Bacterial pathogen emergence required more than direct contact with a novel passerine host

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Running Head: Ecological processes alone cannot explain a host shift

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ABSTRACT

While direct contact may sometimes be sufficient to allow a pathogen to jump into a new host species, in other cases fortuitously adaptive mutations that arise in the original donor host are also necessary. Viruses have been the focus of most host shift studies, so less is known about the importance of ecological versus evolutionary processes to successful bacterial host shifts. Here we tested whether direct contact with the novel host was sufficient to enable the mid-1990s jump of the bacterium *Mycoplasma gallisepticum* from domestic poultry into house finches (*Haemorhous mexicanus*). We experimentally inoculated house finches with two genetically distinct *M. gallisepticum* strains obtained either from poultry (Rlow) or from house finches at epizootic outbreak (HF1995). All 15 house finches inoculated with HF1995 became infected, whereas Rlow successfully infected 12 of 15 (80%) inoculated house finches. Comparisons among infected birds showed that, relative to HF1995, Rlow achieved substantially lower bacterial loads in the host respiratory mucosa and was cleared faster. Furthermore, Rlow-infected finches were less likely to develop clinical symptoms than HF1995-infected birds and, when they did, displayed milder conjunctivitis. The lower infection success of Rlow relative to HF1995 was not, however, due to a heightened host antibody response to Rlow. Taken together, our results indicate that contact between infected poultry and house finches was not, by itself, sufficient to explain the jump of *M. gallisepticum* into house finches. Instead, mutations arising in the original poultry host would have been necessary for successful pathogen emergence in the novel finch host.

[250 words]
INTRODUCTION

Recent outbreaks of novel diseases in humans and domestic animals underscore the critical need to elucidate the factors that enable pathogens to become established in new host species (1-5). Host shifts require not only that pathogens come in direct contact with the novel host, but that they also have the capacity to infect and be transmitted by the new host (6, 7). Contact depends on opportunities for the pathogen to leave the original host and gain access to a novel host and as such is mitigated by the geographic ranges and ecologies of both the hosts and the pathogen (8). For instance, exposure of European rabbits (Oryctolagus cuniculus) to the myxoma virus during an eradication attempt in Australia mid-20th century was sufficient to allow for pathogen emergence, even though the virus’ natural host is a South American leporid rabbit (Sylvagus brasiliensis) (9, 10). Infectiousness and transmission, in contrast, will primarily be determined by pathogen and host genotypes (11, 12). For example, humans have long been in contact with pathogens of Himalayan palm civets (Paguma larvata), which are traditional food items in China. Despite this, there had been no known host shifts from civets into humans until the emergence of the severe acute respiratory syndrome (SARS) virus in 2002, then made possible by adaptive genetic changes in the virus’ receptor binding domain (13, 14). The extent to which hosts shifts are limited by opportunities for contact with novel hosts versus by fortuitous mutations that predispose the pathogen to infect the novel host remains, however, understudied despite the potential impacts on humans and livestock of hosts shifts by pathogens.

In recent decades, molecular analyses have revealed that host shifting by bacterial pathogens may be occurring more frequently than previously thought (15-18). For example, phylogenetic analyses suggest Wolbachia bacteria independently colonized multiple species of
arthropods via horizontal transmission (15, 19, 20). *Staphylococcus aureus* similarly exhibits a diverse host range including poultry, ruminants, and other mammals, likely the result of host shifting from humans (17, 18). Indeed, the jump of *S. aureus* from humans to rabbits only required a single mutation in a gene encoding an integral membrane protein (1). Yet, for other bacteria, there is evidence of more restrictive host ranges despite regular contact with other potential host species. For example, wood mice (*Apodemus sylvaticus*) and bank voles (*Myoedes glarionus*) in the United Kingdom harbor unique variants of *Bartonella*, despite fleas carrying bank vole-specific variants being collected from wood mice and vice versa (21). Experimental studies on cotton rats (*Sigmodon hispidus*) and white-footed mice (*Peromyscus leucopus*) similarly found that *Bartonella* infections were successful only when bacteria originated from the same host species or from their close phylogenetic relatives (22). An important limitation to understanding the role of contact versus host suitability in bacterial host shifts is that a majority of studies have focused on viral pathogens (11). Yet bacterial host shifts may be subject to different constraints than viral host shifts; unlike viruses, bacteria must also be able to extract essential metabolic substrates, nutrients, and enzymatic cofactors such as iron from their host and may face a suite of different host immune defenses (23). As a result, further studies are required to better understand the role of ecological versus evolutionary factors in bacterial host shifts.

