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Pathogen Diversity and Hidden Regimes of Apparent Competition

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ABSTRACT: Competition through cross-reacting host immune responses, a form of apparent competition, is a major driver of pathogen evolution and diversity. Most models of pathogens have focused on intraspecific interactions to explain observed patterns. Two recent experiments suggested that *Haemophilus influenzae*, a common nasopharyngeal colonizer of humans, might alter the immune environment in a way that favors otherwise less fit serotypes of another common pathogen, pneumococcus. Using a computational model, we demonstrate that *H. influenzae*, if it consistently raises the fitness of the less fit serotypes, can strongly promote pneumococcal diversity. However, the effects of *H. influenzae* are so sensitive to the prevalence of *H. influenzae* that this species is unlikely to be the main driver of serotype coexistence. Interactions that significantly affect diversity could furthermore be extremely difficult to detect through co-occurrence analysis alone. These results suggest that small differences in strains' adaptations to different immunological regimes, which are shaped by coinfections with other pathogens, can have dramatic effects on strain dynamics and patterns of phenotypic variation. Studies of microbial communities might therefore benefit from the use of varied approaches to infer the presence of indirect interactions.

Keywords: apparent competition, pneumococcus, *Haemophilus influenzae*, microbiome, immunity, pathogen.

Introduction

Apparent competition—indirect competition between two or more species through a shared enemy (Holt 1977)—can be a major force shaping ecological communities (Holt and Lawton 1994; Bonsall and Hassell 1997; Chaneton and Bonsall 2000). In some cases, apparent competition may have stronger effects than interference or exploitation competition on species coexistence (Holt 1984; Price et al. 1986; Holt and Lawton 1993). Its role might be especially large in systems where consumers (predators or parasites) and resources (prey or hosts) have comparable generation times, allowing consumers to respond rapidly to changes in the population size of resources. Extinction of one or more resource species is a common outcome (Holt 1977, 1984; Holt and Lawton 1993), although apparent competition can also enable coexistence (e.g., Bonsall and Hassell 1997).

In host-pathogen interactions, coexistence of species, or of genetic types within a species, can depend on apparent competition (Holt and Dobson 2006; Pedersen and Fenton 2007; Mideo 2009; Fenton and Perkins 2010). From one perspective, pathogens are consumers and hosts resources (Holt and Pickering 1985; Price et al. 1986; Bowers and Turner 1997; Tompkins et al. 2000). Host populations sharing a common pathogen can indirectly compete and be regulated by the pathogen through, for example, pathogen-induced mortality or a reduction in host fecundity (Anderson and May 1978; May and Anderson 1978). Especially when transmitted by a vector or environmental reservoir, a pathogen shared by multiple hosts can depress host populations enough that the hosts effectively do not compete for resources (Price et al. 1986; Holt and Lawton 1993). On another scale, the populations of cells and factors that comprise the host immune response might be viewed as predators of pathogenic prey (Mideo 2009; Fenton and Perkins 2010). Immune responses may be particularly powerful because they are not limited by the traditional energetic constraints of classical predators. From this perspective, the question is how shared immune responses affect the outcome of competition among pathogens within an individual host or host population. Competition for nonimmune hosts can be thought of as competition for enemy-free space (Holt and Lawton 1993).

Theoretical models (e.g., Bowers and Turner 1997; Gupta et al. 1998; Gog and Grenfell 2002; Andreasen 2003; Ferguson et al. 2003; Koelle et al. 2006) have demonstrated

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that this second kind of apparent competition can shape pathogen diversity. In the absence of other effects such as spatial structure, the intrinsic reproductive numbers of different strains (R_0) and their degree of antigenic relatedness, often referred to as cross immunity, have been the focus of models that examine strain diversity. The majority of such models incorporate cross-immunity that arises from acquired immunity, such as T cells and B cells, which are at least somewhat specific to particular antigens expressed by pathogens and can persist in the host's immune memory. In general, the specificity of these responses promotes negative frequency-dependent selection, so that strains that have been seen more often before (by an individual host or a host population) are more easily targeted, promoting the growth of rarer, less cross-reactive strains. These dynamics can generate strain coexistence over long time periods. However, if strains share common immunological "predators" but differ substantially in their R_0, coexistence is less certain (Omori et al. 2010).

