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HOST MICROBE INTERACTIONS

Variation in Plumage Microbiota Depends on Season and Migration

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Abstract Migratory birds can be efficient dispersers of pathogens, yet we know little about the effect of migration and season on the microbial community in avian plumage. This is the first study to describe and compare the microbial plumage community of adult and juvenile migratory birds during the annual cycle and compare the plumage community of migrants to that of resident birds at both neotropical and nearctic locations. We used length heterogeneity PCR (16S rRNA) to describe the microbial assemblage sampled from the plumage of 66 birds in two age classes and from 16 soil samples. Resident birds differed significantly in plumage microbial community composition from migrants ($R \geq 0.238$, $P < 0.01$). Nearctic resident birds had higher plumage microbial diversity than nearctic migrants ($R = 0.402$, $P < 0.01$). Plumage microbial composition differed significantly between fall premigratory and either breeding ($R \geq 0.161$, $P < 0.05$) or nonbreeding stages ($R = 0.267$, $P < 0.01$). Six bacterial operational taxonomic units contributed most to the dissimilarities found in this assay. Soil microbial community composition was significantly different from all samples of plumage microbial communities ($R \geq 0.700$, $P < 0.01$). The plumage microbial community varies in relation to migration strategy and stage of the annual cycle. We suggest that plumage microbial acquisition begins in the first year at natal breeding locations and reaches equilibrium at the neotropical wintering sites. These data lead us to conclude that migration and season play an important role in the dynamics of the microbial community in avian plumage and may reflect patterns of pathogen dispersal by birds.

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Introduction

Every year, 12–20 billion birds migrate between the neotropics and the nearctic. We now recognize that avian migration can be an efficient mode of transport for avian microorganisms [16, 33] and a catalyst for infection by endoparasites and pathogens [12]. Furthermore, with the emergence of highly pathogenic diseases such as avian influenza [16] and West Nile virus [20], avian migration has become a topic of multidisciplinary concern (e.g., [13, 26]).

Avian plumage is an interesting, understudied microbial ecosystem. Feathers harbor a diverse microbiota [3, 6, 17, 18, 31], which includes bacteria of the *Pseudomonas* group, feather-degrading bacilli (*Bacillus licheniformis*), and fungi (e.g., *Arthroderma* spp.) Microorganisms in the plumage of migratory birds are exposed to many different habitats

within a single year because the bird migrates between nearctic breeding and neotropical wintering sites and stops at numerous sites enroute. Currently, we do not know if or how long-distance migration of the plumage ecosystem facilitates microbial dispersal or affects the microbial community in the plumage.

In an assay of feather-degrading bacilli in the plumage of over 1,600 birds, Burtt and Ichida [6] found that the occurrence of the bacilli varied with season and the behavioral ecology of the birds. For example, ground foraging birds had a higher occurrence of *Bacillus* spp. than aerial insectivores. Burtt and Ichida [5], Lucas et al. [18], and Peele et al. [22] showed that feather-degrading bacilli and plumage microbial communities varied geographically and locally among habitats. Although focusing on fecal microbes, Waldenström et al. [33] found that *Campylobacter* spp. were more common in short-distance than long-distance migratory birds. Recently, Bisson et al. [3] showed that the abundance of similar types of bacteria (e.g., *Pseudomonas* spp.) in the plumage community differed between neotropical and nearctic sites in five migratory avian species. The plumage ecosystem is a nutrient-poor, dry environment, which raises questions about colonization by new microbes as the bird migrates. However, Gunderson et al. [10] have shown that feather-degrading bacilli actively degrade feather keratin and change the spectral reflectance of the feathers in Eastern Bluebirds (*Sialia sialis*) during the breeding season. Taken together, these studies strongly suggest that the microbial community is a dynamic, functioning ecosystem open to the influence of local habitats. Therefore, we expect that migration will affect the plumage microbial community as the plumage ecosystem travels between temperate and tropical locations. Our study aims to describe and compare the microbial plumage community of adult and juvenile migratory birds during the annual cycle and compare the plumage community of migrants to that of resident birds at both neotropical and nearctic locations. We also make comparisons of the plumage (migrant and resident) microbiota to that of the local soil to test for potential associations between soil and plumage microbial communities.

Methods

Study System

We sampled two migratory species, the American Redstart (*Setophaga ruticilla*) and Common Yellowthroat (*Geothlypis trichas*), one resident species at our neotropical site, the Jamaican Vireo (*Vireo modestus*), and one resident species at our nearctic site, the Northern Cardinal (*Cardinalis cardinalis*). Birds were captured using mist-nets, song recordings, and decoys at one neotropical site (March–April 2004) located at the Font Hill Nature Preserve (18°02'N, 77°57'W) in St. Elizabeth Parish (Black River, Jamaica) and one nearctic site (May–June 2004) located in the Patuxent River Park (38°45′N, 76°42′W) in Maryland, USA.