One notable host shift by a bacterial pathogen occurred when *Mycoplasma gallisepticum* emerged in eastern North American house finches (*Haemorhous mexicanus*) in 1994. Comparative genomic analyses confirmed that this epizootic, which caused measurable declines in Eastern US house finch populations, resulted from a single host shift event of *M. gallisepticum* from poultry that occurred mid-1990s (2, 24-28). The subsequent spread of *M. gallisepticum*
throughout North American house finches was uniquely well documented thanks to externally visible symptoms of conjunctivitis, quick identification of *M. gallisepticum* as the causative agent, and active disease monitoring (2, 24). Since then, spillover infections have been documented in numerous other wild bird species (29, 30), although none have led to an epizootic-scale outbreak, the reason for which remains unclear.

To investigate whether direct contact was sufficient for *M. gallisepticum* to jump into house finches, we experimentally inoculated house finches either with a strain *M. gallisepticum* obtained from the original poultry host (Rlow) or with a strain collected at epizootic outbreak in the novel house finch host (HF1995) (26, 30-32). Whole genome comparisons have revealed that HF1995 exhibits widespread genomic changes relative to Rlow (26), but the functional significance of these genomic changes for colonizing the novel host remain unknown. We predicted that if contact with the novel host alone was sufficient for *M. gallisepticum* to infect house finches, then Rlow and HF1995 should display a similar ability to establish an infection and cause clinical disease in house finches. Conversely, if Rlow showed low or no capacity to infect house finches then it would support the hypothesis that mutations arising in the original poultry host would have been necessary for successful pathogen emergence in the novel finch host.

**RESULTS**

Over the course of the experiment, HF1995 successfully established an infection in the tracheal mucosa of all 15 house finches inoculated and Rlow in 12 of 15 (80%) inoculated birds ($\chi^2 = 1.5$, df = 1, p = 0.22). There was, however, a difference in the timing of the establishment
of infection. At 2 days post-infection (dpi), HF1995-inoculated birds were significantly more likely to test positive for infection: *M. gallisepticum* could be detected in 12 out of the 15 (80%) birds inoculated with HF1995, but in only 6 of the 15 (40%) birds inoculated with Rlow (logistic regression: $z = -2.2$, df = 1, $p = 0.03$). By 7 dpi, however, all the birds that became infected (i.e., 15 HF1995-birds and 12 Rlow-birds) tested positive for *M. gallisepticum*.

When considering only the birds that became infected, we found that HF1995-infected finches reached higher peak bacterial loads than those infected with Rlow (Mann-Whitney U Test, $W = 170$, $p < 0.0001$; Fig. 1). The number of days between inoculation and peak bacterial loads, however, did not significantly differ between treatments (Mann-Whitney U Test, $W = 104$, $p = 0.43$; mean ± standard deviation: Rlow = 9.3 ± 4.6 days, HF1995 = 11.2 ± 6.4 days). By the end of the experiment (56 dpi), all 12 Rlow-infected finches had cleared the infection, whereas 3 of 15 (20%) HF1995-infected birds remained positive for *M. gallisepticum* ($\chi^2 = 14.2$, df = 1, $p < 0.0002$). Additionally, Rlow-infected birds cleared the infection significantly faster than HF1995-infected ones (linear model: $t = -4.5$, $p < 0.001$; mean ± standard deviation: Rlow = 22.8 ± 13.5 days, HF1995 = 41.5 ± 5.3 days).

While 14 out of 15 finches (93%) inoculated with HF1995 developed clinical symptoms (i.e., conjunctivitis), only 5 out of 12 (42%) Rlow-infected individuals exhibited conjunctivitis. This difference was significant: Rlow-infected individuals exhibited a significantly lower probability of developing clinical symptoms than those inoculated with HF1995 ($z = -2.5$, $p = 0.01$). Furthermore, when considering symptomatic birds only, birds that were infected with
HF1995 developed significantly more severe conjunctivitis than birds infected with Rlow (Mann-Whitney U Test, \( W = 69, p < 0.001 \); Fig. 2).

Overall, there was a significant quadratic relationship between the production of \( M. \) gallisepticum-specific antibodies and time (linear mixed model; time: \( F_{1,53.9} = 35.8, p < 0.0001 \); time\(^2\): \( F_{1,54.5} = 8.2, p < 0.0001 \); Fig 3). However, the strength and pattern of this relationship differed significantly between the two treatment groups, leading to a significant treatment by time interaction (\( F_{1,53.9} = 8.2, p = 0.045 \)). For example, while HF1995 triggered antibody responses to increase at a rate of 0.06 ELISA units (EU)/ml between 7 and 14 dpi, Rlow did so at a rate of 0.01 EU/ml over that same duration; generating a 130% increase in the amplitude of the response of HF1995-inoculated birds relative to Rlow-inoculated ones (treatment: \( F_{1,58.8} = 18.6, p = 0.022 \)).

