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Commentary

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FEATHER-DEGRADING BACTERIA: A NEW FRONTIER IN AVIAN AND HOST–PARASITE RESEARCH?

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BIRDS ARE IMPORTANT models for the study of host–parasite interactions (Loye and Zuk 1991, Clayton and Moore 1997). Much of this research has focused on arthropod ectoparasites that feed on feathers (e.g., Clayton et al. 2003, Proctor 2003), because feathers are so important to avian life-history traits. Feathers function in thermoregulation (Stettenheim 2000), communication (Andersson 1994, Shuster and Wade 2003), and flight (Rayner 1988). Damaged feathers have reduced abilities to perform these functions (Booth et al. 1993, Swaddle and Witter 1997, Ferns and Lang 2003, Williams and Swaddle 2003), so there are likely fitness consequences for individuals possessing damaged feathers. A subset of plumage bacteria that can degrade feathers has garnered interest, because it may impose significant evolutionary selection pressures on birds, as arthropod ectoparasites do. Aspects of avian morphology, behavior, and life history may be influenced by a coevolutionary battle between birds and feather-degrading bacteria (FDB) that damage their plumage.

Research on FDB and birds is in its nascent stages; however, a substantial body of literature has attempted to understand how birds and these microbes interact. Here, I synthesize what we currently know, highlight important gaps in our knowledge, and suggest next steps for the field, while focusing on three fundamental questions: What are FDB and how do they degrade feathers? How prevalent are FDB on birds? And finally, how can FDB and birds influence one another?

WHAT ARE FEATHER-DEGRADING BACTERIA?

Feather-degrading bacteria are a polyphyletic group related only by the ability to decompose feathers (Onifade et al. 1998). They are phylogenetically and physiologically diverse (Table 1) and appear to be cosmopolitan. The ability to decompose feathers is uncommon among bacteria, because feathers contain >90% β-keratin by mass (Onifade et al. 1998). β-keratins are extensively cross-linked within and between polypeptides through hydrogen and disulfide bonds, which makes them compact and resistant to degradation by most proteolytic enzymes (Gupta and Ramnani 2006). How FDB decompose feathers is not fully understood, but the process likely involves two steps. First, the disulfide bonds of β-keratin are reduced, possibly by the production of disulfide reductases (Yamamura et al. 2002b) or sulfite (Ramnani et al. 2005). Second, proteolytic keratinases specialized in hydrolyzing keratins break the remaining bonds (Gupta and Ramnani 2006).

HOW PREVALENT ARE FEATHER-DEGRADING BACTERIA ON BIRDS?

Feather-degrading bacteria are common within plumage. Burtt and Ichida (1999) opportunistically sampled temperate birds and found FDB of the genus *Bacillus* on 32 of 83 species, and on 89% of species with high sample sizes ($n > 20$; Burtt and Ichida 1999). Within species, the prevalence (percentage of individuals contaminated) ranged from 0 to 29% (mean: 8.4 ± 0.2% [SD]; Burtt and Ichida 1999). The authors found that ground-foraging and water birds have a higher prevalence of *Bacillus* than aerial or bark-probing species, which suggests that FDB are acquired through contact with environmental substrates rather than from conspecifics (Burtt and Ichida 1999); however, their analyses did not control for sampling effort and, thus, are preliminary. Whitaker et al. (2005) surveyed eight temperate bird species and found FDB on all of them, with a mean FDB prevalence of 39%.

These studies indicate that FDB are pervasive among birds and suggest considerable among-species and among-population variation in FDB prevalence; however, they likely underestimated the prevalence of FDB (Clayton 1999, Shawkey et al. 2007). Both studies used highly selective cultivation protocols to isolate FDB

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Table 1. Bacteria with keratinolytic activity. Unless noted, see references for specific strains identified. This list is conservative, because many bacteria have not been tested for keratinolytic activity and many cannot currently be tested because they are unculturable. Keratinolytic bacteria unlikely to be found on birds, such as those from hot springs (Kim et al. 2004), are not included.

Bacterium	Source	Bacterial Phylum	Reference
Bacillus licheniformis	Wild bird	Firmicutes	Burtt and Ichida 1999, Whitaker et al. 2005
B. subtilis	Wild bird	Firmicutes	Burtt and Ichida 1999, Whitaker et al. 2005
B. pumilis	Wild bird	Firmicutes	Burtt and Ichida 1999
B. pseudofirmus	Poultry farm soil	Firmicutes	Gessesse et al. 2003, Kojima et al. 2006
B. cereus	Poultry waste	Firmicutes	Kim et al. 2001
Staphylococcus epidermidis	Wild bird	Firmicutes	Shawkey et al. 2003
S. hemolyticus	Wild bird	Firmicutes	Shawkey et al. 2003
S. hominis	Wild bird	Firmicutes	Shawkey et al. 2003
Enterococcus faecalis	Wild bird	Firmicutes	Shawkey et al. 2003
Kocuria rosea	Wild bird	Actinobacteria	Shawkey et al. 2003
K. rhizophila	Wild bird	Actinobacteria	Shawkey et al. 2003
Micrococcus nishinomyaensis	Wild bird	Actinobacteria	Shawkey et al. 2003
Streptomyces sp. (OWU 1441)	Wild bird	Actinobacteria	Tiquia et al. 2005
Streptomyces sp. 594	Soil	Actinobacteria	Azeredo et al. 2006
Nesterenkonia sp. AL-20	Soil	Actinobacteria	Gessesse et al. 2003
Pseudomonas stutzeri	Wild bird	Proteobacteria	Shawkey et al. 2003
P. fulva	Wild bird	Proteobacteria	Shawkey et al. 2003
Stenotrophomonas sp.	Deer fur	Proteobacteria	Yamamura et al. 2002a, b
Vibrio sp. kr2	Poultry waste	Proteobacteria	Sangali and Brandelli 2000
Chryseobacterium sp. kr6	Poultry waste	Bacteroidetes	Riffel et al. 2003, Brandelli 2005, Brandelli and Riffel 2005
Flavobacterium sp.	Poultry waste	Bacteroidetes	Riffel and Brandelli 2002

