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Effects of *Mycoplasma gallisepticum* infection on preening behaviors and feather quality in House Finches (*Haemorhous mexicanus*)

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Word count: 4,152

Abstract

Pathogen infections can have far-reaching sublethal effects on wildlife, including reduced maintenance of external structures. For many wildlife taxa, daily maintenance of external structures (termed "preening" in birds) is critical to fitness, but few studies have examined how pathogen infections alter such maintenance. *Mycoplasma gallisepticum* is a common pathogen in free-living house finches (*Haemorhous mexicanus*), where it causes mycoplasmal conjunctivitis. Despite documented behavioral changes associated with *M. gallisepticum* infections in finches, no studies have examined how preening behavior changes with infection, and how potential differences in preening affect feather quality. To test this, we experimentally inoculated captive house finches with *M. gallisepticum* or a control treatment, and collected behavioral and feather quality data to detect potential changes in feather maintenance due to infection. We found that finches infected with *M. gallisepticum* preened significantly less often, and within the infected treatment, birds with the highest conjunctivitis severity preened the least often. However, there was no difference in the quality scores for secondary flight feathers collected from control versus infected birds. We also assayed feather water retention, and found that the degree of water retention correlated with our feather quality scores, such that feathers with poor scores retained more water. However, as with quality scores, feather water retention did not differ with infection treatment, which may be due to the controlled environment that birds experienced while in captivity. Our data suggest that, in addition to sickness behaviors previously observed in finches, *M. gallisepticum* infection decreases other behaviors critical to survival such as preening. While the consequences of reduced preening on feather maintenance were not apparent in captive

conditions, further work is needed to determine whether house finches in the wild that are infected with *M. gallisepticum* experience a fitness cost, such as increases in ectoparasite loads, due to this reduced feather maintenance.

Keywords: feather quality, *Haemorhous mexicanus*, house finch, *Mycoplasma gallisepticum*, pathogen infection, preening behavior, sublethal effects

Introduction

When wildlife are actively infected with a pathogen, there can be pronounced energetic trade-offs (Sheldon and Verhulst 1996; Brownlee-Bouboulis and Reeder 2013). These tradeoffs may affect several systems within an organism, including behavioral maintenance of external structures (Leclaire et al. 2014). For example, vampire bats (*Desmodus rotundus*) injected with bacterial lipopolysaccharide to mimic infection spend significantly less time self-grooming (Stockmaier et al. 2018). On the other hand, in some systems, active infection can lead to increased grooming, as occurs for little brown bats affected by white nose syndrome (Brownlee-Bouboulis and Reeder 2013), or in insects affected by cutaneous pathogens (Qiu et al. 2014). Because maintenance of external structures can have effects on organismal fitness, such as by keeping ectoparasite loads low (Mooring et al. 1996), it is important to understand how acute infections alter behavioral investment in external structures, and the potential fitness consequences of such changes in behavior. More broadly, reduced investment in behavioral maintenance of external structures such as skin, fur, and feathers may represent an important source of sublethal effects of pathogens on hosts.

Birds are an interesting taxonomic group for understanding effects of infection on behavioral grooming, because feathers serve diverse and critical functions for birds including thermoregulation, flight effectiveness, and communication (Stettenheim 2000). Feathers are dead structures and require constant maintenance, and the act of behavioral grooming ("preening" in birds) maintains these structures. Preening in birds typically involves the rearrangement of feathers, direct removal of ectoparasites, and the deposition of oily secretions from their preen (or "uropygial") gland onto their plumage (Delius 1988). Experimental studies have shown that preening behavior is key to aspects of avian fitness, with birds prevented from preening showing increases in ectoparasite load and reductions in overall feather quality (e.g. Clayton et al. 2005). However, preening is energetically expensive in birds, with king penguins (*Aptenodytes patagonicus*) estimated to spend ~5% of their daily average energy expenditure on this behavior during breeding, despite energetic fasting during this part of their annual cycle (Viblanc et al. 2011). The time required for preening also carries "opportunity" costs, with a meta-analysis finding that preening represents almost 9% of daily average time budgets across 62 bird species (Clayton and Cotgreave 1994). Overall, prior work demonstrates that preening behaviors have key benefits for wild birds, but also carry significant costs in terms of time and energy.

The key benefits and costs of preening in birds make it important to understand how acute infections by pathogens influence the extent of behavioral preening in birds. Because acute infection in itself is energetically costly (Hawley et al. 2012), birds may significantly reduce preening during pathogen infection, as was observed in juvenile Apapane (*Himatione sanguinea*) that were experimentally infected with avian malaria (Yorinks and Atkinson 2000). Effects of acute infection on preening could constitute important sublethal effects of infection that ultimately contribute to the long-term reductions in survival or reproductive success associated with many infections in birds (Dunn et al. 2021). Further, effects of acute infection on preening behavior could alter the likelihood that avian hosts become simultaneously co-infected with ectoparasites and pathogens, which could together have synergistic effects on host fitness, as has been shown for co-infections in other wildlife systems (Thumbi et al. 2013).

