PLUMAGE MICRO-ORGANISMS AND PREEN GLAND SIZE IN AN URBANIZING
CONTEXT

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ABSTRACT

Urbanization of Earth's habitats has led to considerable loss of biodiversity, but the driving ecological mechanism(s) are not always clear. Vertebrates like birds typically experience urban alterations to diet, habitat availability, and levels of predation or competition, but may also be exposed to greater or more pathogenic communities of microbes. Birds have been popular subjects of urban ecological research but, to our knowledge, no study has assessed how urban conditions influence the microbial communities on bird plumage. Birds carry a large variety of microorganisms on their plumage and some of them have the capacity to degrade feather keratin and alter plumage integrity. To limit the negative effects of these feather-degrading bacteria, birds coat their feathers with preen gland secretions containing antibacterial substances. Here we examined urban-rural variation in feather microbial abundance and preen gland size in house finches (*Haemorrhous mexicanus*). We found that, although urban and rural finches carry similar total-cultivable microbial loads on their plumage, the abundance of feather-degrading bacteria was on average three times higher on the plumage of urban birds. We also found an increase in preen gland size along the gradient of urbanization, suggesting that urban birds may coat their feathers with more preen oil to limit the growth or activity of feather-degrading microbes. Given that greater investment in preening is traded-off against other immunological defenses and that feather-degrading bacteria can alter key processes like thermoregulation, aerodynamics, and coloration, our findings highlight the importance of plumage microbes and microbial defenses on the ecology of urban birds.

Keywords: feather microbes, *Haemorrhous mexicanus*, uropygial gland, urbanization

INTRODUCTION

Urbanization continues to threaten natural landscapes and maintenance of biodiversity worldwide (Aronson et al. 2014). Species richness declines from rural areas toward urban centers in several taxonomic groups, including birds, mammals, and insects (Marzluff 2001, McKinney 2008). Over the last decade, there has been a scientific push to understand the mechanisms behind this process and, due to their broad life-history effects on their hosts, parasites have been proposed as one of the reasons explaining urban loss of vertebrate and invertebrate biodiversity (Bradley and Altizer 2007). In line with this hypothesis, recent studies have shown increased parasite loads in urban compared to rural animal populations (Giraudeau *et al.* 2014a, Becker et al. 2015), a pattern that may be due to urban shifts in parasite transmission/virulence or in host susceptibility (e.g. condition and immunocompetence; Audet et al. 2015, Martin *et al.* 2010).

Typically, urban studies of animal parasites have centered on endogenous pathogens, and less emphasis has been placed on aspects of environmental transmission of microbes. Birds offer an interesting urban microbiological study system, due to the fact that they carry a large variety of microorganisms on their plumage and some of them, the feather-degrading bacteria, have the capacity to alter plumage integrity through their ability to degrade β -keratin, a protein which composes more than 90% of feather mass (Onifade *et al.* 1998, Burt and Ichida 1999, Gunderson 2008). Feather damage caused by these feather-degrading bacteria can alter the plumage insulative efficiency, causing thermoregulatory stress, or reduce aerodynamic efficiency (Clayton, 1999) and plumage coloration (Shawkey *et al.* 2007, 2008, Gunderson *et al.* 2009, Leclaire et al. 2014). To limit the negative effects of feather bacteria, birds have evolved several lines of defenses, including sun and dustbathing, specialized feather structure, seasonal/annual feather molt, or preening behavior (Gunderson 2008). The preen gland is present in nearly every bird species and the secretions produced by this gland are coated on feathers during preening

sequences. Preen oil contains antibacterial substances that have been shown to inhibit the growth of feather-degrading and non-feather-degrading bacteria (Shawkey et al. 2003). These observations raise the questions of whether urban birds may harbor greater plumage microbial communities and whether such ecological conditions may shape their investment in the preen gland or preening behavior.

Surprisingly, no studies to date have examined if or how feather-degrading bacterial loads vary in urban vs. rural birds and how urban birds might respond to increased exposure to these pathogenic bacteria (i.e., investment in preen gland or preening). Recently, Chatelain *et al.* (2015) have shown that an experimental exposure to trace metals (pollutants emitted by anthropogenic activities) led to modifications of plumage bacterial load, bacterial community composition and preening behavior; suggesting that urban conditions might influence plumage micro-organisms and their hosts.

Here, we compared total and feather-degrading cultivable microbial loads in urban and rural house finches (*Haemorrhous mexicanus*). Moreover, we captured 830 house finches from 8 sites along an urban-rural gradient over a period of 9 months to assess if and how preen gland size (a proxy for plumage protection from microbes; Martin-Vivaldi *et al.* 2009) varies as a function of habitat urbanization.

METHODS

Preen gland size

Birds were captured at each of 8 study sites along a gradient of urbanization (see Giraudeau *et al.* 2014a,b for a detailed description of the sites) from January-September 2011 using basket and Potter traps baited with sunflower seeds (N=830, all males); trapping sessions lasted from 0700-

1200 hrs. At capture, each bird was leg banded with a numbered United States Geological Survey metal tag for individual identification. We also determined tarsus length (to the nearest 0.1 mm) and preen gland size (length and width, to the nearest 0.01 mm; Jacob et al. 2014). Afterwards, we used preen gland length and width to estimate an elliptical area (mm²; Galvan and Sanz 2006).

