Prior exposure to pathogens augments host heterogeneity in susceptibility and has key epidemiological consequences

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Abstract. Pathogen epidemics are key threats to human and wildlife health. Across systems, host protection from pathogens following initial exposure is often incomplete, resulting in recurrent epidemics through partially-immune hosts. Variation in population-level protection has important consequences for epidemic dynamics, but whether acquired protection influences host heterogeneity in susceptibility and its epidemiological consequences remains unexplored. We experimentally investigated whether prior exposure (none, low-dose, or high-dose) to a bacterial pathogen alters host heterogeneity in susceptibility among songbirds. Hosts with no prior pathogen exposure had little variation in protection, but heterogeneity in susceptibility was significantly augmented by prior pathogen exposure, with the highest variability detected in hosts given high-dose prior exposure. An epidemiological model parameterized with experimental data found that heterogeneity in susceptibility from prior exposure more than halved epidemic sizes compared with a homogeneous population with identical mean protection. However, because infection-induced mortality was also greatly reduced in hosts with prior pathogen exposure, reductions in epidemic size were smaller than expected in hosts with prior exposure. These results highlight the importance of variable protection from prior exposure and/or vaccination in driving host heterogeneity and epidemiological dynamics.

Author Summary. Individuals in a population can be highly variable in terms of whether or not they get sick during a pathogen outbreak. This individual variability in susceptibility has important consequences for how widely a disease can spread in a population. Therefore, it is key to understand what drives such variability in susceptibility among individuals. One possibility is that variable levels of standing immunity in a population, whether from vaccination or previous infection, lead to variability in susceptibility among individuals. We tested whether acquired immunity creates more variability in susceptibility among individuals in a host population, using a songbird disease system as a model. We found that birds that had acquired immunity to a

51 bacterial pathogen were far more variable in their susceptibility. We also show that this

52 population-level variability in itself can strongly suppress disease outbreaks.

53

54 **Introduction**

55 Pathogen epidemics are increasing in frequency in humans and other animals (1,2),
56 underscoring the need to characterize key sources of heterogeneity that influence both
57 epidemic and evolutionary dynamics for pathogens. There is growing appreciation among
58 infectious disease epidemiologists and ecologists for both the extent and downstream
59 importance of individual variation in traits such as contact rates (e.g. 3,4) and host
60 infectiousness (e.g. 5). In contrast, the heterogeneity present among individual hosts in
61 pathogen susceptibility (defined here as the probability of infection given exposure), and the
62 factors that influence the degree of such population-level heterogeneity in susceptibility, has
63 received relatively less attention (but see (6,7)). Given the documented importance of host
64 heterogeneity in susceptibility for both epidemiological and evolutionary dynamics (e.g. 7–11), it
65 is critical to understand how factors such as acquired protection from prior pathogen exposure
66 influence the degree of among-individual variation present in a given population.

67 Host reinfection is a common but understudied feature of many host-pathogen systems
68 (12–17), including SARS-Cov-2 (18), pneumococcal disease (19), malaria (20), and other
69 diseases of agricultural and wildlife significance. Because the protection acquired from prior
70 pathogen infection is often incomplete and/or wanes over time, reinfections occur even in
71 systems where hosts have lower mean susceptibility during secondary exposures, relative to
72 individuals exposed for the first time (21–24). While there is growing appreciation for the
73 pervasiveness of heterogeneity in acquired host protection in response to vaccination or
74 infection across systems (23,25,26), prior work has largely focused on how host protection from
75 vaccination or prior infection influences mean population traits, rather than variability among
76 individuals in a given population (27). As such, it remains unknown how host protection acquired
77 from prior pathogen exposure alters the degree of inter-individual heterogeneity in susceptibility.

78 Prior pathogen exposure can modify population-level heterogeneity in susceptibility via
79 several mechanisms. First, prior exposure to pathogens could generate incomplete (or “leaky”)

protection similarly for all exposed hosts (23), such that all hosts receive identical protection from reinfection. Alternatively, prior pathogen exposure could result in relatively complete acquired protection for some proportion of hosts, while others remain relatively unprotected (termed “all-or-nothing” immunity (23)). Epidemiological models often select either wholly leaky protection of hosts that allow every host an identical rate of reinfection or all-or-nothing protection such that some hosts remain in the recovered class while a subset is routed back to the susceptible class, representing two extremes of how heterogeneity is incorporated into disease models (6,27). Whether and how prior exposure to pathogens alters population-level heterogeneity in susceptibility in a given system likely depends on the extent of prior pathogen exposure that hosts experience (28) and the mode of action of such protection [“leaky”, “all-or-nothing” (6,27), or some intermediate (26)]. For example, host exposure to low pathogen doses, which can occur during the natural transmission process for many pathogens (e.g. 29–31), might be more likely than higher exposure doses to generate incomplete host protection (32,33) and thus to induce population-level heterogeneity in susceptibility.

Regardless of the underlying mechanisms, effects of prior exposure on population-level heterogeneity in host susceptibility are particularly important to characterize because this heterogeneity can have key epidemiological consequences, including lower predicted outbreak sizes (6) and higher rates of reinfection (10). This is in part due to the process of cohort selection, through which the most susceptible individuals are infected first in any given epidemic time step, leaving lower mean susceptibility among remaining uninfected hosts in a population (34). Experimental quantification of population-level heterogeneity in susceptibility draws on quantitative microbial risk assessment approaches (35) to estimate susceptibility distributions using a series of increasing pathogen challenge doses. Because only the most susceptible hosts become infected at low challenge doses, whereas even the least susceptible hosts become infected at the highest challenge doses, dose-response models can be used to estimate the degree of host heterogeneity in susceptibility in a given population (6). Robustly

106 quantifying changes in host heterogeneity that occur with prior pathogen exposure is
107 challenging due to large sample size requirements, but nonetheless critical for adequate
108 characterization of host-pathogen dynamics, including the potential downstream effects of inter-
109 individual heterogeneity on pathogen strain coexistence and virulence evolution (7,9,36).
110 Here we investigate effects of host prior exposure on inter-individual heterogeneity in
111 susceptibility using a tractable wildlife-pathogen system: a common North American songbird
112 species (house finch; *Haemorhous mexicanus*) and its widespread bacterial pathogen,
113 *Mycoplasma gallisepticum* (MG), which causes severe conjunctivitis. The potential effects of
114 prior pathogen exposure on host heterogeneity are particularly relevant in this system because
115 MG exposure doses are variable across free-living hosts: susceptible birds contact MG that was
116 deposited onto bird feeders in variable amounts by infected individuals (37,38). Further,
117 although the conjunctivitis caused by MG indirectly results in significant mortality in finches,
118 free-living hosts recover at high rates from initial infection (39), leaving a pool of recovered hosts
119 in natural populations. Experimental studies show that recovered hosts have significant
120 acquired protection relative to pathogen-naive hosts, but can still be successfully reinfected at
121 high rates, even with homologous pathogen strains (28,40). Finally, our work to date suggests
122 that experimental variation in the degree of prior exposure to MG (in both dose and number of
123 exposures) results in heterogeneous protection from reinfection among finches, as measured by
124 conjunctival pathogen loads following reinfection challenge (28,32,33). Whether prior exposure
125 to MG specifically alters inter-individual heterogeneity in susceptibility, a population-level trait
126 that requires dose-response approaches to robustly quantify, has not yet been examined.
127 To test how variation in prior pathogen exposure alters population-level heterogeneity in
128 susceptibility, we first experimentally varied the degree of prior pathogen exposure categorically
129 (none, low-dose, or high-dose) in wild caught-finches that we documented had no exposure to
130 MG prior to capture. After recovery from prior exposure treatments, we measured host
131 susceptibility (1|0) to secondary dose challenge (Figure 1), fitting dose-response models for

each treatment group to determine whether host prior exposure treatment altered population-level heterogeneity in susceptibility. Specifically, for each treatment group, we considered population-level distributions of susceptibility that represent either identical infection probabilities (homogeneous model) or models that represent inter-host variation in rates of infection (heterogeneous model, where numerical susceptibility values were estimated according to a fitted gamma distribution (6,11,41,42)). Importantly, our models allowed us to disentangle effects of prior exposure on mean susceptibility from changes in heterogeneity in susceptibility. Lastly, to determine effects of exposure-induced heterogeneity on resulting epidemic dynamics, we parameterized an SIR model for this system with susceptibility distributions and expected mortality rates in the wild estimated from our experimental data.

