total bacteria; B: FDB), preening, scaled mass index. F-values and their associated p-values (in parentheses) are reported.

Sex		df	Brightness	Chroma	Hue UV	Hue VIS
(A) Total bacterial						
Females	Total bacteria	13	2.45 (0.14)	0.08 (0.77)	0.03 (0.84)	0.12 (0.72)
	Preening	13	1.94 (0.18)	0.08 (0.77)	0.15 (0.70)	0.06 (0.80)
	Total bacteria × Preening	13	0.07 (0.78)	2.02 (0.17)	0.55 (0.46)	0.01 (0.98)
	Scaled mass index	13	0.20 (0.65)	1.23 (0.28)	0.89 (0.36)	4.08 (0.06)
Males	Total bacteria	6	0.78 (0.41)	2.21 (0.18)	0.01 (0.91)	0.13 (0.72)
	Preening	6	0.41 (0.54)	2.22 (0.18)	0.03 (0.86)	0.08 (0.78)
	Total bacteria × Preening	6	0.51 (0.50)	2.09 (0.19)	0.01 (0.94)	0.19 (0.67)
	Scaled mass index	6	0.35 (0.57)	1.97 (0.20)	0.74 (0.42)	1.29 (0.29)
(B) FDB						
Females	FDB	13	0.09 (0.76)	0.18 (0.67)	0.07 (0.78)	0.29 (0.59)
	Preening	13	0.23 (0.63)	0.01 (0.92)	0.10 (0.74)	0.17 (0.68)
	FDB × preening	13	0.01 (0.89)	1.29 (0.27)	0.20 (0.60)	0.03 (0.86)
	Scaled mass index	13	0.77 (0.39)	2.17 (0.16)	0.55 (0.46)	5.01 (0.04*)
Males	FDB	6	0.01 (0.92)	4.03 (0.09)	0.05 (0.82)	0.33 (0.59)
	Preening	6	0.01 (0.94)	4.28 (0.08)	0.14 (0.71)	0.76 (0.41)
	FDB × Preening	6	0.01 (0.99)	4.03 (0.09)	0.03 (0.85)	0.29 (0.60)
	Scaled mass index	6	0.12 (0.74)	5.83 (0.05)	0.84 (0.39)	1.91 (0.21)

how FDB impacts the keratin structure of feathers on a microscopic level may explain variation in the results that have been found across studies.

The birds used in this study were in a captive environment and may be exposed to lower abundances of microorganisms than what could cause damage to the feathers. However, a study with wild house finches found a range of bacterial abundances as well as differences in feather coloration that were similar to our captive peafowl (Shawkey et al. 2009). We also found that the birds spent approximately 7% of the observation time preening, which is within the range found of wild and free-ranging peafowl populations (Walther 2003, Harikrishnan et al. 2010). We collected preening data over the course of the breeding season but collected bacterial abundance and feather color data only at one time point. This may have obscured the relationship between bacterial abundances and feather color, and it would be interesting to investigate if bacterial abundance and color change correlate over the course of the breeding season or the lifetime of a molt.

We also note that in female birds, the hue of the feathers was correlated with bird size. Smaller female birds had greener plumage that was unrelated to preening or feather bacteria. This is likely not related to dominance, as body size and hue were not found to be related to dominance in female peafowl (Earl et al. 2022). The birds had ad libitum access to feed, which makes nutritional stress an unlikely cause of variation in hue and body size. The birds in this study were captured from the wild as adults and therefore we do not know their specific ages, so we cannot determine whether age may be a factor. Therefore, future studies would be needed to determine why hue is correlated with body size in females.

Removal of bacteria on the feathers may have important functions other than impacting feather color. A study in zebra finch *Taeniopygia guttata castanotis* found that feather bacteria influenced reproductive performance. Experimentally reducing feather bacterial abundances increased fledging success due to decreased mortality of the chicks (Burley et al. 2022). Our measurements took place during the breeding season, so female birds may be removing bacteria not to preserve their feathers but to prevent spreading bacteria to eggs or chicks. The peafowl population sampled in this study do not raise offspring (eggs are removed after laying), therefore we are unable to compare our results with hatching or fledging success. However, because males do not raise offspring in this species, this could contribute to differences we found between the sexes (Petrie and Williams 1993).

While we measured the amount of time the birds spent preening, we did not account for possible variation of usage of preen oil during preening. Preen oil is spread on the feathers during preening and provides benefits such as waterproofing feathers and controlling bacteria via antimicrobial compounds (Shawkey et al. 2003, Giraudeau et al. 2010, Alt et al. 2020). Preen oil is not used during all preening bouts and its chemical make-up can vary among individuals of the same species (Whittaker et al. 2010, Tuttle et al. 2014). Therefore, variation in preen oil usage as well as variation in composition may have impacted the relationships between preening, bacteria abundances, and feather color. Accounting for the variation in preen oil usage and composition between individuals would be a valuable addition to futures studies on this topic.