Plumage bacterial load

Nine urban males and eight rural males were captured in July 2014 at each of 2 urban and 2 desert study sites (rural: Estrella Mountain Park and South Mountain Reserve, urban: Phoenix downtown and the Arizona State University campus). We selected this subset of sites because they comprise the most rural- and urban-like of our original 8 sites and therefore capture the full range of environmental variation due to human activities (Hasegawa *et al.* 2014). At capture, the crown, breast, back, belly, and rump feathers of each bird were swabbed with a sterile cotton tipped applicator (Puritan medical products) to collect the plumage microbiota. Plumage bacteria can be distinguished into two ecological types (free-living and attached, Alt *et al.* 2015) and only free-living bacteria have been collected with this method. The swabs were then kept in sterile 1.5 ml screw-cap microtubes containing 0.5 ml of tryptic soy broth with 20% glycerol and bacterial load was measured within 2 hours after sample collection.

To separately quantify total-cultivable and feather-degrading bacterial loads, we used two different growth media (Giraudeau *et al.* 2010, 2013, Czirjak *et al.* 2013). Firstly, Trypticase Soy Agar (TSA, #22091, Fluka) is a rich medium on which many heterotrophic bacteria grow, thus enabling assessment of the total cultivable microbe loads on the feathers. Secondly, we quantified the feather-degrading bacterial load using Feather Meal Agar (FMA) media (Sangali

and Brandelli 2000, Shawkey et al. 2003, 2007, Møller et al. 2009). One hundred microliters of the microbial solution were spread on each of the two growth media and the plates were incubated at 37°C, for 48 hrs in the case of TSA, and for 72 hrs for the FMA. After incubation, the numbers of visible colony-forming units (CFU) on each plate were counted. All counts were performed by one of us (RS) without knowledge of bird origin.

Metrics of urbanization

Following Giraudeau *et al.* (2014b), we extracted eight urbanization parameters from a local database that is part of the Central Arizona-Phoenix Long-Term Ecological Research program: (1) human population density within a radius of 1 km around each trapping site, estimated from the 2010 US Census data; (2) land-use and land-cover (LULC, in 2007) variables within the same 1 km radius. From satellite images, we determined percentage of land dedicated to 7 land uses: cultivated vegetation and cultivated grass, river gravel and water, vegetation, disturbed-commercial/industrial and asphalt, undisturbed, disturbed-compacted soil, disturbed-mesic and xeric vegetation residential. We then generated urbanization scores using PCA for the eight variables cited above. PC1 loaded strongly and negatively with the amount of undisturbed land cover, and strongly and positively with the amount of disturbance caused by yards and industry. PC2 was loaded strongly and positively with both the amount of compacted soil and vegetation (Giraudeau et al. 2014b. see ESM).

Statistics

Statistics were completed in the R statistical computing environment (R Core Team, 2014) with α set to 0.05. To test for urban and seasonal variation in preen gland size (Spring: March – May,

Summer: June – August, Fall: September – November, Winter: December – February), we created linear models initially loaded with habitat PC1, habitat PC2, season, and the two-way interactions of season with both habitat PCs. Preen gland elliptical area was not significantly related to tarsus length (a good indicator of skeletal size in passerines; Senar and Pascual, 1997), meaning that larger birds did not have larger preen glands ($\beta = -0.10$, $p = 0.20$). We thus used uncorrected values of preen gland elliptical area. Non-significant interactions ($p > 0.05$) were sequentially removed from the initial models (Zuur *et al.* 2009) to eventually provide a final model. Non-significant lower-order effects were not removed if they were contained in a significant higher-order interaction (Zuur *et al.* 2009). We used multiple imputation to recover a single missing value, which was determined to be missing at random, in the bacterial load data set with the *mice* package in R and combined all imputed datasets for further analyses (Little and Rubin 2002). We chose this method because only a single datum was missing, and alternatives such as mean replacement and serial deletion are thought to skew and bias variances (Little and Rubin 2002).

RESULTS

We found a significant positive relationship between uropygial gland size and urban PC1 (but not urban PC2; Table 1; Figure 1; PC1 standardized $\beta = 0.084$). Uropygial gland size varied across seasons as well, with it being larger during spring and summer compared to fall and winter (Table 1).

Urban and rural birds did not differ in the abundance of total cultivable plumage bacteria (TSA media, *t*-test: $t = -0.08$, $df = 15$, $p = 0.93$; Figure 2a). However, we did find that feathers of urban finches harbored a far greater abundance of feather-degrading bacteria than rural birds

(FMA media, Welch's Two-sample t -test: $t = -2.36$, $df = 8.8$, $p = 0.043$, power = 0.49, Hedge's $g = -1.02$; Figure 2a). Additionally, we found that the ratio of feather-degrading bacteria to total-cultivable bacteria was significantly different between populations, with urban birds having proportionally more feather-degrading bacteria per total-cultivable bacteria (Mann-Whitney U test: $U = 53$, $p = 0.028$, Hedge's $g = 0.93$; Figure 2b).