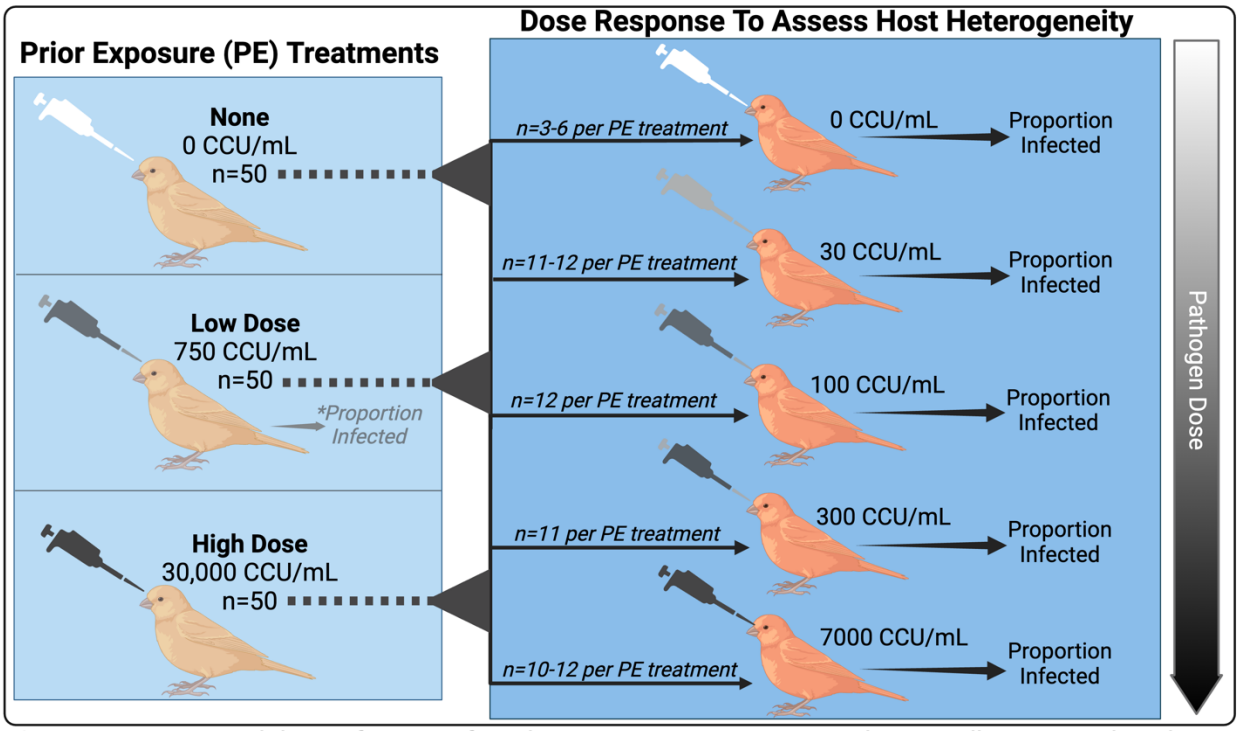

Figure 1. Experimental design for quantifying how prior exposure to *Mycoplasma gallisepticum* alters host heterogeneity in susceptibility in house finches (n=150 for final analysis). Five weeks after one of three prior exposure (PE) treatments (none, low, or high dose; left panel), all birds received a secondary dose challenge (right panel) to assess heterogeneity in susceptibility. Our primary data set was whether birds became infected (0|1) in response to a given secondary dose (right). *However, to improve model fits, we also used bird responses to low-dose PE treatment (left, asterisk), which fell intermediate to our highest secondary challenge doses (300 and 7000 Color Changing Units [CCU]/mL), to quantify the proportion of birds with no prior exposure (at the time of low-dose PE) that become infected at a 750 CCU/mL dose. Made in Biorender.

Results

Prior exposure to pathogens augments population-level heterogeneity in susceptibility. Birds with no prior pathogen exposure at the time of secondary dose challenge had low variability in host susceptibility (coef of variation [CoV] from gamma distribution = 0.899) and there was no support that heterogeneous dose-response models were an improvement over models assuming homogeneous host susceptibility (defined here as identical infection probabilities across hosts in a population) (Fig 2a; deviance homogeneous = 1.248; deviance heterogeneous = 3.307). Birds with no prior pathogen exposure also had high mean susceptibility to infection (mean susceptibility [fitted gamma distribution]=1.181). In contrast, dose-response curves were shallower and fewer birds were infected upon secondary exposure in both the low (mean susceptibility = 0.446 [fitted gamma], CoV [fitted gamma] = 1.630; Fig 2b), and high-dose (mean susceptibility = 0.192 [fitted gamma], CoV [fitted gamma] = 2.511; Fig 2c) prior exposure groups. Both prior exposure treatments were better described by models assuming heterogeneous versus homogeneous susceptibility (Fig 2; likelihood ratio tests heterogeneous versus homogeneous (35), low dose: P = 0.020, high dose: P = 0.033).

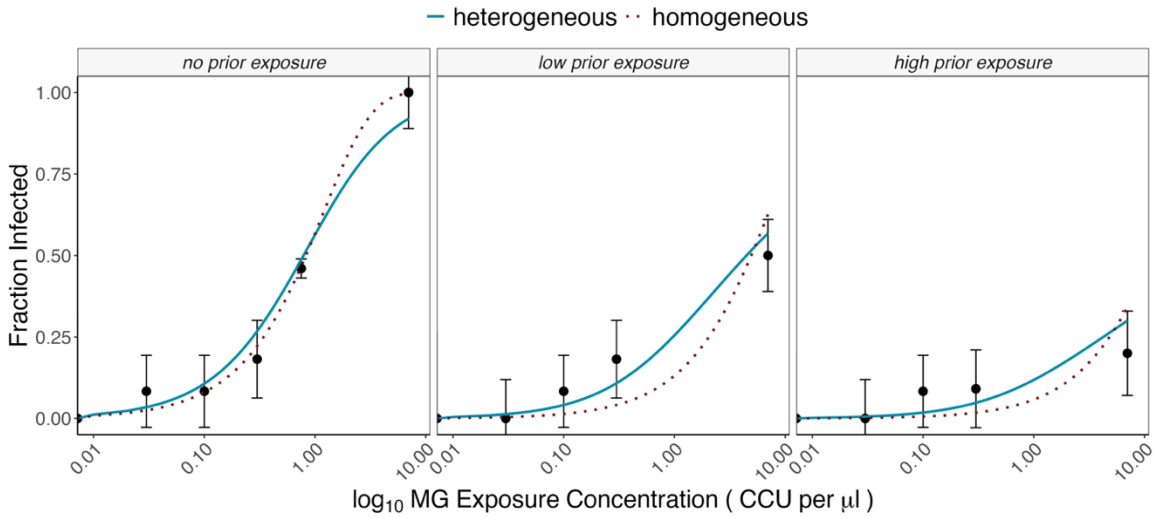

Figure 2. Dose response curves for house finch susceptibility to secondary challenge with *Mycoplasma gallisepticum* across prior exposure treatments. Points (+/- 1SE) show the fraction of individual birds (n=10-12 birds for most points; individual responses are 1|0) infected at each secondary exposure dose, shown as Color Changing Units (CCU)/uL. Lines indicate model fits, with blue indicating gamma (heterogeneous) model fits, and red dashed lines indicating homogeneous model fits. Panel labels show prior exposure treatment (birds in the no prior

exposure treatment were pathogen-naive at the time of secondary dose challenge). In hosts with prior pathogen exposure (low and high-dose prior exposure groups), the gamma model (which accounts for inter-individual heterogeneity) was better supported via likelihood ratio tests.

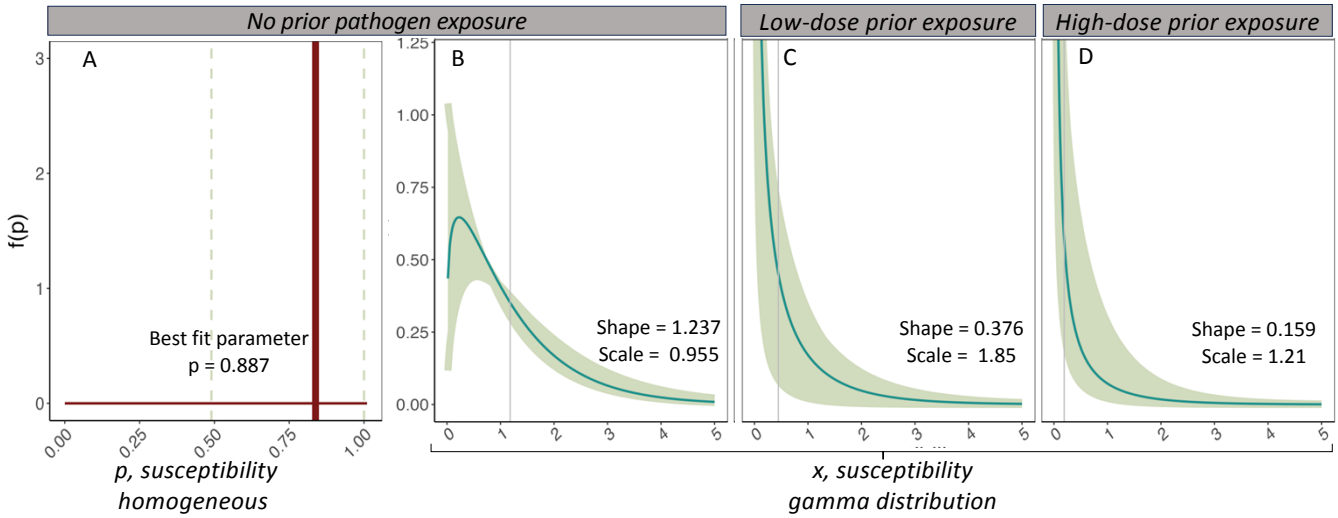

Fig 3. Host susceptibility distributions for house finches from variable prior exposure treatments: no prior exposure (A,B); low-dose (C); or high-dose (D). Colored lines show estimated susceptibility distributions from either homogeneously (A) or gamma-distributed (B-D) models (note distinct axes for the two models). In (A), susceptibility (*p*) is shown as the single best fit parameter *p* (dotted vertical lines represent 1 standard error) for the homogeneous model, which was the best fit model for the no-prior exposure group (see *Results*). In (B-D), the best fit parameters (shape and scale) for gamma distributions (teal line) are listed for each group, and vertical gray lines indicate mean susceptibility (*x*) for that treatment. Lighter shading represents 95% confidence regions for gamma distributions, obtained by bootstrapping chi-squared residuals to create 1,000 pseudoreplicates of infection data and then refitting the model to pseudoreplicates, as per (6,35). The gamma model was the best fit for only the low-dose and high-dose groups. Gamma estimates are also shown for the no prior exposure group (B) because this allowed more equivalent comparisons for certain SIR simulations (see *Methods*).

