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House Finch Populations Differ in Early Inflammatory Signaling and Pathogen Tolerance at the Peak of *Mycoplasma gallisepticum* Infection

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ABSTRACT: Host individuals and populations often vary in their responses to infection, with direct consequences for pathogen spread and evolution. While considerable work has focused on the mechanisms underlying differences in resistance—the ability to kill pathogens—we know little about the mechanisms underlying tolerance—the ability to minimize fitness losses per unit pathogen. Here, we examine patterns and mechanisms of tolerance between two populations of house finches (*Haemorhous* [formerly *Carpodacus*] *mexicanus*) with different histories with the bacterial pathogen *Mycoplasma gallisepticum* (MG). After infection in a common environment, we assessed two metrics of pathology, mass loss and eye lesion severity, as proxies for fitness. We calculated tolerance using two methods, one based on pathology and pathogen load at the peak of infection (point tolerance) and the other based on the integrals of these metrics over time (range tolerance). Alabama birds, which have a significantly longer history of exposure to MG, showed more pronounced point tolerance than Arizona birds, while range tolerance did not differ between populations. Alabama birds also displayed lower inflammatory cytokine signaling and lower fever early in infection. These results suggest that differences in inflammatory processes, which can significantly damage host tissues, may contribute to variation in tolerance among house finch individuals and populations. Such variation can affect pathogen spread and evolution in ways not predictable by resistance alone and sheds light on the costs and benefits of inflammation in wild animals.

Keywords: disease ecology, emerging infectious disease, house finches, inflammation, tolerance, resistance, *Mycoplasma gallisepticum.*

Introduction

The mechanisms underlying the extraordinary variation in how individuals, populations, and species respond to infectious agents have fascinated the medical and biological communities for decades (Schmid-Hempel 2011). Uncovering these mechanisms can reveal predictive markers of disease susceptibility, clearance, or transmission (Long et al. 2008; Poland et al. 2008; Vollmer-Conna et al. 2008). Despite progress on the molecular and immunological basis of resistance, or a host's ability to reduce pathogen numbers, relatively less attention has focused on an alternative yet fundamental host strategy for responding to infectious agents: tolerance, or the ability to minimize the per-pathogen reduction in host fitness (Jokela et al. 2000; Rausher 2001; Råberg et al. 2007, 2009). While empirical and theoretical interest in tolerance has grown in recent years (Jokela et al. 2000; Rausher 2001; Restif and Koella 2003, 2004; Råberg et al. 2007, 2009), few studies have examined variation in tolerance for ecologically relevant diseases of wild animals (but see Rohr et al. 2010 and Tobler and Schmidt 2010). Moreover, the physiological mechanisms that enable animals to tolerate infection remain unclear. Because the extent to which hosts tolerate rather than resist infections has far-reaching implications for pathogen evolution and spread (Roy and Kirchner 2000; Pfennig 2001; Ferrari et al. 2004; Lloyd-Smith et al. 2005; Miller et al. 2006; Carval and Ferriere 2010; Little et al. 2010; Long and Boots 2011), understanding the mechanisms that underlie variation in resistance and tolerance in naturally occurring host-parasite systems is particularly important.

One mechanism proposed to drive variation in resistance and tolerance in animals involves the balance between pro- and anti-inflammatory immunological signaling (Råberg et al. 2009; Sears et al. 2011). Inflammatory responses include both local and systemic processes, such as localized swelling and infiltration of white blood cells, production of reactive oxygen species, and fever (Janeway et al. 2005; Medzhitov 2008). While such responses effectively kill diverse types of pathogens, they carry high risks of immunopathology, or damage to a host's own tissues

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(Kluger 1991; Graham et al. 2005; Medzhitov 2008; Ashley et al. 2012). When engaging inflammatory responses, hosts must balance the damage resulting directly from the parasite's actions against the damage resulting from the immune system itself (Rolff and Siva-Jothy 2003; Graham et al. 2005, 2010, 2011; Viney et al. 2005). By reducing the degree of inflammation, hosts may be able to reduce immunopathology, limiting total damage and moving toward a more tolerant phenotype (Råberg et al. 2009; Sears et al. 2011). For diseases involving inflammation, vertebrate hosts may increase tolerance by reducing production of proinflammatory signals, including the cytokines interleukin 1β (IL-1β), interferon-γ, and tumor necrosis factor-α (TNF-α), or by increasing production of anti-inflammatory signals, including cytokines such as interleukin 10 (IL-10) and transforming growth factor-β (Råberg et al. 2009; Sears et al. 2011).

Here, we ask whether links between resistance, tolerance, and early inflammation exist in a recently emerged host-pathogen system: house finches (*Haemorhous mexicanus*) infected with *Mycoplasma gallisepticum* (MG). This bacterial pathogen first jumped from poultry to house finches in the early 1990s (Ley et al. 1996; Dhondt et al. 1998). MG causes severe conjunctivitis in house finches and has been related to population declines in the eastern half of North American (Hochachka and Dhondt 2000). In house finches, as well as chickens, this pathogen is known to induce proinflammatory responses at both systemic and local levels, including proinflammatory cytokine production, local infiltration of lymphocytes and heterophils, and fever, all of which can increase damage to host cells (Luttrell et al. 1998; Mohammed et al. 2007; Hawley et al. 2012). Therefore, this system is particularly well suited to examining pro- and anti-inflammatory responses in relation to resistance and tolerance.

The house finch–MG system is also particularly interesting for examining population differences in tolerance because of the contrasting histories of host populations with this pathogen. Since its initial detection in house finches, MG spread rapidly through populations east of the Rocky Mountains (Dhondt et al. 1998) but did not reach western North America until approximately 10 years after emergence (Ley et al. 1996; Dhondt et al. 2006). Thus, eastern populations of house finches have a distinctly longer history with MG than do western populations. Recent work on two house finch populations, one in the West with no history of MG (Arizona) and one in the East with a 12-year history of MG at the time of the study (Alabama; Nolan et al. 1998), documented differences in resistance to MG consistent with their histories with this pathogen (Bonneaud et al. 2011). Bonneaud and colleagues (2011) showed that after 2 weeks of infection, Alabama birds showed significantly lower pathogen load, or higher resistance, than did Arizona birds. Given the high potential for costly inflammation during MG infection, we hypothesized that Alabama birds should also exhibit a phenotype more tolerant than that of Arizona birds. Importantly, theoretical work suggests that higher tolerance and higher resistance are not necessarily mutually exclusive if the relative costs of these responses are similar (Restif and Koella 2003, 2004).

Here, we utilize birds from the same eastern and western populations studied by Bonneaud and colleagues (2011), Alabama (AL) and Arizona (AZ), to determine whether tolerance and components of the costly inflammatory response to MG infection differ between populations. Because many *Mycoplasma* species express immunomodulatory and evasive mechanisms in their coevolved hosts, including the downregulation of inflammatory signaling and suppression of both B- and T-cell responses (Razin et al. 1998; Ganapathy and Bradbury 2003; Gaunson et al. 2006; Mohammed et al. 2007), we sought to minimize any such effects in our study. We therefore used the earliest MG isolate available from house finches, obtained in 1994, shortly after the pathogen's initial jump from chickens (Ley et al. 1996). This method limited the chance that differences between hosts were the result of pathogen adaptation to a specific house finch population.

To assess differences in tolerance and inflammation between these populations, we experimentally infected MG-naive captive finches and measured pathogen load and two components of pathology, eye lesion severity and mass loss, which served as proxies for fitness costs of infection. To ascertain how pro- and anti-inflammatory signaling differ, we measured relative messenger RNA (mRNA) expression of the proinflammatory cytokine IL-1β and the anti-inflammatory cytokine IL-10 in peripheral blood. We also measured fever and sickness behavior, two whole-body components of the early inflammatory response to infection (Hart 1988; Kluger 1991). Finally, because antibody responses against *Mycoplasma* can be important for both pathogen clearance and immunopathology (Razin et al. 1998) and thus potentially contribute to both resistance and tolerance, we measured MG-binding immunoglobulin Y (IgY) production.

Methods

Host Species and Capture Locations

House finches are socially monogamous passerines whose range covers most of North America (Badyaev et al. 2012). The species was originally native to western North America. A small, introduced population on the East Coast has expanded since the mid-1900s to cover most of the United States east of the Rockies (Badyaev et al. 2012). Studies of

neutral genetic markers and major histocompatibility complex (MHC) diversity reveal differences between eastern and western populations that are consistent with genetic isolation (Hawley et al. 2006*a*, 2008; Hawley and Fleischer 2012). In addition, western populations are largely sedentary (with no known migration in AZ; Badyaev et al. 2012), whereas limited migration has been documented in southeastern-US populations along a southwest-northeast axis (Able and Belthoff 1998).

In February 2010, eight adult house finches were captured in feeder traps near Arizona State University, in Tempe (33°25′39.30″N, 111°56′55.96″W). In September 2010, 11 additional adults were captured in feeder traps on or near the Auburn University campus, Auburn, Alabama (32°25′29.78″N, 85°29′13.59″W). Birds were transported to Virginia Tech via commercial aircraft (Arizona) or automobile (Alabama) and housed in groups of two or three in indoor cages (76 cm × 46 cm × 46 cm) on a 12L : 12D cycle for at least 4 months before the start of experiments. All animals used in this experiment showed no clinical signs of MG infection and were seronegative for anti-MG antibodies. A subset of birds captured in Alabama for other experiments (~10%–20%) showed clinical signs of MG during a 2-week quarantine, which is typical for birds from eastern-US populations (J. S. Adelman and D. M. Hawley, personal observation). No birds captured in Arizona showed any signs of MG or anti-MG antibodies. On the basis of plumage estimates, all birds were adults at the time of the experiment. The sex ratio of experimentally infected animals (females : males) was 4 : 3 in AZ and 5 : 3 in AL. Three females from AL and one female from AZ were not infected and served as controls (see "Analyses" below). Animals were captured and experiments were performed under the following permits: Virginia Tech Institutional Animal Care and Use Committee (10-059-BIOL), Arizona Game and Fish Department (SP573456), Alabama Department of Conservation and Natural Resources (5436), US Fish and Wildlife Service (MB158404-1), and United States Geological Survey Bird Banding Lab (23513).