of the genus *Bacillus* (Burtt and Ichida 1999, Whitaker et al. 2005), which are mildly thermotolerant, halotolerant, and Gram-positive. Isolating bacteria with these characteristics narrows the range of bacteria that can be detected. More inclusive cultivation methods detected FDB on 88% of male Eastern Bluebirds (*Sialia sialis*; Shawkey et al. 2007) and found a phylogenetically diverse assemblage of FDB on House Finches (*Carpodacus mexicanus*; Shawkey et al. 2003). Similar methods isolated 13 strains of putative FDB from soil, which suggests that birds can encounter a high diversity of FDB in the environment (Lucas et al. 2003). Feather-degrading bacteria are physiologically diverse, and this diversity must be accommodated in culture-based surveys to determine the exposure of birds to FDB as a group.

Culture-independent methods may also be useful in detecting FDB on birds. Approximately 99% of bacterial species are unculturable because of their ability to enter nonculturable states or because no culture methods have been established (Amann et al. 1995). Thus, a significant portion of FDB species could go undetected in the culture-based surveys that have dominated this field thus far. Several molecular techniques can be employed, typically involving sequencing of ribosomal RNA (rRNA) genes extracted directly from cells in a microbial community sample (Head et al. 1998). However, this cannot identify nonculturable FDB, because there is no direct observation of keratin degradation by the bacteria, which occurs in culture-based surveys. A more direct method would be to amplify the keratinase genes present in a sample of the plumage microbial community, which could detect the presence of FDB that cannot be grown in culture. However, all keratinases are not homologous, and primers that have been developed so far come mostly from *Bacillus* (Gupta and Ramnani 2006); thus, this technique would not identify a phylogenetically diverse range of FDB. To construct the most effective primer sets for the amplification of keratinase genes in bacterial community samples, direct DNA sequencing of keratinase genes from a diverse assemblage of culturable FDB is needed. Ultimately, for future surveys of FDB on birds, culture-dependent and independent methods should be combined, because particular bacteria may be detectable using only one method or the other (Shawkey et al. 2005).

Surveys of the prevalence, diversity, and quantity of FDB on birds will help determine broad geographic, ecological, and phylogenetic patterns of avian contamination with FDB. Importantly, one or several model systems for the study of birds and FDB could emerge. Large-scale, multispecies sampling of birds using standardized sampling techniques would be beneficial. At the very least, researchers working with their own avian model systems should begin to characterize the bacteria that live on their birds.

Importantly, surveys of FDB on birds have generally not addressed variation in FDB intensity (the number of parasite individuals associated with a host individual) among individuals within a population (but see Shawkey et al. 2007). High among-individual variation in FDB intensity, coupled with a correlation between FDB intensity and fitness, is expected if FDB are mediating selection (Goater and Holmes 1997). Surveys that collect quantitative, rather than simply presence–absence, FDB data from sampled birds will help to determine whether FDB are currently a selective force, which has largely been assumed (rather than demonstrated) in the current literature.

Have Birds Evolved Defenses against Feather-degrading Bacteria?

There is consensus that FDB commonly inhabit avian plumage. Therefore, it is relevant to ask whether birds have evolved mechanisms to combat FDB. Several lines of evidence suggest that this has occurred.

Feather structure and color.—Feather biochemistry is a bird's first line of defense against bacterial feather degradation. The tightly folded keratins of feathers cannot be cleaved by most proteolytic enzymes. Selection exerted by FDB is probably not responsible for the utilization of keratin in feathers; however, the action of FDB may favor the evolution and maintenance of biochemical feather characteristics that inhibit the action of FDB. As a corollary to this selection, the deposition of particular feather pigments may be selected because of their protective value against FDB.

Melanin pigments are responsible for most of the black and earth-toned colors of bird feathers (McGraw 2006) and are important for signaling (Griffith et al. 2006) and crypsis. Feathers colored by melanins are also more resistant to FDB than unpigmented feathers (Goldstein et al. 2004, Gunderson et al. 2008; but see Grande et al. 2004). How melanized feathers resist FDB is unknown. Melanized feathers are harder and more resistant to physical abrasion than unmelanized feathers (Burtt 1986, Bonser 1995), and melanins can bind to proteolytic enzymes (Kuo and Alexander 1967). One or both of these mechanisms may protect melanized feathers from FDB. It is important to consider that results from one species or strain of FDB cannot be generalized to all FDB. Some FDB could be inhibited by feather melanization, whereas others could be unaffected or adapted to feeding on melanized feathers. The two types of feather melanin, eumelanin and phaeomelanin, may also differ in their influence on FDB. Future studies need to be conducted with multiple species of FDB and with feathers from several different species of birds to determine the generality of this trend. *In-vivo* experimental studies are now needed to determine whether feather melanization reduces bacterial growth and bacterially induced feather damage on live birds.

