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Incomplete host immunity favors the evolution of virulence in an emergent pathogen

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Abstract

Immune memory evolved to protect hosts from reinfection, but incomplete responses that allow future reinfection might inadvertently select for more harmful pathogens. We present empirical and modeling evidence that incomplete immunity promotes the evolution of higher virulence in a natural host-pathogen system. We performed sequential infections of house finches with *Mycoplasma gallisepticum* strains of varying virulence. Virulent bacterial strains generated stronger host protection against reinfection than less virulent strains, and thus excluded less virulent strains from infecting previously-exposed hosts. In a two-strain model, the resulting fitness advantage selected for an almost two-fold increase in pathogen virulence. Thus, the same immune systems that protect hosts from infection can concomitantly drive the evolution of more harmful pathogens in nature.

One sentence summary:

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Supplementary Materials:

Materials and Methods

Supplementary Text: Pathogen traits and key terminology, Modeling framework, Model fitting to empirical data, Invasion analysis, Numerical sensitivity analysis

Figures S1 to S4

Tables S1 to S5

References (36–49)

By inducing strong protection against later infection, virulent pathogen strains are favored by incomplete immunity in a natural songbird system.

Imperfect vaccines promote the evolution of more harmful pathogens by creating a fitness advantage for virulent strains in vaccinated hosts, such as high rates of infectivity or transmission (1–5). By preventing disease-induced host death and subsequent removal of virulent strains from a population, imperfect vaccines also reduce the overall costs of virulence to pathogens (5). We asked whether incomplete immune responses to natural infections can similarly favor the evolution of more virulent pathogen strains that cause greater host mortality. The bacterial pathogen *Mycoplasma gallisepticum* emerged in the 1990s in free-living house finches (*Haemorrhous mexicanus*), causing severe conjunctivitis that indirectly reduces finch survival via visual impairment and reduced ability to escape predators (6, 7). Following emergence in the eastern United States, *M. gallisepticum* spread throughout the house finch range (8), transmitted by direct contact and contaminated surfaces, such as bird feeders (9). Soon after the pathogen became endemic on each coast of the USA, there was a rapid increase in the virulence of circulating *M. gallisepticum* strains, as measured by disease severity produced in common-background hosts (10). More than half of free-living house finches can recover from *M. gallisepticum* infection (6), generating pools of recovered hosts in wild populations. Furthermore, recovered hosts show significant but incomplete immune protection, and thus can become reinfected with homologous or heterologous pathogen strains (11–13). Here we tested whether incomplete host immunity drives the evolution of greater virulence in this system.

We used sequential-inoculation experiments to quantify how incomplete immunity generated from experimental prior exposure alters host responses relevant for pathogen fitness. House finches naïve to *M. gallisepticum* at capture (n=120) were individually housed and sequentially exposed to pairs of *M. gallisepticum* strains that were either identical (“homologous”) or of differing virulence (“heterologous”), allowing clinical recovery between exposures. We performed two identical experiments in successive years, each using three distinct pathogen strains in a completely randomized design (Table 1). Positive controls received sterile media during primary inoculation and thus had no pathogen exposure prior to secondary inoculation with one of the six strains. We quantified strain virulence as the degree of within-host replication (e in Table 1) using conjunctival pathogen loads measured by quantitative polymerase chain reaction (qPCR) (10) across eight weeks following primary inoculation of immunologically-naïve birds (see supplementary materials). To examine how virulence of the primary and secondary strains affected host responses relevant for pathogen fitness, we measured conjunctivitis severity and pathogen load for five weeks following secondary inoculation. Conjunctivitis severity, which correlates with disease-induced mortality risk in the wild (6, 7), was scored on a 0 to 3 ordinal scale, and conjunctival pathogen load was quantified using qPCR (10).