**DISCUSSION**

If the host shift of \( M. \) gallisepticum from poultry to house finches had simply been the result of house finches coming into contact with infected chickens, then we predicted that a virulent strain of poultry \( M. \) gallisepticum (Rlow; 31) should be able to successfully infect house finches upon inoculation. Contrary to this prediction, however, Rlow displayed a poor capacity to infect house finches relative to the 1995 house finch epizootic outbreak strain (HF1995). Indeed, compared to HF1995, Rlow achieved substantially lower bacterial loads following experimental inoculation, caused less severe clinical disease, and elicited weaker specific (i.e., antibody) immune responses in house finches. Additionally, house finches were able to clear Rlow much faster than HF1995. Taken together, our results indicate that contact with the novel...
host alone was insufficient to explain the host shift of *M. gallisepticum* from poultry and, instead, that genetic change(s) were also necessary for emergence into house finches.

Poultry and house finch strains are known to exhibit a number of genomic differences (26, 33), but identifying the specific mutation(s) underlying the host shift is challenging. For instance, relative to Rlow, HF1995 (cross-listed as GA_1995; (26)) displays a reduced CRISPR repeat diversity, numerous fixed, non-synonymous single nucleotide polymorphisms, and loss of 52 (or 8.6% of) known protein-coding genes through genomic deletions, disruption by novel IS insertion elements, or mutations leading to pseudogenization (26). However, in the light of our findings of a lower ability of Rlow to colonize the house finch respiratory mucosa and/or replicate within the novel finch host, functional divergence in genes coding for proteins involved in cytoadhesion (33) are of particular interest. We know from numerous other bacterial host-pathogen systems that mutations in genes that influence adherence to the host mucosal epithelium and host cell uptake of intracellular bacterial pathogens impact disease progression (34-37). Accordingly, the attenuated poultry strain of *M. gallisepticum*, Rhigh, exhibits low levels of host cell adherence and minimal pathology in poultry relative to Rlow (38). This is thought to result, in part, from the loss of expression of GapA and CmrA, which encode proteins involved in cytadherence (32, 38). Consistent with a dual role of adhesin molecules, Rhigh is also compromised in its ability to invade host cells, cross the poultry respiratory mucosa, and spread systemically (39-41). Other genes of potential interest include those that encode factors affecting metabolic capacity and that may play a role in the low pathogen load observed in this study. For example, transposon mutagenesis experiments revealed the metabolic enzyme dihydrolipoamide dehydrogenase, a subunit of a multi-enzyme involved in glycolysis, as a
That contact was not sufficient to allow *M. gallisepticum* to jump from poultry into house finches may explain why this pathogen, which is often found in other avian host species, seems unable to persist within these hosts (30, 43, 44). Infections of other passerine species are indeed thought to reflect spillover events from natural house finch host reservoirs (45). In support, a phylogenetic analysis of 107 *M. gallisepticum* strains from poultry, house finches, and other songbirds revealed that all isolates obtained from non-house finch songbirds clustered with house finch rather than poultry isolates (46). Furthermore, while house finch *M. gallisepticum* can infect a diverse array of passerines, it is pathogenic (i.e., causes conjunctivitis) only to closely related species within the family Fringillidae, such as purple finches (*Haemorhous purpureus*) and American goldfinches (*Carduelis carduelis*) (30, 43). Indeed, evidence for *M. gallisepticum* exposure, either via positive PCR-based detection of *M. gallisepticum* or positive antibody tests, was found in 27 species of wild birds representing 15 families, but clinical disease signs were rare or completely absent in species outside of family Fringillidae (29). Taken together, these studies suggest that transmission is occurring regularly from house finches to other avian species as a result of contact, but that contact alone is insufficient to enable MG to jump into any of these novel hosts. This is consistent with our findings: poultry *M. gallisepticum* is able to colonize mucosal surfaces of individual house finches, but is somehow compromised in its ability to replicate, persist within, and/or cause pathology to the novel house finch host.
Our findings may also shed light on a phylogenetic study that found a singular *M. gallisepticum* strain collected from an asymptomatic house finch to be more closely related to poultry *M. gallisepticum* strains than house finch *M. gallisepticum* strains (46). Indeed, if contact alone was sufficient for *M. gallisepticum* emergence in house finches, then this occurrence should be more common; yet house finch *M. gallisepticum* strains examined to date have been shown to be derived from a single ancestor (26, 33). Given that the sampling of *M. gallisepticum* strains from house finches is both sporadic and conducted randomly, finding a *M. gallisepticum* strain closely related to poultry in house finches should be extremely unlikely unless spill-overs of poultry *M. gallisepticum* into house finches through contact occur more frequently than expected. The unique outbreak of severe conjunctivitis in house finches attests to the fact that these spill-overs are generally unsuccessful and that the genetic changes required for host shifts are themselves extremely rare.

