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

Introduction

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

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

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

80 protection similarly for all exposed hosts (23), such that all hosts receive identical protection
81 from reinfection. Alternatively, prior pathogen exposure could result in relatively complete
82 acquired protection for some proportion of hosts, while others remain relatively unprotected
83 (termed “all-or-nothing” immunity (23)). Epidemiological models often select either wholly leaky
84 protection of hosts that allow every host an identical rate of reinfection or all-or-nothing
85 protection such that some hosts remain in the recovered class while a subset is routed back to
86 the susceptible class, representing two extremes of how heterogeneity is incorporated into
87 disease models (6,27). Whether and how prior exposure to pathogens alters population-level
88 heterogeneity in susceptibility in a given system likely depends on the extent of prior pathogen
89 exposure that hosts experience (28) and the mode of action of such protection [“leaky”, “all-or-
90 nothing” (6,27), or some intermediate (26)]. For example, host exposure to low pathogen doses,
91 which can occur during the natural transmission process for many pathogens (e.g. 29–31),
92 might be more likely than higher exposure doses to generate incomplete host protection (32,33)
93 and thus to induce population-level heterogeneity in susceptibility.
94 Regardless of the underlying mechanisms, effects of prior exposure on population-level
95 heterogeneity in host susceptibility are particularly important to characterize because this
96 heterogeneity can have key epidemiological consequences, including lower predicted outbreak
97 sizes (6) and higher rates of reinfection (10). This is in part due to the process of cohort
98 selection, through which the most susceptible individuals are infected first in any given epidemic
99 time step, leaving lower mean susceptibility among remaining uninfected hosts in a population
100 (34). Experimental quantification of population-level heterogeneity in susceptibility draws on
101 quantitative microbial risk assessment approaches (35) to estimate susceptibility distributions
102 using a series of increasing pathogen challenge doses. Because only the most susceptible
103 hosts become infected at low challenge doses, whereas even the least susceptible hosts
104 become infected at the highest challenge doses, dose-response models can be used to
105 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.