There is some preliminary evidence of coevolution between FDB and feather coloration. With a subjective measure of bacterial activity, *B. licheniformis* strains isolated from a dark subspecies of Song Sparrow (*Melospiza melodia morphna*) were found to degrade unpigmented chicken (*Gallus gallus domesticus*) feathers faster than *B. licheniformis* strains isolated from a light subspecies of Song Sparrow (*M. m. fallax*; Burtt and Ichida 2004). It was assumed that the darker subspecies had a higher concentration of melanin in its feathers. More effective FDB on birds with higher feather-melanin concentrations suggest that an evolutionary "arms race" may be occurring, with increases in bacterial efficiency selecting for birds with increased melanin deposition and vice versa (Burtt and Ichida 2004). However, bacterial activity on the birds themselves was not considered, and how variation in bacterial degradation on unpigmented chicken feathers relates to variation in bacterial activity on melanized Song Sparrow feathers is unclear. More direct assessments of bacterial activity on birds with melanin color variation would be beneficial.

Preen oil and preening.—Birds may manipulate the bacterial composition of their plumage by the selective use of preen oil on feathers. Plumage condition deteriorates with surgical removal of the preen gland (Moyer et al. 2003), and it is assumed that preen oil maintains feather condition by waterproofing, by maintaining feather flexibility, or both (Jacob and Ziswiler 1982). However, to my knowledge, there is no direct experimental evidence to support either of these assumptions. Preen oil may maintain feather condition by inhibiting FDB. Removal of the preen gland from chickens shifted the structure and composition of microbial communities on the birds' skin (Bandyopadhyay and Bhattacharyya 1996). Notably, *Bacillus* became the second most prevalent genus of bacteria on glandless birds but was never found on birds with uropygial glands (Bandyopadhyay and Bhattacharyya 1996). *In vitro*, House Finch preen oil inhibits the growth of several species of FDB (Shawkey et al. 2003), and Green Wood Hoopoe (*Phoeniculus purpureus*; Burger et al. 2004) and Red Knot (*Calidris canutus*; Reneerkens et al. 2008) preen oils inhibit *B. licheniformis*.

There are at least three modes by which preen oil could influence FDB. First, preen oil may simply form a physical barrier that prevents FDB from getting access to the feather surface (Reneerkens et al. 2008). Second, the lipids composing preen oil could be antibiotic. The wax 3,7-dimethyloctan-1-ol, isolated from Northern Gannet (*Morus bassanus*) preen oil, inhibits the growth of several bacteria *in vitro* (Jacob et al. 1997). Third, antibiotic-producing bacteria could be cultivated within the uropygial gland and then applied to feathers with preen oil. *Enterococcus feacalis*, isolated from Green Wood Hoopoe preen oil, produces antibiotic bacteriocins that are effective against *B. licheniformis* and several other bacteria (Martin-Platero et al. 2006). It is not known whether the antibiotics produced by *E. feacalis* affect plumage (or egg and nest) bacterial communities, but the possibility is intriguing.

Preen oil can clearly affect FDB. Whether these effects are adaptive is unclear, however. The antibacterial properties of preen oil could be byproducts of its composition that do not influence fitness. It is worth mentioning that some feather mites feed on preen oil and possibly on feather microbes (Proctor and Owens 2000, Proctor 2003) and could influence the relationship between birds and FDB. Longitudinal studies that monitor FDB communities, feather wear, and fitness metrics before and after removal of preen glands would be powerful in determining whether preen oil influences FDB *in vivo*. Also, the act of preening, irrespective of preen oil, could physically dislodge or damage bacteria (Clayton 1999).

Anting, dustbathing, and sunbathing.—Dustbathing and sunbathing are behaviors that have eluded explanation but may influence FDB (Burtt and Ichida 1999, Clayton 1999). Dustbathing dries the plumage but would also expose birds to FDB, which are common in soil (Lucas et al. 2003). This behavior could also expose plumage to microorganisms that displace or otherwise influence FDB. Sunlight reduces the number of viable FDB on feathers *ex vivo* (Saranathan and Burtt 2007), which suggests that birds could use sunbathing to destroy FDB. Tracking FDB load and feather damage of birds experimentally exposed to different sunlight treatments could reveal whether sunbathing functions to inhibit FDB.

Anting may serve an antimicrobial function (Ehrlich et al. 1986), given that some passerines ant with ants that produce formic acid as a defense mechanism. However, extracts from five species of formicine ant (Formicidae: Hymenoptera) did not inhibit FDB growth in culture (Revis and Waller 2004). Birds also "ant"

with other objects that contain antimicrobial compounds, including snails (VanderWerf 2005) and fruit (Clayton and Vernon 1993, VanderWerf 2005). Experimental tests of anting behavior, such as that conducted by Lunt et al. (2004), could determine whether anting influences FDB.

Choice of nest materials.—Many birds line their nests with fresh green vegetation. The nest-protection hypothesis proposes that birds place fresh plant material in their nests to protect against parasites (Clark 1991). In Corsican Blue Tits (*Cyanistes caeruleus ogliastrae*) and European Starlings (*Sturnus vulgaris*), preferred nest plants are high in volatile compounds that inhibit bacterial growth (Clark and Mason 1985, Petit et al. 2002). Corsican Blue Tits use olfactory cues to determine when to bring fresh plant material to the nest, which suggests that birds use fresh plants for the volatile compounds they contain (Petit et al. 2002). No study has addressed the topic of nest plant material in relation to FDB, yet it seems an area worthy of consideration.

Feather molt.—Molt may have evolved to replace worn and damaged feathers (Williams and Swaddle 2003) and, thus, FDB may have selected for the evolution of molt (Burtt and Ichida 1999, Clayton 1999). Molt may also reduce plumage loads of FDB. Preliminary evidence suggests that birds harbor fewer *B. licheniformis* during the spring and fall molts (Burtt and Ichida 1999), though this has not been addressed systematically. Studies that measure the intensity of FDB on individuals before, during, and after molt could indicate whether or not molt reduces FDB load.

