Nested inference of pairwise interactions from strain frequency data across settings and context-dependent mutual invasibilities

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Abstract

How does coexistence of multiple species or pathogen strains arise in a system? What do coexistence patterns in time and space reveal about the epidemiology, ecology and evolution of such systems? Species abundance patterns often defy fully mechanistic explanations, especially when compositional variation and relative taxa abundances differ across settings. To link such variation to deterministic biological processes in a *cause-and-effect* fashion requires modeling frameworks that are general in spirit, simple enough to understand and implement, and easily-applicable to multi-site data and their environmental gradients. Here, we propose a method to study variation in serotype frequencies of *Streptococcus pneumoniae* bacteria across different geographic endemic settings. We use the framework of replicator dynamics, derived for a multi-strain *SIS* model with co-colonization, to extract from 5 countries data fundamental parameters of inter-strain interactions, based on pairwise invasion fitnesses and their context-dependence. We integrate serotype frequency distributions and serotype identities (SAD + identities) collected from cross-sectional epidemiological surveys in Denmark, Nepal, Iran, Brazil and Mozambique. The snapshot observations are modelled under the same nested framework, by which we present a rationale for mechanistically linking and fitting multi-strain distributions across sites. Besides yielding an effective numerical estimation for more than 70% of the 92×92 (α_{ij}) in the pneumococcus serotype interaction matrix, this study offers a new proof-of-concept in the inference of random multi-species interactions. We show that in pneumococcus the vast majority of standardized interaction coefficients in co-colonization are concentrated near zero, with a few serotype pairs displaying extreme deviations from the mean. This statistical pattern confirms that the co-colonization coefficients in pneumococcus display a random probability distribution governed by a limited set of parameters, which are slightly modulated in each epidemiological context to shape coexistence. We also discuss key assumptions that must be carefully balanced in the estimation procedure. Our study paves the way for a deeper qualitative and quantitative understanding of the high-dimensional interaction landscape in multi-strain co-colonization systems.

1 Introduction

Diversity is ubiquitous in nature. Quantifying diversity, its generative processes and consequences is a major aim in biology today, with applications in ecology, conservation, epidemiology

and biomedicine. Although our ability to gather diversity data has increased in recent years to unprecedented levels of resolution, our ability to interpret such data and mechanistically explain and cross-link species abundances in different studies and systems remains limited. It has been long recognized that the structure and magnitude of species interactions can drastically affect the complexity and stability of ecosystems [May, 1972, McCann, 2000], and patterns of species abundances [Motomura, 1932, MacArthur, 1960, Cody and Diamond, 1975, Hubbell, 2011, Tokita, 2004, 2006, Yoshino et al., 2008]. However uncovering the rules of interactions and of multispecies coexistence from data remains still challenging empirically.

One such example in the microbiology literature are the microbial species (OTU taxa) populating the human or murine gut, often tackled with generalized Lotka-Volterra systems applied to longitudinal time-series data of species abundances in the same host over time to infer pairwise species interactions [Stein et al., 2013, Marino et al., 2014, Coyte et al., 2015, Alshawaqfeh et al., 2017, Lo and Marculescu, 2017].

Another example, from the epidemiology literature, comes from multi-strain dynamics models, where epidemiological diversity included in structural assumptions, is used to predict interactions between strains, either in the form of cross-immunity coefficients e.g in influenza [Koelle et al., 2006], or infer competition coefficients between strains, from co-occurrence of different strains, e.g. in the context of pneumococcus serotype data, [Gjini et al., 2016], or in simulations of SIS endemic systems [Man et al., 2023, 2019]. Some other epidemiological models focus on interactions between two different diseases or pathogens, e.g. between influenza and pneumococcus [Shrestha et al., 2013].

More often than mechanistic models, statistical (non-mechanistic) models are used to infer strain interactions, based on Markov chain description of colonization dynamics in a cohort of individuals tracked over time to estimate conditional hazards for acquisition of different strain pairs [Lipsitch et al., 2012] or statistical odds-ratios, e.g. among Dengue serotypes [Aguas et al., 2019].

In general, so far, most existing methods for detecting multi-strain pathogen interactions have been limited to assuming uniform interactions across strains (i.e. all modeled strains interact with one another with the same coefficient), or described only marginal interactions between pairs of pathogen strains, without accounting for other co-occurring strains) [Alizon et al., 2019, Chaturvedi et al., 2011, Mejlhede et al., 2010, Auranen et al., 2010, Gjini, 2017].

Thus, numerical quantification of pairwise strain-interactions from *real* epidemiological data for a *high* strain number remains a hard and mostly untackled problem in the field of endemic microbial ecosystems. In this study, inspired by pneumococcus, we will propose a method for quantifying a high-dimensional interaction matrix between strains, from cross-sectional relative abundance data, based on the replicator equation [Madec and Gjini, 2020, Hofbauer and Sigmund, 1998] .

Pneumococcus: a case study for linking different cross-sectional diversity data

A very well-studied interacting multi-strain system in epidemiology is the system of *Streptococcus pneumoniae* bacteria, their serotype structure, and their global epidemiology of colonization, and co-colonization. *Streptococcus pneumoniae* bacteria are typically asymptomatic colonizers of the human nasopharynx. Yet, it is known that carriage is a pre-requisite for disease, and that these bacteria can cause pneumonia, invasive disease, otitis, meningitis and other complications, in children or vulnerable adults. Single and multiple carriage and invasive pneumococcal disease are typically most prevalent in children under 5 years old, and vary across settings, depending

on demographic and environmental factors, many of which are not understood [Dekaj and Gjini, 2022, Garcia Quesada et al., 2021].

Underneath the global variation of total carriage and co-colonization, lie the questions of serotype variation [Imöhl et al., 2010, Moore, 2009]. Why do more than 90 pneumococcus serotypes and strains coexist, and why are particular types present at particular dominant or sub-dominant frequencies in certain locations? This question has intrigued epidemiologists and modelers of pneumococcus dynamics for a long time, especially after the introduction of PCV vaccines. While it is known that each serotype is defined by a unique polysaccharide capsule [Bentley et al., 2006, Geno et al., 2015], hence presenting slightly different within-host growth properties and potentially-varying antigenic properties, it remains still unclear how and to what extent such micro-scale differences translate to higher-level phenotypes to mediate population coexistence [Weinberger et al., 2009], furthermore when compounded with variation in other life-history traits of the microorganism itself, or of the host population [Cobey et al., 2017] - a question not unique to pneumococcus [Koelle et al., 2006, Wikramaratna et al., 2015].