Overall, we found partial support for our prediction that preening would correlate with abundances of bacteria on peafowl feathers, but our prediction that bacterial abundances would correlate with feather color was not supported. More time spent preening correlated with lower abundances of bacteria on female but not male peafowl, possibly due to differences in their use of feathers as signals or their roles in reproduction. We did not find relationships between feather color and bacterial abundances. These results suggest that preening may modify feather bacterial abundance, but these bacteria may not significantly impact feather coloration in this species.

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Author contributions

Melanie R. Florkowski: Conceptualization (equal); Formal analysis (lead); Funding acquisition (lead); Methodology (equal); Writing – original draft (lead); Writing – review and editing (equal). **Kirstin M. DeBlonk:** Data curation (lead); Methodology (supporting); Writing – review and editing (supporting). **Jessica L. Yorzinski:** Conceptualization (equal); Formal analysis (supporting); Methodology (supporting); Resources (lead); Supervision (lead); Writing – review and editing (equal).

Transparent peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/jav.03209.

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.1jwstqk18 (Florkowski et al. 2024).

References

Alt, G., Mägi, M., Lodjak, J. and Mänd, R. 2020. Experimental study of the effect of preen oil against feather bacteria in passerine birds. – Oecologia 192: 723–733.

Barbosa, A., Merino, S., Lope, F. and Møller, A. P. 2002. Effects of feather lice on flight behavior of male barn swallows (*Hirundo rustica*). – Auk 119: 213–216.
Bonser, R. H. C. 1995. Melanin and the abrasion resistance of feathers. – Condor 97: 590–591.
Bonser, R. H. C. 1996. The mechanical properties of feather keratin. – J. Zool. 239: 477–484.
Burley, N. T., Campos, F. A., Chien, E., Duarte, S., Kirshman, N., Phan, A. T. T. N. and Wilson, K. M. 2022. Experimentally reduced feather microbial loads improve reproductive performance in captive zebra Finches. – Ornithology 139: ukac021.
Burtt, E. H. and Ichida, J. M. 1999. Occurrence of feather-degrading bacilli in the plumage of birds. – Auk 116: 364–372.
Clayton, D. H. and Cotgreave, P. 1994. Comparative analysis of time spent grooming by birds in relation to parasite load. – Behaviour 131: 171–187.
Cristol, D. A., Armstrong, J. L., Whitaker, J. M. and Forsyth, M. H. 2005. Feather-degrading bacteria do not affect feathers on captive birds. – Ornithology 122: 222–230.
Dakin, R. and Montgomerie, R. 2013. Eye for an eyespot: how iridescent plumage ocelli influence peacock mating success. – Behav. Ecol. 24: 1048–1057.
Daub, B. C. 1989. Behavior of common loons in winter. – J. Field Ornithol. 60: 305–311
Earl, A. D., Simpson, R. K. and Yorzinski, J. L. 2022. Dominant females have brighter ornamentation in a sexually dimorphic lekking species. – Ethology 128: 85–93.
Fitzpatrick, S. 1998. Colour schemes for birds: structural coloration and signals of quality in feathers. – Ann. Zool. Fenn. 35: 67–77.
Florkowski, M. R., DeBlonk, K. M. and Yorzinski, J. L. 2024. Data from: Preening correlates with lower feather bacteria abundance but not feather coloration in a lek-breeding bird. – Dryad Digital Repository, https://doi.org/10.5061/dryad.1jwstqk18.
Giraudeau, M., Duval, C., Guillon, N., Bretagnolle, V., Gutierrez, C. and Heeb, P. 2010. Effects of access to preen gland secretions on mallard plumage. – Naturwissenschaften 97: 577–581.
Griggio, M., Zanollo, V. and Hoi, H. 2010. UV plumage color is an honest signal of quality in male budgerigars. – Ecol. Res. 25: 77–82.
Grindstaff, J. L., Lovern, M. B., Burtka, J. L. and Hallmark-Sharber, A. 2012. Structural coloration signals condition, parental investment, and circulating hormone levels in eastern bluebirds (*Sialia sialis*). – J. Comp. Physiol. A 198: 625–637.
Gunderson, A. R. 2008. Feather-degrading bacteria: a new frontier in avian and host–parasite research? – Auk 125: 972–979.