Most studies have focused on mechanisms of competition between strains of the same species or genus (e.g., *Plasmodium* spp. [Buckee and Gupta 2010; Gupta et al. 1998], influenza viruses [Andreasen 2003; Ferguson et al. 2003; Koelle et al. 2006], and dengue viruses [Nagao and Koelle 2008; Cummings et al. 2009; Recker et al. 2009]) to explain strain dynamics. The presence of other pathogen species can add complexity by changing the immunological environment in which pathogens interact. Indirect positive interactions between species are one possible outcome. By reducing the $CD4^+$ T cell population, for example, HIV facilitates coinfection with malaria; malaria also increases HIV viral loads (Abu-Raddad et al. 2006). Infection with helminths may predispose human hosts to acquisition of bacterial and viral pathogens via a trade-off between the T_h1 and T_h2 responses (Ezenwa and Jolles 2011). Indirect competition can also result through shared inflammatory responses (Brown et al. 2008; Graham 2008). These examples demonstrate that pathogen abundance can be determined partly by indirect interactions, either positive or negative, with other species.

An open question is the extent to which the presence of one pathogen species can change the outcome of intraspecific competition in another pathogen species by modifying the immunological environment. In this scenario, the presence of one species alters the type of apparent competition experienced by strains of another species. If different outcomes are possible, then attempts to understand or manage the dynamics of any single pathogen species may need to consider the broader within-host community.

Recently, it has been suggested that colonization with *Haemophilus influenzae* might affect competition among individual serotypes of *Streptococcus pneumoniae* in humans (Lysenko et al. 2010; Margolis et al. 2010). Both *H. influenzae* and *S. pneumoniae* are extremely common nasopharyngeal colonizers (fig. B1, available online; table A1). The >90 serotypes of *S. pneumoniae*, also called pneumococcus, have a stable rank order: the same serotypes tend to be common everywhere and over time (Cobey and Lipsitch 2012). Pneumococcus is typically found in the human nasopharynx, where it is said to be carried. The most common serotypes have relative long durations of carriage (Hogberg et al. 2007; Lipsitch et al. 2012). The most common serotypes are also better at preventing cocolonization with other serotypes (Lipsitch et al. 2012) and resisting phagocytosis by neutrophils (Weinberger et al. 2009). These observations raise the question of how the rarer and less obviously fit serotypes persist. Elsewhere, we proposed that coexistence can result from a combination of acquired non-serotype-specific immunity, which disproportionately reduces the fitness of the commonest serotypes, and a small amount of acquired serotype-specific (anticapsular) immunity (e.g., an ~30% reduction in susceptibility to a serotype if a host has carried it before; Cobey and Lipsitch 2012). This amount of serotype-specific immunity is consistent with the few available observations (McCool and Weiser 2004; Goldblatt et al. 2005; Hill et al. 2008; Weinberger et al. 2008). An alternative or additional hypothesis is that *H. influenzae* indirectly promotes diversity by changing the immunological environment in which pneumococcal serotypes compete. Two limited experiments in mice have suggested that in the presence of *H. influenzae*, the outcome of competition between a common (presumably more fit) and rare (presumably less fit) serotype might be reversed: the common serotype outcompetes the rare serotype when *H. influenzae* is absent, and the rare serotype outcompetes the common serotype when *H. influenzae* is present (Lysenko et al. 2010; Margolis et al. 2010). This reversal is thought to arise from a trade-off between a serotype's ability to resist opsonic and nonopsonic phagocytosis. In nonopsonic phagocytosis, phagocytes such as neutrophils consume pneumococci directly. When phagocytosis is enhanced by opsonization, immune effectors such as complement protein or antibodies bind to the surface of a pathogen and make it easier to destroy. *Haemophilus influenzae* increases the rate of opsonization in a complement-dependent manner, which appears to harm the common serotypes disproportionately (Lysenko et al. 2005, 2010; Hyams et al. 2010). Thus, *H. influenzae* might change the kind of apparent competition experienced by serotypes.

In this study, we first consider whether species might coexist due to shifts in apparent competition induced by another species. Specifically, we ask how a change in apparent competition between pneumococcal serotypes would affect serotype diversity, assuming the impact of *H.*

influenzae observed for the three serotypes examined in animal models were fully general across a range of pneumococcal serotypes. We computationally investigate whether trade-offs in the survival of pneumococcal serotypes in hosts colonized or uncolonized with *H. influenzae* might explain the coexistence of pneumococcal serotypes.