When considering parametrization of pneumococcus phenotypic diversity we must inevitably face the conundrum of how universal these parameters really are, i.e. how much do they vary across settings, and what are the drivers and implications of such variation? How can we use the serotype interaction parameters estimated in one context to understand or predict epidemiology in another? Furthermore, even when assuming consensus on the parameters, the question of how those values impact on the quality of coexistence dynamics of pneumococcus serotypes, their hierarchies, the number and identities of strains composing a given system, the stability and complexity of epidemiological equilibria in a given setting, still remains largely unexplored. The literature is replete with descriptive reports of serotype frequencies, and distribution variation (e.g. see references in [Dekaj and Gjini, 2022] and recent reports [Garcia Quesada et al., 2021, Xu et al., 2024]). However, such empirical picture has not been complemented with a theoretical underpinning based on which such data can be interpreted mechanistically and quantitatively.

Here, we propose a model-based method to tackle this problem. Our approach builds on a general SIS colonization model derived for microbial ecosystems such as endemic pneumococci [Madec and Gjini, 2020], which uses time-scale separation and similarity between strains to simplify coexistence dynamics. This framework arrives down to a very simple replicator equation for strain frequencies over time, expressed in terms of pairwise invasion fitnesses. In the following, we show how this model, with evolutionary-game theory [Hofbauer and Sigmund, 1998] and adaptive dynamics at its core, can be used to integrate context-dependent mechanisms of pneumococcus coexistence across systems, and applied for inter-serotype interaction parameter estimation. Beyond pneumococcus, this estimation method suggests an avenue for similar applications also in multi-site microbiota data.

2 Method

2.1 SIS colonization and co-colonization dynamics model for N strains

Our modeling framework is based on the quasi-neutrality (similarity) assumption for multiple strains co-circulating in a population. The transmission of N strains, follows at its core *Susceptible-Infected-Susceptible* dynamics, with coinfection, described in detail in [Madec and Gjini, 2020] and extended in [Le et al., 2023], for strain variation along many traits. The variables are: S, I_i and I_{ij}, referring respectively to the proportion of susceptible hosts, those singly-colonized by strain i and those co-colonized by strains i and j. How these variables change

Table 1: **Five epidemiological *S. pneumoniae* contexts with different global parameters.** Here we report mean-field parameters of *Streptococcus pneumoniae* endemic prevalence across 5 studies in children populations (< 6 years), estimated by Dekaj and Gjini [2022] using the SIS model with co-colonization [Madec and Gjini, 2020]. The order of presentation in this table follows the increasing number of serotypes reported.

Location / Study site (Nr.serotypes/Sample size)	*Single to cocolonization ratio* $\hat{\mu}$ (95% CI)	*Basic reproduction number* $\hat{R}_0$ (95% CI)	*Mean susceptibility to co-colonization* $\hat{k}$ (95% CI)	*Epidemiological data reference*
Brazil (26/242)	9.85 (4.09-13.93)	2.4 (2.28-2.86)	0.07 (0.06-0.13)	Rodrigues et al. [2017]
Iran (30/1291)	0.93 (0.66-1.06)	1.52 (1.5-1.58)	2.06 (1.9-2.6)	Tabatabaei et al. [2014]
Denmark (33/437)	10.23 (5.44-13.16)	2.3 (2.22-2.56)	0.08 (0.06-0.12)	Harboe et al. [2012]
Mozambique (45/927)	16.8 (10.05-20.38)	6.44 (6.14-7.69)	0.01 (0.009-0.015)	Adebanjo et al. [2018]
Nepal (69/600)	3.95 (2.49-4.71)	1.98 (1.92-2.14)	0.26 (0.23-0.35)	Kandasamy et al. [2015]

over time is given by a system of of ordinary differential equations [Le et al., 2023]:

$$\frac{dS}{dt} = r(1-S) + \sum_{i=1}^{N} \gamma_i I_i + \sum_{i,j=1}^{N} \gamma_{ij} I_{ij} - S \sum_{i=1}^{N} \beta_i J_i, \quad (1)$$

$$\frac{dI_i}{dt} = \beta_i J_i S - (r+\gamma_i) I_i - I_i \sum_{j=1}^{N} k_{ij} \beta_j J_j, \qquad 1 \le i \le N, \quad (2)$$

$$\frac{dI_{ij}}{dt} = k_{ij} I_i \beta_j J_j - (r+\gamma_{ij}) I_{ij}, \qquad 1 \le i,j \le N \quad (3)$$

where J_i is proportion of all hosts transmitting strain i, including singly- and co-colonized hosts and is given explicitly by $J_i = I_i + \sum_{j=1}^{N} \left(p_{ij}^i I_{ij} + p_{ji}^i I_{ji} \right)$. Note that $F_i = \beta_i J_i$ is the force of infection of strain i, for all i in the system, with β_i representing strain-specific transmission rate. Co-colonized hosts with first strain i and then j, transmit i with probability p_{ij}^i and j with probability $1 - p_{ij}^i$. In the system, the parameter γ_i is clearance rate of single colonization episodes by strain i and γ_{ij} is the clearance rate of co-colonization by i and j. The recruitment rate of susceptibles is r. Susceptible birth rate is assumed equal to natural mortality rate. Upon co-colonization, strains can interact with coefficients K_{ij}, which if above 1 indicate pairwise facilitation, while if below 1 reflect competition in co-colonization between i and j. The model thus mirrors that a prior colonization by strain i alters the susceptibility of the host to co-colonization by strain j. There are thus at most 5 dimensions of fitness where similar strains can vary [Le et al., 2023], although in the original model [Madec and Gjini, 2020] only k_{ij} coefficients are assumed to vary, while all the others were assumed equal between strains.

2.2 How the replicator equation arises in this system

The first link with the replicator equation was uncovered in [Madec and Gjini, 2020], by assuming strain similarity in K_{ij}, and expressing each co-colonization vulnerability coefficient as a benchmark value k plus a deviation from it:

$$K_{ij} = k + \epsilon\alpha_{ij}, \quad 0 < \epsilon \ll 1. \tag{4}$$

In this simpler model version, equivalence was assumed in all other epidemiological traits. By applying a slow-fast timescale decomposition of the dynamics, a suitable model reduction revealed that over slow time strain frequency evolution was exactly described by a replicator equation. In the later study [Le et al., 2023] the same replicator equation has been derived for a more general case of N strains varying in transmission rate, clearance rate, coinfection clearance rate, and transmission probability bias from mixed coinfection (see also [Le et al., 2022] for a thorough analysis of such model for $N = 2$).

Within this very general formulation, the N-strain frequency dynamics are neutral over the fast timescale $o(1/\epsilon)$, but over the slow timescale $\tau = \epsilon t$, the frequencies $z_i, \sum_i z_i = 1$, are captured by the N-dimensional replicator system:

$$\frac{d}{d\tau} z_i = \Theta z_i \cdot \left(\sum_{j \neq i} \lambda_i^j z_j - \sum_{k \neq j = 1}^{N} \lambda_j^k z_j z_k \right), \quad i = 1, \cdots, N \tag{5}$$

where λ_i^j denote pairwise invasion fitnesses (pairwise invasibility coefficients) between any two strains, and Θ gives the speed of the dynamics.

