Host-to-host variation of ecological interactions in polymicrobial infections

Sayak Mukherjee\(^1,2\), Kristin E Weimer\(^6\), Sang-Cheol Seok\(^1\), Will C Ray\(^1,2\), C Jayaprakash\(^1,3\), Veronica J Vieland\(^1,2,4\), W Edward Swords\(^6\) and Jayajit Das\(^1,2,3,5\)

\(^1\) Battelle Center for Mathematical Medicine, The Research Institute at the Nationwide Children’s Hospital and, The Ohio State University, 700 Children’s Drive, Columbus, OH 43205, USA
\(^2\) Departments of Pediatrics, The Ohio State University, 700 Children’s Drive, Columbus, OH 43205, USA
\(^3\) Departments of Physics, The Ohio State University, 191 West Woodruff Ave, Columbus, OH 43210, USA
\(^4\) Departments of Statistics, The Ohio State University, 1958 Neil Ave, Columbus, OH 43210, USA
\(^5\) Departments of Biophysics Graduate Program, The Ohio State University, 484 W. 12th Ave., Columbus, OH 43210, USA
\(^6\) Department of Microbiology and Immunology, Wake Forest School of Medicine, Winston-Salem, NC 27101, USA

E-mail: das.70@osu.edu

Keywords: maximum entropy, Lotka–Volterra kinetics, microbial ecology

Supplementary material for this article is available online

Abstract
Host-to-host variability with respect to interactions between microorganisms and multicellular hosts are commonly observed in infection and in homeostasis. However, the majority of mechanistic models used to analyze host–microorganism relationships, as well as most of the ecological theories proposed to explain coevolution of hosts and microbes, are based on averages across a host population. By assuming that observed variations are random and independent, these models overlook the role of differences between hosts. Here, we analyze mechanisms underlying host-to-host variations of bacterial infection kinetics, using the well characterized experimental infection model of polymicrobial otitis media (OM) in chinchillas, in combination with population dynamic models and a maximum entropy (MaxEnt) based inference scheme. We find that the nature of the interactions between bacterial species critically regulates host-to-host variations in these interactions. Surprisingly, seemingly unrelated phenomena, such as the efficiency of individual bacterial species in utilizing nutrients for growth, and the microbe-specific host immune response, can become interdependent in a host population. The latter finding suggests a potential mechanism that could lead to selection of specific strains of bacterial species during the coevolution of the host immune response and the bacterial species.

Introduction
Outcomes of a pathogen exposure often vary from individual to individual in a population. Even in well controlled experiments, it is common to see wide variation in pathogen titers and in biomarkers of host immune response, between animals subject to identical pathogen loads \([1–3]\). Variations in the relative abundances of members of the microbial community residing in homeostasis with the host immune system are also observed between individuals \([4]\).

However, despite the ubiquity of such host-to-host variations of the host–microorganism relationship, our mechanistic understanding of such relationships or ecology of host–microorganisms \([5, 6]\) are based primarily on average values obtained from experiments done on host populations. The variations around the averages are typically assumed to arise from independent inter-host variations of phenomena that affect the host–microorganism relationship, such as the host immune response or availability of nutrients for the microorganisms. Variations between hosts are often represented merely as error bars in data summaries \([7, 8]\). This view overlooks the fact that the differences between hosts themselves may provide valuable clues regarding perturbations of the underlying mechanistic framework in a natural setting, and may relate directly to evolutionary selection of a particular host–pathogen or host–microbiota relationship based on sustaining the observed diversity in a population \([9]\).
Here, we seek mechanistic insights into host-to-host variations of the host–microorganism relationship by analyzing the population kinetics of multiple bacterial species in the well characterized model of polymicrobial otitis media (OM) in adult chinchillas (Chinchilla laniger). OM is a common childhood polymicrobial infection of the middle ear involving one or more of three predominant bacterial species that are normally a part of the microbiota in the upper respiratory tract (URT) [10, 11]: nontypeable Haemophilus influenzae (NTHI), Streptococcus pneumoniae (Sp), and Moraxella catarrhalis (Mcat). OM provides an excellent model system to dissect host–microbiota relationships because of the relatively small number of species in the relevant microbial community, and also because it offers practical advantages such as culturability of the three main bacterial species [11]. While chinchillas are not a natural host for the bacteria or viruses that cause human OM, they can be infected with and colonized by all three of the predominant bacterial OM pathogens [11].

Using an in silico approach based on maximum entropy (MaxEnt) and population dynamics combined with samples recovered from the chinchilla middle ear, we quantified ecological interactions that regulate the kinetics of bacterial infection and the host immune response in individual hosts. We show that the nature of interspecies interactions (e.g., competition, cooperation or neutral) between the bacterial species NTHI and Sp, which is not directly related to the immune response, critically regulates the host-to-host variations of the ecological interactions. More importantly, seemingly independent ecological interactions, such as the ability of the bacterial species to utilize resources and the rate at which the host immune response eliminates specific bacterial species, become interdependent in hosts. This suggests the utility of this method to characterize evolutionary selection of interspecies interactions in microbial communities through host–bacteria interactions.

**Variations of kinetics of polymicrobial infection**

Animal-to-animal variations of kinetics of bacterial species are clearly observed in experiments studying OM in rodents such as rats [1] or chinchillas [2]. For instance, in the experiments reported by Weimer et al [2], the population of Sp showed an almost bimodal behavior three days after inoculation with mixed NTHI and Sp strains; the Sp population fell below the detectable limit in a few animals, but varied between $10^4$ to $10^6$ CFUs in other animals (figure 1). The population kinetics of NTHI, although less dramatic, showed animal-to-animal differences up to three orders of magnitude in animals challenged with single and mixed species inoculations (figure 1). Small (<10%) differences in the initial dose of inoculation could be a minor contributing source to these variations but it is our firm belief that given these chinchillas were outbred animals, the major contribution comes from the inter host differences and not from the small differences in the initial dose. The bacterial species, NTHI and Sp, have been observed to interact with each other and with the host and these interactions affect the growth of the bacterial species. For example, in in vitro cultures, certain strains of Sp eliminate NTHI by secreting toxic hydrogen peroxide generated during aerobic metabolism [12]. Alternatively, NTHI can trigger mobilization of neutrophils in the epithelial layer that eliminate Sp but not NTHI via complement-mediated opsonization [13, 14]. In addition, the secretion of quorum sensing molecules by these bacterial species has been found to affect the growth of multiple bacterial species participating in the infection [15]. The bacterial species also depend on the host for extracting essential nutrients such as metals for their growth. For example, the Gram-negative NTHI and Gram-positive Sp require iron extracted from the serum generated by the host during inflammation [16, 17]. Therefore, it is plausible that variations of these factors across hosts would lead to differences in infection kinetics between hosts. Here, we quantify ecological interactions in the system and model the mechanisms that lead to the infection kinetics observed in the experiments reported by Weimer et al [2]. We have referred the infection experiments done in the chinchilla middle ears as in vivo experiments in the text, and in vitro experiments refer to the culture experiments.