We next examine whether it might be theoretically possible to detect an effect of *H. influenzae* on pneumococcus using available observations. In other systems, the presence of apparent competition has been demonstrated by direct manipulation of predator and prey densities (Grosholz 1992; Bonsall and Hassell 1997; Chaneton and Bonsall 2000; Graham 2008; Balmer et al. 2009; Karvonen et al. 2009), by fitting models to time series (Bonsall and Hassell 1998; Lello et al. 2004; Telfer et al. 2008, 2010), and by analyzing correlations in nature (Chaneton and Bonsall 2000; Tompkins et al. 2000; Byrne et al. 2003; Lello et al. 2004; Behnke et al. 2005; Bottomley et al. 2005; Leung and Poulin 2007; Karvonen et al. 2009; Fenton et al. 2010). A persistent challenge is to distinguish the effects of apparent competition from those of resource and interference competition. Many of the traditional approaches are not amenable to the study of interactions between bacterial pathogens of humans. In mice, a standard animal model of pneumococcus, population sizes of the immune system and concentrations of chemical immune effectors are difficult to manipulate (apart from complete abrogation in genetic knockout mice) and to measure at the site of infection. Even if accurate measurement were possible, it is unclear whether numerical responses are preserved across host species. At the population level, extensive time series of the co-colonization rates of *H. influenzae* and individual pneumococcal serotypes have not been gathered. We were thus interested in whether cross-sectional epidemiological studies might be used to detect an interaction between the species. If *H. influenzae* affects the colonization abilities of pneumococcal serotypes differently, we might expect to see lower rates of co-colonization with serotypes that are more sensitive to its effects.

We find that by altering the type of apparent competition experienced by pneumococcus, *H. influenzae* could strongly promote serotype diversity, but it is not a sufficient explanation for observed coexistence. Additionally, its role would be extremely difficult to infer from cross-sectional observations alone. These results demonstrate that small differences in the competitive ability of pathogen strains under different immunological states can strongly promote diversity, but additional lines of evidence are necessary to identify and quantify these effects. We briefly discuss implications for understanding the complex communities of the human microbiome from cross-sectional observations.

Model

We developed an agent-based model to simulate the transmission of 25 arbitrary pneumococcal serotypes and one strain of *H. influenzae*. The model of pneumococcal transmission has been described elsewhere (Cobey and Lipsitch 2012), but the major features are summarized here, followed by the modeled interactions with *H. influenzae* that are specific to this study. The simulated serotypes differed in their intrinsic durations of carriage and their ability, when already colonizing a host, to prevent colonization by an invading serotype. We assumed that the intrinsic duration of carriage and exclusion ability were positively correlated, so that the serotype with the longest duration of carriage was also best at inhibiting colonization by other serotypes (Weinberger et al. 2008; Lipsitch et al. 2012). The maximum and minimum intrinsic durations of carriage were taken from observations (Gray et al. 1980; Hogberg et al. 2007), and the durations of carriage of the other serotypes were evenly and linearly interpolated between these points. The exclusion abilities (Lipsitch et al. 2012) were also linearly interpolated, with the least fit serotype (which also has the shortest intrinsic duration of carriage) having no ability to exclude invaders. In the absence of evidence to the contrary, all serotypes were assumed to have identical transmission rates (Erasto et al. 2010).

Hosts colonized with pneumococcus developed two kinds of immunity. Serotype-specific immunity reduced susceptibility by a fraction σ if a host had ever been previously colonized with a serotype; for a given simulation, σ was the same for all serotypes. Initially, we assumed that non-serotype-specific (henceforth, "nonspecific") immunity exponentially reduced the duration of carriage to a minimum level (here, 25 days) depending on the number of times a host had carried pneumococcus before. We also tested a linear decline to zero. For each functional form, we varied the slope of the initial decline. For each of these different parameterizations (form and slope) of nonspecific immunity, we also varied the strength of serotype-specific immunity from none ($\sigma = 0$) to strong but imperfect ($\sigma = 0.9$).