The fast variables instead, are the total prevalence of susceptible hosts $S(t)$, singly-colonized hosts $I(t)$, and co-colonized hosts $D(t)$, which reach their equilibrium (S^*, I^*, D^*) on the fast timescale $o(1/\epsilon)$ and stay constant over the slow-timescale $\tau = \epsilon t$ (see [Madec and Gjini, 2020, Gjini and Madec, 2021]):

$$\begin{aligned} S(t) &\rightarrow S^* = \frac{1}{R_0} \\ I(t) &\rightarrow I^* = \frac{R_0 - 1}{R_0 + k(R_0 - 1)} \\ D(t) &\rightarrow D^* = \frac{k(R_0 - 1)^2}{R_0 + k(R_0 - 1)}, \end{aligned} \tag{6}$$

where $R_0 = \beta/(r + \gamma)$ denotes the basic reproduction number, equal among all strains [Madec and Gjini, 2020].

In the simplest model, if strains vary in *only* co-colonization vulnerabilities, as shown in [Madec and Gjini, 2020], the pairwise invasion fitness between strains i and j has a simpler form and is given by:

$$\lambda_i^j = \alpha_{ji} - \alpha_{jj} + \mu\left(\alpha_{ji} - \alpha_{ij}\right), \tag{7}$$

where the parameter

$$\mu = \frac{I^*}{D^*} = \frac{1}{k(R_0 - 1)}$$

describes the ratio of single to co-colonization in the overarching neutral model, and emerges to be a decreasing function of both mean-field parameters R_0 and k.

The speed of the dynamics Θ is also a function of μ and infected host turnover rate $m = \gamma + r$ and given as follows Le et al. [2022]:

$$\Theta = \frac{2m}{2\mu^2 + 3\mu + 2}. \tag{8}$$

Depending on which traits vary among strains in more general model versions, (β, γ, γ_{ij}, K_{ij} and p^i_{ij}), the exact expression for Θ and λ^j_i will change, but essentially the λ^j_i will be a weighted average of all relevant trait variations favouring one over the other strain [Le et al., 2023].

As we have shown in [Madec and Gjini, 2020] and [Gjini and Madec, 2021], depending on the biological details, the emergent structure of the Λ matrix can vary and will have distinct signatures on the qualitative features of coexistence dynamics in the multi-strain system, as expected from the general behavior of replicator dynamics [Allesina and Levine, 2011, Yoshino et al., 2008, Hofbauer and Sigmund, 1998].

In this slow-fast dynamics model approximation, the epidemiological infected compartments are obtained as:

$$I(\tau) = I^* z_i(\tau) \tag{9}$$

$$D(\tau) = D^* z_i(\tau) z_j(\tau). \tag{10}$$

Pneumococcus serotype frequency data

The replicator equation is an explicit model for evolution of strain frequencies over time. To link it with data, we considered serotype frequency data reported in epidemiological cross-sectional surveys of *Streptococcus pneumoniae* in the literature. We use 5 reports from different countries, which quantified also global colonization and co-colonization prevalences, analyzed by us in a previous study [Dekaj and Gjini, 2022].

Although the previous study showed that basic reproduction number R_0 and mean co-colonization vulnerability k inversely co-vary globally to give rise to a generally conserved ratio μ around 6, individual geographic sites do show some empirical variation, which may be relevant, and which was quantified broadly as *low* vs. *high* μ settings.

Our dataset here contains pneumococcus serotype frequency data from both high and low μ settings, namely from five geographic settings (see Figure 1): Denmark Harboe et al. [2012], Iran Tabatabaei et al. [2014], Brazil Rodrigues et al. [2017], Nepal Kandasamy et al. [2015], and Mozambique Adebanjo et al. [2018], with ratios of single-to-colonization between 0.93 (Iran) and 16.8 (Mozambique). These settings differ in the number and identities of serotypes reported, as well as their frequencies, however they display a remarkably similar rank-order-abundance distribution (Fig. 1). Furthermore, a number of seven serotypes appear in all 5 settings, these being 14, 19A, 19F, 3, 4, 6A, 6B. The data-files are provided in the Supplementary Materials.

Context-dependence model for invasion fitness parameters

The case of bottom-up constitution of the pairwise invasion fitness matrix Λ via random normally-distributed α coefficients centered at zero has been explored in depth in [Gjini and Madec, 2021]. This bottom-up Λ structure obtained via fixing the α^j_i coefficient distribution, and possibly varying the ratio of single to co-colonization across settings