Field Sampling of Plumage and Soil Microorganisms

Before removing each captured bird from the mist net, we sterilized our hands with quartenary disinfectant or store-bought hand sanitizer [6]. These disinfectants reduce microbial contamination on hands to barely detectable levels (Burtt and Ichida, personal communication). Latex gloves were not used because of the difficulty they pose in removing birds from mist nets. We disinfected and air-dried our hands before we handled every bird. To ensure that we did not sample the same bird more than once, individuals were uniquely marked with either a combination of a US Fish and Wildlife Service band and two plastic color bands or by cutting the tip of the third left tail feather. Within 3–5 min of capture and removal from the net, we plucked three adjacent feathers from the breast and dorsum using sterile forceps. Feathers from each body region were placed in individual sterile envelopes and stored at 4°C until processed in the laboratory. After sampling, we sexed and aged each bird prior to release. We also collected eight soil samples at each site (Jamaica and Maryland) by removing the top 1 cm of soil using a sterile spoon and placing the soil in individual sterile envelopes at 4°C until processed in the laboratory.

To compare the microbial community of migrant and resident birds at both sites, we sampled the microbial community of adult (after second year), second-year (first breeding season after one winter season in the tropics), and hatch-year plumage of two neotropical–nearctic migratory bird species, American Redstart and Common Yellowthroat; one tropical resident species, Jamaican Vireo; and one nearctic resident species, Northern Cardinal. To assay changes in the plumage microbial community throughout a bird's annual cycle, we sampled the plumage of both neotropical migrants during the breeding season in May–June 2004, during the fall premigratory period in late September 2004, and during the late winter (nonbreeding) in March to early April of the same year for American Redstarts (Fig. 1). Jamaican Vireos were sampled at the neotropical site at the same time as wintering Redstarts and Northern Cardinals were sampled at the nearctic site at the same time as breeding Redstarts and Yellowthroats (Fig. 1).

DNA Extraction and 16S rDNA Length Heterogeneity PCR Fingerprinting

We used culture-independent methods [length heterogeneity (LH) polymerase chain reaction (PCR)] to assay the

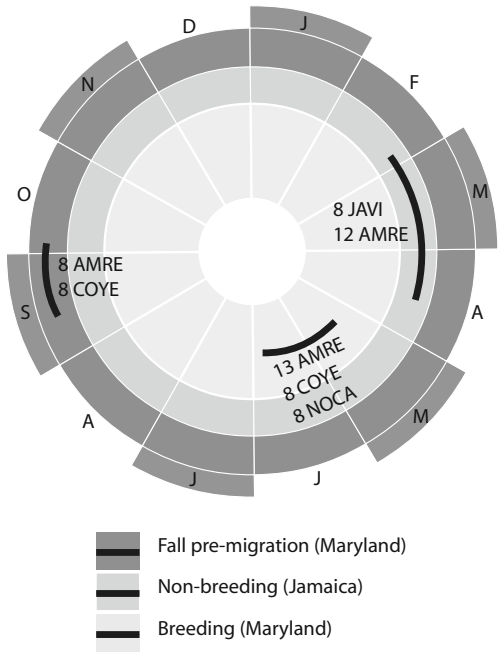

Figure 1 Graphical representation of a bird's annual cycle showing timing of sampling and sample size for birds sampled in Jamaica and Maryland in 2004. Species abbreviations are *AMRE*, American Redstart; *COYE*, Common Yellowthroat; *NOCA*, Northern Cardinal; *JAVI*, Jamaican Vireo

composition of the plumage and soil microbial communities. LH is a PCR analysis that distinguishes different organisms based on natural variations in the length of the 16S ribosomal DNA sequences [operational taxonomic units (OTUs)]. Each amplicon (amplified PCR sequence of the variable region in the 16S rDNA gene) may represent more than one bacterial taxon (genus, species, or strain). LH-PCR is robust and highly replicable for different environmental samples when compared to other methods [15, 21] and, therefore, provides a consistent measure of microbial community composition.

We extracted DNA directly from soil samples (~500 mg) and pooled feather samples (three feathers for each breast and dorsal region) in a sterilized laboratory environment using a FastDNA spin kit for soil (QBiogene) and following the manufacturer's instructions and additional methods detailed in Bisson et al. [3]. The first two variable regions of the 16S rDNA gene were PCR-amplified using about 10-ng DNA in a PCR reaction with fluorescently labeled (6FAM) forward primer (27F 5′-FAM-AGA GTT TGA TCM TCG CTC AG-3′) and unlabeled reverse primer (335R 5′-GCT GCC TCC CGT AGG AGT-3′) following protocols by Bisson et al. [3]. Both primers are universal bacterial primers [14]. We duplicated PCR amplifications to assess reproducibility and replicate PCR products were subsequently processed for LH-PCR profiling. Duplicates were not significantly different at the 99.9% confidence level for all taxa (paired-sampled t test, $|t_{17}|=2.999$, $P=0.008$ after Bonferroni correction). LH-PCR duplicates were therefore highly repeatable, as shown previously [3, 21].