The model also simulated the dynamics of a single strain of *H. influenzae*. Although diverse types of *H. influenzae* circulate in nature, we know of no evidence or reason why these differences should affect interactions with pneumococcus. The acquisition of immunity to *H. influenzae* colonization following exposure to the organism has not been quantified, and we parsimoniously assumed it was similar to that of a pneumococcal serotype. Hosts that had previously cleared *H. influenzae* were less susceptible to future carriage with *H. influenzae* ($\sigma_H = 0.3$), and the duration of carriage declined nonlinearly with past exposure. Consistent with experiment (Lysenko et al. 2005; Margolis

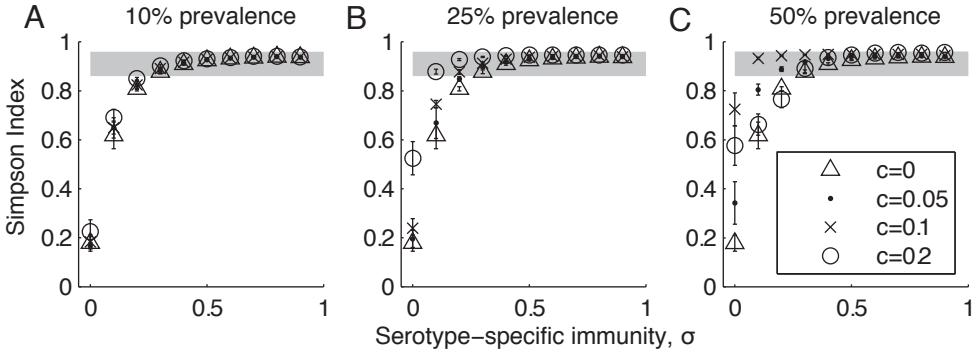

Figure 1: Pneumococcal serotype diversity, as measured by the Simpson index, for different levels of serotype-specific immunity σ, prevalences of *Haemophilus influenzae*, and maximal clearance rates c. Measures were obtained by averaging annual estimates of the Simpson index for each of the last 20 years of each simulation. Gray areas denote the range observed in carriage studies (Cobey and Lipsitch 2012). Error bars show SD from 10 simulations.

et al. 2010), the dynamics of *H. influenzae* were modeled to be independent of the dynamics of pneumococcus: current or past colonization with pneumococcus affected neither the risk of colonization nor the duration of colonization with *H. influenzae*. However, carriage of pneumococcus was sensitive to concurrent carriage of *H. influenzae*. When a host carrying pneumococcus acquired *H. influenzae*, each carried strain of serotype z cleared instantaneously with some probability $h(z)$. When a host carrying *H. influenzae* became colonized with pneumococcus of serotype z, that pneumococcal colonization immediately cleared with the same probability. This rapid clearance is consistent with the observed speed of clearance in hosts who have received the pneumococcal conjugate vaccine, which induces a strong antibody response. For simplicity, we assumed a linear relationship between the probability that a strain of pneumococcus is cleared by colonization with *H. influenzae*, $h(z)$, and the fitness rank (in the absence of *H. influenzae*) of that pneumococcal serotype, $f(z)$. The serotype with the longest duration of carriage, which is always the most fit serotype in the absence of *H. influenzae*, has rank $f(z) = 1$, and the serotype with the shortest duration of carriage has rank $f(z) = Z$, where Z equals the number of serotypes. The probability of clearance of serotype z is given by

$$h(z) = c\left(1 - \frac{f(z) - 1}{Z}\right). \quad (1)$$

The parameter c equals the probability of clearing the otherwise fittest serotype (the one with the longest intrinsic duration of carriage and best exclusion abilities) and scales the sensitivity of all serotypes to clearance by *H. influenzae*. The otherwise least fit serotype (with the shortest intrinsic duration of carriage and no exclusion abilities) always has a positive probability (equal to c/Z) of being cleared.

We simulated the model for different carriage prevalences of *H. influenzae* (10%, 25%, or 50% in children <5 years old), maximum clearance probabilities c ($c = 0, 0.05$, 0.1, and 0.2), and levels of serotype-specific immunity σ ($\sigma \in [0, 0.9]$). For each set of parameters, we refitted pneumococcal transmission rates with *H. influenzae* present so that the total carriage prevalence of pneumococcus in children <5 years old was 40%. This prevalence is approximately the midpoint of the broad range of observed carriage rates in young children (table A1, fig. B1). At pneumococcal carriage rates of 37%-41%, the prevalence of *H. influenzae* varied between 11% and 70% and had a mean of 38% (table A1, fig. B1).