**MaxEnt based method to quantify variations of ecological niches**

**A. Population dynamic model**

We constructed kinetic models based on ordinary differential equation (ODE) to describe the time evolution of populations of NTHI and Sp bacterial cells (figure S1 stacks.iop.org/PB/12/016003/mmedia). The equations are based on Lotka–Volterra (LV) type models [7], which describe the growth of two or more bacterial species interacting with each other to access available resources. These models have been successfully applied to characterize kinetics of bacterial populations in chemostat experiments [7, 18].

We modified the LV models to include the host immune responses during the acute infection phase, which is primarily regulated by innate immunity [11]. In our models, the bacterial species consume nutrients from the local environment and replicate. NTHI and Sp can compete for a common nutrient (e.g., iron) for their growth. Additionally, each species can indirectly help in the growth of the other species by generating more inflammation because the serum generated during inflammation contains iron-containing chemical
compound heme which provides the essential metal iron for growth of the bacterial species [16, 17]. In addition, NTHI and Sp can affect each other’s growth by secreting small molecules, e.g., toxins [12] or quorum sensing molecules [15]. Therefore, NTHI and Sp can potentially oppose, help, or remain uninvolved in each other’s growth depending on the nature of inflammation or the concentration of secreted molecules in the microenvironment. We considered all nine possibilities (see table 1) for interspecies interactions affecting the growth rates of NTHI and Sp.

In addition, both species induce innate immune responses (release of antimicrobial proteins [19] or influx of neutrophils in epithelial layer [14]) in the middle ear. In our coarse-grained phenomenological models, we do not distinguish between antimicrobial proteins and neutrophils, and immune response is represented by a single variable, $I$, that eliminates NTHI and Sp with different rates. The dynamics of the abundances of NTHI and Sp in the presence of the host immune response in the middle ear of a particular animal (indexed by $a$) can be described by a pair of coupled ODEs:

$$\frac{dN_{1(a)}}{dt} = f_{1,1}^{(a)}(N_{1(a)}, N_{2(a)}) - g_{1,1}^{(a)}(N_{1(a)}, N_{2(a)})$$

$$\frac{dN_{2(a)}}{dt} = f_{2,1}^{(a)}(N_{1(a)}, N_{2(a)}).$$

where, $N_{1(a)}$ and $N_{2(a)}$ denote the population sizes of NTHI and Sp, respectively, $f_{1,1}^{(a)}(N_{1(a)}, N_{2(a)})$ and $f_{2,1}^{(a)}(N_{1(a)}, N_{2(a)})$ describe the growth rate of NTHI and Sp, respectively, regulated by available resources and inter/intra species interactions. Both NTHI and Sp interact with the immune response elicited by the host that eliminates the bacteria, and $g_{1,1}^{(a)}(N_{1(a)}, N_{2(a)})$ and $g_{2,1}^{(a)}(N_{1(a)}, N_{2(a)})$ describe the rate of elimination of NTHI and Sp, respectively, by the immune response.

Table 1. List of the models considered.

| Effect on growth | $M_{1+}$ | $M_{1-}$ | $M_{2+}$ | $M_{2-}$ | $M_{10}$ | $M_{20}$ | $M_{1+0}$ | $M_{1-0}$ | $M_{2+0}$ | $M_{2-0}$ |
|------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| NTHI on Sp       | +        | +        | 0        | +        | 0        | 0        | 0        | 0        | 0        | 0        |
| Sp on NTHI       | +        | –        | 0        | +        | 0        | 0        | +        | –        | 0        | 0        |

$+ = \text{counteracts, } - = \text{helps, } 0 = \text{stays neutral.}$

Figure 1. Variations of bacterial kinetics between hosts and culture medium trials. (A) The bacterial kinetics of ‘in silico’ animals are shown with gray lines. An ‘in silico’ animal is represented by a particular set of parameters in table 2 that was drawn from the inferred MaxEnt distribution for the $M_{1+}$ model. The ‘in silico’ animal was co-inoculated or was assigned with initial numbers of NTHI and Sp, and then equation (2) was solved with that initial condition to obtain the population kinetics of NTHI in the middle ear of the ‘in silico’ animal. The in vivo experimental data (each red triangle corresponds to an individual chinchilla middle ear) were taken from [2], where the animals received inocula of $\sim 10^5$ CFU and $\sim 150$ CFU of NTHI and Sp, respectively. (B) Kinetics of Sp for the same set up as in (A) is shown using the same visualization scheme. The experimental data (in vivo) are shown using solid green circles. (C) Kinetics of NTHI (gray lines) generated by solving equation (S1) in individual trials in ‘in silico’ culture experiments. Each ‘in silico’ culture experiment is represented by a set of parameters in table 2, that was drawn from the inferred MaxEnt distribution for the $M_{1+}$ model. The in vitro experimental data for each trial for the co-culture experiments with NTHI and Sp are shown in red triangles. (D) Kinetics of Sp for the same set up as in (C). The in vitro experimental data are shown in solid green circles.
To keep the notation simple, we will drop the superscript in the rest of the equations where all the variables and the parameters describe the kinetics in an individual animal or a trial in culture experiments. Following the LV model for interspecies interaction we use [7], \(f_1(N_1, N_2) = r_1 N_1(K_1 - \alpha_{11} N_1 - \alpha_{12} N_2)\) and \(f_2(N_1, N_2) = r_2 N_2(K_2 - \alpha_{21} N_1 - \alpha_{22} N_2)\). The carrying capacities, \([K_1, K_2]\), determine the maximum values of the population that can be sustained by the available resources [7]. \([\alpha_{11}, \alpha_{22}]\) denotes the competition for resources between bacteria of the same species and \([\alpha_{12}, \alpha_{21}]\) parametrizes interspecies interaction between NTHI and Sp. We have used \([\alpha_{11}, \alpha_{22}] > 0\), implying that the bacteria of the same species always compete with each other for resources. We considered positive, negative, and zero values for \([\alpha_{12}, \alpha_{21}]\) to describe competing, cooperating, and neutral nature of interspecies interactions respectively. The interspecies interactions are generally not reciprocal, i.e., \(\alpha_{ij} \neq \alpha_{ji}\).