First, we found that hosts with prior experimental exposure to any *M. gallisepticum* strain showed reduced severity of the clinical signs that predict mortality risk in the wild (6, 7), compared to hosts with no prior exposure (Fig. 1A). Thus, consistent with prior work using vaccines (5), host immunity reduced the costs of virulence to the pathogen by protecting

hosts from the disease-induced host mortality that prevents onward pathogen transmission and thus reduces pathogen fitness to zero. Second, the greatest reduction in pathogen load and clinical signs, and thus the strongest protection against reinfection, was observed in hosts previously exposed to higher virulence strains (Fig. 1, Table 1). Primary exposure to a homologous strain also generated significant host protection (Fig. 1), suggesting that adaptive immune responses, either alone or in combination with innate priming mechanisms (14), likely underlie the detected incomplete protection against reinfection (see also Fig. S1).

We used the empirical responses to secondary inoculation to fit two key pathogen traits, disease-induced mortality and susceptibility, as continuous functions of the virulence of both the primary and secondary strain (see supplementary materials). Disease-induced mortality rates were inferred to scale linearly with conjunctivitis severity, which predicts mortality risk in the wild (7); susceptibility to infection was inferred from the presence of a pathogen load above 10^2 in the conjunctiva at any post-inoculation qPCR sampling point. As expected, disease-induced mortality and susceptibility increase with the virulence of the currently-infecting strain (Fig. 2; Tables S1–S2). Thus, virulent strains are better able to successfully infect hosts, but also cause higher disease-induced mortality than less virulent strains. However, the host immune status generated by primary treatment strongly modifies the degree of disease-induced mortality and susceptibility for all strains. Hosts with prior exposure to high virulence strains (red lines, Fig. 2) have the lowest disease-induced mortality and susceptibility relative to all other primary treatments. Thus, by generating stronger host protection during primary infection (Fig. 1), high virulence strains effectively exclude low virulence strains from future infections of that host.

We next asked how the observed relationship between virulence and protection against reinfection alters the optimal or evolutionarily stable level of virulence in a pathogen population (Fig. 3). We used a two-strain SIRS model with the empirically-fit mortality and susceptibility functions (eqns.S2; Table S3), and compared this “incomplete immunity” model to one that still allows for higher host mortality and transmission in higher virulence strains (15) but does not include immune protection from prior exposure. We applied an adaptive dynamics approach by simulating the invasion of a new mutant into a population with an endemic resident pathogen strain for a range of virulence levels of both the resident and invading strains (see *Invasion analysis* in supplementary materials). In the resulting pairwise invasibility plots (PIPs), the protective effects of host immunity shift the pathogen’s evolutionary stable strategy to almost two-fold higher virulence relative to a model with no host protection (Fig. 3). This result, which is robust even when additional protective effects of strain homology are added to the model (Fig. S3), is driven by two distinct mechanisms favoring greater virulence. First, because disease-induced mortality is a key cost of virulence, higher-virulence strains benefit the most from the reduction in host mortality (Fig. 2A) generated by incomplete immune protection. Second, the stronger protection provided by higher-virulence strains reduces the pool of previously-infected hosts available for reinfection by lower-virulence strains (Fig. 2B). We evaluated the general applicability of our two-strain model to other study systems by conducting a numerical and analytical global sensitivity analysis. We found that a stronger relationship between virulence and immune protection enhances the competitive advantage of the more virulent strain (Tables S41–S5). Thus, incomplete immunity should favor the evolution of greater virulence in any system in

which higher-virulence strains have a stronger protective effect against reinfection compared to lower-virulence strains (Tables S41–S5).

Our results, combined with high *M. gallisepticum* prevalence (8, 16, 17) and recovery rates in the wild (6), indicate that host immunity plays a prominent role in the evolution of increased *M. gallisepticum* virulence in nature (10). Additional evolutionary processes, such as selection favoring higher transmission rates of more virulent strains (15), might also contribute to the observed increases in virulence. In contrast, adaptation to a novel host is unlikely to explain the virulence increases on both coasts, as the more evolutionarily-derived California strains of *M. gallisepticum* have lower virulence than eastern strains (Table 1 (10, 18)). While evolution of host resistance can lead to increased virulence (19) and may play a role in this system (20), there was no evidence of evolved host resistance in two studies conducted after the detected increases in virulence (21, 22). Thus, our results suggest that *M. gallisepticum* virulence increased in both eastern and western populations as hosts with incomplete immunity became more common in each population.