To estimate effects of prior exposure on host mortality rates in the wild, we used clinical scores of eye disease in response to secondary dose challenge to estimate mean mortality rates for each prior exposure treatment group (as per (22); see Methods). As expected, predicted host mortality rates decreased with the degree of prior host exposure (Table S2), declining from 0.05894/day in birds with no prior exposure, to 0.02806/day in birds with prior low-dose exposure, and to a negligible value ($2.483*10^{-17}$) in birds with prior high-dose exposure.

Exposure-induced heterogeneity in susceptibility reduces epidemic size. Using these empirically-derived susceptibility distributions and predicted mortality rates (Fig 3, Table S2) to

195 parameterize an SIR model of this system, we find that the total epidemic size is largest
196 (90.51% of the population) for a host population with no prior pathogen exposure, while for low
197 and high-dose prior exposure groups, the total epidemic size is significantly reduced (33.67%
198 and 16.13%) of the population, respectively; blue bars, Fig 4A). Because prior pathogen
199 exposure also protects hosts from mortality, there is no mortality during the epidemic in the
200 high-dose prior exposure population, while there is 16.28% mortality in the low-dose prior
201 exposure population, and more than 59.98% mortality in the naive host population (darker
202 colored portion of bars, Fig 4A; dashed lines, Fig 4C).
203
204 We also examined whether the observed reductions in epidemic size with host prior exposure
205 are driven primarily by changes in the susceptibility distribution, versus reductions in mean host
206 susceptibility (blue versus red bars within each prior exposure treatment group, Fig 4A, B). For
207 empirically determined best fit parameters, epidemic size for a paired simulation assuming
208 homogeneous rather than heterogeneous susceptibility was larger by 59% and 56%, under low
209 dose and high dose prior exposure, respectively (low dose, fitted mortality: 49.12 epidemic size
210 difference [hom-het]; high dose, fitted mortality: 56.55 epidemic size difference [hom-het]). We
211 simulated the heterogeneous and homogeneous models using the parameter estimates
212 obtained from bootstrapping chi-squared residuals (see Fig 3). Assuming homogeneous versus
213 heterogeneous susceptibility (while holding mean susceptibility constant for a given prior
214 exposure treatment) results in consistently larger outbreaks (Fig S1; difference in epidemic size
215 = homogeneous [red]-heterogeneous [blue] outbreak size for a given mean susceptibility).
216 Indeed, across all simulations, outbreaks are never smaller in models assuming homogeneous
217 versus heterogeneous susceptibility (all $P < 0.0001$; Fig S1; epidemic size differences [95% CIs
218 of hom-het epidemic size] for each simulation: low dose, fitted mortality [4.009,49.15]; high
219 dose, fitted mortality [0.8994,61.29]; low dose, fixed mortality [0.1198,40.77]; high dose, fixed
220 mortality [0.010,36.18]). Together, these results indicate effects of host heterogeneity *per se* on

221 outbreak size, which act above and beyond the effects of lower mean susceptibility from prior
222 pathogen exposure.
223
224 Reductions in mean susceptibility with host prior exposure also contribute to smaller outbreak
225 sizes, but the extent depends on the assumed level of host mortality. When controlling for
226 mortality differences (i.e., all populations are assumed to have mortality equivalent to the naïve,
227 no-prior exposure population), the outbreak size of both groups with prior pathogen exposure
228 (low or high) is reduced (Fig 4B,D). In the case of simulations assuming homogeneous
229 susceptibility (red bars, Fig 4B) while accounting for differences in mean protection with prior
230 exposure, this reduction is *entirely* due to lower mean host susceptibility resulting from prior
231 pathogen exposure. However, when heterogeneity in susceptibility and reduced mean
232 susceptibility are both accounted for, further reductions in outbreak size and mortality are found
233 for the low-dose prior exposure group (blue bars and lines, Fig 4B,D). Further reductions from
234 heterogeneity *per se* are not possible for the high-dose prior exposure group, for which our
235 model produced an outbreak size of nearly 0 from changes in mean susceptibility alone.
236
237 In the more biologically realistic setting (Fig 4A,C) with changes in susceptibility and host
238 mortality in response to prior exposure both accounted for, we find a more complicated pattern.
239 If we assume a homogeneous population (red bars) with the mean susceptibility found
240 empirically for low and high-dose prior exposure groups (and mortality rates set as empirically
241 determined values), the epidemic sizes drastically increase (82.79% and 72.68% of the
242 population for the low and high prior exposure, respectively) relative to models accounting for
243 host heterogeneity in susceptibility (blue bars and lines; Fig 4A,C). The large outbreak in the
244 high-dose prior exposure population (despite very low mean susceptibility in this group) is due
245 to the absence of disease-induced mortality in this population, resulting in a higher basic
246 reproductive number because all individuals survive the entire infectious period.