Results

Effects of Haemophilus influenzae *Are Sensitive to Its Prevalence*

Depending on its prevalence, *H. influenzae* could exert large or inconsequential effects on the dynamics of pneumococcal serotypes. When colonizing only 10% of young children, *H. influenzae* had no significant effect on pneumococcal serotype diversity for the values of c tested (fig. 1*A*). This diversity is measured by the Simpson index, which equals the probability of randomly picking two different serotypes from the same population with replacement; it was calculated as $1 - D$, where $D = \Sigma p_i^2$, and p_i denotes the frequency of the ith serotype. At *H. influenzae* carriage rates of 25%, maximal clearance rates of $c = 0.1$ and $c = 0.2$ were associated with a large increase in the Simpson index at low levels of serotype-specific immunity, σ (fig. 1*B*). At carriage rates of 50%, a 10%

clearance probability of the fittest serotype in the presence of *H. influenzae* ($c = 0.1$) could produce realistic values of the Simpson index starting at very low serotype-specific immunity ($\sigma = 0.1$; fig. 1*C*). However, a larger maximal clearance probability ($c = 0.2$) at the same prevalence dramatically altered the outcome of competition. Rather than predominating, the serotypes with the longest durations of carriage went extinct, and the most frequent serotypes were the ones best able to resist clearance in the presence of *H. influenzae* (fig. B2, available online). This reversal in rank order has not, to our knowledge, been reported in natural populations (Cobey and Lipsitch 2012).

The rank-frequency distributions provide additional evidence that *H. influenzae* can profoundly alter the outcome of pneumococcal competition, but not in a way consistent with observations (figs. B3-B5, available online). For many combinations of c and σ, simulations generate realistic values of the Simpson index (figs. 1, B3, B4), but the rank-frequency distributions are significantly flatter than observed. There is no combination of c and σ at which *H. influenzae* can significantly increase diversity and maintain a realistic rank-frequency distribution across a wide range of prevalences. Because the maximal rate of clearance c and strength of serotype-specific immunity σ should not vary between populations, the rank-frequency distributions suggest that *H. influenzae* is not the primary driver of serotype coexistence.

The presence of *H. influenzae* can nonetheless promote the diversity of serotypes that would otherwise be unable to coexist without higher levels of serotype-specific immunity, σ. A relevant question is whether, in a population where *H. influenzae* did in fact play such a role, a significant interaction would be detectable in a cross-sectional, observational study of reasonable size.

Detection from Cross-Sectional Data May Be Impractical

Through repeated simulations, we calculated that even when *H. influenzae* exerts substantial selective pressure on serotypes ($c = 0.2$, $\sigma = 0.1$, and 25% prevalence of *H. influenzae*), the probability that a child <5 years old who was colonized with serotype z also carried *H. influenzae* ranged only 2% between the serotypes: the probability of co-colonization with *H. influenzae* was ~24.5% for the commonest serotype and ~26.5% for the rarest. We used these probabilities to simulate binomial random draws for different sample sizes and calculated the probability of inferring, via logistic regression, a significant positive relationship between serotype rank (which correlates linearly with resistance to clearance in the presence of *H. influenzae*) and the probability of being co-colonized with *H. influenzae* (fig. 2). To have a 90% chance of detecting a significant positive relationship would require a sample size of ~90,000 children; sample sizes of several thousand pneumococcus-positive children, exceeding the size of published studies, would provide less than a 10% chance of detecting a true trend. This analysis suggests that epidemiological studies of co-colonization might well find null results, even if another species such as *H. influenzae* is a significant force promoting serotype diversity.

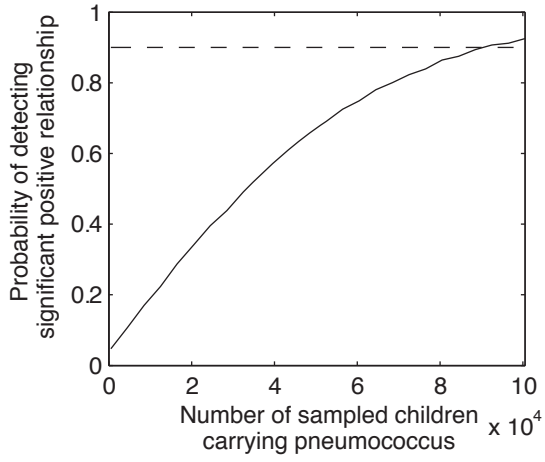

Figure 2: Probability of detecting a significant positive trend between serotype rank and the rate of co-colonization with *Haemophilus influenzae* for different sample sizes when *H. influenzae* significantly increases pneumococcal diversity ($c = 0.2$, *H. influenzae* prevalence = 25%, $\sigma = 0.1$). Serotypes are ranked in descending order of their frequency (or intrinsic duration of carriage), with rank 1 denoting the most common serotype and 25 the rarest. The sample size refers to the number of children <5 years old colonized with pneumococcus. The dashed line shows a 90% probability of detecting a significant positive trend by logistic regression.