Feather-degrading Bacteria and Feather Color Expression

Feather color can communicate information about the nutrition (Hill and Montgomerie 1994), immunocompetance (Saino et al. 1999), endoparasite load (Hamilton and Zuk 1982), age (Siefferman et al. 2005), and dominance (McGraw et al. 2003) of the signaler. However, these mechanisms typically influence color during feather growth. Feather-degrading bacteria may alter feather coloration after the feather is fully formed, and the effects could be positive or negative. Feather degradation could certainly reduce feather color expression. However, many birds acquire breeding plumage coloration after molt by wearing of the ends of feathers (Veiga 1996, Willoughby et al. 2002). Feather-degrading bacteria may aid this process by weakening the ends of feathers.

The effect of FDB on feather color expression could also be more subtle. Structurally colored blue rump feathers of Eastern Bluebirds degraded by FDB *in vitro* are significantly brighter and have greater spectral saturation than feathers not degraded by FDB (Shawkey et al. 2007). Furthermore, bacterial feather damage correlates negatively with ultraviolet (UV) chroma (the percentage of total light reflected in the UV portion of the spectrum; Shawkey et al. 2007). Eastern Bluebird rump feathers may be sexually selected (Siefferman and Hill 2003). Thus, by brightening feathers, the action of FDB might positively influence a sexually selected trait.

Shawkey et al. (2007) found that the abundance of culturable FDB on individual bluebirds did not correlate with feather brightness in the wild. They argued that certain FDB may be more effective at feather degradation than others and, thus, that bacterial damage may not correlate with bacterial abundance. Indeed, there is variation among FDB in their rates of keratinase production, keratinase activity, and rates of feather degradation (Kim et al. 2001, Lucas et al. 2003). Alternative explanations include an important environmental covariate that was not considered and differential susceptibility of individual birds to bacterial feather degradation. Importantly, within-individual feather color change in relation to FDB load has not been addressed. Structural feather coloration can be influenced by factors such as age (Siefferman et al. 2005) and premolt energetic expenditure (Siefferman and Hill 2005). This variation could mask the detection of variation in feather coloration resulting from FDB if feather color is measured at one point in time. Measuring the magnitude of within-individual color change in relation to FDB load would reduce this noise and provide increased power to detect an effect of FDB on feather coloration.

Shawkey et al. (2007) found that feather brightness of wild Eastern Bluebirds positively correlates with total bacterial abundance, inclusive of all bacteria, not just FDB. They suggested that this correlation may result from reduced self-maintenance (i.e., preening) in the more dominant bright males, or that bright males may be able to promote the growth of beneficial bacteria, perhaps by way of preen oil (Shawkey et al. 2007). Dominant males may spend more time defending territories, and they perhaps have to provision more offspring. For instance, European Starlings with experimentally increased broods harbor more bacterial cells (Lucas et al. 2005). However, if birds can promote the growth of certain bacteria, that does not necessitate an increase in total bacterial abundance. More beneficial bacteria would likely come at the expense of other species, particularly if the beneficial bacteria inhibit the growth of detrimental bacteria. This could be seen as a shift in the relative abundance of species present, not as an increase in total bacterial abundance.

Variation in structural feather coloration can be condition-dependent (Keyser and Hill 1999, Doucet 2002, Johnsen et al. 2003) and can influence mate preferences (Bennett et al. 1997, Andersson et al. 1998). Structural color is also important in carotenoid color expression (Shawkey and Hill 2005). If FDB positively influence sexually selected color signals on birds by increasing feather brightness, and these characteristics correlate with condition, it is possible that good condition is partially indicated by the ability to cultivate beneficial exogenous microorganisms (Shawkey et al. 2007). Interestingly, Blue Tits' structural feather color increases in brightness but has reduced UV chroma after molt and throughout the breeding season (Örnborg et al. 2002), a pattern of structural color change remarkably similar to that inflicted by FDB *in vitro*.

Do Feather-degrading Bacteria Affect Feathers of Live Birds?

Do FDB degrade the feathers of live birds? As obvious as this question may seem, it is rarely addressed in the literature. Only one study has attempted to experimentally detect bacterial degradation of feathers on live birds. In two separate experiments, Cristol et al. (2005) inoculated flight feathers of captive birds with *B. licheniformis* and treated control feathers with an antibiotic. One experiment was conducted on Northern Cardinals (*Cardinalis cardinalis*) during winter, the second on European Starlings during summer in experimentally increased humidity. Feather damage did not

differ between the two treatments in either experiment. However, aspects of the experiments may have compromised their ability to detect bacterial feather degradation (Cristol et al. 2005). The cold and dry winter conditions of the first experiment were likely too harsh for the mildly thermophilic *B. licheniformis* to be active (Cristol et al. 2005). The use of European Starlings, whose black feathers are melanized and likely resistant to *B. licheniformis*, may have negated a positive influence of increased temperature and humidity in the second experiment. Perhaps most importantly, only one species of FDB was used in both experiments. Given the complexity of the plumage bacterial communities (Shawkey et al. 2005), inoculation with one species of FDB may not create realistic conditions conducive to FDB activity (see below; Shawkey et al. 2007).

The Next Step Should Be the First Step

Published studies investigating FDB on birds, including the present review, are replete with speculations as to the potential influence of FDB on avian evolution. However, there is a lack of empirical evidence to support these claims, and no demonstration of a direct link between FDB and changes in feather condition. Research on FDB and birds cannot move past speculation until bacterial feather degradation has been demonstrated on a live bird, particularly in the wild (Clayton 1999).