Experimental Time Line

Before the start of experiments, birds were moved to individual cages. On day 7 before inoculation, we took baseline measurements for mass change, eye lesion scores, and conjunctival pathogen load and drew blood for baseline measurement of circulating anti-MG antibody (see below for specific methodologies). As initial mass did not differ between populations ($t_{17} = 0.15$, $P = .88$), we report absolute change rather than proportional change in mass to facilitate ease of interpretation. On day 2 before inoculation, birds were fitted with radio transmitters for baseline temperature measurement. Birds were inoculated with MG or control media on experimental day 0. Twenty-four hours after inoculation, blood was drawn to assess cytokine expression. Fever and sickness behavior were monitored by radio telemetry (Adelman et al. 2010*a*). We limited our use of telemetry to 6 days after inoculation in order to avoid any possible inaccuracies due to the limited battery life of the transmitters. Eye lesion scores, pathogen load, and mass change were measured on days 1, 3, 7, 14, 21, 28, 42, and 56 after inoculation.

Experimental Inoculation

On day 0, birds were inoculated in the palpebral conjunctiva of both eyes with 0.025 mL of *Mycoplasma gallisepticum* suspended in Frey's media or with 0.050 mL of media alone. The MG isolate used here (VA1994) was obtained from a house finch with conjunctivitis in Virginia in 1994 (Ley et al. 1996). We used an expansion of the seventh in vitro passage of VA1994 as inoculum (7994-1 7P 2/12/09; D. H. Ley, North Carolina State University, College of Veterinary Medicine, Raleigh). This passage had a viable count of 2.24×10^7 color changing units (CCU) per milliliter, determined by the most-probable-number method (Meynell and Meynell 1970). Immediately after the inoculum thawed, we diluted it 1 : 7.37 in Frey's media in order to match the target dose of other isolates in a concurrent experiment (D. M. Hawley, unpublished data). Our final dose for inoculation was 3.04×10^6 CCU/mL.

Eye Lesion Scoring

The severity of eye lesions was scored on a three-point scale per eye (Hawley et al. 2011, modified from Sydenstricker et al. 2006). Briefly, a score of 0 indicates no pathology, 1 indicates minor swelling around the eye, 2 indicates moderate swelling and slight eversion of the conjunctiva, and 3 indicates moderate to severe swelling, eversion of the conjunctiva, and noticeable exudate. Scores from both eyes were summed to give a total eye score ranging from 0 to 6 for each individual.

Pathogen Load

We assessed pathogen load by using a quantitative PCR assay that targets the *mgc2* gene of MG by using the primers, probes, and sampling methods of Grodio et al. (2008). Briefly, each bird's conjunctiva was swabbed by the same observer (D. M. Hawley) for 5 s with sterile cotton swabs dipped in tryptose phosphate broth (TPB). Swabs were then swirled in 300 μL of sterile TPB in a microcentrifuge tube. Swabs were wrung out against the side of the mi-

crocentrifuge tube to remove as much liquid as possible and then discarded. Samples were then frozen at −20°C.

We extracted genomic DNA from these samples with Qiagen DNeasy 96 Blood and Tissue kits (Qiagen, Valencia, CA). Quantitative PCR reactions were performed in a total liquid volume of 25 μL, including 12.5 μL iQ Supermix (2X) (Bio-Rad Laboratories, Hercules, CA), 0.65 μL of a 10-mM solution of each primer, 0.35 μL of a 10-mM solution of probe, 5.85 μL of DNase-free water, and 5 μL of template. Reactions were performed on a MyiQ Single Color Real-Time PCR Detection System (Bio-Rad) under the following conditions: 95°C for 3 min, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s, with a ramp rate of 0.5°/s. Standard curves for each run were generated with serial dilutions of a plasmid containing a 303-bp *mgc2* insert, ranging from 1.15×10^2 to 1.15×10^8 copy numbers (Grodio et al. 2008). Consistent with prior work (Grodio et al. 2008), samples for pathogen load taken at the same time from an individual's left and right eyes were highly correlated (in our study: intraclass correlation coefficient = 0.96, based on a total of 170 left-eye and 170 right-eye samples from 19 individuals), suggesting that our method is highly repeatable.

Cytokine mRNA Expression

We measured relative RNA expression of two cytokines, IL-1β and IL-10, in comparison to the reference gene GADPH. Total RNA was isolated from whole-blood samples stored in RNA*later*, with the commercially available RNeasy Mini Kit (Qiagen) according to the manufacturer's recommended protocol for animal tissues with minor modification. Briefly, the samples were centrifuged for 10 min to remove the RNA*later*, and pelleted cells were lysed and homogenized with 915 μL of Buffer RLT. RNA was precipitated with 1 volume of 70% ethanol. RNA was extracted from this lysate and eluted in 30 μL of RNase-free water and stored at −80°C until use. RNA concentrations were quantified with the NanoDrop 2000 (Thermo Scientific, Wilmington, DE) and normalized to equal concentrations before complementary DNA (cDNA) synthesis. The cDNA was synthesized with Applied Biosystems High Capacity cDNA Reverse Transcription Kit (Foster City, CA) according to the manufacturer's instructions.

Real-time quantitative PCR for cytokine mRNA expression was performed with iQ SYBR Green Supermix (Bio-Rad). Each 15-μL reaction consisted of 7.5 μL of iQ SYBR Green Supermix, 0.5 μM each of forward and reverse primers, 3 μL of 1,000-ng cDNA template, and 3 μL of water. Reactions were run on the MyiQ Single Color Real-Time PCR Detection System (Bio-Rad) with the following cycling parameters: one cycle of 95°C for 3 min, 40 cycles of 95°C for 10 s and a combined annealing-extension step of 30 s, ranging from 57.4° to 68.5°C, depending on the primer set (see table A1). Final expression levels are based on threshold cycle values calculated by the MyiQ Optical System Software 1.0 (Bio-Rad).

Radio Telemetry: Fever and Sickness Behavior

Changes in body temperature and locomotor activity were measured with the methods of Adelman et al. (2010*a*). Briefly, a small patch of feathers was trimmed from each bird's back, just lateral to the spine. Then, temperature-sensing radio transmitters (model LB-2NT, Holohil Systems, Carp, Ontario, Canada) were affixed with eyelash adhesive (Andrea, American International Industries, Los Angeles) and Vetbond surgical glue (3M, St. Paul, MN). With an automated receiving unit (model 10–1000, Sparrow Systems, Champaign-Urbana, IL), transmitters were monitored for pulse signal strength and interpulse interval, from which we determined locomotor activity and temperature, respectively. At 2-min intervals across the entire day, a bird was determined to be inactive if the signal strength from its transmitter fluctuated by less than ±4 dB from the prior interval (Kjos and Cochran 1970). The temperature-to–interpulse interval relationship was calibrated for each transmitter by the manufacturer. For 2 days before inoculation, we monitored temperature from each individual's transmitter at 2-min intervals and calculated the average temperature across both preinoculation days for each interval. As transmitters most accurately reflect changes in core body temperature, rather than absolute core temperature (Adelman et al. 2010*b*), we report temperature changes after inoculation, calculated as current temperature minus the baseline at the same time of day.

Antibody Responses

MG-binding IgY levels were assessed with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (FlockChek *M. gallisepticum* ELISA kit, IDEXX, Westbrook, ME). Manufacturer's instructions were followed, with the addition of a blocking step: incubation of all wells with 300 μL of a 1% solution of bovine serum albumin in phosphate-buffered saline for 40 min at room temperature (Pierce 10X BSA; Thermo Fisher Scientific, Rockford, IL). Samples were plated in duplicate. Absorbance was measured at 630 nm with a microplate reader (ELx800, Bio-Tek, Winooski, VT). MG-specific IgY levels were assessed as (sample mean-blank)/(positive control-blank). Additional details can be found in Hawley et al. (2011).

Measuring Tolerance and Resistance

Although pathology as a proxy for fitness has become standard in measuring tolerance in vertebrates (Råberg et al. 2009; Little et al. 2010; Graham et al. 2011), it is important to consider the relationships between fitness and specific measures of pathology. The eye lesions associated with MG likely impede vision, the ability to locate food, and the ability to avoid predators (Nolan et al. 1998). Indeed, wild birds exhibiting eye lesions have a lower survival probability than birds without lesions (Faustino et al. 2004). While field studies cannot assess whether eye lesions per se reduce survival, they support the use of eye lesions as an indicator of fitness. Mass loss during infection, our other proxy for fitness, represents a decrease in available physiological reserves, including water, protein, or fat. These reserves have been shown to increase various fitness components, including survival and breeding success, for diverse passerines in the wild (Labocha and Hayes 2012). Consequently, the amount of mass lost during infection represents a reasonable proxy of an animal's ability to increase other fitness components. The measures of pathology used in this study are therefore likely to provide valid surrogates for fitness in house finches.

While recent animal studies have used peak levels of pathogen load and pathology to quantify tolerance, the duration of pathology may also predict fitness (Mackinnon and Read 2004; Råberg et al. 2007). Therefore, we analyzed tolerance in two ways: (1) using peak levels of pathology and pathogen load and (2) using the area under the curves of pathology and pathogen load over time (hereafter the "integral" of each), which incorporates both infection duration and intensity.