Dilutions of PCR products were made based on the quantization on 1% agarose gel and ethidium bromide, and the diluted product was mixed with ILS-600 (Promega) size standard (1/20 in Hi Di Formamide) and run on a SCE9610 (Spectrumedix LLC) capillary sequencer. The raw data were then analyzed with Genospectrum software (Spectrumedix LLC), which performs color deconvolution and size interpolation of the resulting electropherogram peaks. The peaks of the electropherograms represent different taxa of microbiota and generally indicate different genera or species of bacteria. A custom PERL script was used to calculate relative peak areas (normalized abundance) and interleave the profiles from various samples. We refer to each amplicon as an OTU, and the number associated with the OTU refers to its base pair length.

Statistical Analyses

Analysis of Similarity

We used the Bray–Curtis index [4] to construct similarity matrices for subsequent nonparametric statistical tests in Primer v.5.2.9 [8]. We tabulated the relative abundance (normalized abundance calculated from the relative peak areas using PERL scripts) of each taxon from the LH-PCR analyses into a data matrix and then applied a square-root transformation of the data. We then used the Bray–Curtis similarity index to measure bacterial community similarity. One-way and two-way nested analysis of similarity (ANOSIM) [7] tests were performed subsequently to compare the plumage bacterial composition and diversity among (1) migratory and resident birds, (2) different stages of the annual life cycle for migratory species, (3) soil and plumage samples, and (4) between-age classes for Redstarts and Yellowthroats. ANOSIM uses similarity matrices to conduct an approximation of the standard univariate analysis of variance testing for among-group differences. ANOSIM tests provide the R-test statistic (analogous to the analysis of variance F-test statistic) together with a probability value. R values are equally, if not more, important to consider when evaluating the outcome of ANOSIM analyses. Specifically, the R-test statistic can be defined as

$$R = \frac{(\bar{r}_B - \bar{r}_W)}{\frac{1}{2}M},$$

where $\bar{r}_B$ is the average of all rank similarities among samples within groups and $\bar{r}_W$ is the average of rank similarities from all sample pairs between groups, $M = n(n-1)/2$. The R value represents the absolute value of how similar or

dissimilar the groups are. If the similarities among samples between and within groups are the same, $R=0$ and we accept the null hypothesis. If the similarities among samples are greater within groups than between groups, $R=1$ and we reject the null hypothesis. When significant differences ($P<0.05$) were found at any scale, we used similarity percentages to determine the percentage contribution of each bacterial taxon to the average dissimilarity between groups. All other analyses, including diversity graphs, were conducted using SPSS statistical package v. 12.0.1 (SPSS 2001).

Results

A total of 19 OTUs were amplified from plumage samples. Microorganisms belonging to OTU of base length 336 dominated (79% of total OTUs amplified) the plumage microbial community, as in a previous study [3]. Microbial diversity (Shannon–Wiener index [29]) was highest for Northern Cardinals and lowest (but not significant, $P=0.166$) for Redstarts sampled during the fall premigratory period (Fig. 2). Diversity values differed significantly between Northern Cardinals and the other avian species ($P\leq0.004$, Bonferonni-corrected).

Resident and migratory birds had significantly different microbial community compositions in their plumage. The difference was significant whether all species at both nearctic and neotropical sites were compared ($R=0.238$, $P<0.001$, Table 1) or only the species at each site (nearctic: $R=0.402$, $P<0.001$; neotropical: $R=0.294$, $P<0.001$, Table 1). The higher R value at the nearctic site (Table 1) indicates more pronounced differences in the plumage microbiota of resident and migratory birds at that site than at the neotropical site. Differences at the nearctic site were between Northern Cardinals, a Nearctic resident, and Common Yellowthroats, or American Redstarts, neotropical migrants (Table 1). OTUs of base pair length 336, 310, 344, 357, and 333 (in order of percent contribution) explained >50% of the dissimilarities between Cardinals and Redstarts and Cardinals and Yellowthroats (Fig. 3). Bacterial OTU 336 was more abundant in the plumage of migratory species than in the plumage of Cardinals (Fig. 3). Bacterial OTU 357 was most abundant in the plumage of Yellowthroats; however, all other microbial taxa were more abundant in the plumage of Cardinals (Fig. 3). The highest within-species microbial similarity was among Redstarts sampled at the neotropical site (79.9%), whereas Cardinals showed the lowest within-species similarity (50.4%), indicating higher variability in microbial composition of the plumage among Cardinals (Table 1). The plumage of Redstarts and Jamaican Vireos differed primarily in the abundance of OTUs 344, 357, and 333 (in order of % contribution), explaining >50% of the dissimilarity found (Fig. 3). Redstarts had a higher abundance of OTUs 344 and 357.