$$\mu_{site} = \frac{I_{site}}{D_{site}}$$

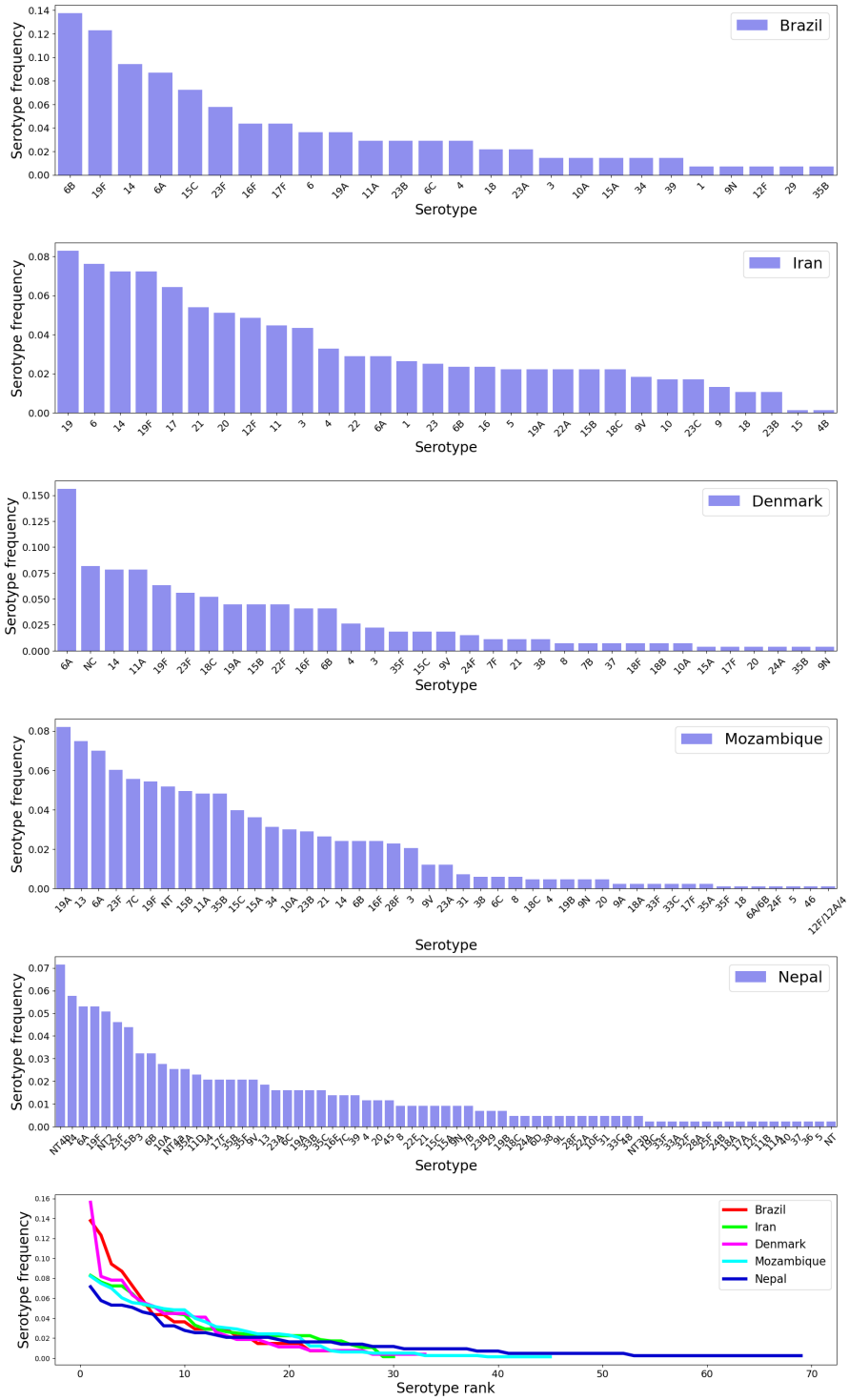

Figure 1: **Rank-abundance data for pneumococcus serotype frequencies across 5 geographical sites, presented in the order of increasing number of serotypes.** The cross-sectional data are reported in the following epidemiological studies conducted in children populations: Brazil Rodrigues et al. [2017], Iran Tabatabaei et al. [2014], Denmark Harboe et al. [2012], Mozambique Adebanjo et al. [2018] and Nepal Kandasamy et al. [2015]. See also Table 1 for more details.

has the interesting feature of interpolating between regimes of stable coexistence and oscillatory dynamics among serotypes, in the limits of $\mu \to 0$ and $\mu \to \infty$, respectively, when $\alpha_{ij} \sim \mathcal{N}(0, \sigma^2)$.

As this Λ matrix structure encodes context-dependence explicitly, we will consider it as the plausible governing mode of mutual invasibility among multiple pneumococcus serotypes, and without loss of generality, since many different types of replicator dynamics can be captured by this formulation [Gjini and Madec, 2021]. Thus we will assume that, on one hand, the α matrix is fixed (universally conserved):

$$\alpha_{ij} = \frac{K_{ij} - k}{\epsilon},$$

and on the other, the Λ matrix may be different in each system, and can be obtained by substituting the site-specific value of μ (Table 1) into Eq.7. Thus, pairwise invasion fitness matrix in each setting is given by:

$$\Lambda_{site} = f(\alpha, \mu_{site}),$$

applying the model where strains vary just in vulnerabilities to co-colonization (Eq. 7), while being equivalent in other traits [Gjini and Madec, 2021]. This is a quite general and flexibile representation because mathematically it has the capability to generate all types of complex replicator dynamics. Our goal will be to estimate the matrix of α_{ij} in a way that is consistent with serotype composition and frequencu data, and μ values across all countries.

Equilibrium or non-equilibrium assumption?

We do not have multiple time-points from the same epidemiological setting. Instead we have a snapshot from each site. How much can we infer from single cross-sectional patterns of serotype frequency variation across settings? The first modeling choice will be how to interpret data in each system, as an equilibrium representation or as a more arbitrary snapshot of the dynamics. To answer this question, we resorted to the most-likely scenario predicted by the model for that empirical μ value (Figure 5 in [Gjini and Madec, 2021] for $N = 10$). According to the values estimated for these 5 geographical settings, we concluded that Brazil, Denmark and Mozambique having high values of μ above 10 on average, are more likely to display non-equilibrium dynamics, whereas Nepal and Iran, having lower values of μ are more likely to display a steady-state scenario. This means that these data should be interpreted under these different assumptions, and hence fitted via optimization of slightly different functions. Besides considering whether frequency data reflect a steady state or dynamic snapshot, we must also make a decision in the former case, whether we must impose the strict stability property on the steady-state. Mathematically this means that it is not sufficient to fit the frequency as a steady state but also to make sure that the Jacobian of the replicator evaluated at that fixed point has a dominant eigenvalue with negative real part. We chose to impose the stability criterion whenever a steady-state was assumed.

2.3 Estimating strain interaction parameters in co-colonization

We now describe the process estimating parameters of the matrix (α_{ij}) in detail. There are some conditions:

1. We assume strain variations are only in co-colonization interactions k_{ij} (sufficient for describing all regimes of replicator dynamics).

2. We assume all the strains observed in the study were present at equal frequencies at some unknown initial state in the past (in the Dyn assumptions cases).

3. We assume the standardized co-colonization vulnerability coefficients of the same strain pair are the same in all the countries $\alpha_{ij} = \frac{k_{ij}-k}{\epsilon}$ for all (i, j) pairs co-occurring across sites.

4. Once the α_{ij} is estimated for one pair of serotypes, it stays fixed at that value for all other subsequent sites in the fitting procedure.

5. The range of α_{ij} is fixed to be $[-10, 10]$ across all studies.

6. Not all pairs of strains will co-occur in our dataset, so for the unobserved serotype pairs i, j the associated coefficient α_{ij} cannot be estimated and will remain unknown.

Site ordering for the nested α_{ij} estimation

Our goal is to estimate the matrix of α_{ij} in a way that is consistent with serotype frequency data $\mathbf{z}$ and μ values across all systems. We adopt 2 ways of ordering the sites of pneumococcus serotype frequency observations: i) one which is just purely based on the increasing number of strains among sites, and ii) another one, which prioritizes stability for the site with plausible stable coexistence between the largest number of strains (see Figures 2-3 for an illustration).