House finches (*Haemorhous mexicanus*) provide a tractable system for understanding how acute infection with a pathogen affects feather maintenance in birds. The bacterial pathogen *Mycoplasma gallisepticum* (MG) emerged in the mid-1990s in house finches and continues to cause annual epidemics in this species. Infection with MG causes mycoplasmal conjunctivitis, which is associated with significant mortality in free-living finches (Faustino et al. 2004), largely through enhanced predation risk for infected birds (Adelman et al. 2017). Free-living finches with mycoplasmal conjunctivitis show distinct behaviors such as reduced movement (Hawley et al. 2007), and in captivity, experimental MG infection causes extreme lethargy and inactivity (Kollias et al. 2004; Love et al. 2016). Notably, the expression of these sickness behaviors can last for up to four weeks post-inoculation (Kollias et al. 2004). Further, the sickness behaviors generated by acute MG infection result in behavioral trade-offs important for predator avoidance and fitness (Adelman et al. 2017). However, it is unknown whether MG infection also influences other behaviors important for survival, such as preening to maintain feather quality. To date, direct effects of MG infection on feather maintenance have not been examined. However, a field study found higher ectoparasite loads on wild-caught house finches with mycoplasmal conjunctivitis (Davis and Cornelius 2013), providing potential indirect support for the idea that MG infection alters preening behavior in ways relevant for ectoparasite control in finches.

We used an experimental approach to examine how MG infection alters preening behavior, activity levels, and feather quality of wild-caught house finches in captivity. Given that

MG results in significant energetic tradeoffs (Hawley et al. 2002) and reduces activity levels (Love et al. 2016), we hypothesized that MG infection would reduce the amount of time that birds invest in maintaining feathers. We predicted that experimentally infected birds would spend less time preening, more time inactive, and consequently have lower feather quality. Because our captive-housed birds did not have notable levels of ectoparasites, here we measured feather quality using two proxies: overall feather appearance and the propensity of feathers to retain water.

Materials and Methods

Experimental Design, Inoculation, and Data Collection

House finches (n = 33) were captured in Montgomery County, Giles County, and the City of Radford, Virginia in the summer of 2019 using baited traps. To standardize for age, only hatch-year birds (aged via plumage) were retained. Birds were quarantined for two weeks post-capture and examined every 3-5 days for the presence of MG clinical signs such as swelling of the conjunctiva (eye score; as per Sydenstricker et al. 2006). All experimental birds showed no clinical signs (pathology score > 0) prior to beginning the experiment. Birds were also blood sampled and tested for anti-MG antibodies in the plasma approximately two weeks after capture, and no birds that were seropositive (as per Hawley et al. 2011) were included in the study. Beginning 13 days before inoculation, all birds were single-housed in wire-mesh cages (76 x 46 x 46 cm) with a constant 12:12 light: dark cycle and provided food and water throughout the day *ad libitum*. To ensure no MG spread between cages, plastic sheets were hung between each cage stack.

As part of a separate study (Weitzman et al. 2021), birds were assigned to one of three MG treatment groups: a high ($3x10^4$ color-changing units/mL, CCU/mL; n = 11) or mid ($3x10^3$ CCU/mL, n = 12) dose of MG, or sham control treatment with sterile media (n = 10). Birds were inoculated with 70 uL total (approximately 35 uL/eye) by droplet installation into the conjunctiva with either Frey's medium alone (control treatment) or the VA1994 MG isolate (7994-1 6 P 9/17/2018) diluted in Frey's medium to the appropriate concentration. Because of the large number of birds in the associated study (Weitzman et al. 2021), birds were divided into two temporal groups that were inoculated and sampled four weeks apart but otherwise treated identically. Both temporal groups included birds in all experimental treatments.

We measured disease severity to determine if individuals' responses to infection predict their behaviors, and consequently, their feather quality. Pathology was measured on a scale from 0–3 for each eye and summed between the two sides as previously described (Sydenstricker et al. 2006; Hawley et al. 2011). Briefly, a score of 0 indicates no clinical signs of conjunctivitis, 1 signifies minor swelling and minor conjunctival eversion, 2 signifies moderate swelling and eversion, and 3 represents severe swelling, eversion, and exudate. We collected pathology data at multiple time points throughout infection (3, 8, 13, 20, and 27 days post-inoculation, referred to hereafter as DPI).

We collected behavioral data for each bird both pre- and post-treatment, using a duration recording approach to quantify the percentage time each bird engaged in certain behaviors, including preening (Table 1). We recorded each bird twice: once on a single morning before inoculation ("pre"), as well as on a single morning 12 days post-inoculation ("peak", because it falls within the peak infectious period for MG in house finches; Dhondt et al. 2008). Thus, we had two total videos for most individuals. However, due to technological constraints, one bird was not recorded at either timepoint, and two additional birds were not recorded at peak infection only. Thus, sample sizes for videos were reduced to n=62 videos from n=32 unique birds. All video recordings were begun between 0800-0830 for all birds, a time of peak activity, to account for potential variation in grooming behavior throughout the day. We minimized potential effects of recent handling on behavior by timing recordings such that birds were not handled for at least three full days prior to all videotaping.

Birds were video-recorded for a total of 71-121 minutes per sampling day and a single observer (D.A.) who was blind to the treatment group at the time watched all videos and quantified the total time each bird engaged in one of three behaviors: preening, feeding, or inactivity (Table 1). Variation in the length of videos was accounted for in our analyses by weighting each model by the total time each bird was video recorded (see Statistical Analyses). Time spent preening included behaviors such as a bird reaching toward the preen gland on its rump, or using its beak to comb through the plumage (Table 1). Time spent inactive was only recorded if a bird spent more than five seconds in a single location, while not perched on the food dish. Time inactive was thus mutually exclusive from active behaviors such as preening; however, because we analyzed time spent preening or inactive as proportions of the total time each bird was recorded on that day (see Statistical Analysis), these two variables were not entirely independent.