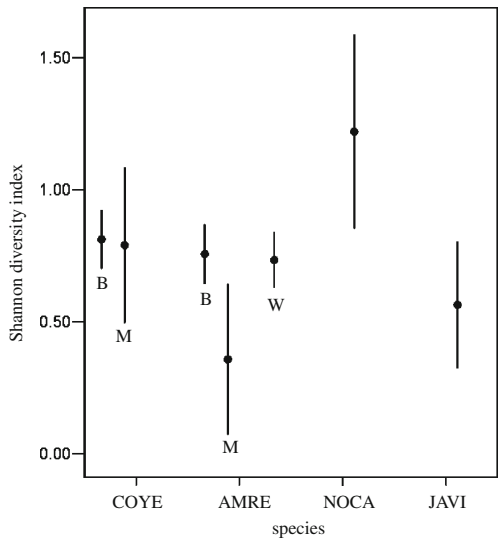

Figure 2 Means and 95% confidence intervals for Shannon–Wiener diversity index calculated for Common Yellowthroats (*COYE*) and American Redstarts (*AMRE*) during the breeding (*B*), fall premigration (*M*), and winter (Redstarts only) stages of the life cycle and for resident species sampled at the nearctic site (Northern Cardinals, *NOCA*) and neotropical site (Jamaican Vireo, *JAVI*) in 2004

The microbial community in the plumage of Redstarts varied significantly among breeding, nonbreeding, and fall premigration periods (Table 2), but the low R value suggests some similarity in plumage bacteria among stages (Table 2). When multiple comparisons were performed, the only difference was between bacterial community composition in the plumage of fall premigrating and nonbreeding Redstarts and, to a lesser extent (lower R value), between fall premigrating and breeding Redstarts (Table 1). Wintering Redstarts harbored a greater abundance of OTUs 343 and 357 and a lower abundance of OTU 336 (Fig. 3) than fall premigrating Redstarts. The microbial community diversity of Redstart plumage was lowest during the fall premigration period, but this difference was not significant (Fig. 2; one-way ANOVA, $F_{(2,30)}=1.908$, $P=0.166$). Although most males sampled during the fall premigration period were hatch-year, we did not find a significant age effect ($R=0.024$, $P=0.230$). Hatch-year males sampled at the neotropical site had a significantly different microbial community composition than that in the plumage of hatch-year males sampled at the Nearctic site ($R=0.388$, $P<0.001$), but had a similar microbial community composition to after-second-year males at the neotropical site ($R=-0.046$, $P=0.614$).

Like Redstarts, the plumage microbial community composition of Yellowthroats differed significantly between breeding and fall premigration stages (Table 2). We did not sample the plumage of Common Yellowthroats at their wintering site. Bacterial OTUs 344 and 333 (in order of percent of contribution) accounted for >50% of the average

Table 1 Comparison of the microbial community in the plumage of neotropical migrants (American Redstart and Common Yellowthroat) and residents at a nearctic breeding site and a neotropical nonbreeding site (Northern Cardinal and Jamaican Vireo, respectively)

Main effect	Global R[a]	Average dissimilarity between groups (%)	Average similarity within groups (%)
Neotropical migrants vs residents			
Across both sites	0.238**	37.2	70.8 (Redstart and Yellowthroat)/57.8 (Cardinal and vireo)
Within breeding site	**0.402****	45.3	68.8 (Redstart and Yellowthroat)/ 50.4 (Cardinal)
Within nonbreeding site	0.294**	27.9	79.9 (Redstart)/71.7 (vireo)
Species in nearctic site			
All species	0.164**	–	
Redstart vs Yellowthroat	0.013 ns	30.9	64.6 vs 75.1
Redstart vs Cardinal	0.344*	47.9	64.6 vs 50.4
Yellowthroat vs Cardinal	**0.355****	41.9	75.1 vs 50.4

The comparison is based on ANOSIM and Similarity Percentages. Bold R values indicate the greatest differentiation among samples for each analysis. A dash (–) indicates that similarity percentages tests were not performed because comparisons involved more than two groups.