We considered nine different models, each denoting a specific type of interspecies interaction (see table 1 for the list). For example, \(M_{12}\) describes the model where the interspecies interactions are given by \(\alpha_{12} > 0\) and \(\alpha_{21} < 0\). The immune responses \(I\) are described by monotonically increasing functions of \(N_1\) and \(N_2\) representing concentrations of antimicrobial proteins or neutrophils attracted to the infection site, i.e., \(g_1(N_1, N_2) = k_{I1} N_1 I\) and \(g_2(N_1, N_2) = k_{I2} N_2 I\). The immune response, \(I\), is generated due to the immune response \(I_1\) and \(I_2\) induced by \(N_1\) and \(N_2\) respectively, and is assumed to be additive, i.e., \(I = I_1 + I_2\). We assume the immune response to depend on \(N_1\) and \(N_2\) as, \(I_1 = k_1 N_1 / (K_{M1} + N_1)\) and \(I_2 = k_2 N_2 / (K_{M2} + N_2)\). We have also studied the effect of immune responses as different functions of \(N_1\) and \(N_2\) which produced qualitatively similar results as the forms we have used here (figures S4–S5). We write \(g_1(N_1, N_2)\) as, \(g_{i1}(N_1, N_2) = k_{Ii1}(N_1)^{d_1} / (K_{MI} + N_1) + k_{Ii12} N_1 N_2 / (K_{MM} + N_2)\), and \(g_{i2}(N_1, N_2) = k_{Ii21} N_1 N_2 / (K_{MI} + N_1) + k_{Ii22} (N_1)^{d_2} / (K_{MM} + N_2)\), where, \(k_{Ii1} = k_{Ii1} \cdot \alpha_{ij}\) (\(i, j \in \{1, 2\}\)). Thus the kinetics is described by the following ODEs

\[
\frac{dN_1}{dt} = r_1 N_1 \left( K_1 - \alpha_{11} N_1 - \alpha_{12} N_2 \right) - \frac{k_{I11} N_1^2}{K_{M1} + N_1} - \frac{k_{I12} N_1 N_2}{K_{M1} + N_1},
\]

\[
\frac{dN_2}{dt} = r_2 N_2 \left( K_2 - \alpha_{21} N_1 - \alpha_{22} N_2 \right) - \frac{k_{I21} N_1 N_2}{K_{M2} + N_2} - \frac{k_{I22} N_2^2}{K_{M2} + N_2}.
\]  

(2)

Depending on the values of the parameters, the kinetics described by the ODEs in equation (2) produce multiple fixed points. For example, \(N_1\) is present but \(N_2\) is absent, \(N_1\) is absent but \(N_2\) is present, or both \(N_1\) and \(N_2\) are present. With appropriate choices of parameter values, these fixed points can become stable fixed points that the system will eventually reach if the initial values are appropriately chosen to be within the domain of attraction (details in the supplementary material). The values of \(N_1\) and \(N_2\) at the stable fixed points as well as the kinetics of \(N_1\) and \(N_2\) leading to those fixed points vary as the parameters in the ODEs are changed. Since the parameters in the ODEs (representing the nature of resource utilization, inter- and intra-species interaction, host immune responses and their effect on the bacterial population) describe the role of the environment and interspecies interactions on the size of the bacteria population, we call these parameters \(\{\epsilon_i\}\) ‘ecological interactions’ (see table 2 and S1 for further details). We hypothesize that these ecological interactions vary from animal to animal, resulting in different populations of bacterial species infecting/colonizing middle ears in individual animals. Here, we address the following questions: (1) What can we deduce about any relationship between ecological interactions in individual animals from the experimentally observed host-to-host variations in bacterial populations? (2) Does the extent of variation of the other ecological interactions depend on the interspecies interactions between the bacterial species? (3) Is it possible that seemingly unrelated ecological interactions are interdependent, and does this occur in response to selective pressures on specific bacterial strains in a host population?

B. MaxEnt formalism to quantify host–host variations

MaxEnt is widely used in statistical physics [20–22], information theory [23], and statistics [24] to infer distributions of variables based on available measurements. The MaxEnt based approach enables us to estimate the distributions based solely on the available data and is free of any additional assumptions. Thus, the estimated distribution quantifies the minimal structure required in the distribution to describe the available data and could point to general principles in the system [25]. In addition, the approach enables systematic incorporation of new data, and therefore continual improvement of the estimation as new data becomes available. Recently we used MaxEnt to quantify functional implications of cell-to-cell variations of chemotactic protein abundances [26, 27]. Here, we use MaxEnt to infer the distribution of the ecological interactions in individual animals, using the observed populations of NTHI and Sp in OM as constraints. We introduce a parameter vector, \(\{\epsilon_i\}\), that represents the parameters in the ODE models and use our MaxEnt based method to estimate the distribution, \(\hat{P}(\{\epsilon_i\})\). We outline our method for a simple example below and provide further details regarding the full calculation in the supplementary material available at stacks.iop.org/PB/12/016003/mmedia. The experimentally measured values of NTHI (or \(N_1\)) and Sp (or \(N_2\)) populations at different time points in single infections (where the middle ear is infected with a single bacterial species) or coinfections (where the middle ear is infected with both NTHI and Sp) provide us with average values and variances of NTHI and Sp population. For example, the average values of the NTHI and Sp populations can...
Table 2. Summary of the physical relevance and the range of variation of the parameters used in in silico models.