The effects of incomplete immunity described here are arguably a specific case of a broader phenomenon whereby increased virulence is favored by quantitative host variation in susceptibility, whether due to host genetic variation (23), imperfect vaccines (5), or innate immune priming (24). Disentangling differences in susceptibility versus infectiousness is a difficult problem in any system, and we do not attempt to do so here. Our model attributed differences in infection rates to what we term host susceptibility (η in eqns. S2), as infection occurred through inoculation with equal pathogen doses. It is likely that lower pathogen loads in hosts with prior exposure (Fig. 1B) will also lead to lower infectiousness and thus less transmission by those individuals. Although our models allowed for higher transmission rates of more virulent strains in both scenarios (Fig. 3), we did not vary transmission rates with host prior exposure. Further, although our data support reduced infection length with prior exposure (Fig. S2), we were unable to robustly quantify this effect and thus assumed equal infection lengths. Thus, our model is likely conservative, as selection for higher virulence should be even stronger if infectiousness and infection length also vary with host prior exposure.

Previous studies argue for great care in designing vaccines because incomplete protection can select for increased virulence in the targeted pathogens (1, 3, 5). Our study suggests that pathogens could readily evolve towards higher virulence simply due to the imperfect nature of host immune memory, whether via adaptive or innate responses (14). Despite historical focus on the small subset of pathogens that confer complete and lifelong immunity, incomplete immunity following infection is widespread in humans (25–28) and other animals (29–32). Because there are likely many systems where more virulent pathogen strains stimulate stronger immune responses and thus provide stronger protection (33, 34), the fitness advantages to higher virulence demonstrated here are relevant for a range of host and pathogen systems, including humans. Overall, our results show that the same immune systems that evolved to protect hosts from infection can drive the evolution of more harmful pathogens in nature.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1: Clinical signs of conjunctivitis (A), which predict mortality in the wild, and conjunctival pathogen load (B) in hosts with no previous exposure (positive controls) or previous exposure to *Mycoplasma gallisepticum* strains that were either homologous or of differing virulence. Negative controls received sterile media for both exposures. Host protection was strongest when the primary exposure strain was of higher virulence than the secondary strain, indicating an effect of strain virulence separate from strain homology effects. Boxplots show the maximum observed lesion score (0–3 scale per eye, summed within sampling date for a maximum of 6) or conjunctival pathogen load for each of 120 individuals from 6 post-exposure measurements. Virulence of previous exposure strain is grouped categorically here for clarity (differences among treatments: Kruskal-Wallis test;

eye score: $H = 68.16$, $df = 4$, $p < 0.0001$; pathogen load: $H = 76.80$, $df = 4$, $p < 0.0001$), but was treated as a continuous variable in the model and analysis.

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Fig. 2:

Model parameters as functions of pathogen virulence, fit to empirical data from Experiments 1 (circles) and 2 (triangles). Higher virulence of the currently infecting (i.e., secondary) strain is associated with higher host mortality (A) and host susceptibility (B), but host prior exposure (colored lines) reduces disease-associated mortality and susceptibility. Prior exposure to a high virulence strain (red) provides the most protection and thus results in the lowest host mortality and susceptibility. The effect of virulence of the primary strain was fit as a continuous function, but to visualize effects, lines and points show three categories of

primary strain virulence: low (blue), intermediate (purple) or high (red) virulence. Shading around lines give bootstrapped 95% confidence intervals incorporating error in virulence estimates. Mortality rates (A) were inferred to scale linearly with conjunctival lesion scores; susceptibility (B) was fit to binomial infection status (Y/N) inferred from conjunctival pathogen presence. Because distinct strains were used in each experiment (Table 1), lines show the function averaged over the two experimental strains in each virulence category (e.g., 'low virulence'). See Fig S4 for functions and data separated by Experiment.