Peak, or "point," tolerance. As in other recent studies of vertebrate tolerance (Råberg et al. 2007), peak pathology lagged behind peak pathogen load (fig. 1), so these metrics were obtained on different dates for a given individual. Because peak pathogen levels showed little variation among individuals, we assessed pathology at essentially two points along a possible continuum: preinfection (always 0 pathogen load) and postinfection peak (very similar pathogen load for all infected individuals). These comparisons are therefore most consistent with the concept of point tolerance, assessing fitness at a single pathogen load, rather than range tolerance, assessing fitness across many different pathogen loads (Little et al. 2010).

Integral, or "range," tolerance. Because the integral of pathogen load showed substantial variation among individuals, we calculated the slope of the relationship between integrals of pathology and pathogen loads, thus capturing a metric of range tolerance (Little et al. 2010). In order to calculate integral metrics, we assumed a linear change in response variables between time points.

We assessed resistance, or the ability to kill pathogens, by using multiple metrics. First, we assessed pathogen load with mixed-effects models (see "Analyses") that ascertained differences between populations in mean pathogen load over time. In addition, we compared peak pathogen load and the integral of pathogen load, since we do not know the most appropriate single metric for this system.

Analyses

In order to minimize the number of wild-caught vertebrates used in experimental infections (National Research Council 2011), we took baseline measurements of each individual to serve as its own control. Consequently, we included only four individuals as negative controls (those receiving media-only inoculations), three females from AL and one female from AZ, to ensure that any observed pathology was related to infection and not to the time elapsed since treatment. In this study, with the exception of locomotor activity, which increased slightly for all controls by day 6 after treatment (see fig. 4*B*; table A2), control animals showed no treatment-related changes in pathology, pathogen load, or other responses (see table A2). We therefore limited most analyses to the individuals who received MG (8 from AL, 7 from AZ), with the exception of cytokine mRNA analyses (see below). Metrics of range tolerance also included control individuals, because with only one time point before infection, we could not calculate the integral of the preinfection pathology and pathogen load. Because of our limited sample size, we had little power to detect sex differences in response variables and therefore did not include sex as a covariate in our analyses. Prior studies in this species have shown small to no effects of sex on pathology or pathogen load and no effects of sex on responses to novel antigens (Altizer et al. 2004; Kollias et al. 2004; Hawley et al. 2006*b*, 2007*b*).

Data on disease progression (pathology, pathogen load, tolerance, fever, sickness behavior, and antibodies; figs. 1, 2, 4) were analyzed with linear mixed-effects models in R, version 2.11 (R Development Core Team 2010). These models included individual ID as a random effect, with population, days after inoculation, and their interaction as fixed effects. We controlled for temporal autocorrelation by including autocorrelation functions selected with the Akaike information criterion corrected for small sample sizes (AICc) from among the options available in the nlme package for R (Pinheiro and Bates 2000; Burnham and Anderson 2002). Data on pathogen load were $\log_{10}$ transformed (after adding 1 to the raw pathogen load), and data on the proportion of time spent active were arcsine square root transformed to ensure normality and equality of variances. Parameter estimates from these models were used to determine the days on which populations differed

from each other. In addition to the mixed-effects model of pathogen load described above, we also assessed differences in resistance between populations by using *t*-tests of peak pathogen load and the integral of pathogen load. Two MG-treated birds from Alabama were excluded from the analysis of fever because of malfunctions in the temperature sensors of their radio transmitters.

Point tolerance was analyzed with linear mixed-effects models on MG-treated birds that used peak pathology as the dependent variable, with individual ID as a random effect and population and measurement number (pre- vs. postinfection) as fixed effects. Range tolerance was analyzed with general linear models on all birds, including controls, using the integral of pathology over time as the dependent variable, with population, the integral of pathogen load over time, and their interaction as predictors. Models were simplified by removing terms with $P > .05$, beginning with interactions.

Models of cytokine expression were analyzed with general linear models, with population and treatment (control vs. infected) as predictors (fig. 3). The addition of a function that modeled different variances for each treatment group improved the model's fit (assessed by AICc) and was retained (for details, see Pinheiro and Bates 2000 and Carroll and Ruppert 1988). We grouped controls from both populations to increase statistical power because the single Arizona control bird fell within 1 standard error of the Alabama control mean for both IL-1β and IL-10 expression.

Results

Pathology

In both populations, eye score and mass change varied with time after inoculation (fig. 1; eye score: day after inoculation: $F_{8,104} = 32.52$, $P < .0001$; mass change: day after inoculation: $F_{8,104} = 6.67$, $P < .0001$). On average, eye score did not differ between populations (fig. 1*A*; population: $F_{1,13} < 0.01$, $P = .98$), but infected Alabama birds gained mass, on average, while Arizona birds lost mass (fig. 1*B*; population: $F_{1,13} = 8.20$, $P = .01$). Peak pathology was less severe among birds from Alabama than among birds from Arizona in terms of both eye score and mass loss. The peak in eye score was lower and later in Alabama birds (4.13 ± 0.48 on day 14) than in Arizona birds (5.79 ± 0.14 on day 7; fig. 1*A*; day after inoculation × population: $F_{8,104} = 4.84$, $P < .0001$). The greatest mass loss occurred on day 7 in both populations, with Alabama birds having lost only 0.0125 ± 0.16 g and Arizona birds having lost 0.90 ± 0.22 g (fig. 1*B*; day after inoculation × population: $F_{8,104} = 2.51$, $P = .02$).

Resistance (Pathogen Load)

Contrary to our predictions, Alabama birds did not, on average, show lower resistance than Arizona birds, as measured by mean pathogen load (population: $F_{1,13} = 1.41$, $P = .26$). There was a significant interaction between population and time, with higher pathogen loads in Alabama birds on day 21 (mean $\pm$ 1 SE: 5.47 ± 0.42, $\log_{10}$ *mgc2* copies) than in Arizona birds (2.60 ± 1.10, $\log_{10}$ *mgc2* copies; fig. 1*C*; day after inoculation × population: $F_{8,104} = 4.83$, $P < .001$; parameter estimate for day 21 × AL = 2.87, SE = 1.03, $P = .01$). However, this interaction, which indicates transiently higher pathogen loads in the population with a longer history of exposure to MG, was in the opposite direction of our prediction and the findings of previous work (Bonneaud et al. 2011). Alternative quantifications of resistance, based on peak pathogen load and the integral of pathogen load over time, did not differ between populations (peak: $t_{13} = 0.18$, $P = .86$; integral: $t_{13} = 1.38$, $P = .19$).

Point Tolerance (Peak Pathology and Pathogen Load)

In keeping with our predictions, birds from the population with a longer history of MG presence, Alabama, showed higher point tolerance than did birds from Arizona, where MG had not been detected at the time of study initiation (fig. 2*A*, 2*B*). Both peak eye score and minimum mass (expressed as change from preinfection values) were less pronounced in Alabama birds, despite similar peak levels of pathogen load (eye score: parameter estimate for measurement number [peak] × AL = −1.44, SE = 0.40, $t_{13} = -3.61$, $P = .003$; mass: parameter estimate for measurement number [peak] × AL = 0.76, SE = 0.25, $t_{13} = 3.07$, $P = .01$).

Range Tolerance (Integrals of Pathology and Pathogen Load over Time)

When tolerance was assessed with the integrals of pathology and pathogen load, no significant differences were detected between populations (eye score [fig. 2*B*]: integral of $\log_{10}$ *mgc2* copies × population: $F_{1,15} = 3.11$, $P = .10$; mass loss [fig. 2*D*]: integral of $\log_{10}$ *mgc2* copies × population: $F_{1,15} = 0.32$, $P = .59$). In fact, no significant slope existed between the integral of pathogen load and the integral of mass change (integral of $\log_{10}$ *mgc2* copies: $F_{1,15} = 2.94$, $P = .11$), suggesting that these metrics are unrelated when measured over the entire course of infection.