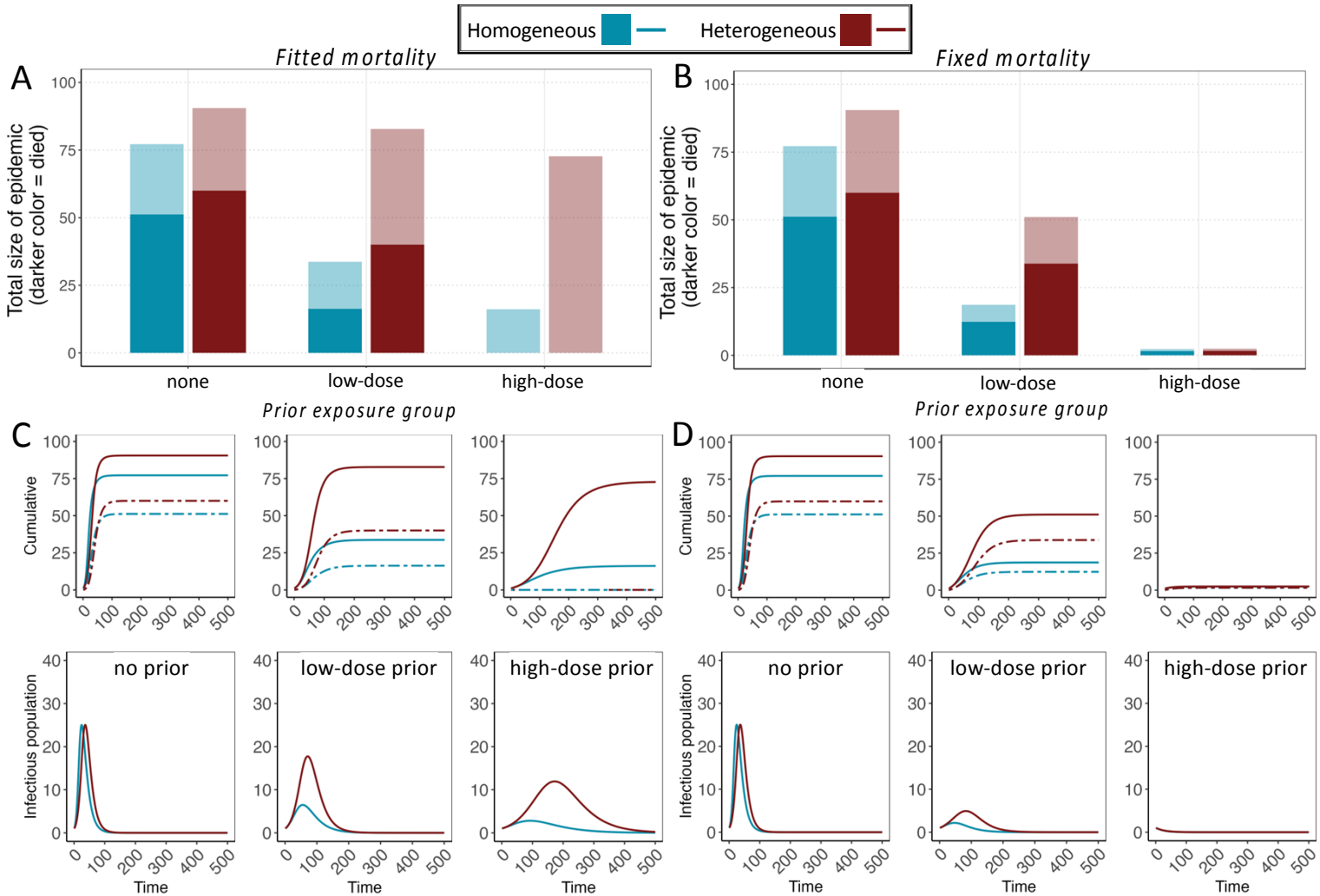

Fig 4. Cumulative epidemic size (proportion of individuals who ever entered the infectious class) (A,B); dynamics of cumulative infections and deaths (top: C, D; deaths: dashed lines) over time, and numbers of infectious hosts over time (bottom: C, D) in our SIR model, as a function of prior exposure treatment (x-axis for A,B; columns for C,D). Total starting population was 100; darker shading within color represents individuals (within cumulative totals) that did not survive the epidemic. Blue bars and lines use fitted heterogeneous susceptibility (gamma distribution) for all prior exposure groups (Fig 3, Table S2). Red bars and lines assume homogeneous susceptibility for all groups, with mean susceptibility for each group equal to that of the fitted heterogeneous distribution for that treatment group. Left panels (A,C) use fitted mortality rates, whereby estimated mortality rates declined with prior exposure, while right panels (B,D) assume the fixed mortality rate of all groups is equal to that of the no prior exposure birds.

Discussion

Here we use a naturally-occurring host-pathogen system characterized by high rates of reinfection (32) to test how acquired protection from variable degrees of prior pathogen exposure influences host heterogeneity in susceptibility. Using experimental infection data for birds across three distinct prior exposure treatments, we tested for population-level heterogeneity by statistically comparing dose response models that assume either identical susceptibilities among hosts in a population (homogeneous model) or inter-host variation in susceptibility (heterogeneous model). We find that prior exposure to either low or high doses of pathogen significantly augments population-level heterogeneity in susceptibility relative to a pathogen-naive host population, with the extent of population-level heterogeneity increasing with the degree of prior pathogen exposure, from none, low, to high-dose. In addition, our SIR model demonstrates that the observed changes in host susceptibility with prior exposure have key effects on epidemic dynamics. After accounting for estimated effects of prior exposure on host mortality, changes in population-level heterogeneity had stronger effects on outbreak dynamics than reductions in mean host susceptibility, resulting in dramatically lower outbreak sizes.

The past several decades have brought growing recognition that many host-pathogen systems are characterized by reinfection potential, whether shortly following initial infection and recovery, or over longer timescales after any host acquired protection from prior infection has waned (12–15,18,43–46). Despite the ubiquity of highly specific immune memory that allows hosts to respond rapidly to and effectively resist reinfection with the same pathogen, the acquired protection generated by prior pathogen exposure is often incomplete, even in vertebrate taxa with highly specific antigen repertoires and associated immune memory (47). Thus, some degree of reinfection is still possible, despite lower mean susceptibility in hosts with acquired protection (7,21). Given the importance of reinfection in many systems, it is critical to characterize variability in key epidemiological traits such as susceptibility for host populations

273 made up of individuals with some degree of acquired protection, because this population-level
274 heterogeneity can strongly influence the likelihood and severity of outbreaks (3,27,48,49), as
275 well as the evolution of virulence for pathogens (7,9,36,50).

276 One intuitive prediction is that host acquired protection from prior exposure to pathogens
277 should homogenize variation in susceptibility in a population by reducing susceptibility similarly
278 for all individuals. Instead, we found that the protection acquired from prior exposure to a
279 naturally-occurring bacterial pathogen, whether at low or high doses, significantly augmented
280 host heterogeneity in susceptibility in house finches. Importantly, while our dose-response
281 estimates for birds with no prior exposure to MG are well supported by models assuming
282 homogeneous susceptibility, this does not indicate that there is no biological variation in
283 susceptibility present within this treatment group. Instead, this result suggests that variation in
284 susceptibility among house finches without prior exposure is of sufficiently low magnitude, which
285 is further supported by the low coefficient of variation of the estimated gamma distribution for
286 this group. Prior studies in this system find variation among both individuals and populations in
287 other types of host responses to experimental MG infection, including infection severity
288 (pathogen loads), disease severity (eye scores), and tolerance (per-pathogen disease severity)
289 (e.g. 51–54), even for birds with no prior exposure to MG infection. Whether host susceptibility
290 *per se* tends to be less variable than other types of host responses, both in this system and
291 others, is an interesting topic for future inquiry, particularly given the key epidemiological
292 consequences of host susceptibility *per se*.

293 Interestingly, the only other study to examine effects of acquired protection (from
294 vaccination rather than active infection) on host heterogeneity in susceptibility (6) found similar
295 results: vaccination of rainbow trout against a virus augmented host heterogeneity in
296 susceptibility relative to unvaccinated individuals. Although the protection that hosts acquire
297 from active infection can be stronger and/or more variable in strength or duration than that
298 acquired by vaccination (e.g. 55,56), our susceptibility distributions suggest that host protection

generated by prior exposure to live pathogen, whether low or high doses, significantly augments host heterogeneity in susceptibility, akin to patterns found for vaccination of trout. Further, the detected pattern of increasing heterogeneity in susceptibility with higher degrees of prior pathogen exposure, from no, low-dose, to high-dose prior exposure treatments, suggests that stronger stimulation of host acquired immune responses by high pathogen doses (in this case, 30000 CCU/mL) can, at least in this system, lead to even more variability across individuals in a population. Intriguingly, we observed highly variable responses to prior exposure despite using an MG strain (VA1994) isolated from free-living house finches over 25 years ago. Because significant host evolution has occurred since then (51,54), one might expect less variable host responses to the strain used here, compared to a more recent MG strain, if host resistance traits have been favored or even fixed in the host population. Nonetheless, prior exposure to an older, basal house finch MG strain still induced significantly higher levels of host heterogeneity in susceptibility, even at low prior exposure doses. Because strain traits such as virulence may influence the degree of host heterogeneity in a population (7), future work should examine whether prior exposure to MG strains more virulent and derived than the strain used here generates even higher degrees of heterogeneity in susceptibility in a host population.

We used an SIR model parameterized empirically to predict how variation in host prior exposure would influence epidemic dynamics in this system. Consistent with prior work using SIR (6,10) and dynamic network approaches (57), we show that population-level heterogeneity in susceptibility (here as a result of prior pathogen exposure) suppresses overall outbreak size relative to models that assume homogeneous host susceptibility. Importantly, under either set of mortality assumptions used in our model, outbreak size is always reduced when prior exposure generates heterogeneity in susceptibility, compared to the homogeneous case that still accounts for reductions in mean host susceptibility with prior exposure. These results indicate direct effects of exposure-induced heterogeneity *per se* on outbreak size, effects which act above and beyond effects of mean susceptibility from prior pathogen exposure on outbreak size. Moreover,

325 under the more realistic assumption of host protection from mortality after prior pathogen
326 exposure, the reductions on overall epidemic size in our models appear to be driven
327 predominantly by increased host heterogeneity in susceptibility.

328 An earlier SIR model of vaccine-induced heterogeneity also found that augmented host
329 heterogeneity in susceptibility reduced outbreak size (6), but this model did not incorporate
330 potential changes in host mortality rate. Reductions in mortality rate in hosts with prior pathogen
331 exposure, which we estimated were significant in the house finch system (Table S2), are likely
332 common in hosts with acquired protection, as such protection often reduces host disease and
333 mortality more strongly than infection risk (e.g. 58,59). In our model results, the degree to which
334 prior exposure-induced reductions in mean susceptibility drive outbreak size depends on
335 assumptions of mortality rates in our model. In our model with empirically-derived mortality
336 rates, infected birds in the prior exposure groups are more likely to survive infection (lighter
337 color sections of bars; Fig 4A). Intriguingly, these higher survival rates nearly outweigh the
338 protective effects of reduced mean susceptibility in previously-exposed hosts, resulting in
339 relatively little reduction in epidemic size in both prior exposure groups (assuming
340 homogeneous susceptibility for both groups; red bars, Fig 4A), despite significantly lower mean
341 host susceptibility in birds with prior exposure. In contrast, when mortality rates are held
342 constant regardless of prior exposure treatment, exposure-induced reductions in mean host
343 susceptibility alone result in substantially smaller outbreak sizes (red bars; Fig 4B). These
344 results indicate that effects of prior exposure on mortality rate and susceptibility distributions are
345 both critical to account for in epidemiological models, as they may interact to drive outbreak
346 size.