DISCUSSION

Here we show that urban and desert birds carry similar total-cultivable bacteria loads on their plumage but that the abundance of feather-degrading bacteria was on average three times higher on the plumage of urban birds. This result is consistent with a previous study from our group, in which we found that the prevalence and severity of infection by a gut protozoan (coccidians from the genus *Isospora*) and a virus (genus *Poxvirus*) increased along the same gradient of urbanization in house finches (Giraudeau *et al.* 2014a). More generally, this result adds to the growing literature showing increased parasite loads in urban habitats (Bradley *et al.* 2008, Lehrer *et al.* 2010, Goulson *et al.* 2012, Kellner *et al.* 2012).

Microorganisms compete with each other for space and resources and can optimally thrive under particular environmental conditions, but surprisingly little is known about the competition and colonization efficacy of feather-degrading bacteria relative to other bacteria. Our results suggest that urban conditions are favorable for feather-degrading bacteria in finches. Another hypothesis to explain our result would be that the higher densities of birds in cities and the increased contact rate between birds would favor the transmission of plumage bacteria. In line with this hypothesis, Møller *et al.* (2009) found that the abundance of feather-degrading bacteria was positively related to colony size in barn swallows (*Hirundo rustica*). While we

cannot rule out the idea that feather-degrading bacteria might have a higher transmission rate than commensal bacteria, a higher transmission of plumage bacteria in dense populations (urban populations in our case) seems unlikely given that no difference in total-cultivable bacterial loads have been found in both studies.

We also found an increase in preen gland size along the gradient of urbanization, suggesting that, under the assumption that larger glands produce more oil (Galvan et al. 2008) urban finches have the ability to coat their feathers with more preen oil to combat the comparatively more abundant feather-degrading bacterial community. An increase of preen gland size has been recently measured in response to an experimental increase of plumage bacteria density in great tits (*Parus major*, Jacob et al. 2014). Similarly, an experimental reduction of the feather bacteria load in feral pigeons (*Columbia livia*) led to a decreased investment in preen oil secretions and in preening behavior (Leclaire et al. 2014). Taken together, these results show that preen gland size and putative oil production is a plastic trait that can be adjusted according to the bacterial community present on the plumage. However, investment in this antimicrobial defense seems to be traded-off against components of the immune system (Leclaire et al. 2015), possibly influencing the host resistance to others parasites and the evolution of the avian host-pathogen interactions.

Despite their increased investment in antimicrobial defenses, urban birds still carried three times more feather-degrading bacteria than rural birds, and this has potentially large fitness consequences, in terms of reduced thermoregulation, aerodynamics, and plumage coloration. To our knowledge, no studies have ever compared plumage quality (proportion of damaged vs. undamaged barbs, Shawkey et al. 2007) between urban and rural birds, but an increased and faster feather degradation caused by feather-degrading bacteria might be an important

component explaining the decreased coloration recurrently found in urban birds (Hörak *et al.* 2000, Isaksson *et al.* 2005, Giraudeau *et al.* 2015, Hutton and McGraw 2016). Most of the plumage colors that have been studied in urban ecological contexts result from the combination of a keratin-based background structure that reflects light and pigments that absorb light (Jacot *et al.* 2010). Thus, degradations of the feather keratin structure by bacteria might influence a large suite of color-producing mechanisms in urban bird plumage (Shawkey *et al.* 2007) and thus strongly influence the inter- and intra-sexual interactions in the urban environment.

To conclude, we have shown for the first time that urban house finches carried more feather-degrading bacteria and had larger preen glands than rural finches. Given that greater investment in preening can be traded-off against immunological investments (Leclaire *et al.* 2015) and that feather-degrading bacteria can alter key fitness traits like aerodynamics and coloration (via effects on plumage integrity; Gunderson *et al.* 2009), our findings highlight the potential functional importance of plumage microbes and microbial defenses on the ecology of urban birds.

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Ethical approval: All applicable institutional and/or national guidelines for the care and use of animals were followed

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TABLES AND FIGURES

Table 1. Final linear model including predictors for uropygial gland size.

Response	Predictor	Degrees of freedom (n, d)	F-value	P-value
Uropygial gland area	Season	3, 824	9.44	< 0.0001
	PC1	1, 824	5.99	0.015
	PC2	1, 824	0.37	0.52
	PC1*Season	-	-	NS
	PC2*Season	-	-	NS

Figure 1. Urban PC1 predicts variation in preen gland. Points and whiskers represent means and standard errors of the mean for each study site.

Figure 2. Urban-rural variation in plumage microbes. (a) Amounts of total-cultivable bacteria and feather-degrading bacteria in urban and rural finches. (b) Ratio of total-cultivable bacteria to feather-degrading bacteria. Bars represent means and whiskers represent standard errors significant. Asterisks denote for which comparisons there were significant differences between urban and rural finches ($p < 0.05$).