On day 34 post-inoculation, the sixth secondary flight feather on each bird's left side was clipped close to the base of the rachis. Feathers were examined under a compound microscope while blind to treatment, to score the barbule structure and level of degradation. Each feather was scored on a scale of 1–4 using a scoring system modified from Burtt and Ichida's (2004) 1–6 scale, with 1 representing the least amount of degradation and 4 representing the most degradation (Figure 1).

Using the secondary feathers, we also measured the amount of water each feather retained, which can be influenced by variation in barb or barbule structure (Bormashenko et al. 2007) or the amount of deposited preen oil (Moreno-Rueda 2017), both of which should be direct functions of an individual's recent preening behavior. Each feather was dry weighed to 0.001 g, then dipped in distilled water for three seconds. The feather was removed from the water, hung for two seconds to allow excess water to escape, and then weighed again to 0.001 g. The feather was then placed back in the water, repeating the procedure to obtain a second and third wet mass of each feather. Water retention was analyzed as the feather's maximum wet mass divided by the average dry mass (Ribak et al. 2005). Two feathers were removed from analyses due to a high coefficient of variation in the average dry mass.

Statistical analysis

Our analyses tested the hypothesis that birds experimentally infected with MG would spend less time preening and more time inactive, and have lower quality feathers as a consequence. Initial analyses were run with three separate treatment groups (two MG-inoculated treatments and control), but the two MG-inoculated dose groups never significantly differed from each other, so we combined all the birds inoculated with MG into one category for all presented analyses. We also included covariates of sex and temporal group in all initial analyses, but they were never significant and were discarded in all final models. We conducted all analyses in R v 4.0.2 in RStudio v 1.3.1093 (R Development Core Team 2015, RStudio Team 2020), using Wald's tests in the car package to determine predictor variable significance (Fox and Weisberg 2019).

We analyzed behaviors (proportion of time spent preening or inactive) with binomial generalized linear mixed effects models (GLMM) weighted by total length of each recording, with bird ID as a random effect, in the lme4 package (Bates et al. 2015). In these analyses, we tested whether MG treatment (inoculated or control), sampling period (pre-inoculation or peak infection), and their interaction predicted proportion of time spent in these behaviors, testing the prediction that control and MG-inoculated birds would not differ in their activities before inoculation, but would differ during peak infection. We assessed the results using *post hoc* pairwise contrasts with the emmeans package (Lenth 2021). To determine if the variation in behaviors were predicted by an individuals' degree of pathology, we analyzed the effects of variation in disease severity (eye score) on behaviors at peak infection, using a weighted binomial GLM. Here we limited the analysis to birds in the infected treatment only.

Lastly, we determined if the proportion of time spent preening was correlated with the proportion of time inactive, testing whether reduced preening should be considered as part of the constellation of "sickness behaviors" expressed during MG infection in house finches. This linear model included data from all birds collected at both time points, weighted for total recording time as above.

We analyzed feather scores with a Poisson distribution to detect if feather quality was affected by MG treatment, maximum eye score, or proportion time preening during peak infection. Due to collinearity of the predictor variables, we analyzed each one separately.

Finally, we analyzed log-transformed values of water retention with linear models. We first assessed how well feather water retention can act as a proxy for feather score, with the prediction that feathers of worse quality would retain more water than better quality feathers. We then assessed whether water retention was associated with MG treatment.

Results

The proportion of time that house finches spent preening ($n = 64$ videos from 32 unique birds) was significantly affected by experimental inoculation with MG (estimate ± SE = –0.944 ± 0.308; $z = –3.068$, $p = 0.002$), sampling period (estimate = 0.664 ± 0.022, $z = 30.6$, $p < 0.0001$), and their interaction (estimate = 0.528 ± 0.029, $z = 17.79$, $p < 0.0001$). Specifically, MG-infected birds (with both inoculation doses pooled) spent significantly less time preening than control individuals at the peak of infection, but as expected, birds in the control and infected treatment did not differ prior to inoculation (Figure 2a). Similarly, proportion of time spent inactive was significantly higher in MG-infected birds (treatment estimate = 2.099 ± 0.036, $z = 5.89$, $p < 0.0001$; Figure 2b) and during peak infection (sampling period estimate = -0.130 ± 0.017, $z = -7.63$, $p < 0.0001$), with patterns of inactivity also significantly predicted by the interaction between MG treatment and sampling period (estimate = -2.642 ± 0.021, $z = -125.4$, $p < 0.0001$).

Among infected birds, the proportion of time spent preening during peak infection ($n = 20$ unique infected birds with videos at this timepoint) was significantly associated with individual variation in the degree of pathology, such that birds with more severe conjunctivitis spent the lowest proportion of time preening (estimate = -0.349 ± 0.015, $z = -22.99$, $p < 0.0001$; Figure 2c). MG-inoculated birds with a higher degree of pathology also spent more time inactive at peak infection (estimate = 0.131 ± 0.004, $z = 30.42$, $p < 0.0001$). Finally, the proportion time birds spent preening on day 12, regardless of infection status, was negatively correlated with proportion time inactive ($n = 30$, estimate = -0.07 ± 0.021, $t = -3.45$, $p = 0.002$).

Despite the significant differences in proportion time spent preening across treatments, feather quality score on day 34 post-inoculation was not predicted by MG treatment ($n = 33$, estimate = -0.14 ± 0.22, $z = -0.64$, $p = 0.5$), maximum eye score ($n = 23$ because analysis was limited to infected birds, estimate = 0.071 ± 0.089, $z = 0.80$, $p = 0.4$), or time spent preening during peak infection ($n = 30$, estimate = 1.87 ± 2.64, $z = 0.71$, $p = 0.5$).