ns not significant

*$P<0.05$; **$P<0.01$

[a] R values are significant at $P<0.05$ and $P<0.01$

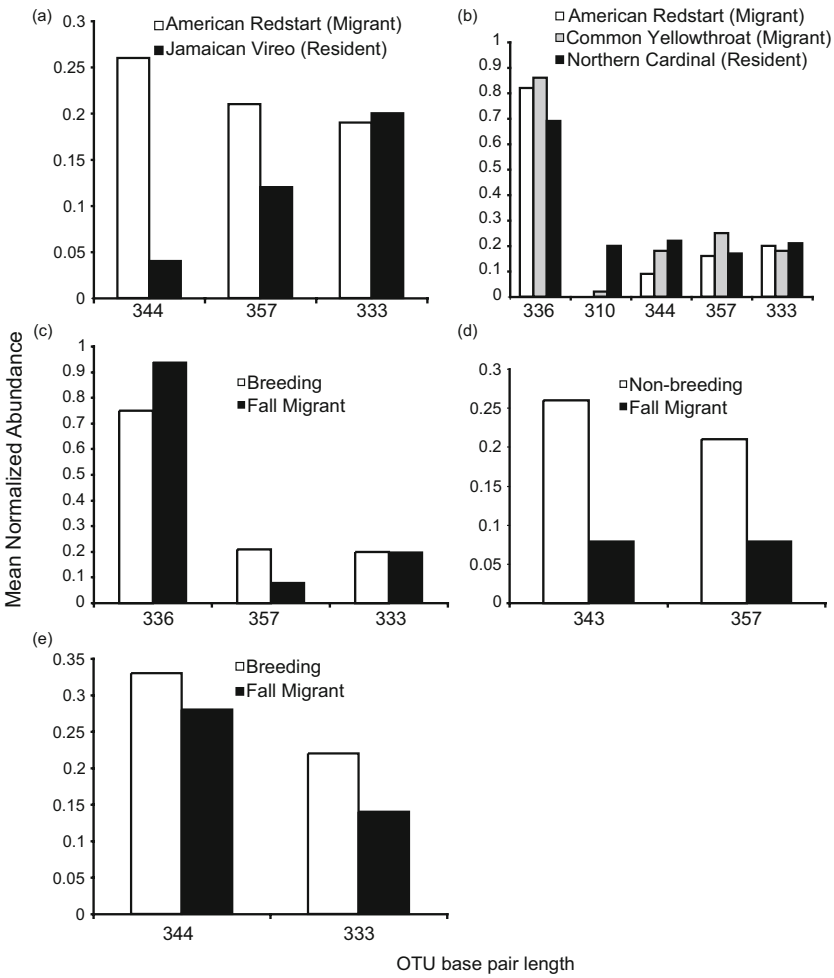

Figure 3 Mean normalized abundance of each OTU that contributed most (>50% cumulative contribution) to dissimilarities detected in the plumage microbial composition of resident vs migrant avian species in **a** the neotropical (Jamaica) and **b** nearctic (Maryland) sites, **c** between fall premigratory and breeding American Redstarts and **d** between fall premigratory and wintering Redstarts, and **e** between fall premigratory and breeding Common Yellowthroats

Table 2 Comparison of the microbial community in the plumage of neotropical migrants (American Redstart and Common Yellowthroat) at the time of breeding, just prior to the fall migration (premigratory), both at the nearctic site and, for Redstarts, at the neotropical nonbreeding site

Stage in annual life cycle	Global R[a]	Average dissimilarity between groups (%)	Average similarity within groups (%)
American Redstarts			
Nonbreeding vs breeding vs premigratory	0.163**	–	
Nonbreeding vs breeding	0.096 ns	32.9	79.9 vs 59.7
Winter vs premigratory	**0.267****	25.1	79.9 vs 80.6
Nonbreeding vs premigratory	0.161*	35.9	59.7 vs 80.6
Common Yellowthroats			
Breeding vs premigratory	0.214*	27.2	86.8 vs 68.6

Common Yellowthroats were sampled only at the nearctic breeding site. The comparison is based on ANOSIM and similarity percentages. Bold R values indicate the greatest differentiation among samples for each analysis. A dash (–) indicates that similarity percentage tests were not performed because comparisons involved more than two groups.

ns not significant

*$P<0.05$; **$P<0.01$

[a] R-values are significant at $P<0.05$ and $P<0.01$

dissimilarity between annual stages. Common Yellowthroat plumage sampled during the breeding stage harbored more of all three groups than plumage sampled during the fall premigration stage (Fig. 3). We sampled both second-year and adult Common Yellowthroats across breeding and fall premigratory stages. Age did not significantly affect the microbial community in the plumage of Common Yellowthroats ($R=-0.114$, $P=0.896$).

The plumage microbial community composition differed significantly from that sampled in the soil at both nearctic and neotropical sites (Table 3). The greatest difference (highest R value, Table 3) was found between the microbial community in the plumage of Yellowthroats and the soil at the nearctic breeding site (Table 1), whereas the greatest similarity (lowest R value, Table 3) was in the microbial community of Cardinal plumage and the soil at the Nearctic site.