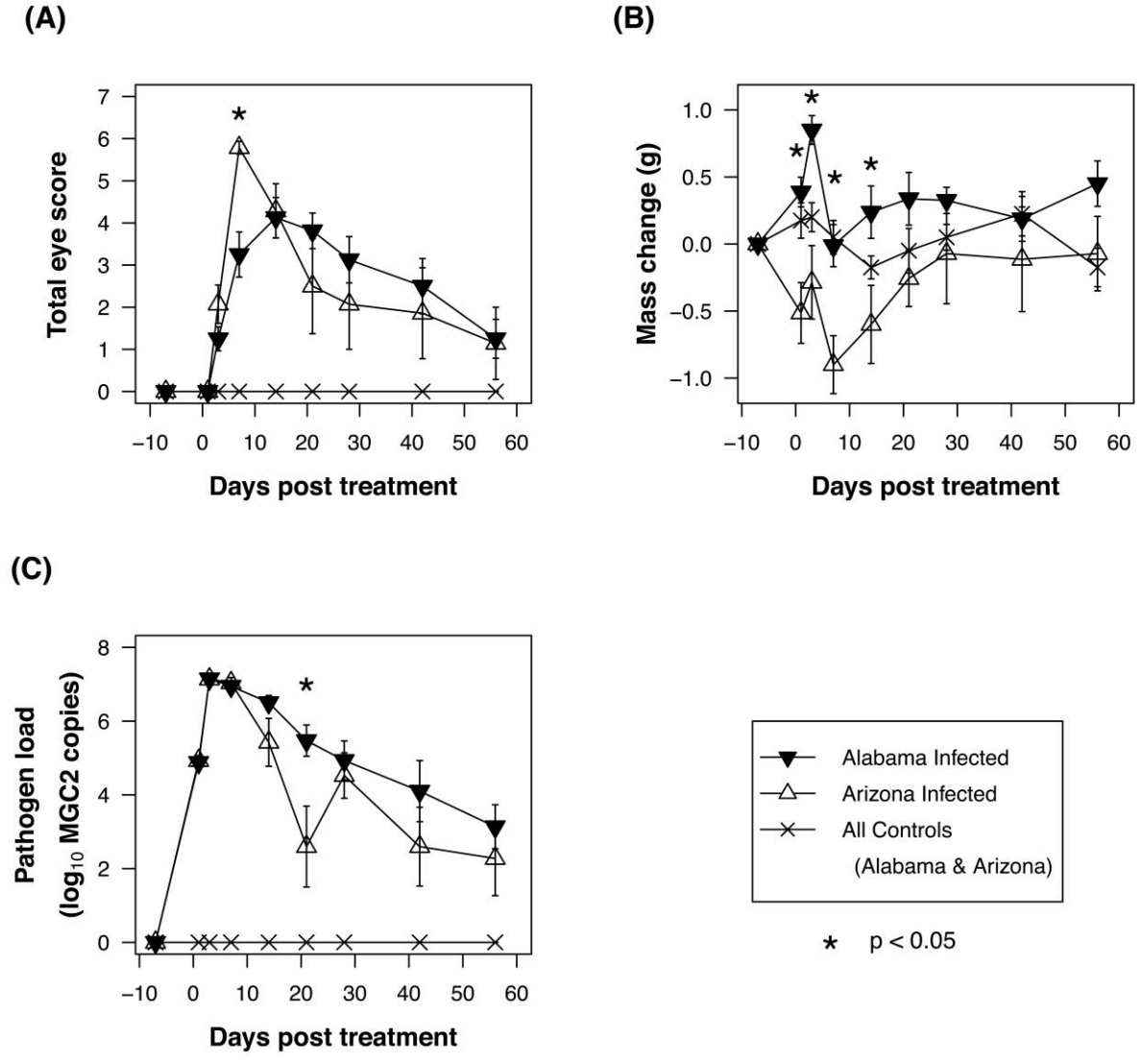

Figure 1: Experimental infection with *Mycoplasma gallisepticum* induces less severe pathology in captive house finches from Alabama than in conspecifics from Arizona. Both peak eye lesion score (*A*) and mass loss (*B*) are significantly less pronounced in Alabama birds, despite similar peak pathogen loads between populations (*C*). Symbols indicate group means ± 1 SE.

Early Inflammatory Signaling: Cytokines

Consistent with our predictions, Alabama birds showed a less inflammatory cytokine profile than did Arizona birds. Expression of the proinflammatory cytokine IL-1β remained at control levels 24 h after inoculation in infected birds from Alabama but was elevated in infected birds from Arizona (fig. 3*A*; parameter estimate for Alabama MG birds = −0.03, SE = 0.28, $P = .92$; parameter estimate for Arizona MG birds = 1.30, SE = 0.56, $P = .037$; difference between Alabama MG and Arizona MG birds: $F_{1,14} = 5.57$, $P = .033$). In addition, expression of the anti-inflammatory cytokine IL-10 was marginally above control levels in infected birds from Alabama but not in those from Arizona (fig. 3*B*; parameter estimate for Alabama MG birds = 0.54, SE = 0.29, $P = .08$; parameter estimate for Arizona MG birds = 0.078, SE = 0.17, $P = .65$; difference between Alabama MG and Arizona MG birds: $F_{1,15} = 2.45$, $P = .13$).

Early Immune Response: Fever and Sickness Behavior

Both populations showed a febrile response to MG infection, with mean nocturnal temperature change rising to approximately 2°C above normal by 2 days after inoculation

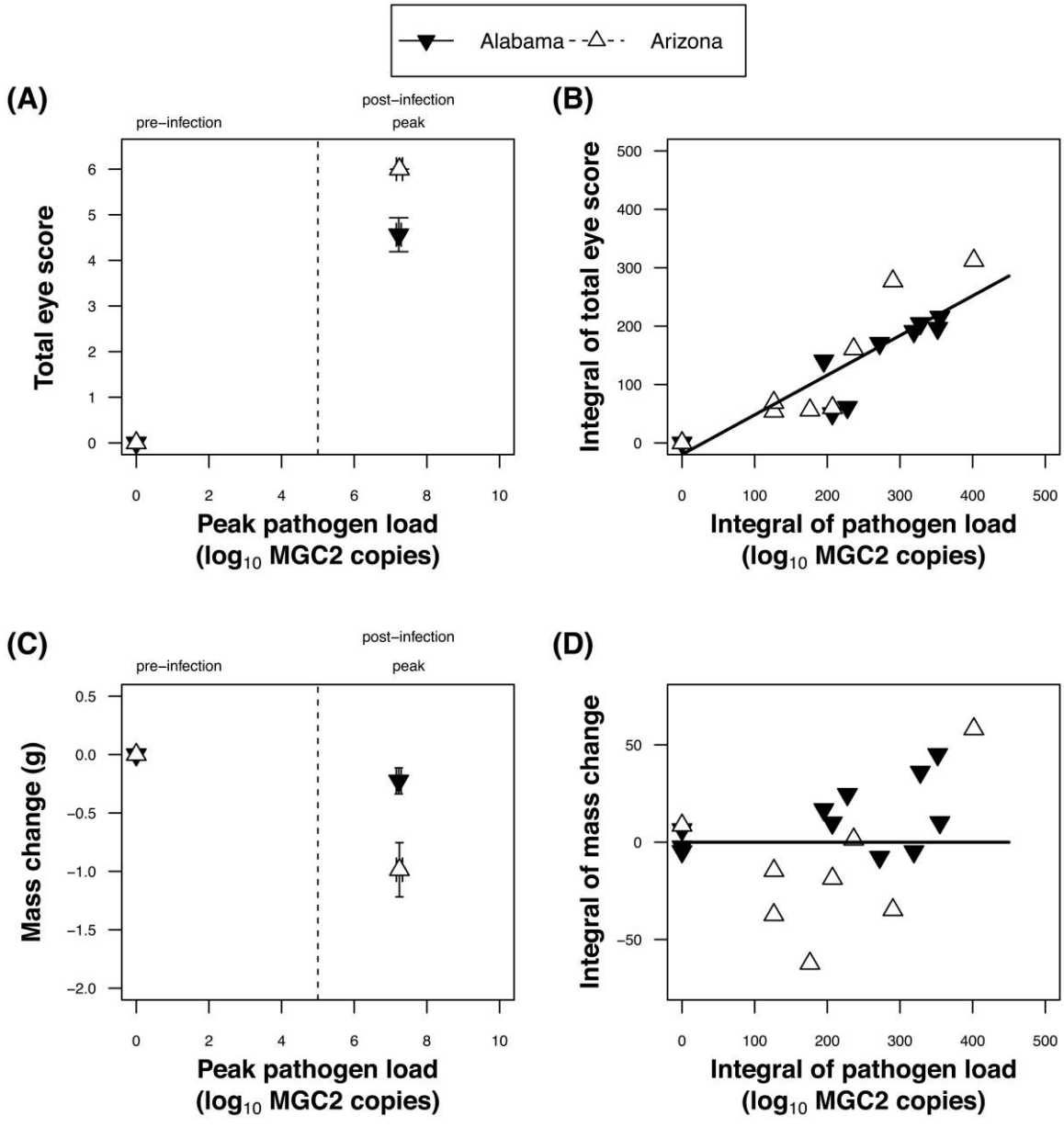

Figure 2: Experimentally infected house finches from Alabama show more pronounced point or peak tolerance of *Mycoplasma gallisepticum*, that is, lower peak pathology per peak pathogen load, when compared with conspecifics from Arizona (*A*, *C*). However, no differences existed between populations in terms of range or integral tolerance, that is, slopes between the integrals of pathogen load and pathology over time (*B*, *D*). Symbols in *A* and *C* show group means ± 1 SE for both axes. Lines in *B* and *D* represent predictions from best-fit general linear models.

(fig. 4*A*; day after inoculation: $F_{6,53} = 58.01$, $P < .001$). In keeping with lower inflammatory signaling at 24 h, body temperature increased later in Alabama birds, which showed an increase in body temperature of 0.71° ± 0.03°C on day 1 after inoculation, half that of Arizona birds (1.44° ± 0.18°C; day after inoculation × population: $F_{6,53} = 3.39$, $P = .01$; parameter estimate for day 1 after inoculation × AL = −0.65, SE = 0.19, $P = .001$).