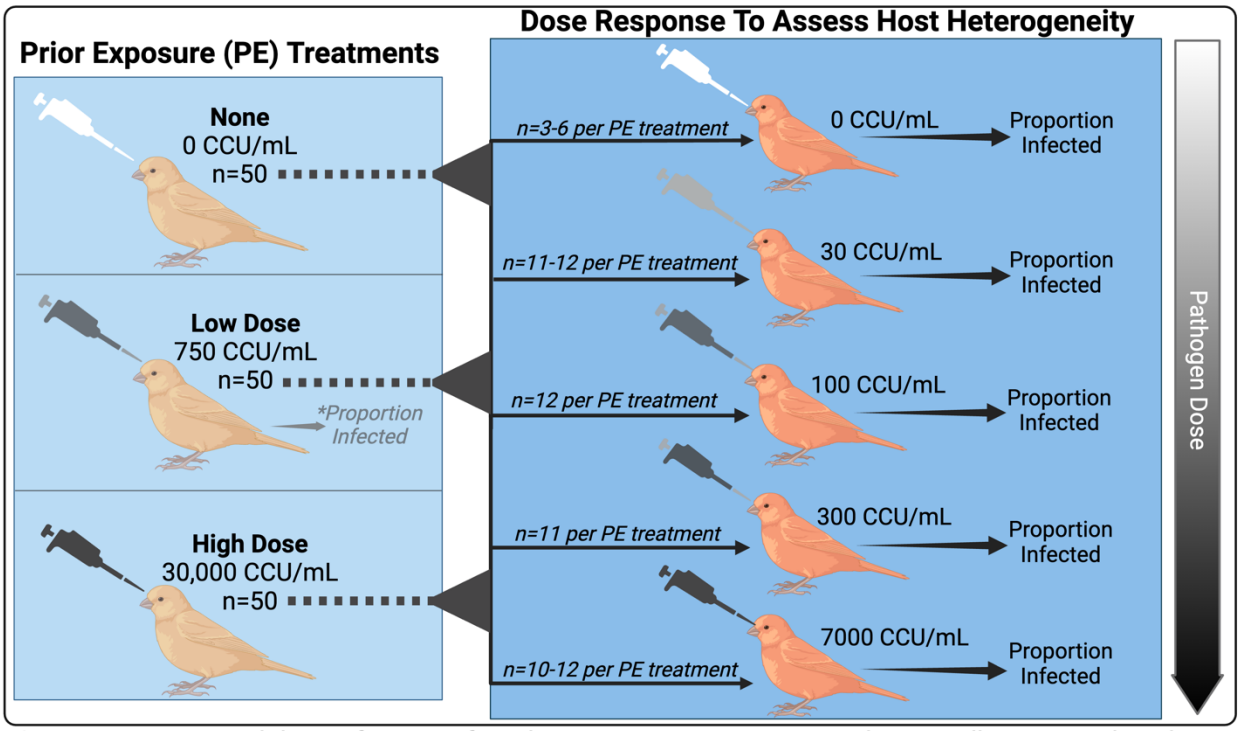

Figure 1. Experimental design for quantifying how prior exposure to *Mycoplasma gallisepticum* alters host heterogeneity in susceptibility in house finches (n=150 for final analysis). Five weeks after one of three prior exposure (PE) treatments (none, low, or high dose; left panel), all birds received 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).

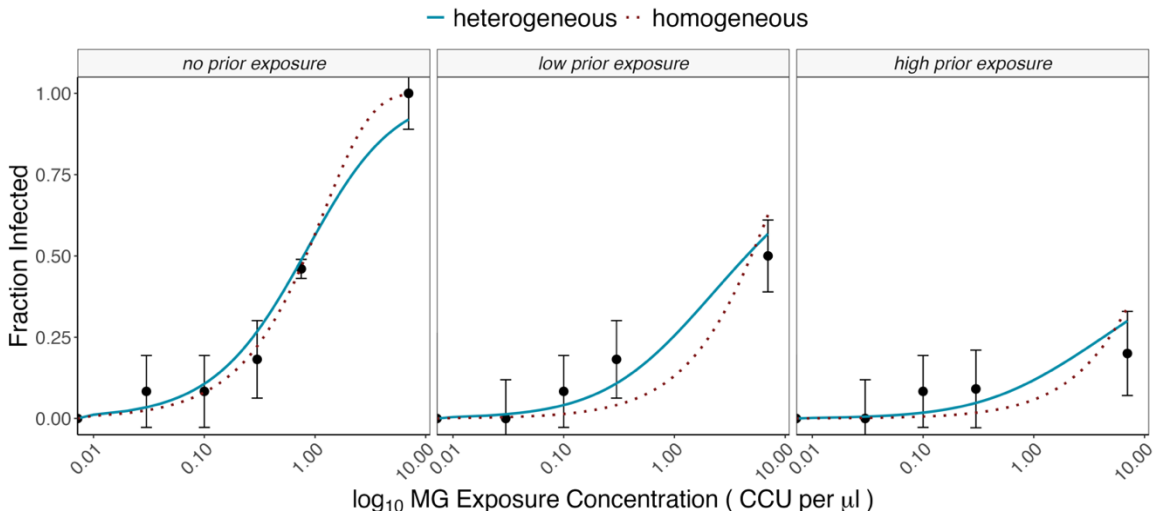

Figure 2. Dose response curves for house finch susceptibility to secondary challenge with *Mycoplasma gallisepticum* across prior exposure treatments. Points (+/- 1SE) show the fraction of individual birds (n=10-12 birds for most points; individual responses are 1|0) infected at each secondary exposure 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.