| Single species parameters [e^{\alpha(t)}] | Range of variation | Physical significance |
|------------------------------------------|--------------------|-----------------------|
| \(\gamma_1, \gamma_2\)                  | From 2 to 10 day\(^{-1}\) for in vivo experiments and from 1 to 10 h\(^{-1}\) for in vitro culture experiments. | Growth rates of \(N_1\) and \(N_2\) respectively (equations S1 and S2). |
| \(\alpha_{11}, \alpha_{22}\)              | From \(10^{-9}\) to \(10^{-7}\) CFU\(^{-1}\) day\(^{-1}\) for in vivo experiments and from \(10^{-7}\) to \(10^{-11}\) CFU\(^{-1}\) h\(^{-1}\) for in vitro culture experiments. | Strength of intraspecies interactions describing competition for resources. |
| \(k_{d1}\)                               | From 0 to 200 day\(^{-1}\) for in vivo experiments. | The rate at which the immune response elicited by \(N_1\) eliminates \(N_2\). |
| \(k_{d2}\)                               | From 0 to 200 day\(^{-1}\) for in vivo experiments. | The rate at which the immune response elicited by \(N_2\) eliminates \(N_1\). |
| \(K_{s11}\)                              | From \(10^9\) to \(10^{11}\) CFU for in vivo experiments. | Michaelis constant (equation (2)) for the immune response elicited by \(N_1\). When \(N_1 \gg K_{s11}\) the response saturates. |
| \(K_{s22}\)                              | From \(10^6\) to \(10^{11}\) CFU for in vivo experiments. | Michaelis constant (equation (2)) for the immune response elicited by \(N_2\). When \(N_2 \gg K_{s22}\) the response saturates. |

Mixed species parameters [e^{\alpha_{12}(t)}]

| \(\alpha_{12}\) | From \(10^{-9}\) to \(10^{-6}\) CFU\(^{-1}\) day\(^{-1}\) for in vivo experiments and from \(10^{-7}\) to \(10^{-11}\) CFU\(^{-1}\) h\(^{-1}\) for in vitro culture experiments. | Strength of the interspecies interaction induced by \(N_2\) on the \(N_1\) growth rate. |
| \(\alpha_{21}\) | From \(10^{-9}\) to \(10^{-6}\) CFU\(^{-1}\) day\(^{-1}\) for in vivo experiments and from \(10^{-7}\) to \(10^{-11}\) CFU\(^{-1}\) h\(^{-1}\) for in vitro culture experiments. | Strength of the interspecies interaction induced by \(N_1\) on the \(N_2\) growth rate. |
| \(k_{d12}\) | From 0 to 200 day\(^{-1}\) for in vivo experiments | The rate at which the immune response elicited by \(N_2\) eliminates \(N_1\). |
| \(k_{d21}\) | Varied from 0 to 200 day\(^{-1}\) for in vivo experiments | The rate at which the immune response elicited by \(N_1\) eliminates \(N_2\). |

Parameters used only for the culture models

| \(K_{\text{log}1}, K_{\text{log}2}\) | \(K_{\text{log}1}\) is varied from \(10^{10}\) to \(10^{16}\) CFU and \(K_{\text{log}2}\) from \(10^9\) to \(10^{14}\) CFU for in vitro experiments. | Effective parameters to incorporate the stunted growth phase of \(N_1\) and \(N_2\). When \(N_1\) and \(N_2\) become much larger than \(K_{\text{log}1}\) and \(K_{\text{log}2}\), the bacteria start to grow exponentially. |

Where \(\gamma_1 = r_1K_1, \gamma_2 = r_2K_2, \alpha_{11} = r_1\alpha_{11}, \alpha_{12} = r_1\alpha_{12}, \alpha_{21} = r_2\alpha_{21}\), and, \(\alpha_{22} = r_2\alpha_{22}\). A new parameter set (see table S1) generated from a combination of these parameters was varied in a uniform distribution.

be described as,

\[
(1/\# \text{ of animals}) \sum_{a=1}^{m} N_{12}^{(a)}(t) = N_{12}^{\text{avg}}(t) = \sum_{\{\ell\}} P(\{\ell\}) N_{12}(\{\ell\}, t),
\]

(3)

where, \(N_{1}^{(a)}(t)\) and \(N_{2}^{(a)}(t)\) refer to the populations \(N_1\) and \(N_2\) in the middle ear of an animal indexed by \(a\) at time \(t\) (e.g., 7 days after inoculation). Thus, the first equality on the LHS defines the average value of \(N_1\) measured at a time \(t\) over multiple animals. The second equality on the RHS equates the model values to the experimental measurements. If the ecological niches \(\{\ell\}\) are distributed according to a distribution \(P(\{\ell\})\) in the animals, and the infection kinetics of \(N_1\) and \(N_2\) follow the ODEs in equation (2), then the average of \(N_1(\{\ell\}, t)\) and \(N_2(\{\ell\}, t)\) over \(P(\{\ell\})\) should reproduce the observed average value at time \(t\). There are many ways to choose a \(P(\{\ell\})\) that will satisfy equation (3). We use a MaxEnt based approach that enables us to infer \(P(\{\ell\})\) solely based on available data without any additional assumptions. This method selects a \(P(\{\ell\})\) that maximizes the Shannon entropy, \(S = -\sum_{\{\ell\}} P(\{\ell\}) \ln P(\{\ell\})\), in the presence of constraints imposed by the available data, such as the second equality in equation (3). Instead of directly maximizing \(S\), we estimate \(P(\{\ell\})\) by minimizing a relative entropy (equation (4)). Further details regarding the method are provided in the methods section and the supplementary material.

Interspecies interactions regulate animal-to-animal variations of microbial kinetics

We quantified the extent of variation of the ecological interactions between animals by calculating the
minimum value of the relative entropy, MinRE defined in equation (4) in materials and methods, for all nine models (figure 2(A)). All the models were constrained to reproduce the average values and variances of NTHI and Sp populations measured at 7 days post inoculation, for animals infected with NTHI and Sp simultaneously.