We also used a measurement of water retention as a proxy for feather quality. Two feathers were removed from these analyses due to high dry mass coefficient of variation. First, we found that water retention correlated with feather quality score, with feathers of poorer quality as ranked on our scoring system retaining more water ($n = 31$, estimate = 0.188 ± 0.078, $t = 2.40$, $p = 0.023$; Figure 3). Similar to the feather score results, the amount of feather water retention was not significantly different between birds with and without MG infection (estimate = -0.18 ± 0.19, $t = -0.94$, $p = 0.36$).

Discussion

We used experimental MG inoculation in captive house finches to test effects of an acute infectious disease on feather maintenance, and found reduced preening and activity in MG-inoculated birds. This result, however, did not extend to two metrics of feather quality, at least over the timescale and under the conditions examined. Overall, our results provide important information on the way that infection can foster a variety of behavioral responses with the potential to affect visible structures and rates of ectoparasitism in songbirds under natural conditions.

Our behavioral results, that infected house finches spent less time preening and more time inactive at peak infection, were consistent with our predictions based on previous work. House finches infected with MG in prior studies showed a suite of sickness behaviors such as lethargy, reduced aggression, and anorexia (Kollias et al. 2004; Bouwman and Hawley 2010; Love et al. 2016; Adelman et al. 2017). Our results here suggest that sickness behaviors in this system also extend to a reduction in behavioral maintenance of external structures, as has been shown in other taxa in response to pathogen infection (Yorinks and Atkinson 2000) or immune stimulation (Stockmaier et al. 2018). In many taxa, animals alter their behavior when they are faced with an active infection, and these sickness behaviors are hypothesized to conserve host energy for immune defense (Hart 1988). Interestingly, however, songbirds do not appear to reduce the degree of preening in response to all infections: experimental coccidial infection in American goldfinches (*Spinus tristus*) in captivity did not result in alterations of preening behavior (Surmacki and Hill 2014), despite prior studies of other species finding that coccidial infections can alter non-preening behaviors (Aguilar et al. 2008) and can even influence flight feather quality (Pap et al. 2013). One possibility raised by Surmacki and Hill (2014) for the lack of detected effect of coccidial infection on preening behavior in goldfinches is that this species may prioritize feather maintenance, even in the face of *Isospora* parasite infection. Future work should examine whether the degree to which pathogen infection alters preening behavior in birds varies with avian species traits such as the importance of feather maintenance, or potentially with parasite-specific traits such as the virulence of infection.

While sickness behaviors may contribute to a finch's ability to respond effectively to MG infection, care of external structures via grooming/preening is essential to fitness in diverse wildlife taxa, from insects to mammals (Sachs 1988; Moore 2002). Feathers often serve as key secondary sexual signals for avian mate choice, and preening can therefore be critical to mate acquisition and reproductive success; for example, blocking access to the preen gland in house finches resulted in reductions in plumage coloration in males (López-Rull et al. 2010) and tail feather damage in magpies was associated with lower reproductive success (Fitzpatrick and Price 1997). In birds, preening is a particularly critical mechanism for defense against ectoparasites (reviewed in Bush and Clayton 2018), and recent evidence suggests that pigeons upregulate preening behavior in the presence of ectoparasites (Villa et al. 2016). Feather damage from parasites also appears to be associated with increased predation risk (al Rubaiee et al. 2017) and decreased flight performance (Barbosa et al. 2002). Thus, the reduced preening behaviors we observed in MG-inoculated birds could have important consequences for other aspects of host health and disease. In particular, our results suggest that reducing time spent preening during acute infection may explain the previously detected higher feather mite loads on free-living house finches with mycoplasmal conjunctivitis (Davis and Cornelius 2013).

In addition to altering preening behavior, pathogen infection can influence other aspects of feather maintenance in birds. For example, song sparrows infected with avian malaria show distinct preen oil chemistry from uninfected sparrows, though it remains unknown whether or how these differences affect the preen oil's effectiveness or overall feather condition (Grieves et al. 2018). Further, avian malaria infection in house sparrows was associated with slower feather growth rates (Coon et al. 2016), suggesting that pathogen infection may compromise the ability of birds to replace feathers during molt. Overall, acute infection in birds has the potential to result in reduced feather quality via several mechanisms, including the reductions in grooming behavior that we observed in infected house finches.

We predicted that the observed reductions in preening would translate to reduced feather quality for house finches infected with MG, but we did not detect any effects of experimental infection on feather quality. Feathers were clipped on day 34 post-inoculation in our experimental timeline, which may have been an insufficient length of time to observe notable changes in feather quality, particularly in the controlled captive setting in which our experiment took place. A second possibility is that baseline differences in feather quality at capture, which we were not able to account for in our study design, made it challenging to detect any treatment effects. The birds in our experiment were all captured in their hatch year (within 1-3 months of fledging given the time of year of capture), which minimized one potential source of variation in baseline feather quality. However, future studies should also score feather quality prior to infection treatment to better account for individual variation in aspects of feather growth and/or maintenance. Finally, our visual scoring of feathers may not have been a good proxy for feather maintenance. However, we did find that lower quality feathers as scored by our 4-point system had greater water retention, indicating that the aspects of feather quality reflected in our scoring system are at least representative of feather waterproofing. Waterproofing in birds is a critical component of thermoregulation and energy conservation, which could affect their ability to fight off an infection (Grémillet 2005). When birds preen, both the maintenance of barbule structure (Liu et al. 2008) and the fatty acid and alcohol components of uropygial oil contribute to feather hydrophobicity, as well as thermal insulation, bacterial defense, and feather hygiene (Shawkey et al. 2003; Salibian and Montalti 2009). Whether our scoring system captures any of these other aspects of feather quality remains unknown, but our results indicate that, at a minimum, the reductions in preening observed in our MG treatment birds did not have immediate effects on feather water retention.