Fig. 3:

The empirically-observed effects of incomplete immunity result in an almost twofold increase in optimal virulence relative to a model with no immunity (A). Pathogen fitness is measured as invasion success, computed from the proportion of resident pathogen parameter space over which an invader of a given virulence level was able to successfully displace the resident pathogen in the PIPs for each model (B,C), scaled to a maximum invasion value of 1 (see supplementary materials and (35)). Shaded areas in the PIPs show parameter space for which a new mutant introduced at very low densities was able to invade a population with equilibrium densities of the resident strain. Non-shaded areas indicate parameter space where the resident strain cannot be competitively displaced, and the asterisks in the center of this space thus mark the Evolutionary Stable Strategy (ESS) for each model. Susceptibility η

and mortality v were both continuous functions of strain virulence (ϵ , Fig. 2); all other parameters were held constant (eqns. S2).

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Table 1.

Treatments and mean $\pm$ 1 SE of $\log_{10}$ conjunctival pathogen load for Experiments 1 and 2, which were identical in design but used distinct strains. All birds (120 total) experienced primary and secondary inoculation with either media or one of six *Mycoplasma gallisepticum* strains that varied in virulence (10). Strains are ordered from low to high virulence (left to right) within experiment, and treatments describe the primary exposure relative to the secondary exposure (e.g., “Less Virulent” indicates primary exposure to a less virulent strain than the secondary strain). Numbers in column headings give strain virulence estimated from a separate data set in naïve hosts (see supplementary materials). Note that strain virulence values ϵ are linear model coefficients (linear mixed effects model fitting strain effects in naïve birds; $LR=172.56$, $df=6$, 124 , $P < 0.001$), while values in tables are raw means of pathogen load ($n=4-5$ each). Shading indicates pathogen load following secondary exposures, and tends to increase top to bottom (with increasing virulence of secondary strain), but decrease left to right within a row (with increasing virulence of primary strain).

Experiment 1		Primary Exposure			
		Media (control)	CA2006 Low ($\epsilon = 1.41 \pm 0.34$)	VA1994 Medium ($\epsilon = 3.31 \pm 0.34$)	NC2006 High ($\epsilon = 4.72 \pm 0.34$)
Secondary Exposure	CA2006	No prior exposure 1.54 $\pm$ 0.23	Homologous 0.47 $\pm$ 0.17	More Virulent 0.20 $\pm$ 0.13	More Virulent 0.27 $\pm$ 0.14
	VA1994	No prior exposure 3.29 $\pm$ 0.35	Less Virulent 1.11 $\pm$ 0.31	Homologous 0.62 $\pm$ 0.20	More Virulent 1.10 $\pm$ 0.18
	NC2006	No prior exposure 4.71 $\pm$ 0.52	Less Virulent 3.30 $\pm$ 0.39	Less Virulent 1.78 $\pm$ 0.18	Homologous 1.02 $\pm$ 0.17
	Media (control)	Negative control 0.044 $\pm$ 0.027			
Experiment 2		Primary Exposure			
		Media (control)	CA2009 Low ($\epsilon = 1.28 \pm 0.34$)	NC1995 Medium ($\epsilon = 1.95 \pm 0.35$)	VA2013 High ($\epsilon = 4.08 \pm 0.34$)
Secondary Exposure	CA2009	No prior exposure 2.82 $\pm$ 0.33	Homologous 0.68 $\pm$ 0.16	More Virulent 1.14 $\pm$ 0.23	More Virulent 1.05 $\pm$ 0.20
	NC1995	No prior exposure 3.67 $\pm$ 0.91	Less Virulent 0.86 $\pm$ 0.33	Homologous 0.56 $\pm$ 0.08	More Virulent 0.74 $\pm$ 0.14
	VA2013	No prior exposure 3.75 $\pm$ 0.37	Less Virulent 3.00 $\pm$ 0.11	Less Virulent 2.64 $\pm$ 0.23	Homologous 1.51 $\pm$ 0.47
	Media (control)	Negative control 0 (n=4)			