Discussion

Avian migration plays an important but little-studied role in the potentially rapid spread of microorganisms from the site of initial acquitision [24]. Understanding how migration affects the composition of the microbial community in the plumage and how this community changes throughout a migrant's annual cycle may provide important insights into microbial dispersal by birds. Our study is the first to compare the diversity and taxonomic composition of the microbial community in the plumage of migratory and resident birds at both neotropical and nearctic locations. Our results indicate that the plumage microbial community of resident birds differs from that of migratory birds (Table 1) and that the plumage microbial community varies across the birds' annual cycle (Table 2). Six of the 19 OTUs

Table 3 Comparison of the microbial community in the soil with that in the plumage of American Redstarts, Common Yellowthroats (both Neotropical migrants), and Northern Cardinals (a resident species) at a nearctic breeding site and a comparison of the microbial community in the soil at a neotropical nonbreeding site with that in the plumage of nonbreeding Redstarts and Jamaican Vireos (a tropical resident) at the same site

Soil vs plumage	Global R[a]	Average dissimilarity between groups (%)	Average similarity within groups (%)
Nearctic breeding site			
Soil vs Redstart plumage	0.750**	79.9	52.3 vs 64.6
Soil vs Yellowthroat plumage	**0.998****	78.8	52.3 vs 75.1
Soil vs Cardinal plumage	0.700**	72.1	52.3 vs 50.4
Neotropical nonbreeding site			
Soil vs Redstart plumage	**0.942****	81.6	38.9 vs 79.9
Soil vs vireo plumage	0.851**	80.3	38.9 vs 71.7

The comparison is based on ANOSIM and similarity percentages. Bold R values indicate the greatest differentiation among samples for each analysis.

*$P<0.05$; **$P<0.01$

[a] R-values are significant at $P<0.05$ and $P<0.01$

amplified contributed most (>50% contribution) to these differences.

Migrant vs Resident Plumage Microbiota

The plumage of migratory birds is exposed to many different habitats, as well as potentially different climatic conditions as the bird moves between neotropical wintering and nearctic breeding sites. Previous studies strongly suggest that habitat type affects bacterial community composition of the plumage [3, 5, 6, 22]. If bacteria can rapidly colonize the plumage of birds, migrant birds, which contact different habitats during their travels and in their summer and winter residences, should harbor a more diverse and abundant microbiota in their plumage than resident species, which occupy the same habitat year-round. Our results show that the plumage microbial community of migrant birds differed significantly from that of local resident birds at both the nearctic and neotropical locations. Bacterial taxa that contributed most to these differences were more prevalent in migratory than in resident birds (Fig. 3). This suggests that migratory behavior contributes to variation of plumage microbiota. Although habitat may strongly influence plumage microbial community composition [6, 18, 22], other factors, such as preening behavior [17], preen (uropygial) oil composition [27, 30], and nutritional conditions of the plumage environment may play important roles in composition of the microbial plumage community of migratory birds. For example, preen oils, which exhibited antimicrobial properties [2, 30] in laboratory experiments, have been shown to change seasonally [27, 32] and among different stages of the breeding season in shore birds (e.g., courtship and incubation [28]). Such changes in chemical or nutritional conditions of the plumage could cause a change in plumage microbial community composition.

Although some bacterial taxa were more abundant in the plumage of migrant birds, the plumage microbial community was not more diverse in migrants; Northern Cardinals (nearctic resident) had twice as many microbial species as all migratory birds sampled (Fig. 2). We offer two possible explanations for this result: (1) microbial acquisition is correlated with foraging behavior, and (2) plumage microbial diversity may be positively correlated with body size. Foraging behavior is thought to influence the abundance of culturable bacteria living in the plumage. However, we do not know how foraging strategies affect plumage bacteria that are not easily cultured [30]. Birds sampled in this study represent at least two different foraging guilds. Cardinals and Common Yellowthroats forage in low shrubs and on the ground ([11]; Bisson, personal observation), where they come in close contact with soil microorganisms, whereas Redstarts and Jamaican Vireos are almost exclusively foliage gleaners [23]. In a culture-dependent assay, Burtt and Ichida [6] showed that ground-foraging birds were more likely than birds foraging in foliage to harbor feather-degrading bacilli (*B. licheniformis*) in their plumage and that bacilli species were more abundant in ground-foragers [6]. Although our results show a pronounced difference between soil and plumage microbiota, they also suggest that the microbiota in Cardinal plumage are more similar to that sampled in local soil than the plumage microbiota of migrant birds are. However, foraging strategy alone cannot explain the high diversity of microbes in Cardinals because diversity values for Common Yellowthroats, also a ground-forager, are comparable to those of foliage-gleaners. Alternatively, the large size of Cardinals may have skewed our results—Cardinals weigh 42–48 g compared to 6–9 g for Redstarts and 9–10 g for Common Yellowthroats and Jamaican Vireos. Large size means more body surface and larger feathers, which means more feather surface that could harbor more bacteria. Size of the bird is an interesting variable when placed in the context of island biogeography [19]. One prediction of island biogeography theory is that species diversity and island size are positively correlated. The application of predictions from island biogeography to microbial ecosystems in avian plumage may be a productive avenue for understanding the interactions of birds and their plumage microorganisms as it has been for aquatic ecosystems (e.g., lake area [25]). Additional sampling of plumage in birds of different sizes and foraging guilds is needed to test both the foraging and body/feather size explanations.