We predict that in the wild, the reduced preening in infected house finches may have a more apparent effect on feather quality for two reasons: first, variation in humidity, temperature, and feather parasites was essentially absent in our captive environment, but in the wild, may present considerable challenges to feather maintenance that become apparent during MG infection, when birds are unable to preen. For example, reductions in preening during infection may explain why free-living finches with mycoplasmal conjunctivitis harbor higher mite loads than finches without conjunctivitis (Davis and Cornelius 2013), though other mechanisms could explain this correlation. Future studies could challenge birds with or without MG infection with ectoparasites to determine whether reductions in preening during infection have consequences for ectoparasite loads. Interestingly, Heylen et al. (2020) challenged house finches with and without mycoplasmal conjunctivitis with ticks, and found no notable differences in tick infestation. However, preening behavior in birds is often unsuccessful at removing ticks, which strongly prefer to embed on the head regions of avian hosts that are inaccessible to preening (Fracasso et al. 2019).

Overall, we showed that house finches experimentally inoculated with MG expressed sickness behaviors that included reductions in preening. In addition to other ways that MG infections affect house finch fitness, such as inducing lethargy, anorexia, and visual impairment, reduced preening in wild birds may have sublethal effects on house finch fitness by affecting diverse aspects of feather functioning including flight ability (Pap et al. 2013), mate attraction (López-Rull et al. 2010), and bacterial and ectoparasitic defense (Bush and Clayton 2018). While we did not detect these differences in our captive birds, examination of feather quality in wild or aviary-held house finches with and without conjunctivitis would provide further insight into less understood ways in which disease affects host fitness. Finally, reduced preening during acute infections could represent a broadly important behavioral mechanism augmenting co-infection in birds and other taxa that rely on grooming to control costly ectoparasitic infections.

Acknowledgments

We thank Allison Rowley, Sara Teemer Richards, Guadalupe Ceja, Isabel Boyce, Drew Hynes, Hannah Farrell, Ricardo Acevedo-Siaca, and Cynthia Harrison for assistance with this project. This work was funded by the Stacey Smith Research Excellence Award from the Department of Biological Sciences to DA and NSF grants IOS 1754872 and 1755051 to DMH.

Literature Cited

Adelman JS, Mayer C, Hawley DM. 2017. Infection reduces anti-predator behaviors in house finches. *J Avian Biol* 48:519–528.

Aguilar TM, Maia R, Santos ESA, Macedo RH. 2008. Parasite levels in blue-black grassquits correlate with male displays but not female mate preference. *Behav Ecol* 19:292–301.

Barbosa A, Merino S, Lope F, Møller AP. 2002. Effects of feather lice on flight behavior of male Barn Swallows (*Hirundo Rustica*). *Auk* 119:213–216.

Bates, D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1-48.

Bormashenko E, Bormashenko Y, Stein T. 2007. Why do pigeon feathers repel water? Hydrophobicity of pennae, Cassie–Baxter wetting hypothesis and Cassie–Wenzel capillarity-induced wetting transition. Elsevier. *J Colloid Interface Sci* 311:212-216.

Bouwman KM, Hawley DM. 2010. Sickness behaviour acting as an evolutionary trap? Male house finches preferentially feed near diseased conspecifics. *Biol Lett* 6:462–465.

Brownlee-Bouboulis SA, Reeder DM. 2013. White-nose syndrome-affected Little Brown Myotis (*Myotis lucifugus*) increase grooming and other active behaviors during arousals from hibernation. *J Wildl Dis* 49:850–859.

Burtt EH, Ichida JM. 2004. Gloger's rule, feather-degrading bacteria, and color variation among song sparrows. *Condor* 106:681–686.

Bush SE, Clayton DH. 2018. Anti-parasite behaviour of birds. *Philos Trans R Soc B* 373:20170196.

Clayton DH, Cotgreave P. 1994. Comparative analysis of time spent grooming by birds in relation to parasite load. *Behaviour* 131:171–187.

Clayton DH, Moyer BR, Bush SE, Jones TG, Gardiner DW, Rhodes BB, Goller F. 2005. Adaptive significance of avian beak morphology for ectoparasite control. *Proc R Soc B* 272:811–817.

Coon CAC, Garcia- Longoria L, Martin LB, Magallanes S, Lope F, Marzal A. 2016. Malaria infection negatively affects feather growth rate in the house sparrow *Passer domesticus*. *J Avian Biol* 47:779–787.

Davis AK, Cornelius E. 2013. Do infections lead to higher feather mite loads in birds? A test with mycoplasmal conjunctivitis in House Finches (*Haemorhous mexicanus*). *Auk* 130:708–714.

Delius JD. 1988. Preening and associated comfort behaviour in birds. *Ann NY Acad Sci* 525:40–55.

Dhondt AA, Dhondt KV, McCleery BV. 2008. Comparative infectiousness of three passerine bird species after experimental inoculation with *Mycoplasma gallisepticum*. *Avian Pathol* 37:635–640.

Dunn JC, Hawley DM, Huyvaert KP, Owen JC. 2021. Fitness Effects of Parasite Infection in Birds. In: *Infectious Disease Ecology of Wild Birds*, Owen JC, Hawley DM, Huyvaert KP, editors. Oxford University Press, Oxford, UK, pp. 99–120.