The model in which Sp helps NTHI to access nutrients but NTHI competes with Sp (model $M_{-+}$) produces the smallest MinRE, i.e., this model is consistent with the broadest parameter variations. The next best (MinRE) model was $M_{+0}$, in which Sp stays neutral to NTHI growth and NTHI competes with Sp for resources. In contrast, the models $M_{++}$ and $M_{0-}$, in which NTHI helps Sp to access resources, are consistent with only a very small amount of variation in the parameters. The results can be understood in the following way. The experiments show that at 7 days after coinoculation with initial bacterial concentrations NTHI $\sim 1000$ CFU and Sp $\sim 150$ CFU, the average population of NTHI ($\sim 10^7$ CFU) is substantially higher than that of Sp ($\sim 10$ CFU). In contrast, when the animals are infected with either NTHI or Sp alone, both species reach high population ($\sim 10^7$ CFU). Therefore, the models that produce high growth for NTHI and low growth for Sp at later times (\sim 7 days) across a wider range of parameter variations will be the models with smaller MinRE. In the model $M_{-+}$, the interspecies interaction supports a higher NTHI and a lower Sp growth since Sp cooperates with NTHI in its growth, but the presence of NTHI counteracts Sp growth. The immune response, regardless of the type of interspecies interaction, can also support a larger NTHI population than Sp population by killing Sp at a higher rate compared to NTHI. In the model $M_{+}$, even when the elicited immune response kills NTHI at a higher rate than it eliminates Sp, which could lead to kinetics opposite to those observed in experiments, higher values of the interspecies interactions (e.g., $\alpha_{12}$ and $\alpha_{13}$) can counteract the effects induced by the immune response and produce a pattern similar to that observed in experiments. In contrast, in the model $M_{++}$, the interspecies interaction supports a higher population number for Sp compared to that of NTHI, so that the MaxEnt probability distribution is heavily concentrated on the subset of vectors of the ecological interaction parameters for which the immune response is able to counteract this effect and produce higher growth in NTHI compared to Sp. These patterns also indicate how seemingly unrelated ecological interactions, such as the interspecies interactions and the immune response, can become correlated. This is discussed in greater detail in the next section.

Next we compared the nature of variations of the ecological interactions explaining the infection data against the in vitro culture experiments (figure 2(B)). In vitro, populations of both NTHI and Sp grew in the medium, whether they were inoculated separately or simultaneously (figure S2). The population size of NTHI is similar to that of Sp when the bacterial species are cultured individually, and the NTHI population is slightly larger than that of Sp when cultured together. The models where NTHI competes (model $M_{+0}$) or stays neutral (model $M_{00}$) with Sp for utilizing resources produce the smallest MinREs, whereas the model...
with only competitive interspecies interactions (model $M_{++}$) produces the highest MinRE. When both species are competing with each other for the common resources, the species can coexist only within a small range of parameter values, as a small difference between $\alpha_{12}$ and $\alpha_{21}$ can lead to elimination of one species (see the analysis of the ODEs in equation S1 in the supplementary material stacks.iop.org/PhysBio/12/016003/mmedia). In contrast, when a species is not interacting with another species it always reaches a population size determined by the carrying capacity. Therefore, the models that contain neutral interactions between the species allow for more variation in underlying parameters compared to the other models.

**Testing predictions**

We used the estimated MaxEnt distributions to generate predictions for measurements that were not used as constraints in fitting the MaxEnt models. Specifically, we predicted the average values of populations of NTHI and Sp at day 3 when the animals were co-inoculated with these species. In addition, we also predicted the correlation between NTHI and Sp at day 7. The predictions from model (M$_{--}$), which was the best (MinRE) model under the original set of constraints, were in reasonable agreement with the data (table S2). The models with larger MinRE values produced less agreement to model M$_{--}$ (table S2). In general, predictions were better for NTHI than Sp. The disagreement between the model predictions and the data for Sp could point to the importance of spatial structures such as biofilms in regulating the bacterial kinetics. This point is further deliberated in the discussion section.

**Specific ecological interactions become interdependent**

Because, $N_1$ and $N_2$ in the ODEs (equation (2)) are complicated nonlinear functions of the parameters $\{e_i\}$, it is not obvious that the inferred distribution $\hat{P}(\{e_i\})$ would follow a normal distribution. We evaluated whether the inferred distribution $\hat{P}(\{e_i\})$ of the model parameters could be well approximated by a multivariate normal distribution (equation (5)). Since the distribution appeared to be well approximated by a multivariate normal distribution (figure S3), the average values and pair-correlations between the parameters capture the majority of the variations in the system and we quantified the interdependencies between the model parameters by using the inverse matrix, $[\Omega]_{ij} = [C^{-1}]_{ij}$, where, $C_{ij}$ denotes the correlation between the model parameters $e_i$ and $e_j$. The correlation matrix, $C$, is defined as, $C_{ij} = (\bar{e}_i - \mu_i)(\bar{e}_j - \mu_j)$ and $\mu_i = \bar{e}_i$, where, the over bar indicates the average over $\hat{P}(\{e_i\})$. We further quantified the strength of the interdependence or relationship between the model parameters by calculating a metric $([\Omega]_{ij})$ for every pair of parameters $e_i$ and $e_j$ using the $\Omega$ matrix (see the methods section for details). A larger magnitude of $[\Omega]_{ij}$ implies a greater contribution of the pair of parameters to determining animal-to-animal (or trial-to-trial) variations (figure 3), and a negative or a positive value indicates whether the members of the pair vary in the same or opposite direction while keeping the response unchanged.

Analysis of the interdependencies using $([\Omega]_{ij})$ for $M_{--}$, showed (figure 3(B)) that parameters that are not directly related to the immune response, such as the carrying capacity or the strength of interspecies interactions, can become dependent on parameters directly related to the immune response, such as the rate of killing of Sp by the immune system. The number of such dependencies with higher magnitudes of $([\Omega]_{ij})$ increases for the higher MinRE models (consistent with less variation in the parameters) in the niches considered here(figures 3(C) and (E)). This result can be intuitively understood as follows: requiring increased interdependence between the parameters imposes greater restrictions on the sets of parameter vectors that are able to reproduce the measured average values. The majority of the dependences can be understood qualitatively by analyzing the ODEs, for example, the increase in the NTHI bacterial load required to induce the maximum immune response that favors an increase in the NTHI population is compensated for by a corresponding decrease in the available resources or the carrying capacity (figure 3(B)). This implies that a particular strain in NTHI that is less efficient in stimulating the immune response will also undergo changes that reduce its capability to utilize the nutrients for growth. Further explanations regarding the other interdependencies are provided in the supplementary material available at stacks.iop.org/PhysBio/12/016003/mmedia (table S3).