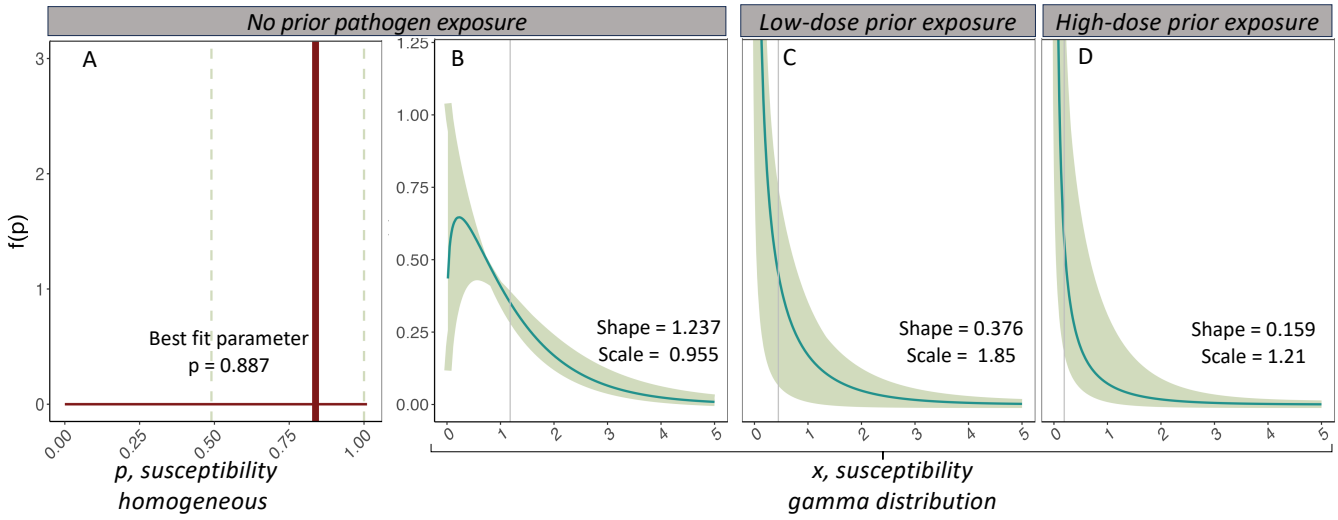

Fig 3. Host susceptibility distributions for house finches from variable prior exposure treatments: no prior exposure (A,B); low-dose (C); or high-dose (D). Colored lines show estimated susceptibility distributions from either homogeneously (A) or gamma-distributed (B-D) models (note distinct axes for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