347 One limitation of our SIR model is that it assumes that epidemics begin in host
348 populations entirely composed of individuals of one prior exposure type. While such models are
349 likely realistic representations of host populations at certain times of the year (e.g., epidemics in
350 late summer that occur in juvenile flocks composed of fully susceptible finches (60,61)), future

351 models should also consider scenarios whereby populations are composed of some individuals
352 with no prior exposure, and others with varying degrees of prior pathogen exposure. Developing
353 a simulated population to such a state would require knowing how the level of susceptibility of
354 individual birds is altered by prior exposure, for which no data exists. Another limitation is the
355 choice of an SIR rather than an SIRS model that allows for loss of immunity over time, as is the
356 case in the house finch-MG model system, in which protection wanes over approximately one
357 year (40). To disentangle effects of population-level heterogeneity of susceptibility induced by
358 prior exposure, we focus this study on a single, short timescale epidemic while ignoring
359 demographic effects and re-infection, similar to previous work (26,62,63). Allowing for
360 reinfection in our model would require an understanding of how susceptibility and infectivity are
361 linked, because such correlations have an impact on the progression of epidemics (62,63).
362 However, such correlations are particularly challenging to quantify in natural host-pathogen
363 systems. Overall, our work represents an important first step in incorporating one key source
364 (prior exposure) of individual variation in susceptibility into mathematical models. Although the
365 importance of individual variation in infection-derived immunity for epidemiological dynamics is
366 increasingly recognized (23,64), to our knowledge, our study is the first to explicitly consider
367 how host prior exposure to pathogens influences population-level heterogeneity in susceptibility.
368 In summary, our results highlight the key importance of variable protection from prior
369 exposure and/or vaccination in driving host heterogeneity in susceptibility, a population-level
370 trait with key downstream consequences for epidemiological and evolutionary dynamics
371 (7,10,48). Because the host protection acquired from a prior infection (or vaccination) is
372 incomplete and/or wanes over time in diverse vertebrate systems (e.g. 18,46), exposure-
373 induced heterogeneity in susceptibility may be a common phenomenon for many animal hosts,
374 including humans. While work to date has largely focused on innate sources of host
375 heterogeneity in susceptibility [sex (65), genotype (66), etc.], our results show that population-

376 level heterogeneity in susceptibility can be readily “induced” by prior pathogen exposure, with
377 key downstream consequences.
378
379 **Methods**
380
381 **Bird Capture and Housing**
382 We captured 157 mixed-sex, hatch-year house finches (aged by plumage (67)) in
383 Montgomery County, Virginia using a combination of cage traps and mist nets in June-Aug
384 2021. Capture and collection were approved by Virginia Department of Game and Inland
385 Fisheries (066646) and USFWS (MB158404), and all handling and care procedures were
386 approved by the Virginia Tech (VT) IACUC. Birds were housed in pairs for a two-week minimum
387 quarantine period in an indoor animal facility, and captured every 3-4 days to assess potential
388 clinical signs of mycoplasmal conjunctivitis. Any birds with signs of conjunctivitis (see below)
389 were immediately isolated, along with their cage-mates, and were not used in experiments.
390 For all birds that never showed clinical signs of conjunctivitis during quarantine, blood
391 samples were collected 14-18 days post-capture and run for ELISA via a commercial IDEXX kit
392 (99-06729) using methods previously described (68). Only birds seronegative via ELISA on
393 days 14-18 post-capture were assigned to MG-exposed treatment groups (Table S1; all birds
394 except n=3 “prior sham-0 challenge” birds; see Supplement). All birds were housed individually
395 and then resampled one week before prior exposure treatments for final confirmation of
396 seronegativity just prior to experiment initiation.
397
398 **Experimental Design and Timeline**
399 We created variation in prior exposure using three categorical treatments (Figure 1,
400 Table S1): no prior exposure to MG (inoculation with the same volume of sterile Frey’s media as
401 a “sham” treatment), low-dose prior exposure (dose of 750 Color Changing Units [CCU]/mL of

402 MG), or high-dose prior exposure (dose of 30,000 CCU/mL of MG). Birds were inoculated with 70
403 uL of their assigned treatment concentration directly into the conjunctivae via micropipette
404 droplets, and then allowed to recover (with recovery quantified at day 41 post-infection; see
405 below). Birds were infected with an MG strain (VA1994 isolate of MG; [7994-1 (6P) 9/17/2018])
406 that was isolated shortly following the pathogen's emergence in house finches; despite
407 significant host and pathogen evolution since then (51,69,70), this 1994 strain still results in
408 pathogen load and eye scores trajectories that are qualitatively similar to later-isolated strains
409 (VA2013 (51)).

410 Forty-two days after prior exposure treatment, birds were given a secondary dose
411 challenge with the same MG strain (again, 70uL directly inoculated into the conjunctivae). Using
412 a fully factorial design (Table S1), we inoculated birds from each prior exposure treatment with
413 one of five MG exposure doses to assess heterogeneity in susceptibility (Figure 1). We selected
414 prior exposure treatments and challenge doses (also termed "secondary doses") most likely to
415 generate and detect differences in susceptibility based on our published work on conjunctival
416 loads (33), and we minimized other inherent sources of variation in susceptibility in our wild-
417 caught birds by using same-age birds, excluding birds with detectable prior exposure at capture,
418 and randomizing assignments of sex within treatment groups, such that sex ratios were similar
419 across treatment combinations. Because of the potential for birds to remain infected from prior
420 exposure treatments until the time of secondary challenge (42 days post-prior exposure), we
421 included a set of controls (n=10 total birds) that received prior exposure doses of pathogen
422 (either low or high-dose) and then received a control inoculation of sterile media at day 42.
423 These 10 birds were all qPCR negative for MG infection by day 41 and during all three
424 subsequent sample periods post-secondary challenge (see Supplement).

425 Because our main interest was in how prior exposure treatment alters host susceptibility
426 (1|0) to secondary dose challenge, we generally do not analyze or present data collected prior
427 to secondary challenge (day 0), with one exception. To improve our dose response model fits

428 for the “no prior exposure” treatment group, we included susceptibility data generated in
429 response to the initial, low-dose prior exposure treatment. We use only the low-dose prior
430 exposure treatment for this purpose, and not the high-dose prior exposure treatment (30000
431 CCU / mL) because the low-dose treatment uniquely provided an exposure dose (750 CCU /
432 mL) that fell intermediate to those used in our secondary challenge doses (which were
433 otherwise 30, 100, 300, and 7000 CCU/mL), informing a key part of the dose response curve.
434 Thus, each of the 50 finches given a low-dose prior exposure treatment and then one of five
435 secondary challenge doses (Table 1) contributed two data points to the analyses: (i) their
436 susceptibility (1|0) to the “no-prior exposure” dose of 750 CCU/mL, with data collected on days -
437 42 to -28 prior to secondary challenge; and (ii) their susceptibility (1|0) to one of five secondary
438 doses, with data collected on days 4-14 post-secondary challenge. All other birds contributed
439 only one data point to the data-set: susceptibility (1|0) to one of five secondary doses, with data
440 collected on days 4-14 post-secondary challenge.
441
442 **Final sample sizes**
443 We began with a balanced design (n=12 birds for all non-0 MG challenge doses in Table
444 S1; n=157 total birds), but one individual died of unknown causes prior to secondary dose
445 challenge. A second individual was excluded from all analyses after study completion because,
446 when ELISA assays on frozen blood samples were completed, that individual was discovered to
447 have been MG-seropositive just before prior exposure treatments were given. Finally, five
448 additional individuals were later determined to be qPCR positive one day prior to secondary
449 challenge (see *Susceptibility and disease sampling*), and thus were eliminated from all analyses
450 to ensure that our susceptibility data represent true responses to secondary challenge rather
451 than residual effects of prior exposure treatment. Final sample sizes for all analyses were n=150
452 individuals (Table S1).
453

454 **Susceptibility and disease sampling**

455 We quantified whether birds were susceptible to infection or not (Y/N, 1|0) at a given MG
456 exposure dose via qPCR of conjunctival swab samples collected post-exposure. We also
457 visually scored clinical signs of conjunctivitis (all scoring was done blind to a bird's treatment) to
458 use as a proxy for the likelihood of mortality during infection (see below). At each sampling
459 point, we first scored clinical signs of conjunctivitis on a scale of 0 to 3 for left and right
460 conjunctiva separately, as per (71), with 0.5 score intervals used when clinical signs were
461 intermediate between two integer scores. We summed the scores (left plus right conjunctiva)
462 within a given sample day for a total maximum score of 6 for a given bird per day. After scoring,
463 each bird's conjunctiva was swabbed for 5s with a sterile cotton swab dipped in tryptose
464 phosphate broth (TPB). Swabs were swirled in 300uL of sterile TPB and then wrung out into the
465 sample collection tube. Samples from both eyes were pooled within sampling date for a given
466 individual and frozen at -20°C until processing. Genomic DNA was extracted from samples
467 using Qiagen DNeasy 96 Blood and Tissue kits (Qiagen, Valencia, CA) as previously
468 described(69). Extracted genomic DNA from each sample was used to measure overall
469 numbers of MG present in the conjunctiva using a qPCR assay targeting the *mgc2* gene of MG
470 using the primers from (72) and qPCR methods outlined in (69).