**MATERIALS AND METHODS**

**House finch capture, housing, and experimental infection.** We trapped male house finches at bird feeder sites in Alabama, USA, between August-September 2014 (as described in (47)). All birds used in the study were yearlings, having hatched in the spring of the calendar year in which they were collected. We collected birds from three sites in Auburn approximately 1.5 miles apart and two sites in Birmingham separated by 8 miles. Upon capture, a blood sample (~70µl) and choanal swab was collected from each bird. Choanal swabbing consisted of inserting a swab into the bird’s oral cavity and then swabbing the tracheal opening and choanal cleft for approximately 15 seconds. Blood plasma was used for a serum plate agglutination assay to test
for anti-\textit{M. gallisepticum} antibodies, indicating prior \textit{M. gallisepticum} exposure (48). Swabs were used for PCR amplification of \textit{M. gallisepticum} DNA to test for current infection (49). Birds positive for either test were immediately released and not retained for the experiment. The remaining birds then underwent a 30-day quarantine period, during which they were treated for infection by \textit{Trichomonas gallinae} and \textit{Coccidia spp}. Following quarantine, birds were randomly divided into treatment groups. Males in one treatment group (N=15) were inoculated with an epizootic outbreak house finch \textit{M. gallisepticum} strain cultured from a wild-caught house finch in Georgia, USA, in 1995 (HF1995; passage 13; cross-listed as GA_1995 in (26)). Males in the second treatment group (N=15) were inoculated with a poultry \textit{M. gallisepticum} strain (Rlow; passage 17), which was provided by Dr. Naola Ferguson-Noel (University of Georgia). We inoculated birds by dropping 10 μl of the respective \textit{M. gallisepticum} culture into each eye, each containing approximately $1 \times 10^4$ to $1 \times 10^6$ color-changing units/ml of \textit{M. gallisepticum}. To prevent \textit{M. gallisepticum} transmission between treatments, we housed finches in separate rooms under identical conditions. Following inoculation, we monitored finches for the development of infection for 8 weeks (56 days), at which time all birds were humanely euthanized by CO$_2$ narcosis in accordance with the rules established by the 2013 American Veterinary Medical Association Guidelines on Euthanasia. We also took a choanal (tracheal) swab sample on these days to test for the establishment of an \textit{M. gallisepticum} infection and pathogen load using quantitative PCR. All described work was approved by Auburn University’s IACUC under PRN 2014-2517 and Biological Use Authorization 500.

\textbf{Quantification of clinical disease severity.} To document clinical disease signs (conjunctivitis), we photographed the right and left eyes of each bird, with the bird’s eye parallel...
to the camera. We then quantified the area of the conjunctiva swelling in the photographs using the programs TpsUtil ver. 1.46 and TpsDig ver. 2.16 [http://life.bio.sunysb.edu/morph/](http://life.bio.sunysb.edu/morph/) (50, 51).

Bill depth was measured with calipers to 0.1mm. This measurement was then used as a scale in the images so the area of conjunctival swelling in mm^2 could be assessed. The scaled picture files were then duplicated, with one file used for the placement of ten landmarks around the inner ring of the conjunctiva. The duplicate file was used to place twelve landmarks around the outer area of the conjunctiva. Area measurements (mm^2) for the outer and inner rings of the conjunctiva were generated using TpsUtil. The area of the conjunctiva was then calculated as the area of the outer ring minus the area of the inner ring. To determine swelling severity, we subtracted the conjunctiva area at Day 0 (pre-inoculation) for a given individual from the area measured at a given sampling time point for that same individual. We estimated the background variation in our measurements by repeating this process with photographs of control birds, using twice the average background variation as the threshold for considering birds to display clinical conjunctiva swelling. The threshold value was subtracted from all measurements, with any values below the threshold being treated as having no, or zero, change in swelling.

**M. gallisepticum presence and load.** We tested the respiratory epithelium of all house finches for *M. gallisepticum* at 0, 2, 7, 14, 21, 28, 42, and 56 dpi. Choanal swabs were tested for the presence of *M. gallisepticum* via PCR followed by agarose gel electrophoresis (49). Briefly, swabs were placed in 100 μl of sterile nuclease free water. Swabs were then placed at 100 °C for 10 minutes, at -20 °C for 10 minutes, and finally centrifuged at 13000 rpm for 5 minutes. We tested the supernatant of each sample in duplicate for *M. gallisepticum* presence using the forward primer 5’ GCTTCCTTGGTTAGCAAC 3’ and reverse primer 5’
GAGCTAATCTGTAAAGTTGGTC 3’. PCR parameters were as follows: 94° C for 5 minutes, 35 cycles of 94° C for 30 seconds, 55° C for 30 seconds, and 72° C for 30 seconds, and a final 5 minute extension at 72° C (49). In each assay, M. gallisepticum DNA extracted from pure culture served as a positive control.