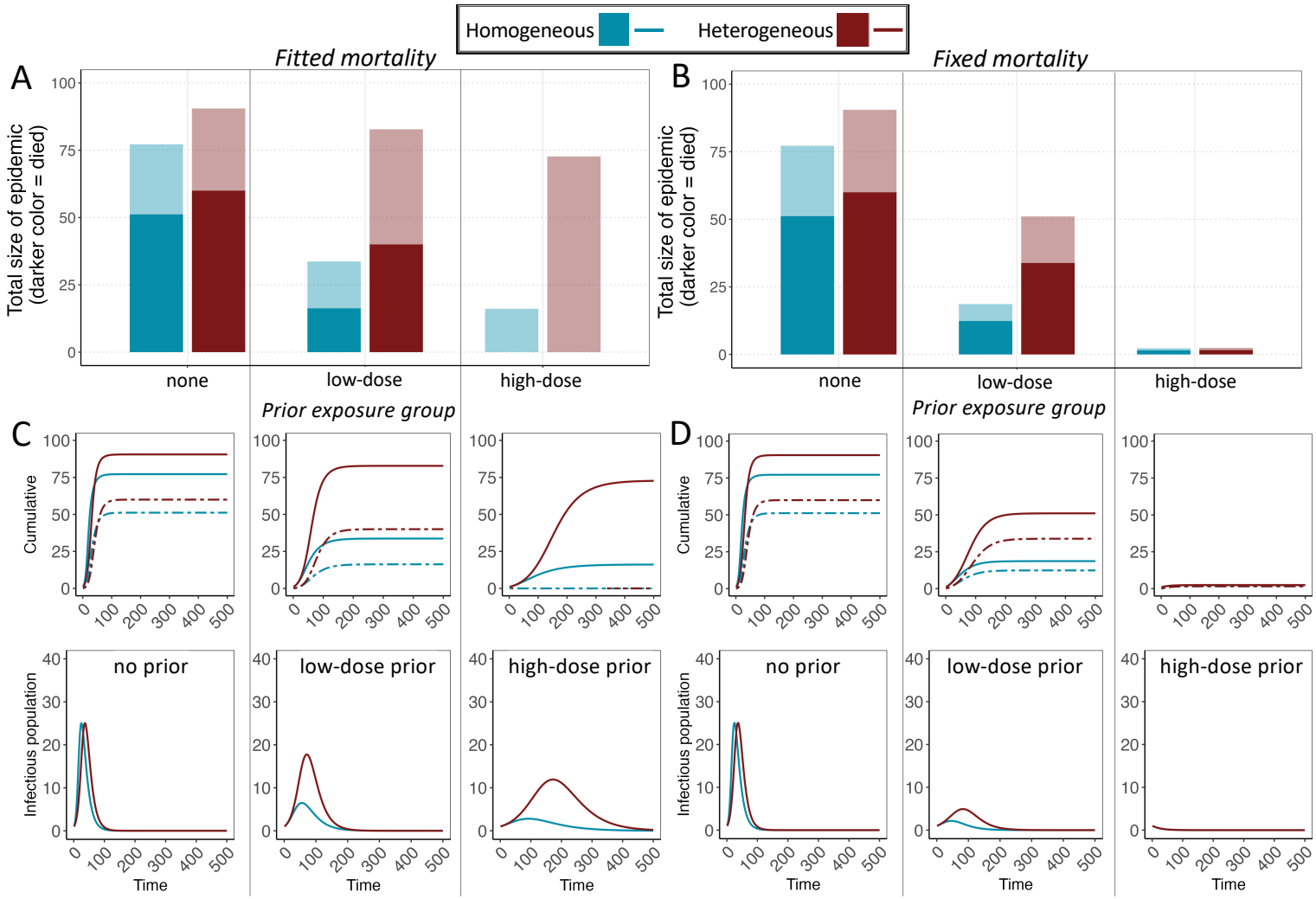

Fig 4. Cumulative epidemic size (proportion of individuals who ever entered the infectious class) (A,B); dynamics of cumulative infections and deaths (top: C, D; deaths: dashed lines) over time, and numbers of infectious hosts over time (bottom: C, D) in our SIR model, as a function of prior exposure 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

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

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

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

303 generated by prior exposure to live pathogen, whether low or high doses, significantly augments
304 host heterogeneity in susceptibility, akin to patterns found for vaccination of trout. Further, the
305 detected pattern of increasing heterogeneity in susceptibility with higher degrees of prior
306 pathogen exposure, from no, low-dose, to high-dose prior exposure treatments, suggests that
307 stronger stimulation of host acquired immune responses by high pathogen doses (in this case,
308 30000 CCU/mL) can, at least in this system, lead to even more variability across individuals in a
309 population. Intriguingly, we observed highly variable responses to prior exposure despite using
310 an MG strain (VA1994) isolated from free-living house finches over 25 years ago. Because
311 significant host evolution has occurred since then (51,54), one might expect less variable host
312 responses to the strain used here, compared to a more recent MG strain, if host resistance traits
313 have been favored or even fixed in the host population. Nonetheless, prior exposure to an older,
314 basal house finch MG strain still induced significantly higher levels of host heterogeneity in
315 susceptibility, even at low prior exposure doses. Because strain traits such as virulence may
316 influence the degree of host heterogeneity in a population (7), future work should examine
317 whether prior exposure to MG strains more virulent and derived than the strain used here
318 generates even higher degrees of heterogeneity in susceptibility in a host population.
319 We used an SIR model parameterized empirically to predict how variation in host prior
320 exposure would influence epidemic dynamics in this system. Consistent with prior work using
321 SIR (6,10) and dynamic network approaches (57), we show that population-level heterogeneity
322 in susceptibility (here as a result of prior pathogen exposure) suppresses overall outbreak size
323 relative to models that assume homogeneous host susceptibility. Importantly, under either set of
324 mortality assumptions used in our model, outbreak size is always reduced when prior exposure
325 generates heterogeneity in susceptibility, compared to the homogeneous case that still accounts
326 for reductions in mean host susceptibility with prior exposure. These results indicate direct
327 effects of exposure-induced heterogeneity *per se* on outbreak size, effects which act above and
328 beyond effects of mean susceptibility from prior pathogen exposure on outbreak size. Moreover,

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

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

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

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

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

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

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

429 Because our main interest was in how prior exposure treatment alters host susceptibility
430 (1|0) to secondary dose challenge, we generally do not analyze or present data collected prior
431 to secondary challenge (day 0), with one exception. To improve our dose response model fits