Discussion

We previously found that some serotype-specific immunity (σ ~0.3 or higher) was needed to match observed levels of pneumococcal diversity (Cobey and Lipsitch 2012). However, adaptation to different immune environments might explain pneumococcal diversity without the need to invoke serotype-specific immunity. This study was designed to determine whether a trade-off in the ability of pneumococcal serotypes to persist in the presence versus the absence of *H. influenzae* might promote the coexistence of serotypes and if such an interaction might be epidemiologically detectable from cross-sectional studies. We found that *H. influenzae* could be a force that strongly promotes coexistence, but that it is not a plausible driver of observed pneumococcal serotype diversity. The extent to which *H. influenzae* increased diversity was sensitive to

its prevalence and the maximal clearance rate c: there was no situation (combination of c and σ) in which *H. influenzae* could substantially increase diversity while maintaining realistic rank-frequency distributions and rank orders over a range of prevalences (10%-50%). Therefore, when c is high, variations in the prevalence of *H. influenzae* have large consequences for serotype coexistence. If *H. influenzae* is an important mediator of pneumococcal diversity in nature, the observed range in prevalence of *H. influenzae* (fig. B1) thus implies there should be more variability in pneumococcal rank-frequency distributions and rank orders than what is actually observed.

This study's second finding is that even if the range of carriage rates of *H. influenzae* for a given prevalence of pneumococcus were narrower in real populations and the hypothesis thereby plausible, an interaction would be hard to detect epidemiologically by studying patterns of association between *H. influenzae* and individual pneumococcal serotypes in carriage. The patterns are subtle and require very large sample sizes to assess statistically.

While arguing against a primary role for an *H. influenzae*-induced shift in apparent competition for explaining pneumococcal serotype coexistence, our results more generally suggest a need to consider hypotheses invoking shifts in apparent competition, potentially induced by other species, to explain intraspecific pathogen diversity. They also point to the need for multiple lines of evidence to detect what might be small differences in pathogens' (or other microbial commensals') adaptations to different immunological environments.

This analysis underscores the challenges of relying on cross-sectional observations of many pathogens to infer what amount to subtle differences between them. Here, the question was not whether pneumococcal serotypes compete intraspecifically but whether the terms of this competition might vary in different environments. A system containing more competing species will generally suffer from smaller sample sizes (the number of hosts carrying each kind of parasite), hindering quantitative inference. This may partly explain why studies reporting correlations have involved fewer species (Lello et al. 2004; Behnke 2008). Pathogen association studies in general can be limited by the presence of hidden confounding factors, such as shared risks for pathogen acquisition, and spurious associations generated by the system's nonlinear dynamics (Behnke et al. 2005; Lello et al. 2008; Karvonen et al. 2009; Fenton et al. 2010). In our simulations, the age-specific hazards were extremely similar for all serotypes and *H. influenzae* (results not shown), interspecific interactions were instantaneous (i.e., occurred only during co-colonization), and the dynamics reached apparently stable point equilibria, but such assumptions might not be easily extended to other multipathogen systems. An alternative approach to measuring the interaction here might involve looking across populations for a positive correlation between serotype evenness and the prevalence of *H. influenzae* at a given carriage rate of pneumococcus. However, this analysis would require many replicates and adjustment for temporal trends, such as pneumococcal vaccination rates, and could be more difficult than a large cross-sectional study. Approaches based on fitting models to time series data (e.g., Shrestha et al. 2011), especially when they contain perturbations and parallel measures of immune function, might be more revealing. Our findings also emphasize the utility of controlled experiments to identify pairwise interactions.