Microbial community ecology will be important in determining whether FDB affect feathers, given that microbially mediated biological processes are often a function of bacterial group composition (e.g., Balser et al. 2002). Most studies have focused on the genus *Bacillus*, and more specifically on *B. licheniformis*. Several other species of FDB can occur within plumage (Table 1), and significant feather degradation may result only from the concerted action of the group. Non-FDB could also inhibit or promote the growth of FDB (Burtt and Ichida 1999, Clayton 1999, Shawkey et al. 2007). Investigation of FDB may benefit from multilevel selection analyses where group and individual bacterial selection is considered in concert with host bird selection. Several techniques are available for assessing microbial community structure and composition (reviewed in Head et al. 1998, Kirk et al. 2004, Dorigo et al. 2005, Sessitsch et al. 2006; for an example of these methods applied to plumage bacteria, see Bisson et al. 2007) and should be employed in *in-situ* studies of FDB.

Studies that look for correlations between FDB load or microbial community composition (or both) and feather damage would be useful. However, because feathers can incur damage in multiple ways, a more direct demonstration of bacterial degradation may ultimately be needed. For instance, scanning electron microscopy could be used to determine whether bacteria aggregate at areas of feather damage. Fluorescent *in-situ* hybridization could be used to locate FDB on feathers, either targeting messenger RNA (mRNA) for keratinase or rRNA specific to FDB. Keratinases can also be probed with fluorescently tagged antibodies (Noronha et al. 2002). Several other techniques, such as environmental functional gene arrays, are available to determine whether a process is bacterially mediated (reviewed in Torsvik and Øvreås 2002, Tringe and Rubin 2005) and could have application in detecting bacterial feather degradation on live birds.

Feather Fungi: A Further Consideration

Along with bacteria, complex communities of fungi exist within plumage and in nests (Apinis and Pugh 1967; Pugh and Evans 1970a, b; Pugh 1972; Hubálek et al. 1973; Hubálek 1976, 1978; reviewed in Hubálek 2000). Many fungi produce antibacterial compounds and, thus, could directly influence the plumage bacterial community. Some plumage and nest fungi can also degrade feathers (referred to as *keratinophilic* fungi). A culture-based survey of a wild bird population isolated keratinophilic fungi from 67% of individuals (Deshmukh 2004). Fourteen species of feather-degrading fungi were isolated from the feathers of 100 live chickens (Kaul and Sumbali 1999). *Chrysosporium georgiae*, a fungus also isolated from chicken feathers, degrades feathers but not the α-keratin of human and bovine hair (El-Naghy et al. 1998). This suggests that *C. georgiae* specializes in degrading the β-keratin in feathers.

No experimental work has addressed the effects of plumage fungi on either plumage bacterial communities or feathers of live birds. However, biochemical (reviewed in Kunert 2000, Gupta and Ramnani 2006) and ecological (see references above) studies of keratinophilic fungi have laid the foundation for such work. Experiments that test for effects of FDB on birds could easily be adapted to test for effects of keratinophilic fungi on birds. The interactions between feather fungi, feather bacteria, and birds are unknown. This is an area of research wide open and ready to be explored.

Conclusion

Demonstrating unequivocally that bacteria (or fungi) are responsible for observed feather wear on live birds will be difficult, because ascribing function to microbes is problematic (Balser et al. 2002, Torsvik and Øvreås 2002). However, tackling this question opens the door for creative interdisciplinary research, with the potential to integrate methods of microbiology with field behavioral ecology. Rigorous experimental studies of FDB and birds are needed to shed light on this system of host–symbiont interaction.

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Literature Cited

Amann, R. I., W. Ludwig, and K. H. Schleifer. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiological Reviews 59:143–169.

Andersson, M. 1994. Sexual Selection. Princeton University Press, Princeton, New Jersey.

Andersson, S., J. Örnborg, and M. Andersson. 1998. Ultraviolet sexual dimorphism and assortative mating in Blue Tits. Proceedings of the Royal Society of London, Series B 265:445–450.

Apinis, A. E., and G. J. F. Pugh. 1967. Thermophilous fungi of birds' nests. Mycopathologia 33:1–9.

Balser, T. C., A. P. Kinzig, and M. K. Firestone. 2002. Linking soil microbial communities and ecosystem functioning. Pages 265–293 *in* The Functional Consequences of Biodiversity: Empirical Progress and Theoretical Extensions (A. P. Kinzig, S. W. Pacala, and D. Tilman, Eds.). Princeton University Press, Princeton, New Jersey.

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 USA 94:8618–8621.

Bisson, I.-A., P. P. Marra, E. H. Burtt, Jr., M. Sikaroodi, and P. M. Gillevet. 2007. A molecular comparison of plumage and soil bacteria across biogeographic, ecological, and taxonomic scales. Microbial Ecology 54:65–81.

Bonser, R. H. C. 1995. Melanin and the abrasion resistance of feathers. Condor 97:590–591.

Booth, D. T., D. H. Clayton, and B. A. Block. 1993. Experimental demonstration of the energetic cost of parasitism in free-ranging hosts. Proceedings of the Royal Society of London, Series B 253:125–129.

Brandelli, A. 2005. Hydrolysis of native proteins by a keratinolytic protease of *Chryseobacterium* sp. Annals of Microbiology 55: 47–50.

Brandelli, A., and A. Riffel. 2005. Production of an extracellular keratinase from *Chryseobacterium* sp. growing on raw feathers. Electronic Journal of Biotechnology 8:35–42.

Burger, B. V., B. Reiter, O. Borzyk, and M. A. du Plessis. 2004. Avian exocrine secretions. I. Chemical characterization of the volatile fraction of the uropygial secretion of the Green Woodhoopoe, *Phoeniculus purpureus*. Journal of Chemical Ecology 30:1603–1611.

Burtt, E. H., Jr. 1986. An analysis of physical, physiological, and optical aspects of avian coloration with emphasis on wood-warblers. Ornithological Monographs, no. 38.