The *in vitro* analyses show (figures 3(D) and (E)) that parameters describing interspecies interactions become more interdependent during the bacterial growth.

**Discussion**

We developed a method based on MaxEnt to quantify host-to-host variations of ecological interactions for two bacterial species, NTHI and Sp, which are responsible for polymicrobial OM infection. A key finding of this analysis is the dependency of the extent of host-to-host variations of the ecological interactions on the nature of the underlying bacterial interspecies interactions. Cooperative–competitive or neutral–competitive interaction models between bacterial species allow for the largest variations (smaller values of MinRE) of the ecological interactions or model parameters, and are likely to be associated with host populations with greater heterogeneity and
environmental perturbations. Interspecies interactions between NTHI and Sp arise via a range of processes, such as secretion of toxins, metabolic byproducts, inflammation and quorum sensing. Since the nature and magnitude of these processes can vary from strain to strain in a bacterial species, it is possible that specific strains of NTHI and Sp with interspecies interactions that are robust to the largest variations in ecological niches in the host population, are selected as the host immune system and the microorganisms co-evolve in an evolutionary arms race [5].

The structure of the inferred variations of ecological interactions reveal that seemingly unrelated ecological variables, such as the carrying capacities and the host immune response, become interdependent. For example, in a model with large ecological variation (M−), a mutually cooperative relationship between the carrying capacity and the rate of bacterial replication is observed.
elimination suggests that an NTHI strain will be selected if it can use equivalent resources more efficiently despite its growth being suppressed by the host immune response (figure 3). These results suggest that if the microbial communities residing in the host have the flexibility to accommodate changes in the ecological interactions, such as, by altering gene expressions [28], these changes are likely to occur in a coordinated manner [29].

The in vitro culture experiments show that both the NTHI and Sp bacterial strains are able to coexist in the culture medium, while in the chinchilla host, the Sp strains are eliminated in the presence of NTHI. This clearly suggests a qualitative difference in ecological niches for growth in the host microenvironment and the in vitro culture. Our MaxEnt based analysis quantitatively characterizes the difference. The analysis showed that the neutral model (M_{00}) produced a wider spread in ecological interactions in vitro over the purely competitive model (M_{++}). This result is consistent with Gause’s law in population dynamics [7], which states that two species competing for the same resources cannot coexist. In contrast, in the presence of the host immune response, the purely competitive model (M_{++}) showed a much wider variation compared to the neutral model (M_{00}). Therefore the models with the smallest MinRE values in vitro have very different interspecies interaction than the models with small MinRE values in vivo. In vivo, the model M_{++} accommodating largest variation in ‘ecological interactions’, contains cooperative and competitive interactions whereas its counterpart (M_{10} or M_{01}) in vitro contains non-interacting bacterial species. These differences emphasize the importance of the immune response in manipulating ecological niches and evolutionary selection of bacterial strains residing in the host.

We primarily studied models that approximated and simplified interspecies interactions in terms of a relatively small number of parameters. Therefore, these models need to be modified in order to investigate interspecies interactions such as quorum sensing, which increases fitness of the same strain, or the formation of spatial structures such as biofilms, which help bacterial species to evade the host immune response. The importance of these effects, in particular biofilm formation, becomes apparent from the disagreements between the model predictions and the experimental data (table S2). The two-species model could be extended in order to include additional strains associated with biofilms found in the chinchilla middle ear [2]. Investigation of the role of these additional strains in host-to-host variations of infection kinetics would be an interesting future direction. Stochastic fluctuations in bacterial growth [30, 31], especially when bacterial population sizes are small, for example, at early phases of infection or when a species is nearing clearance (e.g., Sp population in figure 1(B)) could potentially play a role in animal-to-animal variation of bacterial kinetics. We plan to study these effects in the future.

Our analysis showed that host-to-host variations of polymicrobial infection kinetics can provide valuable clues regarding evolutionary selection of bacterial strains and the role of the host immune response in shaping the fitness landscape of the polymicrobial community. A possible test of the results presented here could be analysis of gene expression from bacterial isolates obtained from the middle ear pre- and post-co-inoculation. If genes responsible for metabolism of essential metals are upregulated during coinfection in NTHI but not in Sp, this would lend additional support to our conclusion that specific attributes promoting NTHI growth are selected due to the combined effect of the presence of Sp and the host immune response. The modeling approach proposed here represents a general method, not limited to OM, which can be utilized to understand mechanisms of host–microorganism relationships and their evolutionary origin using measurements delineating host-to-host variations of microbial and host response kinetics.

**Methods and materials**

**Solution of the ODEs**

The ODEs in equation (2) were solved using the software package BIONETGEN [32]. The codes used in the simulations can be found at http://planetx.nationwidechildrens.org/~jayajit/.

**Estimation of ˆP((i,))**

We used measurements from infection and culture experiments studying kinetics of single or two bacterial species for estimating ˆP((i,)). We separate the parameter vector [i] into two sub-sets [i] and [i] (see tables 2 and S1) that represent respectively the parameters solely regulating bacterial kinetics for experiments with single species and the additional parameters required to describe the kinetics for the mixed coinfection/culture experiments. We described the kinetics in terms of a new set of parameters [i] constructed from a particular combination of [i] (table S1 and supplementary material stacks.iop.org/PB/12/016003/mmedia) and carried out all the MaxEnt analysis on new set [i]. Thus, [i] in the rest of the section refer to [i]. We retained the same symbols for simplicity. ˆP((i,)) can be decomposed into ˆP((i,)) = ˆP((i,)) ˆP((i,), [0]) (see the supplementary material for the derivation), where, ˆP((i,)) and ˆP((i,), [0]) describe the distributions of the parameters consistent with experiments done with single or two bacterial species, respectively. We briefly describe the numerical scheme used in estimating ˆP((i,), [0]) and ˆP((i,)).
(A) Infection (in vivo) experiments

\( \hat{P}^{(S)}(\{e_i^{(S)}\}, \{0\}) \) is estimated from the infection experiments where the chinchilla middle ears are infected with either Sp or NTHi. The a priori distribution of the parameters before the maximization of S was assumed to be a uniform distribution in \( \{e_i^{(S)}\} \), since the uniform distribution represents the maximally uncertain state of a system. The parameters related to mixed two species experiments set to zero, i.e.,

\[ q_U(\{e_i\}) = q_U(\{e_i^{(S)}\}) \times \prod_j \delta^{(M)}(a), \]

where, \( \delta^{(M)} = 1 \) (or = 0) when \( a = b \) (or \( a \neq b \)). We constrained the average values of populations of NTHi and Sp in the models, according to their experimentally measured values at two different times (3 and 7 days).

\[ P(\{e_i^{(S)}\}, \{0\}) \]

is estimated by minimizing the relative entropy

\[ \text{MinRE}^{(S)} = \sum_{\{e_i^{(S)}\}} P(\{e_i^{(S)}\}, \{0\}) \times \ln \left[ \frac{P\left(\{e_i^{(S)}\}, \{0\}\right)}{q_U\left(\{e_i^{(S)}\}, \{0\}\right)} \right] \]

subject to the constraints imposed by the average values.