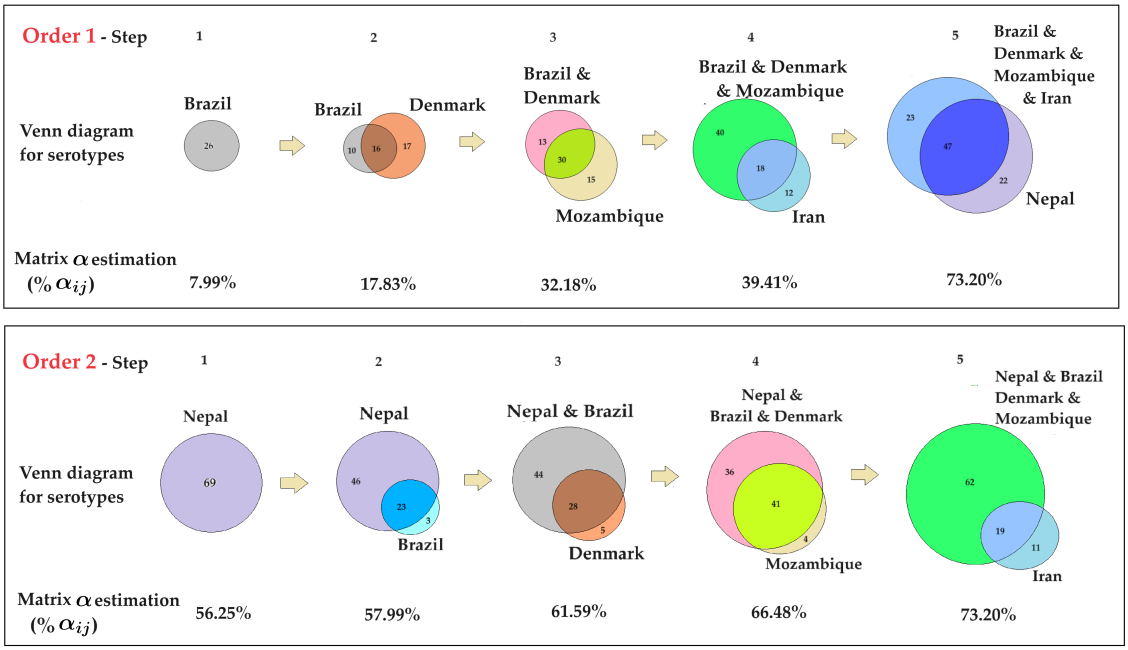

Figure 2: **Venn diagram representation of parameter estimation process.** We visualize the two approaches to parameter estimation using Venn diagrams. At each step, we highlight the key elements, including the countries with the number of strains already included from previous steps, the country and the number of strains being added in the current step, and the number of common strains between them. Additionally, we calculate the percentage of the 92×92 α_{ij} matrix filled at each step.

Order 1: Site-ordering by "increasing strain number" (lowest → highest number of strains) The process is carried in a particular optimized order: from Brazil → Denmark → Mozambique→ Iran → Nepal, respectively, based on their number of empirically-observed strains n, starting from the site with smallest number of reported serotypes. In this ordering, the first 3 countries are assumed to represent a snapshot of the dynamics, whereas for Iran and Nepal (case 1) a stable steady state is more plausible. Since for Nepal $\mu = 3.95$, we also considered the possibility of data reflecting also a snapshot of the dynamics (case 2) but this fit gave higher error. For details please see Supplementary Material S3.

Order 2: Site-ordering by "stability-of-largest-coexistence" first, increasing strain-number second. Here the data are fitted in the following order: Nepal→ Brazil→ Denmark→ Mozambique → Iran, with Nepal data being fitted before all others, as a stable coexistence equilibrium of 69 serotypes. We estimate the parameters for Nepal first, followed by the other countries. This approach is necessary because computing the eigenvalues (to verify stable equilibrium) of the Jacobian matrix of Nepal's fitness matrix (size 69×69) is computationally intensive and challenging due to the lower degrees of freedom. Within remaining countries, the process is carried out in the following order: from Brazil, to Denmark, to Mozambique, Iran, respectively, based on their number of coexisting strains n, starting from the site with smallest number of reported serotypes.

In the second case, we re-scale the α_{ij} variance, immediately after the first country estimation, to match that obtained in Order 1, so that we can compare the resulting interaction parameter distributions between the two methods under the same scale.

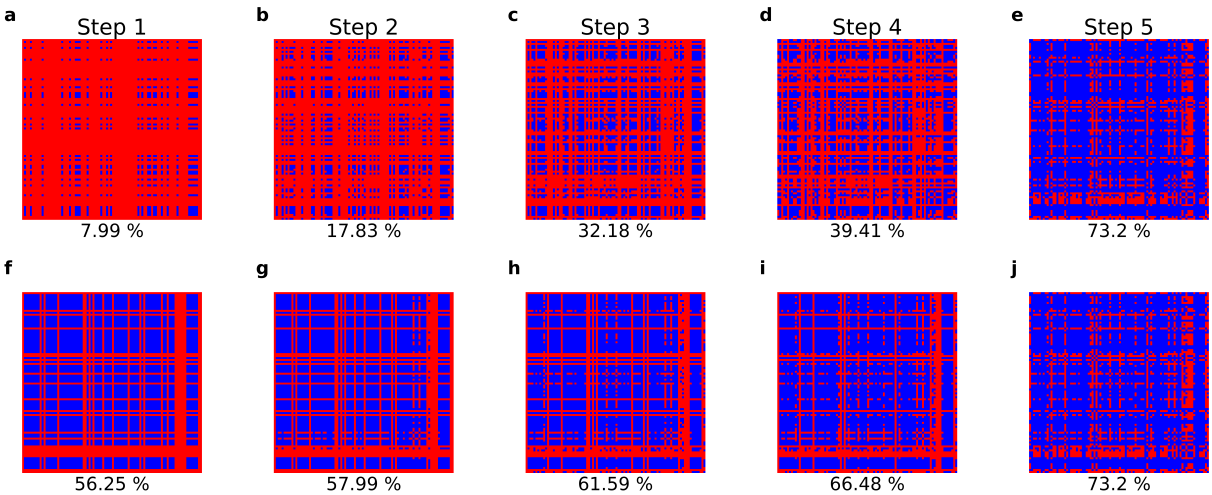

Figure 3: **Stepwise filling of the 92×92 α_{ij} matrix in the orders of parameter estimation: Order 1 (a-e), Order 2 (f-j).** We further investigate the estimation process by examining the step-by-step filling of the 92×92 α_{ij} matrix. In this visualization, red indicates the α_{ij} parameters that have not yet been estimated, while blue indicates those that have been estimated up to the current step (cumulative percentages below the matrix). The upper panel illustrates the process for Order 1, while the lower panel depicts the process for Order 2. This matrix-block visualization is consistent with the Venn diagram in Figure 2.

For details please see Supplementary Material S4-S5.

Error function

In the case of z_{site} representing a dynamic snapshot, the error (root of sum of squares) will be given by:

$$error = \left\| \mathbf{z}^{\mathbf{model}}(50) - z_{\text{site}} \right\|_{\mathbb{R}^n}$$

and in the case of assuming an equilibrium ($dz/d\tau = 0$), the error will be given by:

$$error = \left\| \Lambda z_{\text{site}} - z_{\text{site}}^T \Lambda z_{\text{site}} \right\| .$$

To denote the first case we will use the abbreviation Dyn and for the second case, the abbreviation SS. It should also be noted that, in the estimation process, we assume the speed of the dynamics is consistently set to 1. This assumption will be discussed in detail later.