432 for the “no prior exposure” treatment group, we included susceptibility data generated in
433 response to the initial, low-dose prior exposure treatment. We use only the low-dose prior
434 exposure treatment for this purpose, and not the high-dose prior exposure treatment (30000
435 CCU / mL) because the low-dose treatment uniquely provided an exposure dose (750 CCU /
436 mL) that fell intermediate to those used in our secondary challenge doses (which were
437 otherwise 30, 100, 300, and 7000 CCU/mL), informing a key part of the dose response curve.
438 Thus, each of the 50 finches given a low-dose prior exposure treatment and then one of five
439 secondary challenge doses (Table S1) contributed two data points to the analyses: (i) their
440 susceptibility (1|0) to the “no-prior exposure” dose of 750 CCU/mL, with data collected on days -
441 42 to -28 prior to secondary challenge; and (ii) their susceptibility (1|0) to one of five secondary
442 doses, with data collected on days 4-14 post-secondary challenge. All other birds contributed
443 only one data point to the data-set: susceptibility (1|0) to one of five secondary doses, with data
444 collected on days 4-14 post-secondary challenge.
445
446 **Final sample sizes**
447 We began with a balanced design (n=12 birds for all non-0 MG challenge doses in Table
448 S1; n=157 total birds), but one individual died of unknown causes prior to secondary dose
449 challenge. A second individual was excluded from all analyses after study completion because,
450 when ELISA assays on frozen blood samples were completed, that individual was discovered to
451 have been MG-seropositive just before prior exposure treatments were given. Finally, five
452 additional individuals were later determined to be qPCR positive one day prior to secondary
453 challenge (see *Susceptibility and disease sampling*), and thus were eliminated from all analyses
454 to ensure that our susceptibility data represent true responses to secondary challenge rather
455 than residual effects of prior exposure treatment. Final sample sizes for all analyses were n=150
456 individuals (Table S1).
457

458 **Susceptibility and disease sampling**

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

475 Conjunctival swab samples and clinical scores were taken on days 4, 7, 14, and 21 post-
476 secondary-challenge (and on days 7 and 14 post-prior exposure treatments). However, we only
477 use conjunctival swab data from the first three sample points (days 4, 7, or 14 post-challenge)
478 as most relevant for quantifying susceptibility. To account for potential low-level contamination
479 in our qPCR assay (see Supplement) and the possibility that MG loads from prior exposure
480 treatment might still be present in some birds at the time of secondary dose challenge, we
481 considered a bird as susceptible to a given dose if they had a conjunctival MG pathogen load >
482 50 copies at any of the sample points from day 4 to 14 post-challenge. This cut-off fell above the
483 highest MG load detected among birds that were given a sham secondary challenge treatment

(but had been previously exposed), and thus should not encompass low MG loads that represent residual loads from prior exposure treatments. We also sampled all birds in the study on day 41 post-prior exposure, which was one day prior to secondary challenge treatment (day 42). As noted above, we eliminated 5/155 birds deemed infected on day 41 from all analyses (see Supplement).

Dose response models and parameterization

We used dose-response models to describe the susceptibility of naive and previously exposed birds challenged with MG(6,35). We fit the models to data by minimizing the deviance between the data and the model and compared models using likelihood ratio tests (35). We assumed heterogeneous susceptibility following a gamma distribution (Equation 1) (27). We compared models of homogeneous susceptibility (best supported for the no prior exposure group), with models of gamma distributed susceptibility (best supported for the two prior exposure groups).

Equation 1

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

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

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

509 **Fitting mortality data**

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

523

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

529

530 **Epidemic model**

531 We use a Susceptible-Infectious-Recovered (SIR) model to examine the effect of
532 heterogeneity in susceptibility on outbreak size and impact. For populations with determined
533 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*).

558 We quantify the total size of the epidemic as the cumulative fraction of infected individuals, and
559 simulate for 500 days, after which nearly all epidemics have completely resolved. Because we
560 simulate a short-time scale single epidemic, we ignore demographic effects and assume
561 recovered individuals cannot be reinfected, despite documented waning of immunity to MG over
562 the course of a year (40).
563
564 To disentangle the effects of heterogeneity in susceptibility and changes in mean susceptibility,
565 we simulate outbreaks using the homogeneous model with identical mean susceptibility to the
566 discretized heterogeneous distribution. To generate confidence intervals on outbreak sizes, we
567 used the parameter estimates obtained from bootstrapping the chi-squared residuals of the
568 dose-response estimates. We then estimated the probability of obtaining an epidemic size
569 difference as extreme as observed (hom-het) using the best fit parameters, but with the
570 heterogeneous case resulting in larger epidemics (het-hom).
571
572 **Data Availability Statement**
573 The datasets generated for this study are available in the GitHub repository, at
574 https://github.com/klangwig/mg_dose_response_public. Data and file code will be archived on
575 Dryad upon manuscript acceptance.
576
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