In the next step, we generate the a priori distribution \( q_U(\{e_i\}) \) by choosing parameters \( \{e_i^{(M)}\} \) based on \( \hat{P}^{(M)}(\{e_i^{(S)}\}, \{0\}) \) and the parameters \( \{e_i^{(M)}\} \) were chosen from a uniform distribution, i.e.,

\[ q_U^{(M)}(\{e_i\}) = \hat{P}^{(M)}(\{e_i^{(S)}\}, \{0\}) \times q_U(\{e_i^{(M)}\}). \]

Then we estimate the distribution, \( P^{(M)}(\{e_i\}) \) when \( \{e_i^{(M)}\} \) are not vanishing using the measured values from the coinfection experiments as constraints and minimizing the relative entropy

\[ \text{MinRE} = \sum_{\{e_i\}} P^{(M)}(\{e_i\}) \ln \left[ \frac{P^{(M)}(\{e_i\})}{q_U(\{e_i\})} \right]. \]

where, \( q_U(\{e_i\}) \) denotes a uniform distribution for parameters in both the subsets \( \{e_i^{(S)}\} \) and \( \{e_i^{(M)}\} \). The details regarding sample size and the sampling method are given in the supplementary material available at stacks.iop.org/PB/12/016003/mmedia.

(B) In vitro culture

Since the immune response is absent in the culture experiments, we set \( g_1 = g_2 = 0 \) in the models. The growth of NTHi and Sp are described by the rates, \( f_1(N_1, N_2) = r_1[N_1^2/(K_{lag1} + N_1^2)](K_1 - a_{11}N_1 - a_{12}N_2) \) and \( f_2(N_1, N_2) = r_2[N_2^2/(K_{lag2} + N_2^2)](K_2 - a_{22}N_2 - a_{21}N_1). \)

The terms \([N_1^2/(K_{lag1} + N_1^2)]\) and \([N_2^2/(K_{lag2} + N_2^2)]\) describe the initial lag in the growth of NTHi and Sp. The ODEs are shown in equation (S1). The rest of the parameters are described in the same manner as in the infection models. The ODEs are unable to capture the initial drop in the bacterial populations in the first 3 h (figures 1(G) and (D)), and also produce higher concentrations of bacterial populations at later times with a particular set of values for the parameters. This is because the ODEs use constant rates for bacterial replication and death. A possible solution would be to use time dependent replication and death rates, for example, higher death rate compared to the replication rate at early times and the opposite scenario at later times. Such modifications will complicate the models and we avoid using them here. Therefore, we used the experimental data and the model kinetics at later times in the MaxEnt method which would remain largely unchanged if the models are modified to match the early time kinetics in the experiments. The distribution of the parameters is estimated using the same scheme as described above from the in vitro measurements studying growth of NTHi and Sp growing individually or simultaneously in the medium.

Experimental techniques

*S. pneumoniae* TIGR4 and *H. influenzae* 86-028NP were cultured, alone or together in equivalent ratios, in brain–heart infusion (Difco) supplemented with hemin and NAD, and containing 10% horse serum (HemoStat Laboratories), as previously described in [33]. Bacterial counts were derived by plate-count. Bacterial populations were assessed separately by plating the same serial dilutions on media selective for each species. *Pneumococci* were propagated on blood agar containing 2 micrograms/ml gentamicin, whereas *H. influenzae* was propagated on brain–heart infusion agar (Difco) supplemented with hemin and NAD and containing 3 micrograms/ml vancomycin. All counts are expressed as colony forming units per ml.

Quantification of the relationship between the model parameters

We approximate the distribution \( \hat{P}^{(M)}(\{e_i\}) \) by a multivariate normal distribution (figure S3), i.e.,

\[ \hat{P}^{(M)}(\{e_i\}) \propto e^{-\frac{1}{2} \sum_{\mu} (e_i - \mu_i)^T \Omega_{ij}^{-1} (e_i - \mu_i)}, \]

where, \( \{\mu_i\} \) denote the average values of the parameters \( \{e_i\} \), and, \( \Omega_{ij}^{−1} = C_{ij} \), \( C_{ij} \) denoting the correlation between the niches \( e_i \) and \( e_j \) or \( \mu = \mu_i (e_i - \mu_i), \) where the over bar indicates the average over \( \hat{P}^{(M)}(\{e_i\}) \). The elements of the matrix \( \Omega \) demonstrate the ‘interaction’ between the parameters or the nature of the relationship between the parameters in producing the observed correlations. E.g., a positive (or negative) value \( \Omega_{ij} \) would imply the parameters \( e_i \) and \( e_j \) counter-act (or help) each other in producing the observed population kinetics. A vanishing value of \( \Omega_{ij} \) would imply very little relationship between \( e_i \) and \( e_j \). We evaluated which of the interactions in \( (\Omega_{ij}) \) contribute the most in determining the observed covariance \( C_{ij} \). This was done by not constraining a specific \( C_{ij} \) and then comparing the inferred \( \hat{P}^{(0)}(\{e_i\}) \) with the original inferred distribution, \( \hat{P}(\{e_i\}) \) using the Kullback–Leibler distance,

\[ D_{KL} = \sum_{\{e_i\}} \hat{P}(\{e_i\}) \ln \left[ \frac{\hat{P}(\{e_i\})}{\hat{P}^{(0)}(\{e_i\})} \right]. \]
A larger $|D_{KL}|_{ij}$ implies a greater contribution of a particular $Q_i$ in determining the animal-to-animal variations of the ecological niches (figure 3). Therefore, we use a metric, $I_{int} = \text{sgn}(Q_i) \cdot |D_{KL}|_{ij}$ to quantify inferred interaction strength between the pair of niches, $i$ and $j$.