Like other ecological models, ours makes simplifying assumptions in the presence of incomplete information, and these assumptions should be revisited as more is learned. *Haemophilus influenzae* might interact with pneumococci in ways not examined here. The model assumes a smooth trade-off, that is, $h(z)$ changes linearly with the intrinsic duration of carriage. First, resistance to opsonic phagocytosis might be further mediated by factors other than the capsule, which could make this relationship noisy and even harder to detect (Hyams et al. 2011). Second, some serotypes might be particularly well or poorly adapted to co-colonization with *H. influenzae*. For such serotypes, it might be possible to detect sharper effects in smaller sample sizes. A mechanistic understanding of serotypes' interactions with the immune system could lead the way toward simpler yet more predictive models. For example, is there a positive correlation between a serotype's propensity for opsonic phagocytosis ($h(z)$) and its susceptibility to acquired specific immunity (σ)? Such a relationship might exist if antibody and complement responses were both driven by capsular surface area. It is also possible that strains of *H. influenzae* differ in their effects on opsonic phagocytosis. Ignoring these differences would make a trade-off even more difficult to detect. Our claim that certain regimes of parameter space yielded dynamics that were unrealistically sensitive to *H. influenzae* warrants careful testing, as suggested above: are increases in carriage of *H. influenzae* ever associated with a transient rise in serotype evenness? Further simultaneous surveillance of both *H. influenzae* and pneumococcal serotypes over multiple locations and time points would be helpful to this end.

The challenges posed by these two species suggest hazards for more complex models of commensal microbial communities that interact with host immunity, as occurs in the gut and respiratory tract. There is a shortage of basic information about the biology of these systems, including which direct interactions are present and on what aspects of phenotype they are based. These uncertainties limit the scope of hypotheses that can be tested. For example, the

trade-off described here might be a general phenomenon of Gram-negative bacteria, not just *H. influenzae* (Lysenko et al. 2010). Another Gram-negative species, *Moraxella catarrhalis*, is also a frequent colonizer of the upper respiratory tract, but few epidemiological studies have examined its co-occurrence with *H. influenzae* and individual pneumococcal serotypes. We know of no experiments investigating the interaction of *M. catarrhalis* and individual pneumococcal serotypes in mice or experiments that compare the immune environments induced by each Gram-negative species. This uncertain phenotypic resolution (e.g., whether increased opsonic phagocytosis comes from *H. influenzae* in particular or Gram-negative bacteria in general) pervades research on commensal microbes. Many studies of the human microbiome identify taxonomic units based on 16S RNA, ignoring differences in antigenic phenotype, and effectively omit the consumer (immune) populations. These kinds of data are necessary to develop and test model structure at a rudimentary level, that is, to infer who is interacting directly with whom and how.

A more accurate picture of these communities can be acquired incrementally and iteratively through a combination of experiments, observation, and modeling, but our results support the idea that there may be practical limits to the measurement of indirect interactions in unmanipulated, species-rich communities. Traditional experiments to demonstrate the presence of indirect effects have focused on small motifs of a few species (Wootton 1994). We show that cross-sectional, correlational analyses may be underpowered in detecting differences in the strength of apparent competition in different environments, even when the interaction of interest has a large effect on diversity.

Host-symbiont communities, including the subset of interactions between hosts and pathogens, provide a challenging context for understanding species coexistence. The typical immune response includes components ranging from the nonspecific to the highly specific, and many of these components are unobserved. The presence of less specific components presents an opportunity for apparent competition between different species, and the amount of competition should be influenced by the persistence and functional responses of their immunological predators. Much of the variation observed in pathogens might result from adaptation to different immunological environments, which are continuously shaped by the dynamics of other symbionts (Seppala et al. 2009). Although this study suggests that *H. influenzae* does not play a major role in indirectly regulating the coexistence of pneumococcal serotypes, we have shown that pathogen diversity can in theory be strongly affected by a weak trade-off in escaping different kinds of immunity. Detecting this trade-off only from correlations in natural carriage might be extremely difficult and potentially misleading (Holt 1984; Holt and Lawton 1993; Fenton et al. 2010). Experiments in animal models, longitudinal coinfection studies augmented by immunological data, and interventions such as vaccines that target specific pathogens or strains could help to identify and, critically, quantify the strengths of direct and indirect effects (Behnke 2008; Bradley and Jackson 2008; Telfer et al. 2010).

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APPENDIX A

Table A1: Prevalence or % of positive samples of *Haemophilus influenzae* and *Streptococcus pneumoniae* in carriage studies