471 Conjunctival swab samples and clinical scores were taken on days 4, 7, 14, and 21 post-
472 secondary-challenge (and on days 7 and 14 post-prior exposure treatments). However, we only
473 use conjunctival swab data from the first three sample points (days 4, 7, or 14 post-challenge)
474 as most relevant for quantifying susceptibility. To account for potential low-level contamination
475 in our qPCR assay (see Supplement) and the possibility that MG loads from prior exposure
476 treatment might still be present in some birds at the time of secondary dose challenge, we
477 considered a bird as susceptible to a given dose if they had a conjunctival MG pathogen load >
478 50 copies at any of the sample points from day 4 to 14 post-challenge. This cut-off fell above the
479 highest MG load detected among birds that were given a sham secondary challenge treatment

480 (but had been previously exposed), and thus should not encompass low MG loads that
481 represent residual loads from prior exposure treatments. We also sampled all birds in the study
482 on day 41 post-prior exposure, which was one day prior to secondary challenge treatment (day
483 42). As noted above, we eliminated 5/155 birds deemed infected on day 41 from all analyses
484 (see Supplement).
485
486 **Dose response models and parameterization**
487 We used dose-response models to describe the susceptibility of naive and previously
488 exposed birds challenged with MG(6,35). We fit the models to data by minimizing the deviance
489 between the data and the model and compared models using likelihood ratio tests (35). We
490 assumed heterogeneous susceptibility following a gamma distribution (Equation 1) (27). We
491 compared models of homogeneous susceptibility (best supported for the no prior exposure
492 group), with models of gamma distributed susceptibility (best supported for the two prior
493 exposure groups).
494
495 *Equation 1*

496 $$I_{hom} = 1 - e^{-pd}$$

497 $$I_{het} = 1 - \int_0^1 e^{-xpd} f(x) dx$$

498
499 Here, d (dose) is the exposure concentration in CCU/μl, and I_{hom} or I_{het} is the proportion of
500 individuals infected. The subscript hom denotes a model assuming homogeneous susceptibility
501 where het assumes individuals have susceptibility (x) that is distributed according to a gamma
502 distribution. p is the per bacterial particle concentration rate of host infection. We calculated
503 coefficients of variation for the gamma models as the standard deviation divided by the mean.
504

505 **Fitting mortality data**

506 We use clinical scores of conjunctivitis collected post-secondary challenge (with scores
507 per bird per day ranging from 0 to 6; see above) to estimate mean mortality rates in the wild for
508 each prior exposure treatment group. We assume that these ordinal scores monotonically
509 predict host mortality because several lines of evidence indicate that increasingly severe
510 conjunctivitis and resulting visual and behavioral impairment result in higher mortality risk for
511 house finches in the wild: 1) the presence of conjunctivitis is linked with higher mortality in wild
512 birds (39); 2) higher conjunctivitis severity predicts faster capture time in mock-predation events
513 (i.e., a human capturing the bird by hand) in captivity (73); and 3) indirect mortality from
514 predators appears to be the primary source of mortality for infected birds in the wild, because
515 birds in captivity (a predator-free environment) do not ever succumb to infection, even with
516 highly virulent strains (69). Thus, MG infection *per se* does not directly cause mortality in house
517 finches, but mortality in the wild (via predation or the inability to find food) is directly linked with
518 visual impairment and the associated disease that we measure.

519

520 Mortality rate was fit using nonlinear least squares regression (nls function in R) as the
521 nonlinear inverse logit function, 1/(1+exp(-x)) scaled by 6, where x is the eye lesion score
522 (integers between 0 to 6). This fit was then scaled by vmax (0.25; maximum mortality rate
523 observed in the field from (39)) such that the maximum mortality rate is vmax rather than 1. A
524 similar approach was used in previous work (22).

525

526 **Epidemic model**

527 We use a Susceptible-Infectious-Recovered (SIR) model to examine the effect of
528 heterogeneity in susceptibility on outbreak size and impact. For populations with determined
529 heterogeneity in susceptibility, we consider a continuous distribution of susceptibility, with

susceptibility, *x*, distributed according to the parameters of the gamma distribution obtained from the dose response models.

Equation 2

$$\frac{dS(x)}{dt} = -x\beta IS(x)$$

$$\frac{dI}{dt} = \beta I \int xS(x)dx - \gamma I - \mu I$$

$$\frac{dR}{dt} = \gamma I$$

Here, $S(x)$ is the number of susceptible individuals with susceptibility x, and x is a value between 0 and 10. I is the number of infectious individuals, and R is the number of recovered individuals. Parameters for infectivity (β) and recovery (γ) were taken from a prior empirically-parameterized model in this system (74) (Table S2), while disease-induced mortality (μ) was fit as described above (See *Fitting mortality data*) for each prior exposure group (none, low, high). Infectivity and recovery rates do not depend on prior exposure, while mortality rate does (in the "fitted mortality" model). A density-dependent rather than frequency-dependent transmission term was used due to the nature of transmission in this system, which increases with density (61,75).

For simulation, we discretize the susceptibility distribution of the heterogeneous model into 300 evenly spaced susceptibility classes, represented by the midpoint susceptibility, x, for each class. We assume a population size of 100 and seed infections with a single infected individual, and the initial population in each susceptibility class is determined empirically from the fitted distributions by prior exposure treatment (See *Dose response models and parameterization*).

554 We quantify the total size of the epidemic as the cumulative fraction of infected individuals, and
555 simulate for 500 days, after which nearly all epidemics have completely resolved. Because we
556 simulate a short-time scale single epidemic, we ignore demographic effects and assume
557 recovered individuals cannot be reinfected, despite documented waning of immunity to MG over
558 the course of a year (40).
559
560 To disentangle the effects of heterogeneity in susceptibility and changes in mean susceptibility,
561 we simulate outbreaks using the homogeneous model with identical mean susceptibility to the
562 discretized heterogeneous distribution. To generate confidence intervals on outbreak sizes, we
563 used the parameter estimates obtained from bootstrapping the chi-squared residuals of the
564 dose-response estimates. We then estimated the probability of obtaining an epidemic size
565 difference as extreme as observed (hom-het) using the best fit parameters, but with the
566 heterogeneous case resulting in larger epidemics (het-hom).
567
568 **Data Availability Statement**
569 The datasets generated for this study are available in the GitHub repository, at
570 https://github.com/klangwig/mg_dose_response_public. Data and file code will be archived on
571 Dryad upon manuscript acceptance.
572
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References Cited

1. Smith KF, Goldberg M, Rosenthal S, Carlson L, Chen J, Chen C, et al. Global rise in human infectious disease outbreaks. J R Soc Interface. 2014 Dec 6;11(101):20140950.

2. Schmeller DS, Courchamp F, Killeen G. Biodiversity loss, emerging pathogens and human health risks. Biodivers Conserv. 2020 Aug 13;29(11–12):3095–102.

3. Lloyd-Smith JO, Schreiber SJ, Kopp PE, Getz WM. Superspreading and the effect of individual variation on disease emergence. Nature. 2005 Nov 17;438(7066):355–9.

4. Britton T, Ball F, Trapman P. A mathematical model reveals the influence of population heterogeneity on herd immunity to SARS-CoV-2. Science. 2020 Aug 14;369(6505):846–9.

5. Ke R, Martinez PP, Smith RL, Gibson LL, Mirza A, Conte M, et al. Daily longitudinal sampling of SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness. Nat Microbiol. 2022 May;7(5):640–52.

6. Langwig KE, Wargo AR, Jones DR, Viss JR, Rutan BJ, Egan NA, et al. Vaccine Effects on Heterogeneity in Susceptibility and Implications for Population Health Management. MBio [Internet]. 2017 Nov 21;8(6). Available from: http://dx.doi.org/10.1128/mBio.00796-17

7. Fleming-Davies AE, Dukic V, Andreasen V, Dwyer G. Effects of host heterogeneity on pathogen diversity and evolution. Ecol Lett. 2015 Nov;18(11):1252–61.

8. Hickson RI, Roberts MG. How population heterogeneity in susceptibility and infectivity influences epidemic dynamics. J Theor Biol. 2014 Jun 7;350:70–80.

9. Pugliese A. The role of host population heterogeneity in the evolution of virulence. J Biol Dyn. 2011 Mar;5(2):104–19.

10. Rodrigues P, Margheri A, Rebelo C, Gomes MGM. Heterogeneity in susceptibility to infection can explain high reinfection rates. J Theor Biol. 2009 Jul 21;259(2):280–90.

11. Dwyer G, Elkinton JS, Buonaccorsi JP. Host heterogeneity in susceptibility and disease dynamics: tests of a mathematical model. Am Nat. 1997 Dec;150(6):685–707.

12. Yamamoto T, Nagasawa I, Nojima M, Yoshida K, Kuwabara Y. Sexual transmission and reinfection of group B streptococci between spouses. J Obstet Gynaecol Res. 1999 Jun;25(3):215–9.

13. Nardell E, McInnis B, Thomas B, Weidhaas S. Exogenous reinfection with tuberculosis in a shelter for the homeless. N Engl J Med. 1986 Dec 18;315(25):1570–5.

14. Islam N, Krajden M, Shoveller J, Gustafson P, Gilbert M, Buxton JA, et al. Incidence, risk factors, and prevention of hepatitis C reinfection: a population-based cohort study. Lancet Gastroenterol Hepatol. 2017 Mar;2(3):200–10.

15. Versteegh FGA, Schellekens JFP, Nagelkerke AF, Roord JJ. Laboratory-confirmed reinfections with Bordetella pertussis. Acta Paediatr. 2007 Jan 2;91(1):95–7.