Seasonal Changes in Plumage Microbes or Age-Related Effect?

The composition of the plumage microbial community in the fall premigration period differed significantly from the composition during the Redstart's wintering and breeding stages, which did not differ. Although the differences are generally weak, they were most pronounced between fall premigration and wintering stages of the Redstart's annual cycle. Bacterial taxa that contributed most to these differences (Fig. 3) were less abundant in the plumage of Redstarts that were in their initial migratory movements, and the trend may have been the same in Yellowthroats in which premigratory individuals had significantly fewer OTUs 344 and 333 than breeding individuals (Fig. 3). Wintering Yellowthroats were not sampled. However, most Redstarts sampled during the fall premigratory period were hatch-year males. While age was not a significant variable, newly grown feathers of hatch-year birds may harbor different microbial species. Alternatively, because most nearctic migrants undergo a complete molt after breeding and before migration and because molting is correlated with

reduction in feather-degrading bacilli, molt has been suggested as an adaptation to microbial control in the plumage of birds [6, 9]. Thus, the lower prevalence of some plumage bacteria could be the result of molt rather than changes brought on by initial migratory movements. Sampling migrants during spring when most birds have little or no molt and sampling migrants at stopover sites would enable us to separate the respective contributions of molt and migration to the composition of the microbial community in avian plumage.

Two of our findings may hint at how hatch year birds acquire their microbial community: (1) the bacterial community in the plumage of hatch-year Redstarts in their first winter in Jamaica significantly differed from that of hatch-year Redstarts at the Maryland breeding site, and (2) hatch-year and adult Redstarts in Jamaica shared a more similar microbial community than between hatch-year and adults in Maryland, which differed significantly. These differences and similarities suggest that acquisition of the plumage microbial community begins at the Nearctic natal location, that bacterial species continue to colonize the plumage at stopover sites during migration, and the community reaches adult equilibrium (sensu [19]) within the first winter on the neotropical site. Neotropical habitats may play an important role in microbial colonization of avian plumage. Such an explanation accounts for the closer similarity in plumage microbes between residents and migrants sampled at the neotropical site than at the nearctic site, but it also suggests that freshly molted adult plumage and newly grown juvenile plumage may act as an open habitat for microbial colonization. If that is true, then adult migrants with newly molted plumage and juveniles with fresh, juvenile plumage may be important dispersers of microorganisms.

Conclusions

Avian plumage is an understudied microbial ecosystem. It is thought to be a dry, nutrient-poor environment containing antimicrobial substances but one in which a relatively diverse microbial community thrives. We found variation in the microbial community composition in relation to migratory behavior and season, possibly also in relation to age and body size. Molt may also play an important role in bacterial colonization. Changes in habitat and geographic location associated with migration may be the driving force behind differences in the plumage microbiota of migrant and nonmigrant birds. However, some portions of the microbial community, e.g., attached bacteria, may be little affected by contact with different habitats. Furthermore, the harsh conditions present in the plumage of birds may constrain colonization of some bacteria and differences may occur as a result of intrinsic factors such as preen oil composition or a change in nutritional conditions within the plumage that enable some bacteria, but not others, to thrive [1]. We know little about the nutritional and environmental conditions within the plumage microbial ecosystem and must exercise caution in speculating on observed patterns of variation. Nevertheless, plumage microbial ecology is a research arena with ample room for discovery.

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References

1. Baas Becking LGM (1934) Geobiologie of inleiding tot de milieukunde. WP Van Stockum and Zoon, The Hague
2. Bandyopadhyay A, Bhattacharyya SP (1999) Influence of fowl uropygial gland and its secretory lipid components on the growth of skin surface fungi of fowl. Indian J Exp Biol 37:1218–1222
3. Bisson I-A, Marra PP, Burtt EH, Sikaroodi M, Gillevet PM (2007) A molecular comparison of plumage and soil bacteria across biogeographic, ecological, and taxonomic scales. Microb Ecol 54:65–81
4. Bray JR, Curtis JT (1957) An ordination of the upland forest communities of Southern Wisconsin. Ecol Monogr 27:325–349
5. Burtt EH, Ichida JM (2004) Gloger's rule, feather-degrading bacteria, and color variation among song sparrows. Condor 106:681–686
6. Burtt EH, Ichida JM (1999) Occurrence of feather-degrading bacilli in the plumage of birds. Auk 116:364–372
7. Clarke KR (1993) Non-parametric multivariate analysis of changes in community structure. Aust J Ecol 18:117–143
8. Clarke KR, Gorley RN (2001) Primer v.5: user manual/tutorial. PRIMER-E. Plymouth Marine Laboratory, Plymouth
9. Cristol DA, Armstrong JL, Whitaker JM, Forsyth MH (2005) Feather-degrading bacteria do not affect feathers on captive birds. Auk 122:222–230
10. Gunderson AR, Forsythe MH, Swaddle JP (2009) Evidence that plumage bacteria influence feather coloration and body condition in a passerine. J Avian Biol 40 (in press)
11. Guzy MJ, Ritchison G (1999) Common Yellowthroat (*Geothlypis trichas*). In: Poole, A, Gill, F (eds) The birds of North America, No. 448. The Birds of North America, Philadelphia
12. Gylfe A, Bergström S, Lundström J, Olsen B (2000) Reactivation of *Borrelia* infection in birds. Nature 403:724–725
13. Hubalék Z (2004) An annotated checklist of pathogenic micro-organisms associated with migratory birds. J Wildlife Dis 40:639–659
14. Lane DJ (1991) 16S/23S rRNA sequencing. Nucleic acid techniques in bacterial systematics. E.S. a. M. Goodlfellow. Wiley, West Sussex