For all M. gallisepticum-positive samples, we then quantified bacterial loads using the remaining choanal swab extract and a TaqMan qPCR amplification of the single-copy M. gallisepticum gene, mgc2. To control for variation in the amount of starting material, we also amplified a single copy house finch gene, rag1. The detection limit of this assay was previously reported to be less than 10 genomic copies (52). For HF1995- and Rlow-inoculated individuals, we confirmed the timing or lack of M. gallisepticum colonization by also performing qPCR on samples that were negative for M. gallisepticum at 2 and 7 dpi. To confirm M. gallisepticum clearance, we additionally ran qPCR on the sample collected after the last M. gallisepticum-positive sample for each bird. Before use, we cleaned up extracted swab samples using a Qiagen QIAquick PCR purification kit. All reactions were run on an ABI Prism 7500 (Applied Biosystems). We made a standard curve of pooled genomic DNA to estimate the relative amount of M. gallisepticum between individuals. We then divided the number of mgc2 genes by one half the number of rag1 genes to approximate the ratio of M. gallisepticum cells (haploid) to host cells (diploid).

M. gallisepticum-specific antibody (Immunoglobulin Y, IgY) detection. Comparisons of M. gallisepticum-specific antibody concentrations in plasma at 7, 14, 21, and 28 dpi were made using an enzyme-linked immunosorbent assay (ELISA) and a standard curve of pooled
house finch plasma from experimental house finches. Briefly, the goat anti-passerine IgY secondary antibody (53) was conjugated to horseradish peroxidase (HRP) using a Lightning Link® HRP kit (Innova Biosciences) following the manufacturer’s instructions. Samples and standards were diluted in 1X sample/conjugate diluent (Affinitech, LTD) and then 100ul of each was plated in duplicate onto *M. gallisepticum*-coated plates (Affinitech, LTD). After a one-hour incubation at room temperature (RT), plates were washed three times with 1X wash buffer (50 mM Tris buffered saline, pH 8.0, with 0.05% Tween-20; Bethyl Laboratories). The HRP-conjugated antibody was diluted 1:10,000 in sample/conjugate diluent (50 mM Tris buffered saline, pH 8.0, with 1% bovine serum albumin and 0.05% Tween-20; Bethyl Laboratories) and 100ul of the diluted antibody was then added to each well. Plates were incubated for one hour at RT and then washed three times in 1X wash buffer. 100ul of the enzyme substrate, TMB One Component HRP Microwell Substrate (Bethyl Laboratories), was added to each well and plates were incubated at RT for 15 minutes. The reaction was then stopped with 100ul of ELISA stop buffer (0.18 M H$_2$SO$_4$; Bethyl Laboratories) and plates were read at 450 nm using a BioTek PowerWave XS plate reader. Samples were considered positive for *M. gallisepticum*-specific antibodies if the absorbance at 450nm was at least three times the background. For the pooled standard curve, this cutoff was between a dilution of 1:6,400 and 1:12,800. All samples were run at the same dilution, allowing for comparison of concentrations between treatments based on the pooled standard curve, but not determination of absolute concentrations. As such, antibody concentrations are reported as ELISA Units (EU)/ml, with the starting concentration in the undiluted pooled sample being arbitrarily assigned.
Statistical analyses. All statistical analyses were conducted in R (R Core Team, 2012). We tested for differences in the ability of HF1995 and Rlow to establish an infection using a Chi-squared test. Differences in the probability that birds were infected at 2 dpi were tested using a logistic regression with infection status (infected/not infected) as the response variable and treatment (HF1995 or Rlow) as the explanatory term. Differences in the peak bacterial load and in the time of clearance of the infection were modelled in infected individuals only and by performing Mann-Whitney U tests, with either peak bacterial load or date of clearance as the response variables, and with treatment as the explanatory term. We tested for differences in the ability of HF1995- and Rlow-inoculated birds to clear infection using a Chi-squared test. Differences in the probability of developing clinical symptoms were modelled using a logistic regression with clinical symptoms (0/1) as the response variable and treatment as the explanatory term. We then investigated differences in the severity of conjunctivitis as a function of treatment in symptomatic individuals only using a Mann-Whitney U test with peak conjunctivitis as the response variable and treatment as the explanatory term. To test for differences in circulating levels of anti-*M. gallisepticum* antibodies over time (i.e., between 7 and 28 dpi) in infected individuals, we used lme4 and performed a generalised linear mixed model with antibody concentration as the response term and with time, treatment and their interaction as explanatory terms; individual identity was specified as the random effect (54). All figures were made using ggplot2 (55).

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REFERENCES

1. Viana D, Comos M, McAdam PR, Ward MJ, Selva L, Guinane CM, Gonzalez-Munoz BM, Tristan A, Foster SJ, Fitzgerald JR, Penades JR. 2015. A single natural nucleotide mutation alters bacterial pathogen host tropism. Nature genetics 47:361-366.

2. Ley DH, Berkhoff JE, McLaren JM. 1996. Mycoplasma gallisepticum isolated from house finches (Carpodacus mexicanus) with conjunctivitis. Avian diseases 40:480-483.