Faustino CR, Jennelle CS, Connolly V, Davis AK, Swarthout EC, Dhondt AA, Cooch EG. 2004. *Mycoplasma gallisepticum* infection dynamics in a house finch population: Seasonal variation in survival, encounter and transmission rate. *J Anim Ecol* 73:651–669.

Fitzpatrick S, Price P. 1997. Magpies' tails: damage as an indicator of quality. *Behav Ecol Sociobiol* 40:209–212.

Fox J, Weisberg S. 2018. *An R Companion to Applied Regression*, 3rd Ed. SAGE Publications, Inc, Thousand Oaks, California, 608 pp.

Fracasso G, Matthysen E, Dhondt AA, Heylen D. 2019. Experimental study of micro-habitat selection by ixodid ticks feeding on avian hosts. *Int J Parasitol* 49:1005–1014.

Grémillet D, Chauvin C, Wilson RP, le Maho Y, Wanless S. 2005. Unusual feather structure allows partial plumage wettability in diving great cormorants *Phalacrocorax carbo*. *J Avian Biol* 36: 57-63.

Grieves LA, Kelly TR, Bernards MA, MacDougall-Shackleton EA. 2018. Malarial infection alters wax ester composition of preen oil in songbirds: Results of an experimental study. *Auk* 135:767–776.

Hart BL. 1988. Biological basis of the behavior of sick animals. *Neurosci Biobehav Rev* 12:123–137.

Hawley DM, Davis AK, Dhondt AA. 2007. Transmission-relevant behaviours shift with pathogen infection in wild house finches (*Carpodacus mexicanus*). *Can J Zool* 85:752–757.

Hawley DM, Grodio J, Frasca S, Kirkpatrick L, Ley DH. 2011. Experimental infection of domestic canaries (*Serinus canaria domestica*) with *Mycoplasma gallisepticum*: A new model system for a wildlife disease. *Avian Pathol* 40:321–327.

Hawley DM, Durant SE, Wilson AF, Adelman JS, Hopkins WA. 2012. Additive metabolic costs of thermoregulation and pathogen infection. *Funct Ecol* 26:701–710.

Heylen DJA, Reinoso-Pérez MT, Goodman L, Dhondt KV, Dhondt AA. 2020. Ectoparasitism during an avian disease outbreak: An experiment with *Mycoplasma*-infected house finches and ticks. *Int J Parasitol* 12:53–63.

Kollias GV, Sydenstricker KV, Kollias HW, Ley DH, Hosseini PR, Connolly V, Dhondt AA. 2004. Experimental infection of house finches with *Mycoplasma gallisepticum*. *J Wildl Dis* 40:79–86.

Leclaire S, Pauline P, Chatelain M, Gasparini J. 2014. Feather bacterial load affects plumage condition, iridescent color, and investment in preening in pigeons. *Behav Ecol* 25:1192–1198.

Lenth, RV. 2021. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.5-1. https://CRAN.R-project.org/package=emmeans

Liu Y, Chen X, Xin JH. 2008. Hydrophobic duck feathers and their simulation on textile substrates for water repellent treatment. *Bioinspir Biomim* 3:046007.

López-Rull I, Pagán I, Macías Garcia C. 2010. Cosmetic enhancement of signal coloration: experimental evidence in the house finch. *Behav Ecol* 21:781–787.

Love AC, Foltz SL, Adelman JS, Moore IT, Hawley DM. 2016. Changes in corticosterone concentrations and behavior during *Mycoplasma gallisepticum* infection in house finches (*Haemorhous mexicanus*). *Gen Comp Endocrinol* 235:70–77.

Moore J. 2002. *Parasites and the behavior of animals,* 1st Ed. Oxford University Press, New York, New York, 338 pp.

Mooring MS, McKenzie AA, Hart BL. 1996. Grooming in impala: Role of oral grooming in removal of ticks and effects of ticks in increasing grooming rate. *Physiol Behav* 59:965–971.

Moreno-Rueda G. 2017. Preen oil and bird fitness: a critical review of the evidence. *Biol Rev* 92:2131–2143.

Pap PL, Vágási CI, Bărbos L, Marton A. 2013. Chronic coccidian infestation compromises flight feather quality in house sparrows *Passer domesticus*. *Biol J Linn Soc* 108:414–428.

Qiu H, Lu L, Shi Q, He Y. 2014. Fungus exposed *Solenopsis invicta* ants benefit from grooming. *J Insect Behav* 27:678–691.

R Development Core Team. 2015, February 10. R: a language and environment for statistical computing. http://www.gbif.org/resource/81287.

Ribak G, Weihs D, Arad Z. 2005. Water retention in the plumage of diving great cormorants *Phalacrocorax carbo sinensis*. *J Avian Biol* 36:89–95.

RStudio Team. 2020. RStudio: Integrated Development Environment for R. RStudio, PBC., Boston, MA, USA.

al Rubaiee Z, al Murayati H, Nielsen JT, Møller AP. 2017. Fungi, feather damage, and risk of predation. *Ecol Evol* 7:10797–10803.

Sachs BD. 1988. The development of grooming and its expression in adult animals. *Ann N Y Acad Sci* 525:1–17.

Salibian A, Montalti D. 2009. Physiological and biochemical aspects of the avian uropygial gland. *Braz J Biol* 69:437–446.

Shawkey MD, Pillai SR, Hill GE. 2003. Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. *J Avian Biol* 34:345–349.

Sheldon BC, Verhulst S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol Evol* 11:317–321.

Stettenheim PR. 2000. The integumentary morphology of modern birds—an overview. *Am Zool* 40:461-477.