The algorithm

In either ordering, the algorithm used in the estimation process is **Particle Swarm Optimization** (PSO) with the appropriate range in each country and starting parameter guess at 0. In computational science, particle swarm optimization (PSO) is a powerful computational method that optimizes a problem by iteratively trying to improve a candidate solution with regard to a given measure of quality. It solves a problem by having a population of candidate solutions, here dubbed particles, and moving these particles around in the search-space according to simple mathematical formula over the particle's position and velocity. Each particle's movement is influenced by its local best known position, but is also guided toward the best known positions in the search-space, which are updated as better positions are found by other particles. This is expected to move the swarm toward the best solutions.

The Particle Swarm Optimization (PSO) algorithm was created in 1995 by Russell Eberhart, electrical engineer, and James Kennedy, social psychologist [Eberhart and Kennedy, 1995], and it is inspired by the collective behaviour of flocks of birds or schools of fish. It is one of the bio-inspired algorithms and it is a simple one to search for an optimal solution in the solution space. It is different from other optimization algorithms in such a way that only the objective function is needed and it is not dependent on the gradient or any differential form of the objective. It also has very few hyperparameters. This optimization algorithm performs well on parameter optimization for ordinary differential equation models [Akman et al., 2018], but convergence to the overall optimal solution is not always guaranteed.

3 Results

The process and results of parameter estimation are summarized in Figures 2-3 and Tables S1 and S2.

Fit and quality of fit

Our algorithm is able to fit extremely well the observed serotype prevalences in each study site (Figure 4), via both ordering methods, mean error being less than 1% for each ordering of data (Tables S1-S2). While the nested method based on order 1 is less conservative in terms of dynamics and fits the data in the order of increasing number of serotypes, the second method based on order 2, fits first the largest number of strains adopting the stability constraint. This

means the second method constrains a larger proportion of the alpha matrix from the beginning, an effect that trickles down to all subsequent site fittings. We can see that the estimates obtained by order 2 yield generally lower errors than those obtained with order 1.

When computing the proportion of predicted serotype frequency data within a margin of 10% error for all countries from both orders (Figure S1), we find that in Order 1, the percentage of serotypes predicted within an accuracy of 10% are: Iran 73.33%, Nepal 50.72%, Brazil 65.38%, Denmark 30.3%, and Mozambique 33.33%. Meanwhile, in Order 2, these percentages are higher: Iran 93.33%, Nepal 100.0%, Brazil 80.77%, Denmark 51.52%, and Mozambique 46.67%.

Statistics of the estimated random α matrix

As shown in Figure 5, the distributions of the standardized co-colonization coefficients between serotypes obtained by either nested method have means near 0 and standard deviation near 1. The distribution of α_{ij} in Figure 5a is nearly symmetric, meanwhile in Figure 5b, it is left-skewed, as we can see the outliers near 10. Compared to the kurtosis of normal distribution, which equals 3, both of these distributions have heavier tails, i.e. are leptokurtic. Especially, the distribution in Figure 5b has extremely thin tail, due to the much more dense concentration of α_{ij}'s around 0.

The mutual invasion fitness network that emerges

The pairwise invasion fitness matrix, which is the higher-order fitness modulated by contextual parameters such as the ratio of single and co-colonization, appears to be highly anti-symmetric for pneumococcus serotypes (dominated by $+-$ edges) under the estimation via order 1, and more skewed towards the pairwise coexistence region (dominated by $++$ edges) under the estimation via order 2 (Figure 6 and Figure 7). This means that when imposing few stability constraints for the collective coexistence of subsets of serotypes, the system is more free to vary, and parameter values compatible with observations, reflect more often 'prey-predator' mutual invasion outcomes leading to an oscillatory complex coexistence between serotypes, and more rarely a stable one [Gjini and Madec, 2021]. In contrast, when we start the estimation by imposing stable coexistence first among a large collective of serotypes (Order 2) then, the parameter estimation is more prone to converge towards values compatible with a majority of serotypes pairs in the mutual invasion region, leading to stable coexistence everywhere, independently of contextual parameters or local serotype composition.

Next, we use the estimated invasion fitness matrix to compare invasion resistance Q in each pneumococcus multi-strain system (Figure 8). The mean invasion resistance values for all countries, resulting from Order 1 -estimation, are significantly lower (all below 1) compared to the Q values obtained from Order 2 estimation (all above 7, except for Iran, where the Q value just exceeds 5). It is striking that although the numbers and identities of serotypes vary vastly across settings (Figure 1, the final collective invasion resistance of the pneumococcus multi-strain system is quite similar everywhere.

Quantifying the symmetry of interactions and invasion fitness

The symmetry of the estimated matrices is also important. The symmetry in rescaled interaction coefficients α_{ij} and pairwise invasion fitnesses λ_i^j reveals different properties. To explore the sensitivity of the dynamics to μ (the ratio I/D in each site), we consider the symmetry of the α matrix. To understand the quality of the dynamics, we consider the symmetry of the λ matrix