Treated birds from both populations significantly reduced locomotor activity (i.e., increased sickness behavior) after infection (fig. 4*B*; day after inoculation: $F_{7,81} = 8.29$, $P < .001$). This pattern did not differ between populations (pop-

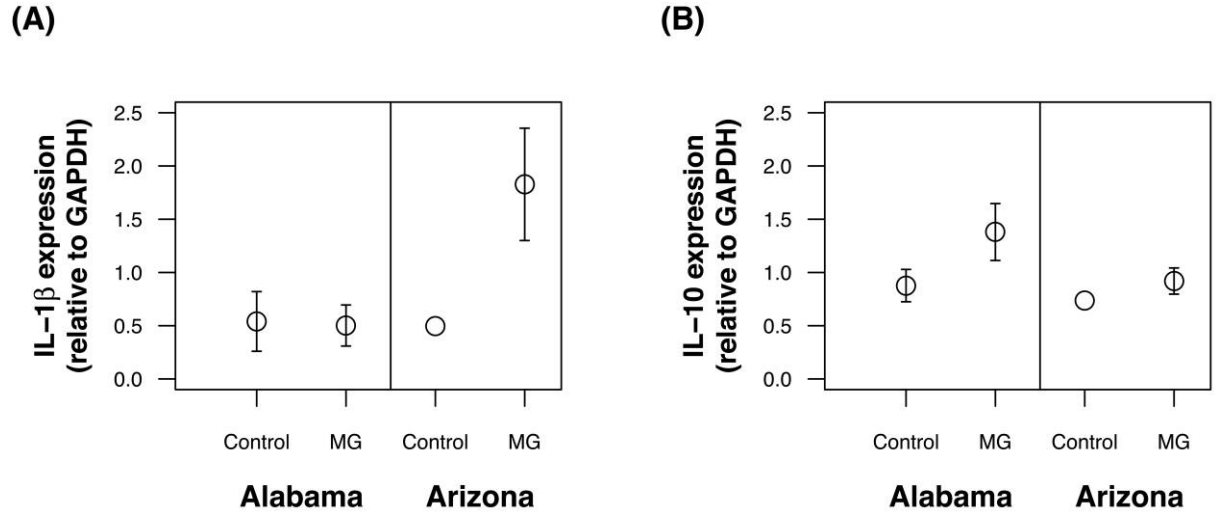

Figure 3: Twenty-four hours after experimental infection with *Mycoplasma gallisepticum* (MG), house finches from Alabama exhibit a less inflammatory signaling profile than do conspecifics from Arizona. Whole-blood messenger RNA expression of the proinflammatory cytokine IL-1β was significantly lower in infected Alabama birds than in infected Arizona birds (*A*). Whole-blood mRNA expression of the anti-inflammatory cytokine IL-10 was marginally higher in infected Alabama birds than in infected Arizona birds (*B*).

ulation: $F_{1,13} = 0.02$, $P = .90$; day after inoculation × population: $F_{7,81} = 0.74$, $P = .63$).

Subsequent Immune Response: Anti-MG Antibodies

Both populations showed increased circulating levels of MG-binding IgY antibodies after infection (fig. 4*C*; day after inoculation: $F_{3,38} = 17.24$, $P < .001$). No significant differences in this pattern were apparent between populations (population: $F_{1,13} = 0.42$, $P = .52$; day after inoculation × population: $F_{3,38} = 1.51$, $P = .23$).

Discussion

We show that in an emerging wildlife disease system, host populations can exhibit differences in pathogen tolerance despite similar levels of resistance: when experimentally infected with *Mycoplasma gallisepticum*, house finches from Alabama showed higher point tolerance than did conspecifics from Arizona (fig. 2*A*, 2*C*). In addition, at 24 h after infection, Alabama birds displayed lower fever, lower levels of proinflammatory signaling, and marginally higher levels of anti-inflammatory signaling (fig. 3). These results lend support to the inflammation-based model of tolerance among wild animals (Råberg et al. 2009; Sears et al. 2011), wherein reductions in inflammatory signaling could help alleviate immunopathological damage to host tissue, minimizing the fitness costs of a given pathogen load. However, when range tolerance was assessed with the integrals of pathology and pathogen load over the entire course of infection, including recovery, we found no differences between populations (fig. 2*B*, 2*D*). Further evaluation of which infection parameters, peak or integral, better reflect the fitness costs of infection will be crucial to predicting how individuals and populations differ in their responses to infectious diseases.

Higher point tolerance in Alabama, the population with a history of exposure to the inflammatory pathogen MG, is consistent with the hypothesis that this population has evolved in response to MG exposure. However, given the small number of populations in our study, we cannot definitively assess whether such differences are truly evolved (Garland and Adolph 1994). Moreover, our limited number of individuals may not reflect the entirety of genetic variation in each population, further limiting our ability to reach evolutionary conclusions. However, because the diversity of loci within the immune-relevant MHC complex changed after the MG epidemic in the eastern United States (Hawley and Fleischer 2012), evolution remains a viable mechanism underlying population differences. Regardless of the ecological or evolutionary drivers, the differences in host responsiveness presented here support a role for anti-inflammatory signaling in tolerance (Råberg et al. 2009; Sears et al. 2011) and illustrate that population differences in tolerance can arise in a recently emerged wildlife disease system. The possibility that tolerance may arise rapidly in response to emerging infectious diseases has received little empirical attention, despite the implications for subsequent pathogen evolution (Miller et al. 2006; Carval and Ferriere 2010; Little et al. 2010) and

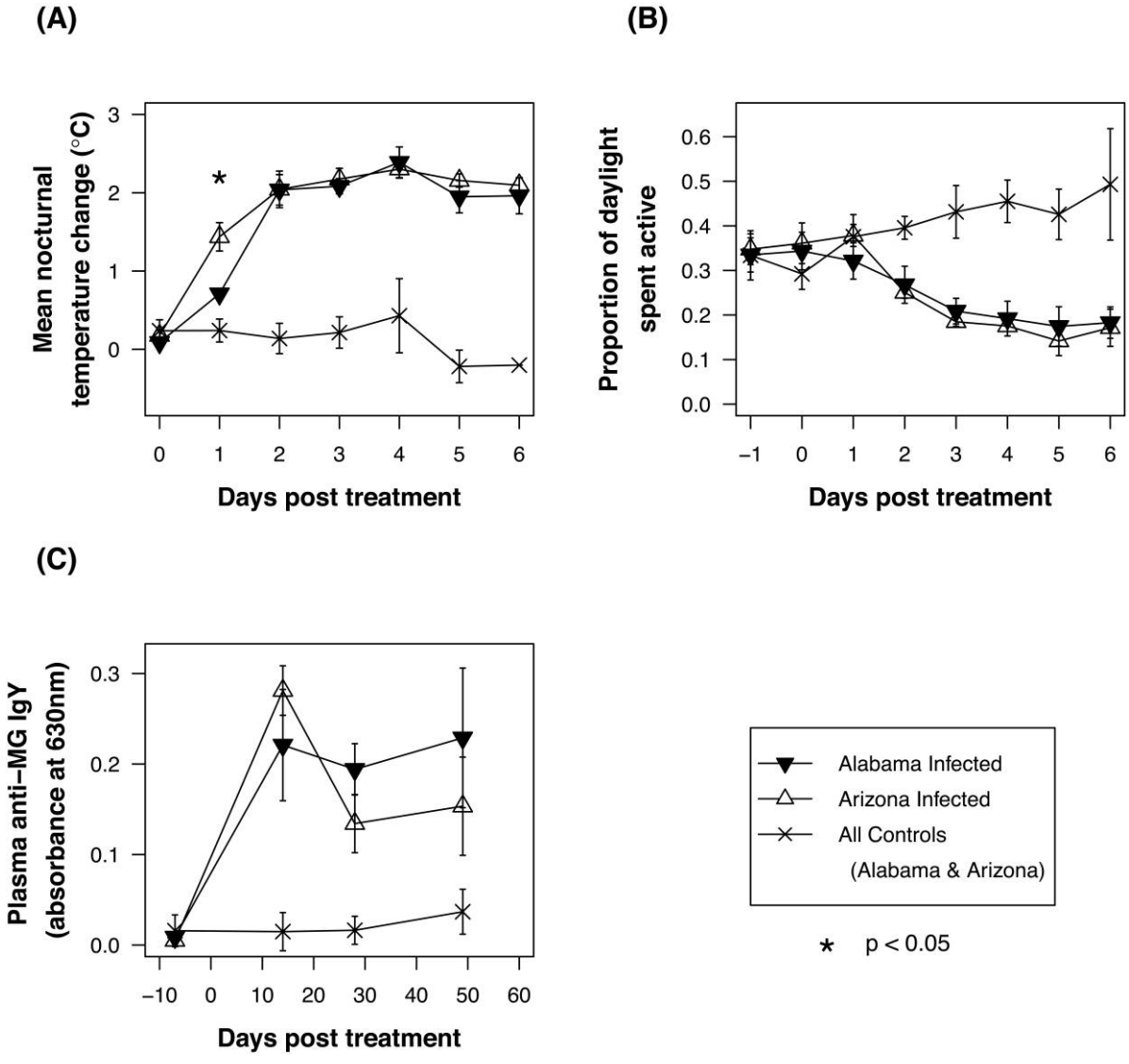

Figure 4: Onset of nocturnal fever in response to experimental infection with *Mycoplasma gallisepticum* is slower in house finches from Alabama than in those from Arizona (*A*), although neither lethargy (*B*), measured as the proportion of time spent active, nor *M. gallisepticum*–binding antibody production (*C*) shows significant differences between populations. Symbols reflect group means ± 1 SE.

prevalence (Roy and Kirchner 2000; Best et al. 2008). Future studies of MG and other emerging wildlife pathogens, such as chytrid fungus (Savage and Zamudio 2011) and avian malaria (Woodworth et al. 2005), could therefore benefit by considering that either tolerance or resistance may arise, allowing host populations to persist in the presence of infectious agents.

Resistance and Tolerance

In contrast to another recent study of Arizona and Alabama house finches (Bonneaud et al. 2011, 2012), we found no evidence for higher resistance, or faster pathogen clearance, in Alabama birds. The only significant difference in pathogen load we detected between populations (higher pathogen load in Alabama on day 21; fig. 1*C*) was in the direction opposite that detected by Bonneaud and colleagues (2011; on day 14 after infection). Such differences between studies may arise for several reasons. First, the strain of MG used in the Bonneaud et al. (2011) study was isolated in 2007, whereas ours was isolated in 1994, and the pathogen's virulence or antigenicity may have evolved between those times. Parasite genotype × host genotype interactions occur commonly in a wide range of

host-parasite systems and can influence the outcome of infection in complex ways (Lazzaro and Little 2009), although such interactions have not been explicitly studied in the house finch–MG system. Second, Bonneaud et al. (2011) did not report measures of pathology, so it may be that tolerance was similar between studies, making our results less divergent. Finally, it is possible that the degree of tolerance and resistance expressed in a given house finch population varies considerably across years and with the currently circulating MG strain (Decaestecker et al. 2007; Lazzaro and Little 2009). Therefore, each of these two studies may provide an accurate snapshot of host phenotypes, but at two distinct points in time (2007 vs. 2010). Regardless of the underlying causes, these differences between studies suggest that we will need more data, from more host populations and pathogen strains, before we can determine whether either resistance or tolerance has evolved in response to MG exposure.