		Prevalence or % positive samples	
Study	Age range	*H. influenzae*	*S. pneumoniae*
Abdullahi et al. 2008	0–4 years	26	57
Almeida Vde et al. 2011	2–59 months	32	58
Aniansson et al. 1992	2 months	5	12
	6 months	6	30
	10 months	13	32
	18 months	24	32
Bakir et al. 2001	0–10 years	22	8
Capeding et al. 1995	6–8 weeks	21	28
	10–12 weeks	30	40
	14–17 weeks	39	47
	18–22 weeks	34	54
	32–39 weeks	50	60
	46–65 weeks	41	54
Chavanet et al. 2011	3 months–3 years	55	48
Christenson et al. 1997 (estimated from fig. 2)	≤2 years	45	60
	3 years	41	40
	4 years	30	40
	5 years	30	25
	6 years	15	24
	7 years	6	21
Dalle et al. 2000	3 months–2 years	33	48
De Lencastre et al. 1999	6 months–6 years	72	46
Dunais et al. 2003	6–36 months	24 (non-DCC)	34 (non-DCC)
		37 (DCC)	55 (DCC)
Factor et al. 2005	2–5 months	45	49
	6–11 months	59	64
	12–23 months	60	66
	24–35 months	60	62
	36–47 months	58	58
	48–69 months	54	51
Gessner et al. 1998, Soewignjo et al. 2001	<2 years	32	48
Greenberg et al. 2006	1–59 months	71	67
Gunnarsson and Ekdahl 1998	0–7 years	13	19
Homoe et al. 1996	0–1 years	77	38
	3–4 years	93	27
	5–8 years	70	23
Huang et al. 2005	0–7 years	17 (in 2001)	26 (in 2001)
		15 (in 2004)	23 (in 2004)
Ito et al. 2002	DCC and 18-month-olds	56	58
Jacoby et al. 2011	<2 years	41 (Abor.)	49 (Abor.)
		11 (non-Abor.)	25 (non-Abor.)
Jain et al. 2005	5–10 years	42	53
Janapatla et al. 2011	≤1 years	3	19
	2 years	6	28
	3 years	7	29
	4 years	2	16
	5 years	12	20

Table A1 (*Continued*)

Study	Age range	Prevalence or % positive samples	
		H. influenzae	*S. pneumoniae*
	Overall	5	23
Jourdain et al. 2011	3–6 years	61	43
Kwambana et al. 2011	<1 years	70	78
Lo et al. 2003, Wang et al. 2008	≤5 years	5	27
Mackenzie et al. 2010	2–15 years	61 (in 2002)	70 (in 2002)
		53 (in 2004)	65 (in 2004)
Madhi et al. 2007	5.7 ± 0.7 years	52	53
Marchisio et al. 2001	1–7 years	13 (fall)	4 (fall)
		18 (spring)	5 (spring)
Marcus and van Dyk 1996	0–8 years	20	41
Mastro et al. 1993	2–59 months	37	62
Masuda et al. 2002	1 month–5 years	53	60
Moulin et al. 1999	6–30 months	45	57
Naaber et al. 2000	2–7 years	17	46
Peerbooms et al. 2002	3–36 months	37 (DCC)	58 (DCC)
		11 (control)	37 (control)
Prymula et al. 2009	15–18 months	18	22
Sa-Leao et al. 2008	14–37 months	87 (mean)	61 (mean)
Stubbs et al. 2005	3–7 years (Abor.)	80	90
	≤4 years (non-Abor. DCC)	41	43
Sulikowska et al. 2004	6 months–5 years	24 (DCC, winter)	57 (DCC, winter)
		58 (orph., winter)	63 (orph., winter)
		11 (home, winter)	26 (home, winter)
		50 (DCC, spring)	54 (DCC, spring)
		70 (orph., spring)	51 (orph., spring)
		3 (home, spring)	19 (home, spring)
Sung et al. 1995	2 months–5 years	6 (Chinese)	10 (Chinese)
		67 (Vietnamese)	56 (Vietnamese)
Torun et al. 2009	6–10 years	32	29
van Gils et al. 2011 (estimated from fig. 1)	6 weeks	15	16
	6 months	35	48
	12 months	43	66
	18 months	57	66
	24 months	52	64
Villasusa Paez et al. 2006	0–6 years	55	78
Vives et al. 1997	1 months	4	3
	12 months	10	19
Wolf et al. 1999	4 months–5 years	41 (Brazil)	19 (Brazil)
		42 (Angola)	35 (Angola)
		70 (Netherlands)	41 (Netherlands)
Zemlickova et al. 2006	3–6 years	25	38

Note: Studies were cross-sectional or obtained isolates over a period of less than 1 year. Studies were excluded if they did not use nasopharyngeal swabs or if the sampled population was chosen based on a suspected or confirmed preexisting condition (e.g., acute otitis media, respiratory illness, or hospitalization). DCC = day-care center; Abor. = Aboriginal population; orph. = orphanage.

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