3. Kramer LD, Bernard KA. 2001. West Nile virus infection in birds and mammals. Annals of the New York Academy of Sciences 951:84-93.

4. Chen H, Smith GJ, Zhang SY, Qin K, Wang J, Li KS, Webster RG, Peiris JS, Guan Y. 2005. Avian flu: H5N1 virus outbreak in migratory waterfowl. Nature 436:191-192.

5. Liu J, Xiao H, Lei F, Zhu Q, Qin K, Zhang XW, Zhang XL, Zhao D, Wang G, Feng Y, Ma J, Liu W, Wang J, Gao GF. 2005. Highly pathogenic H5N1 influenza virus infection in migratory birds. Science 309:1206.
Woolhouse ME, Haydon DT, Antia R. 2005. Emerging pathogens: the epidemiology and evolution of species jumps. Trends in ecology & evolution 20:238-244.

Lambrechts L. 2010. Dissecting the genetic architecture of host-pathogen specificity. PLoS pathogens 6:e1001019.

Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, Delicat A, Paweska JT, Gonzalez JP, Swanepoel R. 2005. Fruit bats as reservoirs of Ebola virus. Nature 438:575-576.

Kerr PJ. 2012. Myxomatosis in Australia and Europe: A model for emerging infectious diseases. Antiviral research 93:387-415.

Kerr PJ, Ghedin E, DePasse JV, Fitch A, Cattadori IM, Hudson PJ, Tscharke DC, Read AF, Holmes EC. 2012. Evolutionary history and attenuation of myxoma virus on two continents. PLoS pathogens 8:e1002950.

Parrish CR, Holmes EC, Morens DM, Park EC, Burke DS, Calisher CH, Laughlin CA, Saif LJ, Daszak P. 2008. Cross-species virus transmission and the emergence of new epidemic diseases. Microbiology and molecular biology reviews : MMBR 72:457-470.

Taubenberger JK, Kash JC. 2010. Influenza virus evolution, host adaptation, and pandemic formation. Cell host & microbe 7:440-451.

Song HD, Tu CC, Zhang GW, Wang SY, Zheng K, Lei LC, Chen QX, Gao YW, Zhou HQ, Xiang H, Zheng HJ, Chern SW, Cheng F, Pan CM, Xuan H, Chen SJ, Luo HM, Zhou DH, Liu YF, He JF, Qin PZ, Li LH, Ren YQ, Liang WJ, Yu YD, Anderson L, Wang M, Xu RH, Wu XW, Zheng HY, Chen JD, Liang G, Gao Y, Liao M, Fang L, Jiang LY, Li H, Chen F, Di B, He LJ, Lin JY, Tong S, Kong X, Du L,
Hao P, Tang H, Bernini A, Yu XJ, Spiga O, Guo ZM, Pan HY, He WZ, Manuguerra JC, Fontanet A, Danchin A, Niccolai N, Li YX, Wu CI, Zhao GP. 2005. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proceedings of the National Academy of Sciences of the United States of America 102:2430-2435.

Li F. 2008. Structural analysis of major species barriers between humans and palm civets for severe acute respiratory syndrome coronavirus infections. Journal of virology 82:6984-6991.

Kraaijeveld K, Franco P, de Knijff P, Stouthamer R, van Alphen JJ. 2011. Clonal genetic variation in a Wolbachia-infected asexual wasp: horizontal transmission or historical sex? Molecular ecology 20:3644-3652.

Guinane CM, Ben Zakour NL, Tormo-Mas MA, Weinert LA, Lowder BV, Cartwright RA, Smyth DS, Smyth CJ, Lindsay JA, Gould KA, Witney A, Hinds J, Bollback JP, Rambaut A, Penades JR, Fitzgerald JR. 2010. Evolutionary genomics of Staphylococcus aureus reveals insights into the origin and molecular basis of ruminant host adaptation. Genome biology and evolution 2:454-466.

Lowder BV, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, Cartwright RA, Simpson AJ, Rambaut A, Nubel U, Fitzgerald JR. 2009. Recent human-to-poultry host jump, adaptation, and pandemic spread of Staphylococcus aureus. Proceedings of the National Academy of Sciences of the United States of America 106:19545-19550.
18. Weinert LA, Welch JJ, Suchard MA, Lemey P, Rambaut A, Fitzgerald JR. 2012. Molecular dating of human-to-bovid host jumps by *Staphylococcus aureus* reveals an association with the spread of domestication. Biology letters 8:829-832.

19. Le Clec'h W, Braquart-Varnier C, Raimond M, Ferdy JB, Bouchon D, Sicard M. 2012. High virulence of *Wolbachia* after host switching: when autophagy hurts. PLoS pathogens 8:e1002844.