Burtt, E. H., Jr., and J. M. Ichida. 1999. Occurrence of feather-degrading bacilli in the plumage of birds. Auk 116:364–372.

Burtt, E. H., Jr., and J. M. Ichida. 2004. Gloger's rule, feather-degrading bacteria, and color variation among Song Sparrows. Condor 106:681–686.

Clark, L. 1991. The nest protection hypothesis: The adaptive use of plant secondary compounds by European Starlings. Pages 205–221 *in* Bird–Parasite Interactions: Ecology, Evolution, and Behaviour (J. E. Loye and M. Zuk, Eds.). Oxford University Press, New York.

Clark, L., and J. R. Mason. 1985. Use of nest material as insecticidal and anti-pathogenic agents by European Starlings. Oecologia 67:169–176.

Clayton, D. H. 1999. Feather-busting bacteria. Auk 116:302–304.

Clayton, D. H., S. E. Bush, B. M. Goates, and K. P. Johnson. 2003. Host defense reinforces host–parasite cospeciation. Proceedings of the National Academy of Sciences USA 100:15694–15699.

Clayton, D. H., and J. Moore. 1997. Host–Parasite Evolution: General Principles and Avian Models. Oxford University Press, Oxford, United Kingdom.

Clayton, D. H., and J. G. Vernon. 1993. Common Grackle anting with lime fruit and its effect on ectoparasites. Auk 110:951–952.

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 122:222–230.

De Azeredo, L. A. I., M. B. de Lima, R. R. R. Coelho, and D. M. G. Freire. 2006. Thermophilic protease production by *Streptomyces* sp. 594 in submerged and solid-state fermentations using feather meal. Journal of Applied Microbiology 100:641–647.

Deshmukh, S. K. 2004. Keratinophilic fungi on feathers of pigeon in Maharashtra, India. Mycoses 47:213–215.

Dorigo, U., L. Volatier, and J.-F. Humbert. 2005. Molecular approaches to the assessment of biodiversity in aquatic microbial communities. Water Research 39:2207–2218.

Doucet, S. M. 2002. Structural plumage coloration, male body size, and condition in the Blue-Black Grassquit. Condor 104:30–38.

Ehrlich, P. R., D. S. Dobkin, and D. Wheye. 1986. The adaptive significance of anting. Auk 103:835.

El-Naghy, M. A., M. S. El-Ktatny, E. M. Fadl-Allah, and W. W. Nazeer. 1998. Degradation of chicken feathers by *Chrysosporium georgiae*. Mycopathologia 143:77–84.

Ferns, P. N., and A. Lang. 2003. The value of immaculate mates: Relationships between plumage quality and breeding success in shelducks. Ethology 109:521–532.

Gessesse, A., R. Hatti-Kaul, B. A. Gashe, and B. Mattiasson. 2003. Novel alkaline proteases from alkaliphilic bacteria grown on chicken feather. Enzyme and Microbial Technology 32:519–524.

Goater, C. P., and J. C. Holmes. 1997. Parasite-mediated natural selection. Pages 9–29 *in* Host–Parasite Evolution: General Principles and Avian Models (D. H. Clayton and J. Moore, Eds.). Oxford University Press, Oxford, United Kingdom.

Goldstein, G., K. R. Flory, B. A. Browne, S. Majid, J. M. Ichida, and E. H. Burtt, Jr. 2004. Bacterial degradation of black and white feathers. Auk 121:656–659.

Grande, J. M., J. J. Negro, and M. J. Torres. 2004. The evolution of bird plumage colouration: A role for feather-degrading bacteria? Ardeola 51:375–383.

Griffith, S. C., T. H. Parker, and V. A. Olson. 2006. Melanin-versus carotenoid-based sexual signals: Is the difference really so black and red? Animal Behaviour 71:749–763.

Gunderson, A. R., A. M. Frame, J. P. Swaddle, and M. H. Forsyth. 2008. Resistance of melanized feathers to bacterial degradation: Is it really so black and white? Journal of Avian Biology 39:539–545.

Gupta, R., and P. Ramnani. 2006. Microbial keratinases and their prospective applications: An overview. Applied Microbiology and Biotechnology 70:21–33.

Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: A role for parasites? Science 218:384–387.

Head, I. M., J. R. Saunders, and R. W. Pickup. 1998. Microbial evolution, diversity, and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms. Microbial Ecology 35:1–21.

Hill, G. E., and R. Montgomerie. 1994. Plumage colour signals nutritional condition in the House Finch. Proceedings of the Royal Society of London, Series B 258:47–52.

Hubálek, Z. 1976. Interspecific affinity among keratinolytic fungi associated with birds. Folia Parasitologica 23:267–272.

Hubálek, Z. 1978. Coincidence of fungal species associated with birds. Ecology 59:438–442.

Hubálek, Z. 2000. Keratinophilic fungi associated with free-living mammals and birds. Pages 93–103 *in* Biology of Dermatophytes and Other Keratinophilic Fungi (R. K. S. Kushwaha and J. Guarro, Eds.). Revista Iberoamericana de Micología, Bilbao, Spain.

Hubálek, Z., F. Balát, I. Touškova, and J. Vlk. 1973. Mycoflora of birds' nests in nest-boxes. Mycopathologia 49:1–12.

Jacob, J., U. Eigener, and U. Hoppe. 1997. The structure of preen gland waxes from pelecaniform birds containing 3,7-dimethyloctan-1-ol: An active ingredient against dermatophytes. Zeitschrift für Naturforschung C 52:114–123.

Jacob, J., and V. Ziswiler. 1982. The uropygial gland. Pages 199–324 *in* Avian Biology, vol. 6 (D. S. Farner, J. R. King, and K. C. Parkes, Eds.). Academic Press, New York.