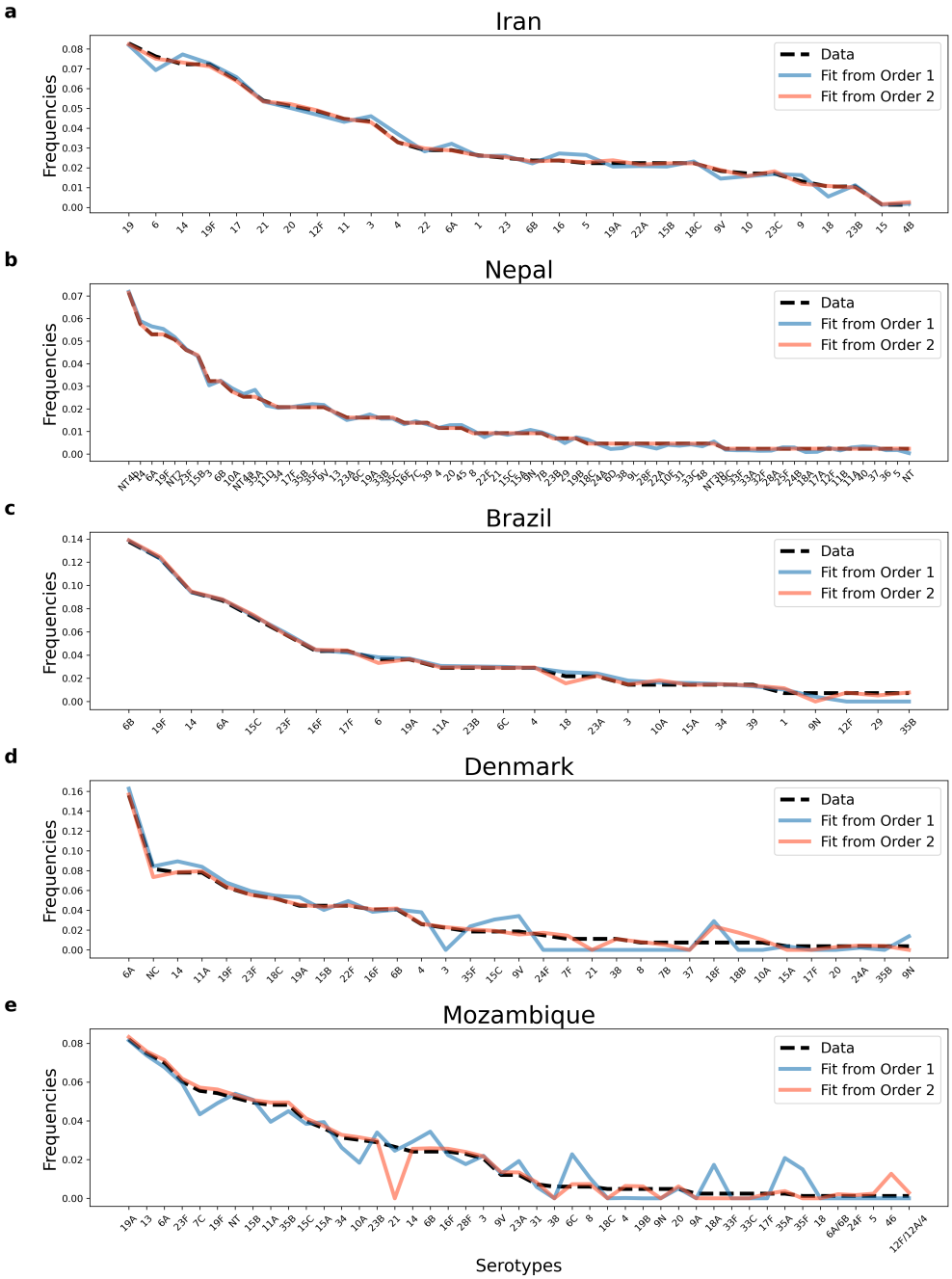

Figure 4: **Relative abundance data and model fits for pneumococcus serotype frequencies in 5 epidemiological contexts (ordered by ratio of single to co-colonization, from lowest to highest).** We plot the data extracted from Denmark Harboe et al. [2012], Iran Tabatabaei et al. [2014], Brazil Rodrigues et al. [2017], Nepal Kandasamy et al. [2015], and Mozambique Adebanjo et al. [2018], as well as the model-predicted relative abundances (estimated from nested data fitting in Order 1 and Order 2), ranked from the most prevalent to the least prevalent serotype. For details on quality of fits see Tables S1-S2.

[Gjini and Madec, 2021]. The level of symmetry of a matrix $M = (m_{ij})_{1 \leq i,j \leq N}$ is calculated as

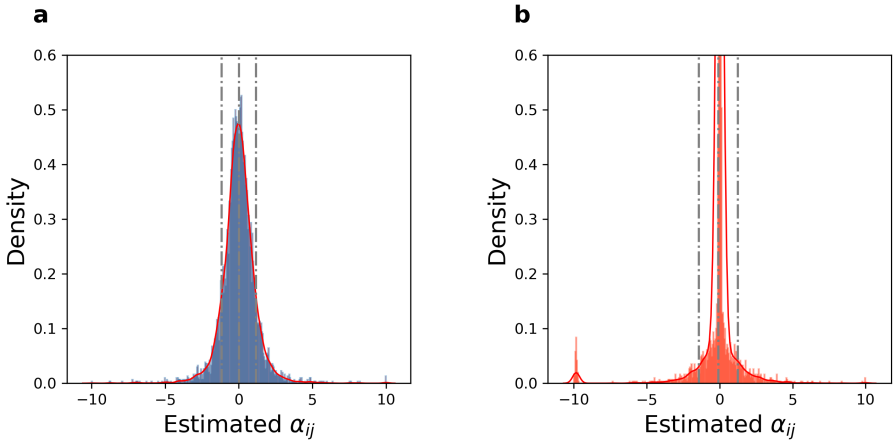

Figure 5: **Final global matrix distribution of rescaled interation coefficients (α_{ij}) for observed serotype pairs across 5 epidemiological contexts of Streptococcus pneumoniae colonization, after the nested fitting of cross-sectional data in Order 1 and Order 2.** The figures illustrate distributions of the all α_{ij}'s (73% of the entire matrix A) estimated in the two estimation approaches. We note that both distributions appear bell-shaped, although the second case is much more highly peaked at 0. The statistics for the two distributions are as follows. **(a)**- Order 1: Mean: 0.0038, Standard Deviation: 1.1610, Skewness: 0.1862, and Kurtosis: 13.2259. **(b)**- Order 2: Mean: -0.0973, Standard Deviation: 1.3389, Skewness: -4.0790, and Kurtosis: 37.8318. For site-specific distributions see Figure S2.

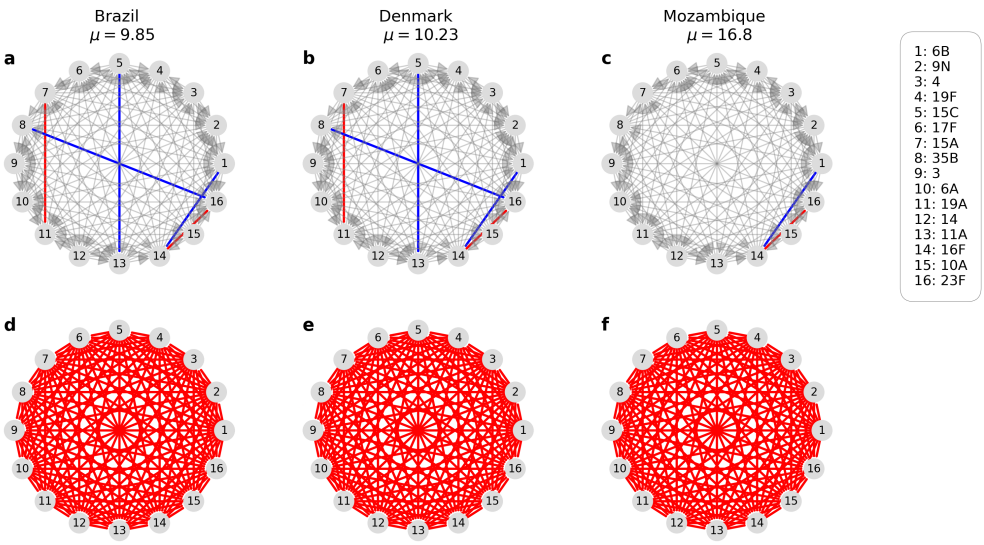

Figure 6: **Emergent invasion fitness network among 16 pneumococcus serotypes in different epidemiological settings.** We show here the obtained mutual invasion fitness network for the co-occurring serotypes in countries where a dynamic snapshot of ecological trajectories is assumed (only common serotypes). The upper panel visualizes the network from Order 1, dominated by $+-$ edges (gray arrows) with rare coexistence (red) or bistability (blue), meanwhile, the lower panel represents the network obtained from Order 2 data fitting, constituted entirely by pairwise coexistence edges $++$ (red lines) for these 16 serotypes.