Stockmaier S, Bolnick DI, Page RA, Carter GG. 2018. An immune challenge reduces social grooming in vampire bats. *Anim Behav* 140:141–149.

Surmacki A, Hill GE. 2014. Coccidial infection does not influence preening behavior in American goldfinches. *Acta Ethol* 17:107–111.

Sydenstricker KV, Dhondt AA, Hawley DM, Jennelle CS, Kollias HW, Kollias GV. 2006. Characterization of experimental *Mycoplasma gallisepticum* infection in captive house finch flocks. *Avian Dis* 50:39–44.

Thumbi SM, de C Bronsvoort BM, Poole EJ, Kiara H, Toye P, Ndila M, Conradie I, Jennings A, Handel IG, Coetzer JAW, Hanotte O, Woolhouse MEJ. 2013. Parasite co-infections show synergistic and antagonistic interactions on growth performance of East African zebu cattle under one year. *Parasitology* 140:1789–1798.

Viblanc VA, Mathien A, Saraux C, Viera VM, Groscolas R. 2011. It costs to be clean and fit: Energetics of comfort behavior in breeding-fasting penguins. *PLoS ONE* 6:e21110.

Villa SM, Campbell HE, Bush SE, Clayton DH. 2016. Does antiparasite behavior improve with experience? An experimental test of the priming hypothesis. *Behav Ecol* 27:1167–1171.

Weitzman CL, Rostama B, Thomason CA, May M, Belden LK, Hawley DM. 2021. Experimental test of microbiome protection across pathogen doses reveals importance of resident microbiome composition. *FEMS Microbiol Ecol* 97: fiab141.

Yorinks N, Atkinson CT. 2000. Effects of malaria on activity budgets of experimentally infected juvenile Apapane (*Himatione sanguinea*). *Auk* 117:731–738.

Table 1. Definitions of the three behaviors (all mutually exclusive) quantified during video sampling. All behaviors were measured in length (seconds) via duration sampling, and then proportions of time preening or inactive were quantified by dividing the time spent in each behavior by the total time the bird was observed via video.

Behavior	**Description**
Preening	Bird reaching toward the preen gland on the rump or using beak to comb through the plumage
Inactive	Bird unmoving (no preening, shaking, or other movement) in single location (other than at the food or water dish) for a minimum of five seconds duration
Eating	Any time bird was perched on the food dish, regardless of whether bird was actively eating

Figure 1. Feather scoring system to quantify the quality of a single secondary flight feather from each house finch (*Haemorhous mexicanus*). The 1-4 scale here, modified from Burtt & Ichida's (2004) six-point scale, is based on the barb attachment to the vane and visible degradation of feather surface as seen under a compound microscope. A score of 1 indicates a higher quality feather and a score of 4 indicates a relatively lower-quality feather. Representative pictures (credit: Danielle Alms) of each score are shown from the study feathers.

Figure 2. Proportion of time that house finches (*Haemorhous mexicanus*) experimentally inoculated with sham media (control; n=10) or with the bacterial pathogen *Mycoplasma gallisepticum* (=MG; n=22) spent (a) preening and (b) inactive both prior to inoculation (Pre = day -2 or -6) and at peak infection (Peak = day 12 post-infection). Boxplots indicate the median, interquartile range, reasonable range of the data, and outliers. Asterisks indicate groups significantly different from all other groups in pairwise contrasts. (c) Proportion of time spent preening by house finches is correlated with individual severity of pathology within the MG-inoculation treatments. Birds with more severe clinical signs spent less time preening. Each point represents an individual (n=20), and the line represents the best fit line from a binomial GLM.

Figure 3. The amount of water that the secondary flight feathers of house finches (*Haemorhous mexicanus*) retained in a lab assay, quantified as the log10 of proportional wet mass of feathers after being dipped into water, was correlated with feather quality scores (higher scores are associated with lower feather quality; Figure 1). Water retention was not influenced by whether finches were experimentally inoculated with sham media (control, open circles) or with the bacterial pathogen *Mycoplasma gallisepticum* (=MG, closed circles)

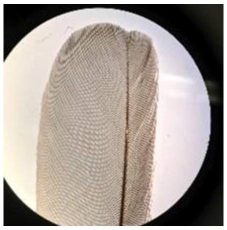
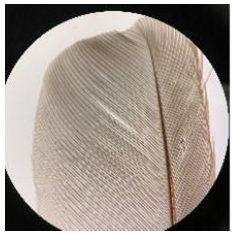
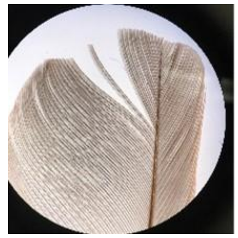
Score 1	Score 2	Score 3	Score 4
			
Fully complete vane, all or almost all barbs (>98%) attached to each other; no detectable feather degradation or loss	Close to complete vane, almost all barbs (>85%) attached to each other; some detectable degradation or loss but <5% of overall vane surface	70% of barbs attached to each other; detectable degradation/loss at (> 5%) of feather surface	50% of barbs attached to each other; detectable degradation/loss at (>15%) of feather surface