Acknowledgments

We are grateful to Lauren Bakaletz for a critical reading of the manuscript. The work is supported by a grant from NIGMS (1R01GM103612-01A1) to JD. JD is also partially supported by the Research Institute at the Nationwide Children’s Hospital and a grant from the Ohio Supercomputer Center (OSC).

References

[1] Margolis E, Yates A and Levin B R 2010 The ecology of nasal colonization of Streptococcus pneumoniae, Haemophilus influenzae and Staphylococcus aureus: the role of competition and interactions with host’s immune response BMC Microbiology 10 59
[2] Weimer K E, Armbruster C E, Juneau R A, Hong W, Pang B and Swords W E 2010 Coinfection with Haemophilus influenzae promotes pneumococcal biofilm formation during experimental otitis media and impedes the progression of pneumococcal disease J. Infectious Diseases 202 1068–75
[3] West E et al 2013 PD-L1 blockade synergizes with IL-2 therapy in reinvigorating exhausted T cells J. Clin. Investigation 123 2604–15
[4] Eckburg P B et al 2005 Diversity of the human intestinal microbial flora Science 308 1635–8
[5] Levin B R and Bull J J 1994 Short-sighted evolution and the virulence of pathogenic microorganisms Trends Microbiology 2 76–81
[6] Smith V H and Holt R D 1996 Resource competition and within-host disease dynamics Trends Ecology Evol. 11 386–9
[7] Kot M 2001 Elements of Mathematical Ecology vol ix (Cambridge: Cambridge University Press) p 453
[8] Nowak M A and May R M 2000 Virus Dynamics: Mathematical Principles of Immunology and Virology vol xii (Oxford, New York: Oxford University Press) p 237
[9] Bolnick D I et al 2011 Why intraspecific trait variation matters in community ecology Trends Ecology Evol. 26 183–92
[10] Klein O J 1997 Role of nontypeable Haemophilus influenzae in pediatric respiratory tract infections Pediatric Infectious Disease J. 16 S5–8
[11] Bakaletz L O 2004 Developing animal models for polymicrobial diseases Nat. Rev. Microbiology 2 552–68
[12] Pericone C D, Overweg K, Herrmans P W and Weiser J N 2000 Inhibitory and bactericidal effects of hydrogen peroxide production by Streptococcus pneumoniae on other inhabitants of the upper respiratory tract Infection Immunity 68 3590–7
[13] Lysenko E S, Lijek R S, Brown S P and Weiser J N 2010 Within-host competition drives selection for the capsule virulence determinant of Streptococcus pneumoniae Curr. Biol. 20 1222–6
[14] Lysenko E S, Ratner A J, Nelson A L and Weiser J N 2005 The role of innate immune responses in the outcome of interspecies competition for colonization of mucosal surfaces PLoS Pathogens 1 e1
[15] Armbruster C E et al 2010 Indirect pathogenicity of Haemophilus influenzae and Moraxella Catarrhalis in polymicrobial otitis media occurs via interspecies quorum signaling mBio 1 e00102–10
[16] Cassat J E and Skaar E P 2012 Metal ion acquisition in Staphylococcus aureus: overcoming nutritional immunity Semin. Immunopathology 34 215–35
[17] Szeleny B R, Heimbich D R, Raffel F K, Justice S S and Mason K M 2013 Haemophilus responses to nutritional immunity: epigenetic and morphological contribution to biofilm architecture, invasion, persistence and disease severity PLoS Pathogens 9 e1003709
[18] Novick A and Sztar I 1950 Experiments with the chemostat on spontaneous mutations of bacteria Proc. Natl. Acad. Sci. USA 36 708–18
[19] Ganz T 2002 Antimicrobial polypeptides in host defense of the respiratory tract J. Clin. Investigation 109 693–7
[20] Jaynes E T 1957 Information theory and statistical mechanics: II Phys. Rev. 108 171–90
[21] Jaynes E T 1957 Information theory and statistical mechanics Phys. Rev. 106 620–30
[22] Bialek W et al 2012 Statistical mechanics for natural flocks of birds Proc. Natl. Acad. Sci. USA 109 4786–91
[23] Cover T M and Thomas J A 1991 Elements of Information Theory vol xxi (New York: Wiley) p 542
[24] Jaynes E T and Bretthorst G L 2003 Probability Theory: the Logic of Science (Cambridge: Cambridge University Press)
[25] Bialek W 2012 Biophysics: Searching for Principles (Princeton, NJ: Princeton University Press)
[26] Mukherjee S, Seok S C, Vieland V J and Das J 2013 Cell responses only partially shape cell-to-cell variations in protein abundances in escherichia coli chemotaxis Proc. Natl. Acad. Sci. USA 110 18531–6
[27] Mukherjee S, Seok S C, Vieland V J and Das J 2013 Data-driven quantification of the robustness and sensitivity of cell signaling networks Phys. Biol. 10 066002
[28] Domka J, Lee J, Bansal T and Wood T K 2007 Temporal gene-expression in Escherichia coli K-12 biofilms Environ. Microbiology 9 332–46
[29] McElroy K E et al 2014 Strain-specific parallel evolution drives short-term diversification during Pseudomonas aeruginosa biofilm formation Proc. Natl. Acad. Sci. 111 E1419–27
[30] Raj A and van Oudenaarden A 2008 Nature, nurture, or chance: stochastic gene expression and its consequences Cell 135 216–26
[31] Gefen O, Galay C, Mumcuoglu M, Engel G and Balaban N Q 2008 Single-cell protein induction dynamics reveals a period of vulnerability to antibiotics in persister bacteria Proc. Natl. Acad. Sci. USA 105 6145–9
[32] Hlavacek W S, Faeder J R, Blinov M L, Posner R G, Hucka M and Fontana W 2006 Rules for modeling signal-transduction systems, Science’s STKE signal transduction knowledge environment Sci. STKE 2006 re6
[33] Weimer K E et al 2011 Divergent mechanisms for passive pneumococcal resistance to beta-lactam antibiotics in the presence of Haemophilus influenzae J. Infectious Diseases 203 549–55