Because birds from both Alabama and Arizona showed little variation in peak pathogen load in our study, the differences we report in tolerance at the peak of infection (fig. 2*A*, 2*C*) are most consistent with the concept of point tolerance: pathology (or fitness) assessed at a single pathogen load (Little et al. 2010). These differences in point tolerance allow us to make the important conclusion that tolerance can differ among host populations in an emerging infectious disease. However, when we assessed tolerance using the integrals of pathology and pathogen load over time (fig. 2*B*, 2*D*), a measure of range tolerance (Little et al. 2010), we found no significant differences between populations. This pattern in range tolerance likely reflects the similar recovery period between populations, with both pathology and pathogen load slowly approaching 0 during weeks 4–8 (fig. 1), which is typical of experimental MG infections (e.g., Hawley et al. 2010). No studies have directly examined whether the peak or the integral of pathology better estimates the fitness consequences of MG infection in house finches. However, cumulative survival probability of infected finches in the wild drops rapidly during the first weeks of infection, suggesting that the majority of fitness costs should be concentrated early in MG infection (Faustino et al. 2004). Therefore, the integral metrics used in this study, which captured 8 weeks of infection, likely dilute the effects of the critical early weeks, whereas peak metrics capture pathology when it may be most important. However, even for murine malaria, which has been studied in greater detail than MG, it is unclear whether peak or integral metrics better reflect fitness, although the two are strongly correlated in that system (Mackinnon and Read 2004; Long et al. 2006, 2008; Råberg et al. 2007). Additional studies are clearly needed to determine the best metrics for use in analyzing tolerance in wild animals.

One seemingly counterintuitive result from our assessment of pathology involves the increase in mass among infected Alabama finches. While infected animals often become anorexic and lose mass (Hart 1988; Exton 1997), increases in mass after MG infection have been observed in captive house finches from the eastern United States (Hawley et al. 2011). In addition, field and laboratory studies of eastern finches have revealed that feeding behavior can remain unchanged or increase after infection (Kollias et al. 2004; Hawley et al. 2007*a*). Although anorexia was not measured in our study, the previously documented ability of eastern birds to maintain food intake, along with the decreased locomotor activity observed in this study (fig. 4*C*), may explain why Alabama birds increased in mass after infection. Moreover, a lack of anorexia would be consistent with the minimal IL-1β expression observed among Alabama birds early in infection (fig. 3), as this cytokine has been shown to reduce feeding in numerous hosts (Dantzer 2004).

Immunological Signaling and Tolerance

At the population level, our results show correlations between point tolerance and expression of cytokines that modulate inflammatory immune responses. When compared with finches from the less tolerant population, Arizona, infected finches from the more tolerant population, Alabama, expressed lower levels of IL-1β mRNA, a proinflammatory cytokine, and tended to express higher levels of IL-10 mRNA, an anti-inflammatory cytokine, at 24 h after infection (figs. 2, 4). While cytokine levels 24 h after infection are unlikely to be the immediate cause of peak pathology or pathogen load 7–14 days later, studies of murine malaria suggest that early inflammatory signaling can predict subsequent pathology. Specifically, experimental downregulation of the proinflammatory cytokine TNF-α from days 4–7 after infection decreases the severity of anemia and mass loss at day 10 (Long et al. 2006, 2008) and increases survival through the fourth week of infection (Grau et al. 1987). Like IL-1β and IL-10, the cytokines measured in our study, TNF-α interacts with a diverse suite of immunological mechanisms (Goldsby et al. 2000) and so cannot itself be cited as the immediate cause of such pathology. However, the patterns reported here and in studies of murine malaria suggest that early levels of such signaling molecules may correlate with subsequent levels of overall inflammation, pathology, and tolerance (Graham et al. 2005; Råberg et al. 2009).

Because cytokine signaling differed significantly between populations in this study, we cannot statistically separate the potential effects of population and cytokine signaling on tolerance. However, viewing our population differences in a two-dimensional trait space, with the ratio of peak pathology to peak pathogen load (point tolerance) on one

axis and the ratio of pro- to anti-inflammatory signaling (IL-1β : IL-10) on the other, our data point toward a potential link between higher tolerance and lower inflammatory signaling (fig. 5). Replacing point tolerance with range tolerance yields qualitatively similar patterns, despite the fact that range tolerance did not differ significantly between populations (data not shown). While levels of pro- and anti-inflammatory signals measured in the periphery (as in this study) may better predict systemic pathology than local pathology, both patterns in figure 5 highlight the need for further tests of the inflammation-based model of tolerance, ideally through experimental manipulations of inflammatory signaling in natural host-pathogen systems.

Other Immune Responses

Consistent with population differences in early inflammatory signaling, Alabama birds showed a delayed onset of fever when compared to Arizona birds, with increases in body temperature half those of Arizona birds on day 1 (fig. 4*A*). Given that fever increases metabolic rate by 15%–30% in other captive birds (Burness et al. 2010; Marais et al. 2011), this represents a significant energetic investment and may contribute to subsequent differences in mass loss (depletion of energy reserves) between populations (fig. 1*B*). Lethargy, on the other hand, did not differ between populations (fig. fig. 3*B*). However, population differences in lethargy can be less pronounced in captivity than in the wild, suggesting that our ability to detect population differences may be limited here (Adelman et al. 2010*a*, 2010*b*). In addition, similar levels of lethargy could reflect an ability of both populations to increase sickness behaviors when their relative fitness and social costs are low (which is likely the case when birds are housed alone in captivity; Aubert et al. 1997; Lopes et al. 2012). Finally, the populations showed no significant differences in MG-binding IgY production (fig. 4). While this suggests that circulating levels of IgY may not be directly linked to tolerance in this system, future studies should address the possibility that another class of antibodies, immunoglobulin A, which is secreted in tears (Grodio et al. 2009), could play an important role in mediating or mitigating local pathology (Javed et al. 2005).

Implications for Host-Pathogen Dynamics

Coupled with theoretical studies of resistance and tolerance, our results and other recent empirical studies (Bonneaud et al. 2011, 2012) suggest that population differences in host responsiveness are likely important for the dynamics of *M. gallisepticum* in the wild. Modeling has shown that increases in tolerance among hosts, alone or in conjunction with increases in resistance, can select for higher pathogen virulence (Restif and Koella 2003; Miller

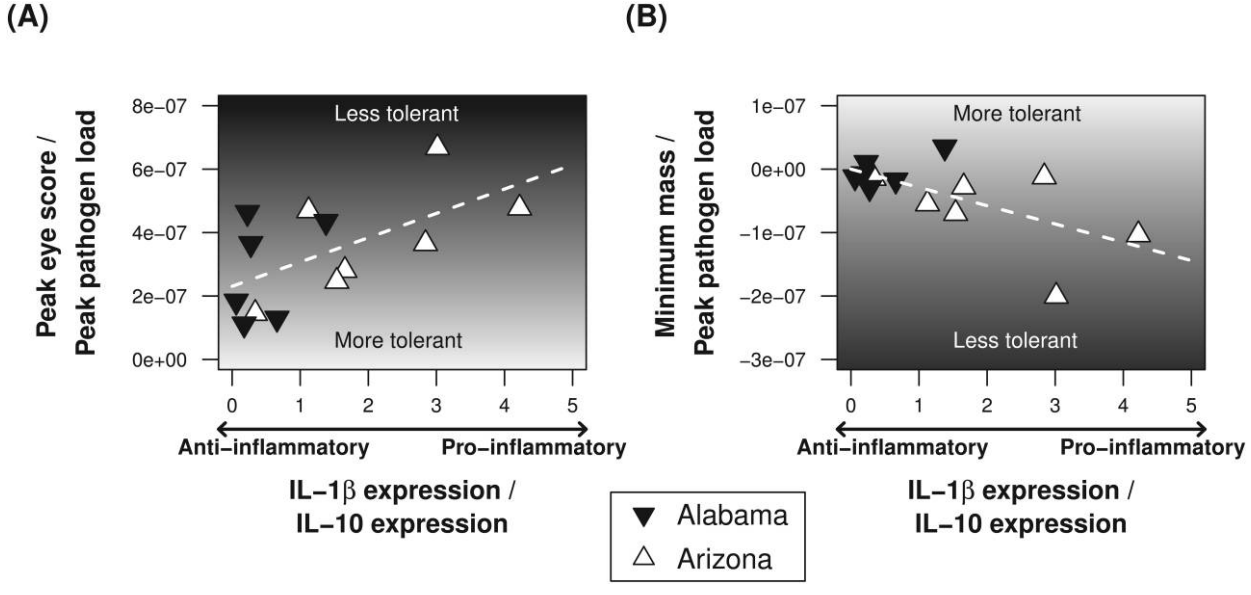

Figure 5: House finches expressing less inflammatory immunological signaling 24 h after experimental infection with *Mycoplasma gallisepticum* show increased point tolerance, that is, lower pathology per unit pathogen at the infection's peak. Lighter shading denotes higher tolerance: a lower eye score per unit pathogen (*A*) indicates the more tolerant response, whereas a higher value of mass change per unit pathogen (*B*) indicates the more tolerant response. Higher IL-1β : IL-10 ratios show more proinflammatory signaling. Dashed white lines are lines of best fit.

et al. 2006; Carval and Ferriere 2010). Additional theory suggests that immunopathology can exacerbate or mitigate the evolution of virulence, depending on how immune-mediated damage to host tissues scales with pathogen exploitation (Day et al. 2007). While Bonneaud and colleagues (2011, 2012) have shown that Alabama house finches have higher resistance to a recent isolate of MG than do finches from Arizona, we have shown here that Alabama finches display higher tolerance at the peak of infection to the earliest available house finch MG isolate. Moreover, our data suggest that tolerance may increase with decreasing inflammatory signaling, a response with high potential for immunopathology (Graham et al. 2005). While our results underscore that complex dynamics are at play, they also suggest that this emerging disease system could prove fertile for uncovering the effects of host tolerance, resistance, and immunopathology on the evolution of pathogen virulence.