Gunderson, A. R., Forsyth, M. H. and Swaddle, J. P. 2009. Evidence that plumage bacteria influence feather coloration and body condition of eastern bluebirds *Sialia sialis*. – J. Avian Biol. 40: 440–447.
Hamilton, W. D. and Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? – Science 218: 384–387.
Harikrishnan, S., Vasudevan, K. and Sivakumar, K. 2010. Behavior of Indian peafowl *Pavo cristatus* Linn. 1758 during the mating period in a natural population. – Open Ornithol. J. 3: 13–19.
Jacob, S., Colmas, L., Parthuisot, N. and Heeb, P. 2014. Do feather-degrading bacteria actually degrade feather colour? No significant effects of plumage microbiome modifications on feather colouration in wild great tits. – Naturwissenschaften 101: 929–938.
Kaul, S. and Sumbali, G. 1999. Production of extracellular keratinases by keratinophilic fungal species inhabiting feathers of living poultry birds (*Gallus domesticus*): a comparison. – Mycopathologia 146: 19–24.
Kilgas, P., Saag, P., Mägi, M., Edenberg, M., Tilgar, V. and Mänd, R. 2012. Variation in assemblages of feather bacteria in relation to plumage color in female great tits. – Condor 114: 606–611.
Lattin, C. R., Reed, J. M., DesRochers, D. W. and Romero, L. M. 2011. Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. – J. Avian Biol. 42: 247–252.
Leclaire, S., Pierret, P., Chatelain, M. and Gasparini, J. 2014. Feather bacterial load affects plumage condition, iridescent color, and investment in preening in pigeons. – Behav. Ecol. 25: 1192–1198.
Leclaire, S., Czirják, G. Á., Hammouda, A. and Gasparini, J. 2015. Feather bacterial load shapes the trade-off between preening and immunity in pigeons. – BMC Evol. Biol. 15: 60.
Lenouvel, P., Gomez, D., Théry, M. and Kreutzer, M. 2009. Do grooming behaviours affect visual properties of feathers in male domestic canaries, *Serinus canaria*? – Anim. Behav. 77: 1253–1260.
Loyau, A., Gomez, D., Moureau, B., Théry, M., Hart, N. S., Jalme, M., Bennett, A. T. D. and Sorci, G. 2007. Iridescent structurally based coloration of eyespots correlates with mating success in the peacock. – Behav. Ecol. 18: 1123–1131.
Lucas, F. S., Broennimann, O., Febbraro, I. and Heeb, P. 2003. High diversity among feather-degrading bacteria from a dry meadow soil. – Microb. Ecol. 45: 282–290.
Maia, R., Eliason, C. M., Bitton, P. P., Doucet, S. M. and Shawkey, M. D. 2013. pavo: an R package for the analysis, visualization and organization of spectral data. – Methods Ecol. Evol. 4: 906–913.
McGraw, K. J. 2008. An update on the honesty of melanin-based color signals in birds. – Pigment Cell Melanoma Res. 21: 133–138.
Meadows, M. G., Morehouse, N. I., Rutowski, R. L., Douglas, J. M. and McGraw, K. J. 2011. Quantifying iridescent coloration in animals: a method for improving repeatability. – Behav. Ecol. Sociobiol. 65: 1317–1327.
Meadows, M. G., Roudybush, T. E. and McGraw, K. J. 2012. Dietary protein level affects iridescent coloration in Anna's hummingbirds, *Calypte anna*. – J. Exp. Biol. 215: 2742–2750.
Møller, A. P. and Nielsen, J. T. 2018. The trade-off between rapid feather growth and impaired feather quality increases risk of predation. – J. Ornithol. 159: 165–171.
Møller, A. P., Czirjak, G. Á. and Heeb, P. 2009. Feather microorganisms and uropygial antimicrobial defences in a colonial passerine bird. – Funct. Ecol. 23: 1097–1102.
Moreno-Rueda, G. 2017. Preen oil and bird fitness: a critical review of the evidence. – Biol. Rev. 92: 2131–2143.
Pap, P. L., Vágási, C. I., Bărbos, L. and Marton, A. 2013. Chronic coccidian infestation compromises flight feather quality in house sparrows *Passer domesticus*. – Biol. J. Linn. Soc. 108: 414–428.
Pehrsson, O. 1987. Effects of body condition on molting in mallards. – Condor 89: 329–339.
Peig, J. and Green, A. J. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. – Oikos 118: 1883–1891.
Petrie, M. and Williams, A. 1993. Peahens lay more eggs for peacocks with larger trains. – Proc. R. Soc. B 251: 127–131.