Johnsen, A., K. Delhey, S. Andersson, and B. Kempenaers. 2003. Plumage colour in nestling Blue Tits: Sexual dichromatism, condition dependence and genetic effects. Proceedings of the Royal Society of London, Series B 270:1263–1270.

Kaul, S., and G. Sumbali. 1999. Production of extracellular keratinases by keratinophilic fungal species inhabiting feathers of living poultry birds (*Gallus domesticus*): A comparison. Mycopathologia 146:19–24.

Keyser, A. J., and G. E. Hill. 1999. Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament. Proceedings of the Royal Society of London, Series B 266:771–777.

Kim, J. M., W. J. Lim, and H. J. Suh. 2001. Feather-degrading *Bacillus* species from poultry waste. Process Biochemistry 37:287–291.

Kim, J.-S., L. D. Kluskens, W. M. de Vos, R. Huber, and J. van der Oost. 2004. Crystal structure of fervidolysin from *Fervidobacterium pennivorans*, a keratinolytic enzyme related to subtilisin. Journal of Molecular Biology 335:787–797.

Kirk, J. L., L. A. Beaudette, M. Hart, P. Moutoglis, J. N. Klironomos, H. Lee, and J. T. Trevors. 2004. Methods of studying soil microbial diversity. Journal of Microbiological Methods 58:169–188.

Kojima, M., M. Kanai, M. Tominaga, S. Kitazume, A. Inoue, and K. Horikoshi. 2006. Isolation and characterization of a feather-degrading enzyme from *Bacillus pseudofirmus* FA30-01. Extremophiles 10:229–235.

Kunert, J. 2000. Physiology of keratinophilic fungi. Pages 77–85 *in* Biology of Dermatophytes and Other Keratinophilic Fungi (R. K. S. Kushwaha and J. Guarro, Eds.). Revista Iberoamericana de Micología, Bilbao, Spain.

Kuo, M.-J., and M. Alexander. 1967. Inhibition of the lysis of fungi by melanins. Journal of Bacteriology 94:624–629.

Loye, J. E., and M. Zuk. 1991. Bird–Parasite Interactions: Ecology, Evolution, and Behavior. Oxford University Press, New York.

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.

Lunt, N., P. E. Hulley, and A. J. F. K. Craig. 2004. Active anting in captive Cape White-eyes *Zosterops pallidus*. Ibis 146:360–362.

Martín-Platero, A. M., E. Valdivia, M. Ruíz-Rodríguez, J. J. Soler, M. Martín-Vivaldi, M. Maqueda, and M. Martínez-Bueno. 2006. Characterization of antimicrobial substances produced by *Enterococcus faecalis* MRR 10-3, isolated from the uropygial gland of the hoopoe (*Upupa epops*). Applied and Environmental Microbiology 72:4245–4249.

McGraw, K. J. 2006. Mechanics of melanin-based coloration. Pages 243–294 *in* Bird Coloration, vol. 1: Mechanisms and Measurements (G. E. Hill and K. J. McGraw, Eds.). Harvard University Press, Cambridge, Massachusetts.

McGraw, K. J., J. Dale, and E. A. Mackillop. 2003. Social environment during molt and the expression of melanin-based plumage pigmentation in male House Sparrows (*Passer domesticus*). Behavioral Ecology and Sociobiology 53:116–122.

Moyer, B. R., A. N. Rock, and D. H. Clayton. 2003. Experimental test of the importance of preen oil in Rock Doves (*Columba livia*). Auk 120:490–496.

Noronha, E. F., B. D. de Lima, C. M. de Sá, and C. R. Felix. 2002. Heterologous production of *Aspergillus fumigatus* keratinase in *Pichia pastoris*. World Journal of Microbiology and Biotechnology 18:563–568.

Onifade, A. A., N. A. Al-Sane, A. A. Al-Musallam, and S. Al-Zarban. 1998. A review: Potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. Bioresource Technology 66:1–11.

Örnborg, J., S. Andersson, S. C. Griffith, and B. C. Sheldon. 2002. Seasonal changes in a ultraviolet structural colour signal in Blue Tits, *Parus caeruleus*. Biological Journal of the Linnean Society 76:237–245.

Petit, C., M. Hossaert-McKey, P. Perret, J. Blondel, and M. M. Lambrechts. 2002. Blue Tits use selected plants and olfaction to maintain an aromatic environment for nestlings. Ecology Letters 5:585–589.

Proctor, H. C. 2003. Feather mites (Acari: Astigmata): Ecology, behavior, and evolution. Annual Review of Entomology 48:185–209.

Proctor, H. [C.], and I. Owens. 2000. Mites and birds: Diversity, parasitism and coevolution. Trends in Ecology and Evolution 15: 358–364.

Pugh, G. J. F. 1972. The contamination of birds' feathers by fungi. Ibis 114:172–177.

Pugh, G. J. F., and M. D. Evans. 1970a. Keratinophilic fungi associated with birds. I. Fungi isolated from feathers, nests and soils. Transactions of the British Mycological Society 54:233–240.

Pugh, G. J. F., and M. D. Evans. 1970b. Keratinophilic fungi associated with birds. II. Physiological studies. Transactions of the British Mycological Society 54:241–250.

Ramnani, P., R. Singh, and R. Gupta. 2005. Keratinolytic potential of *Bacillus licheniformis* RG1: Structural and biochemical mechanism of feather degradation. Canadian Journal of Microbiology 51:191–196.

Rayner, J. M. V. 1988. Form and function in avian flight. Pages 1–66 *in* Current Ornithology, vol. 5 (R. F. Johnston, Ed.). Plenum Press, New York.