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APPENDIX

Supplementary Tables

Table A1: Real-time quantitative RT-PCR primers and the annealing-extension temperatures used for quantification of cytokine expression

RNA target	Primer sequences (5′–3′)	Temperature (°C)[a]
GAPDH		68.5
F	GGAGCGTGACCCCAGCAACA	
R	CACACGCTTGGCACCACCCT	
Interleukin 1β		62.7
F	AAGGATGCCGGTGGCGTTGG	
R	CCCTCCTGATGTCAGCTTCCTCCA	
Interleukin 10		57.4
F	TCTCAGGTTCAGCAGGAGGTTGCC	
R	GAGCCATGAGGAGCAGCAGCCA	

[a] Annealing-extension temperature.

Table A2: Linear mixed-effects models run on data from control animals only

	Effect of time since sham inoculation		
Response variable	*F*	df	*P*
Eye score	1.51	8, 24	.21
Mass loss	1.48	8, 24	.22
Pathogen load	1.48	8, 23	.22
Fever	1.28	6, 14	.33
Activity	3.46	7, 18	.02
Antibody production	.54	3, 9	.67

Note: Results show no measurable pathology or pathogen load following sham treatment. Among all responses measured, only the proportion of daylight spent active varied with time since treatment. In that case, activity increased from 35% to 50% of daylight spent active over 6 days (in contrast to infected birds, who decreased time spent active; fig. 4*B*). For all variables, average values from the single control bird from Arizona fell within 1 SD of the mean for Alabama controls.

Literature Cited

Able, K. P., and J. R. Belthoff. 1998. Rapid "evolution" of migratory behaviour in the introduced house finch of eastern North America. Proceedings of the Royal Society B: Biological Sciences 265:2063–2071.

Adelman, J. S., G. E. Bentley, J. C. Wingfield, L. B. Martin, and M. Hau. 2010*a*. Population differences in fever and sickness behaviors in a wild passerine: a role for cytokines. Journal of Experimental Biology 213:4099–4109.

Adelman, J. S., S. Córdoba-Córdoba, K. Spoelstra, M. Wikelski, and M. Hau. 2010*b*. Radio telemetry reveals variation in fever and sickness behaviours with latitude in a free-living passerine. Functional Ecology 24:813–823.

Altizer, S., A. K. Davis, K. C. Cook, and J. J. Cherry. 2004. Age, sex, and season affect the risk of mycoplasmal conjunctivitis in a southeastern house finch population. Canadian Journal of Zoology 82:755–763.

Ashley, N. T., Z. M. Weil, and R. J. Nelson. 2012. Inflammation: mechanisms, costs, and natural variation. Annual Review of Ecology, Evolution, and Systematics 43:385–406, doi:10.1146/annurev-ecolsys-040212-092530.

Aubert, A., G. Goodall, R. Dantzer, and G. Gheusi. 1997. Differential effects of lipopolysaccharide on pup retrieving and nest building in lactating mice. Brain Behavior and Immunity 11:107–118.

Badyaev, A. V., V. Belloni, and G. E. Hill. 2012. House finch: *Haemorhous mexicanus*. The Birds of North America Online, ed. A. Poole. No. 46. Cornell Lab of Ornithology, Ithaca, NY, http://www.bna.birds.cornell.edu/bna/species/046/articles/introduction.

Best, A., A. White, and M. Boots. 2008. Maintenance of host variation in tolerance to pathogens and parasites. Proceedings of the National Academy of Sciences of the USA 105:20786–20791.

Bonneaud, C., S. L. Balenger, A. F. Russell, J. Zhang, G. E. Hill, and S. V. Edwards. 2011. Rapid evolution of disease resistance is accompanied by functional changes in gene expression in a wild

bird. Proceedings of the National Academy of Sciences of the USA 108:7866–7871.

Bonneaud, C., S. L. Balenger, J. Zhang, S. V. Edwards, and G. E. Hill. 2012. Innate immunity and the evolution of resistance to an emerging infectious disease in a wild bird. Molecular Ecology 21: 2628–2639.

Burness, G., C. Armstrong, T. Fee, and E. Tilman-Schindel. 2010. Is there an energetic-based trade-off between thermoregulation and the acute phase response in zebra finches? Journal of Experimental Biology 213:1386–1394.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York.

Carroll, R. J., and D. Ruppert. 1988. Transformation and weighting in regression. Chapman & Hall, New York.

Carval, D., and R. Ferriere. 2010. A unified model for the coevolution of resistance, tolerance, and virulence. Evolution 64:2988–3009.

Dantzer, R. 2004. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. European Journal of Pharmacology 500:399–411.

Day, T., A. L. Graham, and A. F. Read. 2007. Evolution of parasite virulence when host responses cause disease. Proceedings of the Royal Society B: Biological Sciences 274:2685–2692.

Decaestecker, E., S. Gaba, J. A. M. Raeymaekers, R. Stoks, L. Van Kerckhoven, D. Ebert, and L. De Meester. 2007. Host-parasite "Red Queen" dynamics archived in pond sediment. Nature 450:870–873.

Dhondt, A. A., A. V. Badyaev, A. P. Dobson, D. M. Hawley, M. J. L. Driscoll, W. M. Hochachka, and D. H. Ley. 2006. Dynamics of mycoplasmal conjunctivitis in the native and introduced range of the host. EcoHealth 3:95–102.

Dhondt, A. A., D. L. Tessaglia, and R. L. Slothower. 1998. Epidemic mycoplasmal conjunctivitis in house finches from eastern North America. Journal of Wildlife Diseases 34:265–280.

Exton, M. S. 1997. Infection-induced anorexia: active host defence strategy. Appetite 29:369–383.

Faustino, C. R., C. S. Jennelle, V. Connolly, A. K. Davis, E. C. Swarthout, A. A. Dhondt, and E. G. Cooch. 2004. *Mycoplasma gallisepticum* infection dynamics in a house finch population: seasonal variation in survival, encounter and transmission rate. Journal of Animal Ecology 73:651–669.

Ferrari, N., I. M. Cattadori, J. Nespereira, A. Rizzoli, and P. J. Hudson. 2004. The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse *Apodemus flavicollis*. Ecology Letters 7:88–94.

Ganapathy, K., and J. M. Bradbury. 2003. Effects of cyclosporin A on the immune responses and pathogenesis of a virulent strain of *Mycoplasma gallisepticum* in chickens. Avian Pathology 32:495–502.

Garland, T., Jr., and S. C. Adolph. 1994. Why not to do two-species comparative studies: limitations on inferring adaptation. Physiological Zoology 67:797–828.

Gaunson, J. E., C. J. Philip, K. G. Whithear, and G. F. Browning. 2006. The cellular immune response in the tracheal mucosa to *Mycoplasma gallisepticum* in vaccinated and unvaccinated chickens in the acute and chronic stages of disease. Vaccine 24:2627–2633.

Goldsby, R. A., T. J. Kindt, and B. A. Osborne. 2000. Kuby immunology. W. H. Freeman, New York.

Graham, A. L., J. E. Allen, and A. F. Read. 2005. Evolutionary causes and consequences of immunopathology. Annual Review of Ecology, Evolution, and Systematics 36:373–397.

Graham, A. L., A. D. Hayward, K. A. Watt, J. G. Pilkington, J. M. Pemberton, and D. H. Nussey. 2010. Fitness correlates of heritable variation in antibody responsiveness in a wild mammal. Science 330:662–665.

Graham, A. L., D. M. Shuker, L. C. Pollitt, S. K. J. R. Auld, A. J. Wilson, and T. J. Little. 2011. Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. Functional Ecology 25:5–17.

Grau, G. E., L. F. Fajardo, P. F. Piguet, B. Allet, P. H. Lambert, and P. Vassalli. 1987. Tumor necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. Science 237:1210–1212.

Grodio, J. L., E. L. Buckles, and K. A. Schat. 2009. Production of house finch (*Carpodacus mexicanus*) IgA specific anti-sera and its application in immunohistochemistry and in ELISA for detection of *Mycoplasma gallisepticum*-specific IgA. Veterinary Immunology and Immunopathology 132:288–294.

Grodio, J. L., K. V. Dhondt, P. H. O'Connell, and K. A. Schat. 2008. Detection and quantification of *Mycoplasma gallisepticum* genome load in conjunctival samples of experimentally infected house finches (*Carpodacus mexicanus*) using real-time polymerase chain reaction. Avian Pathology 37:385–391.

Hart, B. L. 1988. Biological basis of the behavior of sick animals. Neuroscience and Biobehavioral Reviews 12:123–137.

Hawley, D. M., J. Briggs, A. A. Dhondt, and I. J. Lovette. 2008. Reconciling molecular signatures across markers: mitochondrial DNA confirms founder effect in invasive North American house finches (*Carpodacus mexicanus*). Conservation Genetics 9:637–643.

Hawley, D. M., A. K. Davis, and A. A. Dhondt. 2007*a*. Transmission-relevant behaviours shift with pathogen infection in wild house finches (*Carpodacus mexicanus*). Canadian Journal of Zoology 85: 752–757.

Hawley, D. M., K. V. Dhondt, A. P. Dobson, J. L. Grodio, W. M. Hochachka, D. H. Ley, E. E. Osnas, K. A. Schat, and A. A. Dhondt. 2010. Common garden experiment reveals pathogen isolate but no host genetic diversity effect on the dynamics of an emerging wildlife disease. Journal of Evolutionary Biology 23:1680–1688.