16. Bazan JA, Tzeng Y-L, Stephens DS, Carter AM, Brown MA, Snyder B, et al. Repeat Episodes of Symptomatic Urethritis Due to a Uropathogenic Meningococcal Clade. Sex

635 Transm Dis. 2020 Jan;47(1):e1–4.

636 17. Barenkamp SJ, Shurin PA, Marchant CD, Karasic RB, Pelton SI, Howie VM, et al. Do
637 children with recurrent Haemophilus influenzae otitis media become infected with a new
638 organism or reacquire the original strain? J Pediatr. 1984 Oct;105(4):533–7.

639 18. Pilz S, Theiler-Schwetz V, Trummer C, Krause R, Ioannidis JPA. SARS-CoV-2 reinfections:
640 Overview of efficacy and duration of natural and hybrid immunity. Environ Res. 2022
641 Jun;209:112911.

642 19. Sanz JC, Rodríguez-Avial I, Ríos E, Ramos B, Cercenado E. Recurrent pneumococcal
643 invasive disease in the region of Madrid during a five-year period. Infection. 2014
644 Jun;42(3):475–83.

645 20. Ranawaka MB, Munesinghe YD, de Silva DM, Carter R, Mendis KN. Boosting of
646 transmission-blocking immunity during natural Plasmodium vivax infections in humans
647 depends upon frequent reinfection. Infect Immun. 1988 Jul;56(7):1820–4.

648 21. Forshey BM, Reiner RC, Olkowski S, Morrison AC, Espinoza A, Long KC, et al. Incomplete
649 Protection against Dengue Virus Type 2 Re-infection in Peru. PLoS Negl Trop Dis. 2016
650 Feb;10(2):e0004398.

651 22. Fleming-Davies AE, Williams PD, Dhondt AA, Dobson AP, Hochachka WM, Leon AE, et al.
652 Incomplete host immunity favors the evolution of virulence in an emergent pathogen.
653 Science. 2018 Mar 2;359(6379):1030–3.

654 23. Le A, King AA, Magpantay FMG, Mesbahi A, Rohani P. The impact of infection-derived
655 immunity on disease dynamics. J Math Biol. 2021 Nov 12;83(6–7):61.

656 24. Altarawneh HN, Chemaitelly H, Hasan MR, Ayoub HH, Qassim S, AlMukdad S, et al.
657 Protection against the Omicron Variant from Previous SARS-CoV-2 Infection. N Engl J
658 Med. 2022 Mar 31;386(13):1288–90.

659 25. Gomes MGM, White LJ, Medley GF. Infection, reinfection, and vaccination under
660 suboptimal immune protection: epidemiological perspectives. J Theor Biol. 2004 Jun
661 21;228(4):539–49.

662 26. Zachreson C, Tobin R, Szanyi J, Walker C, Cromer D, Shearer FM, et al. Individual
663 variation in vaccine immune response can produce bimodal distributions of protection.
664 Vaccine. 2023 Oct 26;41(45):6630–6.

665 27. Gomes MGM, Lipsitch M, Wargo AR, Kurath G, Rebelo C, Medley GF, et al. A missing
666 dimension in measures of vaccination impacts. PLoS Pathog. 2014 Mar;10(3):e1003849.

667 28. Leon AE, Hawley DM. Host Responses to Pathogen Priming in a Natural Songbird Host.
668 Ecohealth. 2017 Dec;14(4):793–804.

669 29. Aiello CM, Nussear KE, Esque TC, Emblidge PG, Sah P, Bansal S, et al. Host contact and
670 shedding patterns clarify variation in pathogen exposure and transmission in threatened
671 tortoise Gopherus agassizii: implications for disease modelling and management. J Anim
672 Ecol. 2016;85(3):829–42.

673 30. Konrad M, Vyleta ML, Theis FJ, Stock M, Tragust S, Klatt M, et al. Social transfer of

674 pathogenic fungus promotes active immunisation in ant colonies. PLoS Biol. 2012 Apr
675 3;10(4):e1001300.

676 31. Stroeymeyt N, Grasse AV, Crespi A, Mersch DP, Cremer S, Keller L. Social network
677 plasticity decreases disease transmission in a eusocial insect. Science. 2018 Nov
678 23;362(6417):941–5.

679 32. Weitzman CL, Ceja G, Leon AE, Hawley DM. Protection Generated by Prior Exposure to
680 Pathogens Depends on both Priming and Challenge Dose [Internet]. Vol. 90, Infection and
681 Immunity. 2022. Available from: http://dx.doi.org/10.1128/iai.00537-21

682 33. Leon AE, Fleming-Davies AE, Hawley DM. Host exposure history modulates the within-host
683 advantage of virulence in a songbird-bacterium system. Sci Rep. 2019 Dec 30;9(1):20348.

684 34. Aalen OO, Valberg M, Grotmol T, Tretli S. Understanding variation in disease risk: the
685 elusive concept of frailty. Int J Epidemiol. 2015 Aug;44(4):1408–21.

686 35. Haas CN, Rose JB, Gerba CP. Quantitative Microbial Risk Assessment. John Wiley &
687 Sons; 1999. 449 p.

688 36. Miller IF, Metcalf CJ. Vaccine-driven virulence evolution: consequences of unbalanced
689 reductions in mortality and transmission and implications for pertussis vaccines. J R Soc
690 Interface. 2019 Dec;16(161):20190642.

691 37. Adelman JS, Carter AW, Hopkins WA, Hawley DM. Deposition of pathogenic Mycoplasma
692 gallisepticum onto bird feeders: host pathology is more important than temperature-driven
693 increases in food intake. Biol Lett. 2013 Oct 23;9(5):20130594.

694 38. Dhondt AA, Dhondt KV, Hawley DM, Jennelle CS. Experimental evidence for transmission
695 of Mycoplasma gallisepticum in house finches by fomites. Avian Pathol. 2007
696 Jun;36(3):205–8.

697 39. Faustino CR, Jennelle CS, Connolly V, Davis AK, Swarthout EC, Dhondt AA, et al.
698 Mycoplasma gallisepticum infection dynamics in a house finch population: seasonal
699 variation in survival, encounter and transmission rate. J Anim Ecol. 2004 Jul;73(4):651–69.

700 40. Sydenstricker KV, Dhondt AA, Ley DH, Kollias GV. Re-exposure of captive house finches
701 that recovered from Mycoplasma gallisepticum infection. J Wildl Dis. 2005 Apr;41(2):326–
702 33.

703 41. Ben-Ami F, Ebert D, Regoes RR. Pathogen dose infectivity curves as a method to analyze
704 the distribution of host susceptibility: a quantitative assessment of maternal effects after
705 food stress and pathogen exposure. Am Nat. 2010 Jan;175(1):106–15.

706 42. Ben-Ami F, Regoes RR, Ebert D. A quantitative test of the relationship between parasite
707 dose and infection probability across different host-parasite combinations. Proc Biol Sci.
708 2008 Apr 7;275(1636):853–9.

709 43. Koopman JS, Henry CJ, Park JH, Eisenberg MC, Ionides EL, Eisenberg JN. Dynamics
710 affecting the risk of silent circulation when oral polio vaccination is stopped. Epidemics.
711 2017 Sep;20:21–36.

712 44. van den Hoogen BG, Herfst S, de Graaf M, Sprong L, van Lavieren R, van Amerongen G,

et al. Experimental infection of macaques with human metapneumovirus induces transient protective immunity. J Gen Virol. 2007 Apr;88(Pt 4):1251–9.

45. Singleton R, Etchart N, Hou S, Hyland L. Inability to evoke a long-lasting protective immune response to respiratory syncytial virus infection in mice correlates with ineffective nasal antibody responses. J Virol. 2003 Nov;77(21):11303–11.

46. Edridge AWD, Kaczorowska J, Hoste ACR, Bakker M, Klein M, Loens K, et al. Seasonal coronavirus protective immunity is short-lasting. Nat Med. 2020 Nov;26(11):1691–3.

47. Boehm T. Evolution of vertebrate immunity. Curr Biol. 2012 Sep 11;22(17):R722-32.

48. Gomes MGM, Aguas R, Lopes JS, Nunes MC, Rebelo C, Rodrigues P, et al. How host heterogeneity governs tuberculosis reinfection? Proc Biol Sci. 2012 Jun 22;279(1737):2473–8.

49. Ferrari MJ, Bansal S, Meyers LA, Bjørnstad ON. Network frailty and the geometry of herd immunity. Proc Biol Sci. 2006 Nov 7;273(1602):2743–8.

50. Leggett HC, Buckling A, Long GH, Boots M. Generalism and the evolution of parasite virulence. Trends Ecol Evol. 2013 Oct;28(10):592–6.

51. Henschen AE, Vinkler M, Langager MM, Rowley AA, Dalloul RA, Hawley DM, et al. Rapid adaptation to a novel pathogen through disease tolerance in a wild songbird. PLoS Pathog. 2023 Jun;19(6):e1011408.

52. Ruden RM, Adelman JS. Disease tolerance alters host competence in a wild songbird. Biol Lett. 2021 Oct;17(10):20210362.