Figure 2

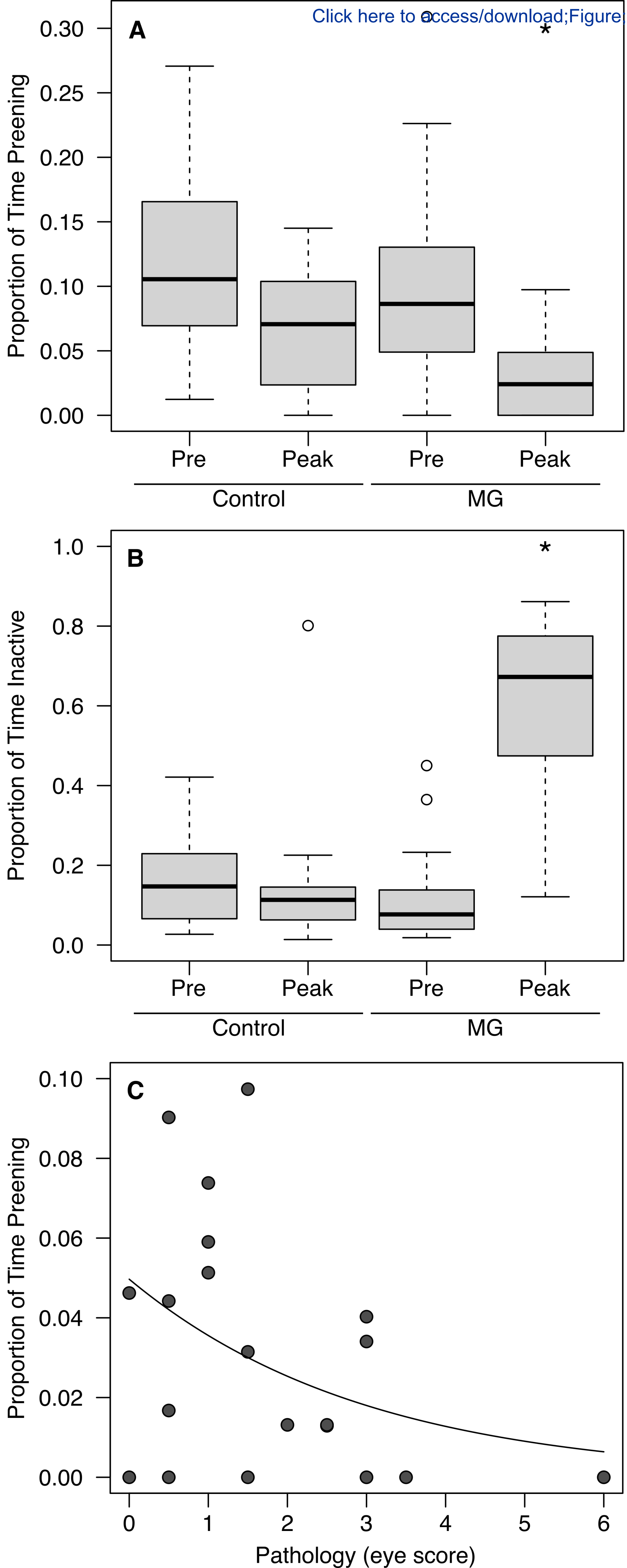
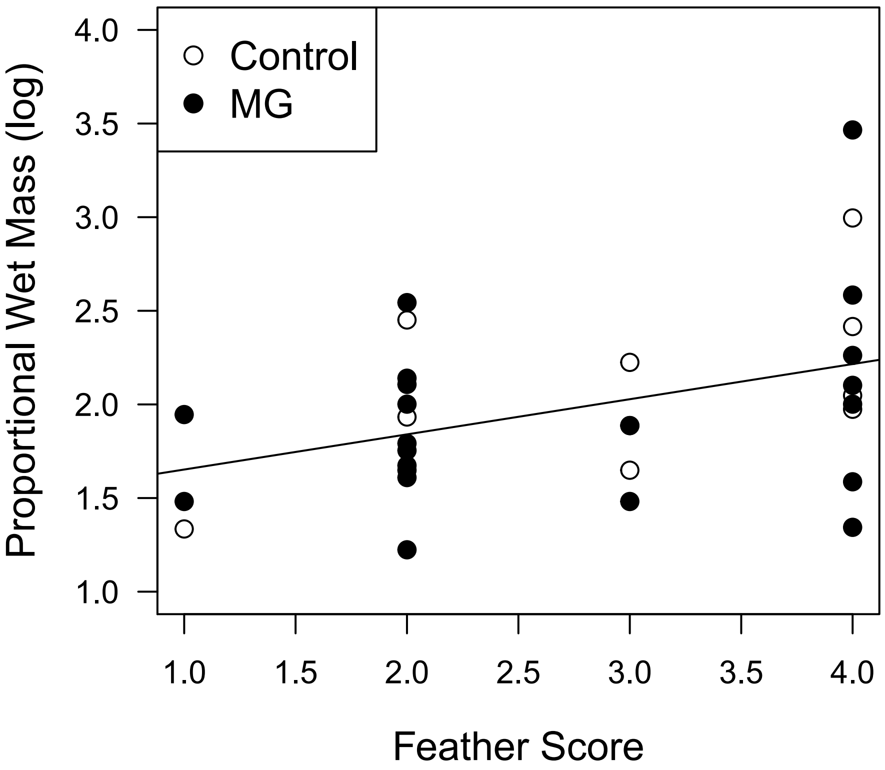

A
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Proportion of Time Preening
*
Pre
Peak
Pre
Peak
Control
MG
B
1.0
0.8
0.6
0.4
0.2
0.0
Proportion of Time Inactive
*
Pre
Peak
Pre
Peak
Control
MG
C
0.10
0.08
0.06
0.04
0.02
0.00
Proportion of Time Preening
0
1
2
3
4
5
6
Pathology (eye score)

Figure 3


Control
MG
Proportional Wet Mass (log)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Feather Score