Hawley, D. M., S. E. DuRant, A. F. Wilson, J. S. Adelman, and W. A. Hopkins. 2012. Additive metabolic costs of thermoregulation and pathogen infection. Functional Ecology 26:701–710.

Hawley, D. M., and R. C. Fleischer. 2012. Contrasting epidemic histories reveal pathogen-mediated balancing selection on class II MHC diversity in a wild songbird. PLoS ONE 7:e30222.

Hawley, D. M., J. Grodio, S. Frasca Jr., L. Kirkpatrick, and D. H. Ley. 2011. Experimental infection of domestic canaries (*Serinus canaria domestica*) with *Mycoplasma gallisepticum*: a new model system for a wildlife disease. Avian Pathology 40:321–327.

Hawley, D. M., D. Hanley, A. A. Dhondt, and I. J. Lovette. 2006*a*. Molecular evidence for a founder effect in invasive house finch (*Carpodacus mexicanus*) populations experiencing an emergent disease epidemic. Molecular Ecology 15:263–275.

Hawley, D. M., C. S. Jennelle, K. V. Sydenstricker, and A. A. Dhondt. 2007*b*. Pathogen resistance and immunocompetence covary with social status in house finches (*Carpodacus mexicanus*). Functional Ecology 21:520–527.

Hawley, D. M., K. Lindström, and M. Wikelski. 2006*b*. Experimentally increased social competition compromises humoral immune responses in house finches. Hormones and Behavior 49:417–424.

Hochachka, W. M., and A. A. Dhondt. 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.

Janeway, C. A., P. Travers, M. Walport, and J. D. Capra. 2005. Immunobiology: the immune system in health and disease. 6th ed. Garland, New York.

Javed, M. A., S. Frasca Jr., D. Rood, K. Cecchini, M. Gladd, S. J. Geary, and L. K. Silbart. 2005. Correlates of immune protection in chickens vaccinated with *Mycoplasma gallisepticum* strain GT5 following challenge with pathogenic *M. gallisepticum* strain R_{low}. Infection and Immunity 73:5410–5419.

Jokela, J., P. Schmid-Hempel, and M. C. Rigby. 2000. Dr. Pangloss restrained by the Red Queen: steps towards a unified defence theory. Oikos 89:267–274.

Kjos, C. G., and W. W. Cochran. 1970. Activity of migrant thrushes as determined by radio-telemetry. Wilson Bulletin 82:225–226.

Kluger, M. J. 1991. Fever: role of pyrogens and cryogens. Physiological Reviews 71:93–127.

Kollias, G. V., K. V. Sydenstricker, H. W. Kollias, D. H. Ley, P. R. Hosseini, V. Connolly, and A. A. Dhondt. 2004. Experimental infection of house finches with *Mycoplasma gallisepticum*. Journal of Wildlife Diseases 40:79–86.

Labocha, M. K., and J. P. Hayes. 2012. Morphometric indices of body condition in birds: a review. Journal of Ornithology 153:1–22.

Lazzaro, B. P., and T. J. Little. 2009. Immunity in a variable world. Philosophical Transactions of the Royal Society B: Biological Sciences 364:15–26.

Ley, D. H., J. E. Berkhoff, and J. M. McLaren. 1996. *Mycoplasma gallisepticum* isolated from house finches (*Carpodacus mexicanus*) with conjunctivitis. Avian Diseases 40:480–483.

Little, T. J., D. M. Shuker, N. Colegrave, T. Day, and A. L. Graham. 2010. The coevolution of virulence: tolerance in perspective. PLoS Pathogens 6:e1001006.

Lloyd-Smith, J. O., S. J. Schreiber, P. E. Kopp, and W. M. Getz. 2005. Superspreading and the effect of individual variation on disease emergence. Nature 438:355–359.

Long, G. H., and M. Boots. 2011. How can immunopathology shape the evolution of parasite virulence? Trends in Parasitology 27:300–305.

Long, G. H., B. H. K. Chan, J. E. Allen, A. F. Read, and A. L. Graham. 2006. Parasite genetic diversity does not influence TNF-mediated effects on the virulence of primary rodent malaria infections. Parasitology 133:673–684.

———. 2008. Blockade of TNF receptor 1 reduces disease severity but increases parasite transmission during *Plasmodium chabaudi chabaudi* infection. International Journal for Parasitology 38:1073–1081.

Lopes, P. C., J. Adelman, J. C. Wingfield, and G. E. Bentley. 2012. Social context modulates sickness behavior. Behavioral Ecology and Sociobiology 66:1421–1428.

Luttrell, M. P., D. E. Stallknecht, J. R. Fischer, C. T. Sewell, and S. H. Kleven. 1998. Natural *Mycoplasma gallisepticum* infection in a captive flock of house finches. Journal of Wildlife Diseases 34:289–296.

Mackinnon, M. J., and A. F. Read. 2004. Virulence in malaria: an evolutionary viewpoint. Philosophical Transactions of the Royal Society B: Biological Sciences 359:965–986.

Marais, M., S. K. Maloney, and D. A. Gray. 2011. The metabolic cost of fever in Pekin ducks. Journal of Thermal Biology 36:116–120.

Medzhitov, R. 2008. Origin and physiological roles of inflammation. Nature 454:428–435.

Meynell, G. C., and E. Meynell. 1970. Theory and practice in experimental bacteriology. 2nd ed. Cambridge University Press, Cambridge.

Miller, M. R., A. White, and M. Boots. 2006. The evolution of parasites in response to tolerance in their hosts: the good, the bad, and apparent commensalism. Evolution 60:945–956.

Mohammed, J., S. Frasca Jr., K. Cecchini, D. Rood, A. C. Nyaoke, S. J. Geary, and L. K. Silbart. 2007. Chemokine and cytokine gene expression profiles in chickens inoculated with *Mycoplasma gallisepticum* strains R_{low} or GT5. Vaccine 25:8611–8621.

National Research Council. 2011. Guide for the care and use of laboratory animals. National Academies Press, Washington, DC.

Nolan, P. M., G. E. Hill, and A. M. Stoehr. 1998. Sex, size, and plumage redness predict house finch survival in an epidemic. Proceedings of the Royal Society B: Biological Sciences 265:961–965.

Pfennig, K. S. 2001. Evolution of pathogen virulence: the role of variation in host phenotype. Proceedings of the Royal Society B: Biological Sciences 268:755–760.

Pinheiro, J. C., and D. M. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer, Berlin.

Poland, G. A., I. G. Ovsyannikova, and R. M. Jacobson. 2008. Immunogenetics of seasonal influenza vaccine response. Vaccine 26S: D35–D40.

Råberg, L., A. L. Graham, and A. F. Read. 2009. Decomposing health: tolerance and resistance to parasites in animals. Philosophical Transactions of the Royal Society B: Biological Sciences 364:37–49.

Råberg, L., D. Sim, and A. F. Read. 2007. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science 318:812–814.

Rausher, M. D. 2001. Co-evolution and plant resistance to natural enemies. Nature 411:857–864.

Razin, S., D. Yogev, and Y. Naot. 1998. Molecular biology and pathogenicity of mycoplasmas. Microbiology and Molecular Biology Reviews 62:1094–1156.

R Development Core Team. 2010. R: a language and environment for statistical computing, version 2.11.0. R Foundation for Statistical Computing, Vienna.

Restif, O., and J. C. Koella. 2003. Shared control of epidemiological traits in a coevolutionary model of host-parasite interactions. American Naturalist 161:827–836.

———. 2004. Concurrent evolution of resistance and tolerance to pathogens. American Naturalist 164:E90–E102.

Rohr, J. R., T. R. Raffel, and C. A. Hall. 2010. Developmental variation in resistance and tolerance in a multi-host–parasite system. Functional Ecology 24:1110–1121.

Rolff, J., and M. T. Siva-Jothy. 2003. Invertebrate ecological immunology. Science 301:472–475.

Roy, B. A., and J. W. Kirchner. 2000. Evolutionary dynamics of pathogen resistance and tolerance. Evolution 54:51–63.

Savage, A. E., and K. R. Zamudio. 2011. MHC genotypes associate with resistance to a frog-killing fungus. Proceedings of the National Academy of Sciences of the USA 108:16705–16710.

Schmid-Hempel, P. 2011. Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics. Oxford University Press, Oxford.

Sears, B. F., J. R. Rohr, J. E. Allen, and L. B. Martin. 2011. The

economy of inflammation: when is less more? Trends in Parasitology 27:382–387.

Sydenstricker, K. V., A. A. Dhondt, D. M. Hawley, C. S. Jennelle, H. W. Kollias, and G. V. Kollias. 2006. Characterization of experimental *Mycoplasma gallisepticum* infection in captive house finch flocks. Avian Diseases 50:39–44.

Tobler, U., and B. R. Schmidt. 2010. Within- and among-population variation in chytridiomycosis-induced mortality in the toad *Alytes obstetricans*. PLoS ONE 5:e10927.

Viney, M. E., E. M. Riley, and K. L. Buchanan. 2005. Optimal immune responses: immunocompetence revisited. Trends in Ecology and Evolution 20:665–669.

Vollmer-Conna, U., B. F. Piraino, B. Cameron, T. Davenport, I. Hickie, D. Wakefield, and A. R. Lloyd. 2008. Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. Clinical Infectious Diseases 47:1418–1425.

Woodworth, B. L., C. T. Atkinson, D. A. LaPointe, P. J. Hart, C. S. Spiegel, E. J. Tweed, C. Henneman, et al. 2005. Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. Proceedings of the National Academy of Sciences of the USA 102:1531–1536.

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House finch. Photo by Bonnie M. Fairbanks.