15. Litchfield CD, Gillevet PM (2002) Microbial diversity and complexity in hypersaline environments: a preliminary assessment. J Ind Microbiol Biot 28:48–55
16. Liu J, Xiao H, Lei F, Zhu Q, Qin K, Zhang X-W, Zhang X-I, Zhao D, Wang G, Feng Y, Ma J, Liu W, Wang J, Gao GF (2005) Highly pathogenic H5N 1 influenza virus infection in migratory birds. Science 309:1206
17. Lucas FS, Moureau B, Jourdie V, Heeb P (2005) Brood size modifications affect plumage bacterial assemblages of European starlings. Mol Ecol 14:639–646
18. Lucas FS, Broenimann O, Febbraro I, Heeb P (2003) High diversity among feather-degrading bacteria from a dry meadow soil. Microb Ecol 45:282–290
19. MacArthur RH, Wilson EO (1967) The theory of island biogeography. Princeton University Press, Princeton
20. Marra PP, Griffing S, Cafree CL, Kilpatrick AM, Mclean R, Brand C, Kramer L, Novak R (2004) West Nile virus and wildlife. Bioscience 54:393–402
21. Mills DK, Fitzgerald K, Litchfield CD, Gillevet PM (2003) A comparison of DNA profiling techniques for monitoring nutrient impact on microbial community composition during bioremediation of petroleum contaminated soils. J Microbiol Meth 54:57–74
22. Peele AM, Burtt EH Jr, Schroeder MR, Greenberg RS (2009) Dark color of Coastal Plains Swamp Sparrow may be an evolutionary response to occurrence and abundance of salt-tolerant, feather-degrading bacilli in its plumage. Auk 126 (in press)
23. Raffaele H, Wiley J, Garrido OH, Keith A, Raffaele JI (1998) A guide to the birds of the West Indies. Princeton University Press, Princeton
24. Rappole JH, Derrickson SR, Hubalek Z (2000) Migratory birds and spread of West Nile virus in the western hemisphere. Emerg Infect Dis 6:319–328
25. Reche I, Pulido-Villena E, Morales-Baquero R, Casamayor EO (2005) Does ecosystem size determine aquatic bacterial richness? Ecology 86:1715–1722
26. Reed KD, Meece JK, Henkel JS, Shukla KS (2003) Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, Influenza A and enteropathogens. Clin Med Res 1:5–12
27. Reneerkens J, Versteegh MA, Schneider AM, Piersma T, Burtt EH (2008) Seasonally changing preen-wax composition: red knots (*Calidris canutus*) flexible defense against feather-degrading bacteria? Auk 125:285–290
28. Reneerkens J, Piersma T, Sinninghe Damste' JS (2002) Sandpipers (*Scolopacidae*) switch from mono- to diester preen waxes during courtship and incubation, but why? Proc R Soc Lond B Biol Sci 269:2135–2139
29. Shannon CE, Weaver W (1949) The mathematical theory of communication. University of Illinois Press, Urbana
30. Shawkey MD, Pilai SR, Hill GE (2003) Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. J Avian Biol 34:345–352
31. Shawkey MD, Mills KL, Dale C, Hill GE (2005) Microbial diversity of wild bird feathers revealed through culture-based and culture-independent techniques. Microb Ecol 50:40–47
32. Soini, HA, Schrock SE, Bruce KE, Wiesler D, Ketterson ED, Novotny MV (2007) Seasonal variation in volatile compound profiles of preen gland secretions of the Dark-eyed Junco (*Junco hyemalis*). J Chem Ecol 33:183–198
33. Waldenström J, Broman T, Carlsson I, Hasselquist D, Achterberg RP, Wagenaar JA, Olsen B (2002) Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrant birds. Appl Environ Microbiol 68:5911–5917