20. Werren JH, Baldo L, Clark ME. 2008. *Wolbachia*: master manipulators of invertebrate biology. Nature reviews. Microbiology 6:741-751.

21. Withenshaw SM, Devevey G, Pedersen AB, Fenton A. 2016. Multihost Bartonella parasites display covert host specificity even when transmitted by generalist vectors. J Anim Ecol 85:1442-1452.

22. Kosoy MY, Saito EK, Green D, Marston EL, Jones DC, Childs JE. 2000. Experimental evidence of host specificity of Bartonella infection in rodents. Comparative immunology, microbiology and infectious diseases 23:221-238.

23. van Baarlen P, van Belkum A, Summerbell RC, Crous PW, Thomma BP. 2007. Molecular mechanisms of pathogenicity: how do pathogenic microorganisms develop cross-kingdom host jumps? FEMS microbiology reviews 31:239-277.

24. Fischer JR, Stallknecht DE, Luttrell MP, Dondt AA, Converse KA. 1997. Mycoplasmal conjunctivitis in wild songbirds: the spread of a new contagious disease in a mobile host population. Emerging infectious diseases 3:69-72.

25. Dondt AA, Tessaglia DL, Slothower RL. 1998. Epidemic mycoplasmal conjunctivitis in house finches from Eastern North America. Journal of wildlife diseases 34:265-280.
26. Delaney NF, Balenger S, Bonneaud C, Marx CJ, Hill GE, Ferguson-Noel N, Tsai P, Rodrigo A, Edwards SV. 2012. Ultrafast evolution and loss of CRISPRs following a host shift in a novel wildlife pathogen, *Mycoplasma gallisepticum*. PLoS genetics 8:e1002511.

27. Nolan PM, Hill GE, Stoehr AM. 1998. Sex, size, and plumage redness predict house finch survival in an epidemic. P Roy Soc B-Biol Sci 265:961-965.

28. Hochachka WM, Dhondt AA. 2000. Density-dependent decline of host abundance resulting from a new infectious disease. Proceedings of the National Academy of Sciences of the United States of America 97:5303-5306.

29. Dhondt AA, DeCoste JC, Ley DH, Hochachka WM. 2014. Diverse wild bird host range of *Mycoplasma gallisepticum* in eastern North America. PloS one 9:e103553.

30. Farmer KL, Hill GE, Roberts SR. 2005. Susceptibility of wild songbirds to the house finch strain of *Mycoplasma gallisepticum*. Journal of wildlife diseases 41:317-325.

31. Yogev D, Levisohn S, Kleven SH, Halachmi D, Razin S. 1988. Ribosomal RNA gene probes to detect intraspecies heterogeneity in Mycoplasma gallisepticum and M. synoviae. Avian diseases:220-231.

32. Papazisi L. 2003. The complete genome sequence of the avian pathogen *Mycoplasma gallisepticum* strain Rlow. Microbiology 149:2307-2316.

33. Tulman ER, Liao X, Szczepanek SM, Ley DH, Kutish GF, Geary SJ. 2012. Extensive variation in surface lipoprotein gene content and genomic changes associated with virulence during evolution of a novel North American house finch epizootic strain of *Mycoplasma gallisepticum*. Microbiology 158:2073-2088.
34. **Leung KY, Finlay BB.** 1991. Intracellular replication is essential for the virulence of *Salmonella typhimurium*. Proceedings of the National Academy of Sciences **88**:11470-11474.

35. **Sieira R, Comerci DJ, Sánchez DO, Ugalde RA.** 2000. A homologue of an operon required for DNA transfer in Agrobacterium is required in *Brucella abortus* for virulence and intracellular multiplication. Journal of bacteriology **182**:4849-4855.

36. **Parsons DA, Heffron F.** 2005. sciS, an icmF homolog in *Salmonella enterica* serovar *Typhimurium*, limits intracellular replication and decreases virulence. Infect. Immun. **73**:4338-4345.

37. **Bhavsar AP, Guttman JA, Finlay BB.** 2007. Manipulation of host-cell pathways by bacterial pathogens. Nature **449**:827-834.

38. **Papazisi L, Frasca S, Gladd M, Liao X, Yogev D, Geary SJ.** 2002. GapA and CrmA coexpression is essential for *Mycoplasma gallisepticum* cytadherence and virulence. Infect. Immun. **70**:6839-6845.

39. **Much P, Winner F, Stipkovits L, Rosengarten R, Citti C.** 2002. *Mycoplasma gallisepticum*: Influence of cell invasiveness on the outcome of experimental infection in chickens. FEMS immunology and medical microbiology **34**:181-186.

40. **Winner F, Rosengarten R, Citti C.** 2000. In vitro cell invasion of *Mycoplasma gallisepticum*. Infect Immun **68**:4238-4244.