Prum, R. O. 2006. Anatomy, physics, and evolution of structural colors. – In: Hill, G. E. and McGraw, K. J. (eds), Mechanisms and measurements, pp. 295–353.
Rayner, J. M. V. 1988. Form and function in avian flight. – Curr. Ornithol. 5:1–66.
Redpath, S. 1988. Vigilance levels in preening dunlin *Calidris alpina*. – Ibis 130: 555–557.
Robicheau, B. M., Adams, S. J., Provencher, J. F., Robertson, G. J., Mallory, M. L. and Walker, A. K. 2019. Diversity and keratin degrading ability of fungi isolated from Canadian arctic marine bird feathers. – Arctic 72: 347–359.
Saino, N., Romano, M., Rubolini, D., Teplitsky, C., Ambrosini, R., Caprioli, M., Canova, L. and Wakamatsu, K. 2013. Sexual dimorphism in melanin pigmentation, feather coloration and its heritability in the barn swallow (*Hirundo rustica*). – PLoS One 8: e58024.
Sangali, S. and Brandelli, A. 2000. Isolation and characterization of a novel feather-degrading bacterial strain. – Appl. Biochem. Biotechnol. 87: 17–24.
Schwab, R. G. and Schafer, V. S. 1972. Avian thermoregulation and its significance in starling control. – In: Proceedings of the vertebrate pest conference, vol. 5 https://escholarship.org/uc/item/396525j3.
Shawkey, M. D., Pillai, S. R. and Hill, G. E. 2003. Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. – J. Avian Biol. 34: 345–349.
Shawkey, M. D., Pillai, S. R., Hill, G. E., Siefferman, L. M. and Roberts, S. R. 2007. Bacteria as an agent for change in structural plumage color: correlational and experimental evidence. – Am. Nat. 169: S112–S121.
Shawkey, M. D., Pillai, S. R. and Hill, G. E. 2009. Do feather-degrading bacteria affect sexually selected plumage color? – Naturwissenschaften 96: 123–128.
Stoddard, M. C. and Prum, R. O. 2008. Evolution of avian plumage color in a tetrahedral color space: a phylogenetic analysis of new world buntings. – Am. Nat. 171: 755–776.
Surmacki, A. 2008. Preen waxes do not protect carotenoid plumage from bleaching by sunlight. – Ibis 150: 335–341.
Tuttle, E. M., Sebastian, P. J., Posto, A. L., Soini, H. A., Novotny, M. V. and Gonser, R. A. 2014. Variation in preen oil composition pertaining to season, sex, and genotype in the polymorphic white-throated sparrow. – J. Chem. Ecol. 40: 1025–1038.
Vezzoli, G., Mullens, B. A. and Mench, J. A. 2015. Relationships between beak condition, preening behavior and ectoparasite infestation levels in laying hens. – Poult. Sci. 94: 1997–2007.
Waite, J. L., Henry, A. R. and Clayton, D. H. 2012. How effective is preening against mobile ectoparasites? An experimental test with pigeons and hippoboscid flies. – Int. J. Parasitol. 42: 463–467.
Walther, B. A. 2003. Do peacocks devote maintenance time to their ornamental plumage? Time budgets of male blue peafowl *Pavo cristatus*. – Lundiana. Int. J. Biodivers. 4: 149–154.
Weaver, R. J., Santos, E. S. A., Tucker, A. M., Wilson, A. E. and Hill, G. E. 2018. Carotenoid metabolism strengthens the link between feather coloration and individual quality. – Nat. Commun. 9: 73.
Whittaker, D. J., Soini, H. A., Atwell, J. W., Hollars, C., Novotny, M. V. and Ketterson, E. D. 2010. Songbird chemosignals: volatile compounds in preen gland secretions vary among individuals, sexes, and populations. – Behav. Ecol. 21: 608–614.
Williams, C. M., Richter, C. S., MacKenzie , J. M. and Shih, J. C. H. 1990. Isolation, identification, and characterization of a feather-degrading bacterium. – Appl. Environ. Microbiol. 56: 1509–1515.
Williams, J. B., Siegfried, W. R., Milton, S. J., Adams, N. J., Dean, W. R. J., du Plessis, M. A. and Jackson, S. 1993. Field metabolism, water requirements, and foraging behavior of wild ostriches in the Namib. – Ecology 74: 390–404.
Yasmin, S. and Yahya, H. S. A. 1996. Correlates of mating success in Indian peafowl. – Auk 113: 490–492.
Yorzinski, J. L. 2019. Conjugate eye movements guide jumping locomotion in an avian species. – J. Exp. Biol. 222: jeb211565.
Yorzinski, J. L., Patricelli, G. L., Babcock, J. S., Pearson, J. M. and Platt, M. L. 2013. Through their eyes: selective attention in peahens during courtship. – J. Exp. Biol. 216: 3035–3046.