53. Adelman JS, Kirkpatrick L, Grodio JL, Hawley DM. House Finch Populations Differ in Early Inflammatory Signaling and Pathogen Tolerance at the Peak ofMycoplasma gallisepticumInfection [Internet]. Vol. 181, The American Naturalist. 2013. p. 674–89. Available from: http://dx.doi.org/10.1086/670024

54. Bonneaud C, Tardy L, Giraudeau M, Hill GE, McGraw KJ, Wilson AJ. Evolution of both host resistance and tolerance to an emerging bacterial pathogen. Evol Lett. 2019 Oct;3(5):544–54.

55. Wendelboe AM, Van Rie A, Salmaso S, Englund JA. Duration of immunity against pertussis after natural infection or vaccination. Pediatr Infect Dis J. 2005 May;24(5 Suppl):S58-61.

56. Hobson D. Resistance to reinfection in experimental mouse typhoid. J Hyg . 1957 Sep;55(3):334–43.

57. White LA, Forester JD, Craft ME. Covariation between the physiological and behavioral components of pathogen transmission: host heterogeneity determines epidemic outcomes. Oikos. 2018 Apr;127(4):538–52.

58. Kapczynski DR, King DJ. Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. Vaccine. 2005 May 16;23(26):3424–33.

751 59. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing
752 antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2
753 infection. Nat Med. 2021 Jul;27(7):1205–11.

754 60. Altizer S, Davis AK, Cook KC, Cherry JJ. Age, sex, and season affect the risk of
755 mycoplasmal conjunctivitis in a southeastern house finch population. Can J Zool. 2004 May
756 1;82(5):755–63.

757 61. Hosseini PR, Dhondt AA, Dobson A. Seasonality and wildlife disease: how seasonal birth,
758 aggregation and variation in immunity affect the dynamics of Mycoplasma gallisepticum in
759 house finches. Proc Biol Sci. 2004 Dec 22;271(1557):2569–77.

760 62. Tkachenko AV, Maslov S, Elbanna A, Wong GN, Weiner ZJ, Goldenfeld N. Time-
761 dependent heterogeneity leads to transient suppression of the COVID-19 epidemic, not
762 herd immunity. Proc Natl Acad Sci U S A [Internet]. 2021 Apr 27;118(17). Available from:
763 http://dx.doi.org/10.1073/pnas.2015972118

764 63. Rose C, Medford AJ, Goldsmith CF, Vegge T, Weitz JS, Peterson AA. Heterogeneity in
765 susceptibility dictates the order of epidemic models. J Theor Biol. 2021 Nov 7;528:110839.

766 64. Carlsson R-M, Childs LM, Feng Z, Glasser JW, Heffernan JM, Li J, et al. Modeling the
767 waning and boosting of immunity from infection or vaccination. J Theor Biol. 2020 Jul
768 21;497:110265.

769 65. Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering how biological
770 sex impacts immune responses and COVID-19 outcomes. Nat Rev Immunol. 2020
771 Jul;20(7):442–7.

772 66. Charbonnel N, Pagès M, Sironen T, Henttonen H, Vapalahti O, Mustonen J, et al.
773 Immunogenetic factors affecting susceptibility of humans and rodents to hantaviruses and
774 the clinical course of hantaviral disease in humans. Viruses. 2014 May 26;6(5):2214–41.

775 67. Al-Bowardi NC, Riddle KE, Bragin EA. Pyle, P. 1997. Identification guide to North. Available
776 from: https://sora.unm.edu/sites/default/files/journals/nabb/v027n01/p0014-p0018.pdf

777 68. Hawley DM, Grodio J, Frasca S, Kirkpatrick L, Ley DH. Experimental infection of domestic
778 canaries (Serinus canaria domestica) with Mycoplasma gallisepticum: a new model system
779 for a wildlife disease. Avian Pathol. 2011 Jun;40(3):321–7.

780 69. Hawley DM, Osnas EE, Dobson AP, Hochachka WM, Ley DH, Dhondt AA. Parallel patterns
781 of increased virulence in a recently emerged wildlife pathogen. PLoS Biol. 2013 May
782 28;11(5):e1001570.

783 70. Bonneaud C, Giraudeau M, Tardy L, Staley M, Hill GE, McGraw KJ. Rapid Antagonistic
784 Coevolution in an Emerging Pathogen and Its Vertebrate Host. Curr Biol. 2018 Sep
785 24;28(18):2978-2983.e5.

786 71. Sydenstricker KV, Dhondt AA, Hawley DM, Jennelle CS, Kollias HW, Kollias GV.
787 Characterization of experimental Mycoplasma gallisepticum infection in captive house finch
788 flocks. Avian Dis. 2006 Mar;50(1):39–44.

789 72. Grodio JL, Dhondt KV, O'Connell PH, Schat KA. Detection and quantification of

790 Mycoplasma gallisepticum genome load in conjunctival samples of experimentally infected
791 house finches (Carpodacus mexicanus) using real-time polymerase chain reaction. Avian
792 Pathol. 2008 Aug;37(4):385–91.

793 73. Adelman JS, Mayer C, Hawley DM. Infection reduces anti-predator behaviors in house
794 finches. J Avian Biol. 2017 Apr;48(4):519–28.

795 74. Williams PD, Dobson AP, Dhondt KV, Hawley DM, Dhondt AA. Evidence of trade-offs
796 shaping virulence evolution in an emerging wildlife pathogen. J Evol Biol. 2014
797 Jun;27(6):1271–8.

798 75. Hochachka WM, Dhondt AA. Density-dependent decline of host abundance resulting from a
799 new infectious disease. Proc Natl Acad Sci U S A. 2000 May 9;97(10):5303–6.

800
801

802 **Figure Captions**

803

804 Figure 1. Experimental design for quantifying how prior exposure to *Mycoplasma gallisepticum*
805 alters host heterogeneity in susceptibility in house finches (n=150 for final analysis). Five weeks
806 after one of three prior exposure (PE) treatments (none, low, or high dose; left panel), all birds
807 received a secondary dose challenge (right panel) to assess heterogeneity in susceptibility. Our
808 primary data set was whether birds became infected (0|1) in response to a given secondary
809 dose (right). *However, to improve model fits, we also used bird responses to low-dose PE
810 treatment (left, asterisk), which fell intermediate to our highest secondary challenge doses (300
811 and 7000 Color Changing Units [CCU]/mL), to quantify the proportion of birds with no prior
812 exposure (at the time of low-dose PE) that become infected at a 750 CCU/mL dose. Made in
813 Biorender.

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815 Figure 2**.** Dose response curves for house finch susceptibility to secondary challenge with
816 *Mycoplasma gallisepticum* across prior exposure treatments. Points (+/- 1SE) show the fraction
817 of individual birds (n=10-12 birds for most points; individual responses are 1|0) infected at each
818 secondary exposure dose, shown as Color Changing Units (CCU)/uL. Lines indicate model fits,
819 with blue indicating gamma (heterogeneous) model fits, and red dashed lines indicating
820 homogeneous model fits. Panel labels show prior exposure treatment (birds in the no prior
821 exposure treatment were pathogen-naive at the time of secondary dose challenge). In hosts
822 with prior pathogen exposure (low and high-dose prior exposure groups), the gamma model
823 (which accounts for inter-individual heterogeneity) was better supported via likelihood ratio tests.

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825 Figure 3. Host susceptibility distributions for house finches from variable prior exposure
826 treatments: no prior exposure (A,B); low-dose (C); or high-dose (D). Colored lines show
827 estimated susceptibility distributions from either homogeneously (A) or gamma-distributed (B-D)

models (note distinct axes for the two models). In (A), susceptibility (*p*) is shown as the single best fit parameter *p* (dotted vertical lines represent 1 standard error) for the homogeneous model, which was the best fit model for the no-prior exposure group (see *Results*). In (B-D), the best fit parameters (shape and scale) for gamma distributions (teal line) are listed for each group, and vertical gray lines indicate mean susceptibility (*x*) for that treatment. Lighter shading represents 95% confidence regions for gamma distributions, obtained by bootstrapping chi-squared residuals to create 1,000 pseudoreplicates of infection data and then refitting the model to pseudoreplicates, as per (6,35). The gamma model was the best fit for only the low-dose and high-dose groups. Gamma estimates are also shown for the no prior exposure group (B) because this allowed more equivalent comparisons for certain SIR simulations (see *Methods*).

Figure 4. Cumulative epidemic size (proportion of individuals who ever entered the infectious class) (A,B); dynamics of cumulative infections and deaths (top: C, D; deaths: dashed lines) over time, and numbers of infectious hosts over time (bottom: C, D) in our SIR model, as a function of prior exposure treatment (x-axis for A,B; columns for C,D). Total starting population was 100; darker shading within color represents individuals (within cumulative totals) that did not survive the epidemic. Blue bars and lines use fitted heterogeneous susceptibility (gamma distribution) for all prior exposure groups (Fig 3, Table S2). Red bars and lines assume homogeneous susceptibility with mean susceptibility equal to that of the fitted heterogeneous distribution. Left panels (A,C) use fitted mortality rates, whereby estimated mortality rates declined with prior exposure, while right panels (B,D) assume the fixed mortality rate of all groups is equal to that of the no prior exposure birds.