41. **Indikova I, Much P, Stipkovits L, Siebert-Gulle K, Szostak MP, Rosengarten R, Citti C.** 2013. Role of the GapA and CrmA cytadhesins of *Mycoplasma gallisepticum* in promoting virulence and host colonization. Infect Immun **81**:1618-1624.
467 42. Hudson P, Gorton TS, Papazisi L, Cecchini K, Frasca S, Jr., Geary SJ. 2006. Identification of a virulence-associated determinant, dihydrolipoamide dehydrogenase (lpd), in Mycoplasma gallisepticum through in vivo screening of transposon mutants. Infect Immun 74:931-939.

471 43. Dhondt AA, Dhondt KV, McCleery BV. 2008. Comparative infectiousness of three passerine bird species after experimental inoculation with Mycoplasma gallisepticum. Avian pathology : journal of the W.V.P.A 37:635-640.

474 44. Stallknecht DE, Luttrell MP, Fischer JR, Kleven SH. 1998. Potential for transmission of the finch strain of Mycoplasma gallisepticum between house finches and chickens. Avian diseases 42:352-358.

477 45. Hartup BK, Dhondt AA, Sydenstricker KV, Hochachka WM, Kollias GV. 2001. Host range and dynamics of mycoplasmal conjunctivitis among birds in North America. Journal of wildlife diseases 37:72-81.

480 46. Hochachka WM, Dhondt AA, Dobson A, Hawley DM, Ley DH, Lovette IJ. 2013. Multiple host transfers, but only one successful lineage in a continent-spanning emergent pathogen. Proceedings. Biological sciences / The Royal Society 280:20131068.

483 47. Hill GE. 2002. A red bird in a brown bag : the function and evolution of colorful plumage in the House Finch. Oxford University Press, Oxford ; New York.

485 48. Luttrell MP, Fischer JR, Stallknecht DE, Kleven SH. 1996. Field investigation of Mycoplasma gallisepticum infections in house finches (Carpodacus mexicanus) from Maryland and Georgia. Avian diseases 40:335-341.
488 49. Roberts SR, Nolan PM, Hill GE. 2001. Characterization of *Mycoplasma gallisepticum*
489  infection in captive house finches (*Carpodacus mexicanus*) in 1998. Avian diseases
490 45:70-75.
491 50. Rohlf FJ. 2010. TpsDig 2, 2.16 ed.
492 51. Rohlf FJ. 2010. TpsUtil: File Utility Program, 1.46 ed.
493 52. Grodio JL, Dhondt KV, O’Connell PH, Schat KA. 2008. Detection and quantification
494  of *Mycoplasma gallisepticum* genome load in conjunctival samples of experimentally
495  infected house finches (*Carpodacus mexicanus*) using real-time polymerase chain
496  reaction. Avian pathology : journal of the W.V.P.A 37:385-391.
497 53. Fassbinder-Orth CA, Wilcoxen TE, Tran T, Boughton RK, Fair JM, Hofmeister
498  EK, Grindstaff JL, Owen JC. 2016. Immunoglobulin detection in wild birds:
499  effectiveness of three secondary anti-avian IgY antibodies in direct ELISAs in 41 avian
500  species. Methods in ecology and evolution 7:1174-1181.
501 54. Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models
502  Using lme4. Journal of Statistical Software 67:1-48.
503 55. Wickham H. 2009. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New
504  York.
FIGURE LEGENDS

FIG 1. Boxplot diagram showing peak bacterial load, estimated as the peak number of bacterial
to host cells over the course of the experiment, in infected house finches following inoculation
with either a poultry strain of *M. gallisepticum* (Rlow) or a house finch epizootic-outbreak
isolate (HF1995). Boxplot show the median and range peak load, with significantly lower peaks
in Rlow- (n=15; median = 0.025, range = 0.0006-0.37) relative to HF1995- (n = 12; median =
5.09, range = 0.001-17.3) inoculated birds. The dots show the raw values.

FIG 2. Boxplot diagram showing clinical symptom severity (in mm²) in infected house finches
following inoculation with either a poultry strain of *M. gallisepticum* (Rlow) or a house finch
epizootic-outbreak isolate (HF1995). Boxplot show the median and range conjunctival swelling,
with significantly lower levels in Rlow- (n=14; median = 0.33, range = 0.05-0.92) than in
HF1995- (n = 5; median = 5.9, range = 0.9-13.5) inoculated birds. The dots show the raw values.

FIG 3. Circulating levels of specific anti-*M. gallisepticum* antibodies in infected house finches
inoculated with either a poultry strain (Rlow) or a house finch epizootic-outbreak isolate
(HF1995) over time (i.e., between 7 to 28 dpi); concentrations are reported as ELISA Units
(EU)/ml based on arbitrarily assigned starting concentration of the undiluted pooled plasma
sample used to create the standard curve. We show raw values of antibody concentrations in
HF1995- (open circles) and Rlow- (full triangles) infected finches, and best fit regression lines.