Reneerkens, J., M. A. Versteegh, A. M. Schneider, T. Piersma, and E. H. Burtt, Jr. 2008. Seasonally changing preen-wax composition: Red Knots' (*Calidris canutus*) flexible defense against feather-degrading bacteria? Auk 125:285–290.

Revis, H. C., and D. A. Waller. 2004. Bactericidal and fungicidal activity of ant chemicals on feather parasites: An evaluation of anting behavior as a method of self-medication in songbirds. Auk 121:1262–1268.

Riffel, A., and A. Brandelli. 2002. Isolation and characterization of a feather-degrading bacterium from the poultry processing industry. Journal of Industrial Microbiology and Biotechnology 29:255–258.

Riffel, A., F. Lucas, P. Heeb, and A. Brandelli. 2003. Characterization of a new keratinolytic bacterium that completely degrades native feather keratin. Archives of Microbiology 179:258–265.

Saino, N., R. Stradi, P. Ninni, E. Pini, and A. P. Møller. 1999. Carotenoid plasma concentration, immune profile, and plumage ornamentation of male Barn Swallows (*Hirundo rustica*). American Naturalist 154:441–448.

Sangali, S., and A. Brandelli. 2000. Feather keratin hydrolysis by a *Vibrio* sp. strain kr2. Journal of Applied Microbiology 89:735–743.

Saranathan, V., and E. H. Burtt, Jr. 2007. Sunlight on feathers inhibits feather-degrading bacteria. Wilson Journal of Ornithology 119:239–245.

Sessitsch, A., E. Hackl, P. Wenzl, A. Kilian, T. Kostic, N. Stralis-Pavese, B. Tankouo Sandjong, and L. Bodrossy. 2006. Diagnostic microbial microarrays in soil ecology. New Phytologist 171:719–736.

Shawkey, M. D., and G. E. Hill. 2005. Carotenoids need structural colours to shine. Biology Letters 1:121–124.

Shawkey, M. D., K. L. Mills, C. Dale, and G. E. Hill. 2005. Microbial diversity of wild bird feathers revealed through culture-based and culture-independent techniques. Microbial Ecology 50:40–47.

Shawkey, M. D., S. R. Pillai, and G. E. Hill. 2003. Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. Journal of Avian Biology 34:345–349.

Shawkey, M. D., S. R. Pillai, G. E. Hill, L. M. Siefferman, and S. R. Roberts. 2007. Bacteria as an agent for change in structural plumage color: Correlational and experimental evidence. American Naturalist 169 (Supplement):S112–S121.

Shuster, S. M., and M. J. Wade. 2003. Mating Systems and Strategies. Princeton University Press, Princeton, New Jersey.

Siefferman, L., and G. E. Hill. 2003. Structural and melanin coloration indicate parental effort and reproductive success in male Eastern Bluebirds. Behavioral Ecology 14:855–861.

Siefferman, L., and G. E. Hill. 2005. Male Eastern Bluebirds trade future ornamentation for current reproductive investment. Biology Letters 1:208–211.

Siefferman, L., G. E. Hill, and F. S. Dobson. 2005. Ornamental plumage coloration and condition are dependent on age in Eastern Bluebirds *Sialia sialis*. Journal of Avian Biology 36:428–435.

Stettenheim, P. R. 2000. The integumentary morphology of modern birds—An overview. American Zoologist 40:461–477.

Swaddle, J. P., and M. S. Witter. 1997. The effects of molt on the flight performance, body mass, and behavior of European Starlings (*Sturnus vulgaris*): An experimental approach. Canadian Journal of Zoology 75:1135–1146.

Tiquia, S. M., J. M. Ichida, H. M. Keener, D. L. Elwell, E. H. Burtt, Jr., and F. C. Michel, Jr. 2005. Bacterial community profiles on feathers during composting as determined by terminal restriction fragment length polymorphism analysis of 16S rDNA genes. Applied Microbiology and Biotechnology 67: 412–419.

Torsvik, V., and L. Øvreås. 2002. Microbial diversity and function in soil: From genes to ecosystems. Current Opinion in Microbiology 5:240–245.

Tringe, S. G., and E. M. Rubin. 2005. Metagenomics: DNA sequencing of environmental samples. Nature Reviews Genetics 6: 805–814.

VanderWerf, E. A. 2005. `Elepaio "anting" with a garlic snail and a *Schinus* fruit. Journal of Field Ornithology 76:134–137.

Veiga, J. P. 1996. Permanent exposure versus facultative concealment of sexual traits: An experimental study in the House Sparrow. Behavioral Ecology and Sociobiology 39:345–352.

Whitaker, J. M., D. A. Cristol, and M. H. Forsyth. 2005. Prevalence and genetic diversity of *Bacillus licheniformis* in avian plumage. Journal of Field Ornithology 76:264–270.

Williams, E. V., and J. P. Swaddle. 2003. Moult, flight performance and wingbeat kinematics during take-off in European Starlings *Sturnus vulgaris*. Journal of Avian Biology 34:371–378.

Willoughby, E. J., M. Murphy, and H. L. Gorton. 2002. Molt, plumage abrasion, and color change in Lawrence's Goldfinch. Wilson Bulletin 114:380–392.

Yamamura, S., Y. Morita, Q. Hasan, S. R. Rao, Y. Murakami, K. Yokoyama, and E. Tamiya. 2002a. Characterization of a new keratin-degrading bacterium isolated from deer fur. Journal of Bioscience and Bioengineering 93:595–600.

Yamamura, S., Y. Morita, Q. Hasan, K. Yokoyama, and E. Tamiya. 2002b. Keratin degradation: A cooperative action of two enzymes from *Stenotrophomonas* sp. Biochemical and Biophysical Research Communications 294:1138–1143.

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