described in Tokita [2023]:

$$s \equiv \frac{\langle m_{ij} m_{ji} \rangle}{\left\langle m_{ij}^2 \right\rangle} = 2 \left(\sum_{i=1}^{N} \sum_{j>i}^{N} m_{ij} m_{ji} \right) \Big/ \left(\sum_{i=1}^{N} \sum_{\substack{j=1 \\ j \neq i}}^{N} m_{ij}^2 \right).$$

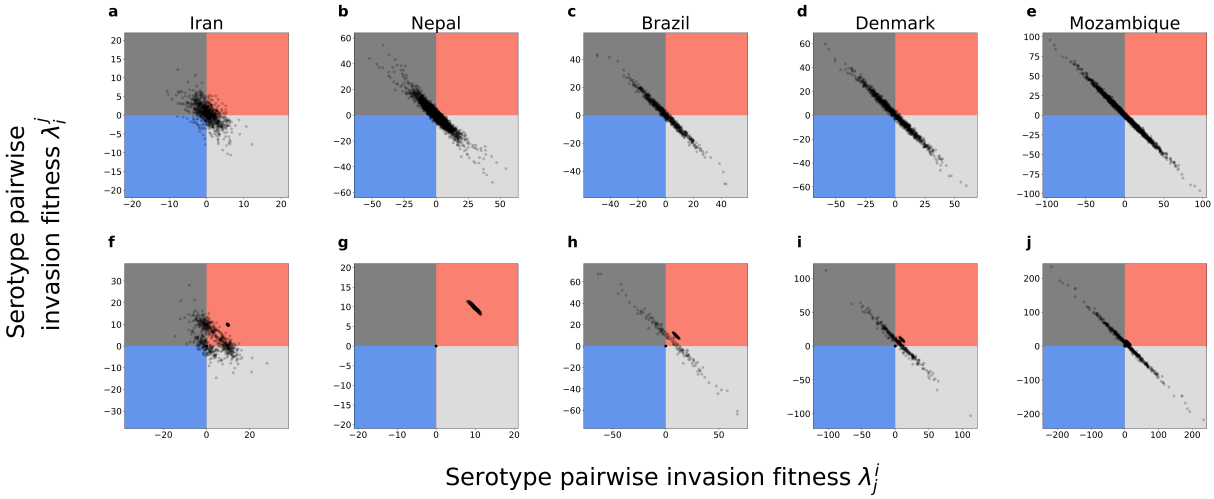

Figure 7: **Quantifying coexistence patterns of different pneumococcus serotype pairs across 5 countries resulted from two approaches.** We plot the resulting pairwise invasion fitnesses between all serotypes present in each study site. **Top row:** obtained by nested method of order 1. **Bottom row:** obtained by nested method of order 2. For more details, we compute the percentages of pairwise outcomes (edges of the network), including exclusion of either strain, bistable outcome, and coexistence, for each country and each estimation order. We report the sequence triple (exclusion of either strain, bistability, coexistence) as the percentage among all pairwise outcomes. We have that, in Order 1: Iran $(69.8\%, 7.6\%, 19.3\%)$, Nepal-Case 1 $(88.2\%, 4.5\%, 5.8\%)$, Brazil $(91.7\%, 2.4\%, 2.1\%)$, Denmark $(94.0\%, 1.7\%, 1.3\%)$, Mozambique $(95.0\%, 1.3\%, 1.5\%)$. Meanwhile, in Order 2: Iran $(41.8\%, 2.9\%, 52.0\%)$, Nepal-Case 1 $(0.0\%, 0.0\%, 98.6\%)$, Brazil $(17.5\%, 0.0\%, 78.7\%)$, Denmark $(21.1\%, 0.0\%, 75.8\%)$, Mozambique $(22.3\%, 0.0\%, 75.5\%)$. This confirms that the second nested approach yields relatively many more coexistence pairwise outcomes.

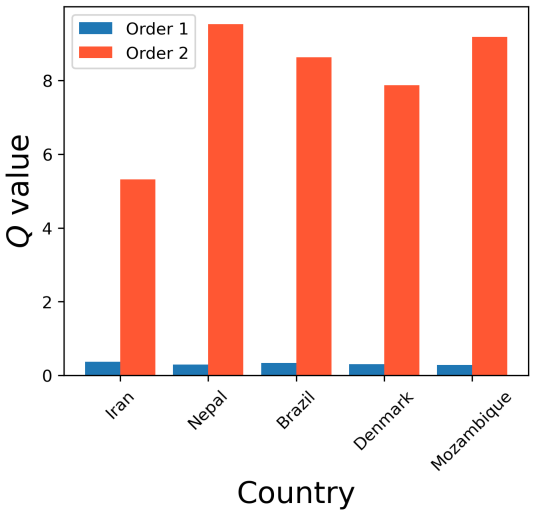

Figure 8: **Resulting mean invasion fitness $Q = \sum_{k \neq j=1}^{N} \lambda_j^k z_j z_k$ for the multi-serotype pneumococcus ecosystem in each country.** In each estimation order, we use the finally-estimated λ's to visualize the $Q = \bar{\lambda}$ values for Iran, Nepal, Brazil, Denmark, and Mozambique, at the z values reported in each setting. Under stable coexistence (Order 2) it is harder for new strains to invade.

The closer the s value is to 1, the more symmetric the matrix is, and hence when this applies to the α_{ij} matrix, the more insensitive to μ will be the resulting λ's, and hence the more insensitive

to 'global context' the quality of the dynamics. We find the matrix α has a symmetry index very close to 0, which indicates a random distribution.

Conversely, the closer s is to -1, the more anti-symmetric the matrix is. Applying this formula to the λ_i^j matrix for each country (Iran, Nepal, Brazil, Denmark, Mozambique), we find that in Order 1, the resulting invasion fitness matrix has a symmetry index very close to -1 in most sites, whereas in order 2 has a symmetry index close to 1. For details see Table 2. This computation is consistent with a picture that the quality of the dynamics is generally sensitive to μ and the quality of the dynamics can be very different if we adopt estimation procedure via order 1 or via order 2, namely more complex and oscillatory in the former, and more stable steady state coexistence in the latter.

Estimation	Matrix	Iran	Nepal	Brazil	Denmark	Mozambique
Order 1	(α_{ij})	0.017	0.003	0.038	-0.011	-0.047
	$\left(\lambda_i^j\right)$	-0.61	-0.96	-0.99	-0.99	-0.99
Order 2	(α_{ij})	-0.035	-0.188	0.164	-0.006	0.003
	$\left(\lambda_i^j\right)$	0.34	0.99	-0.12	-0.34	-0.79

Table 2: **Summary of symmetry levels in (α_{ij}) and Λ matrices across five countries in two Orders.** These computations confirm the long-term behaviors depicted in Figure 9. In Order 1, all five countries exhibit a random distribution of α_{ij} values. A similar pattern is observed in Order 2; however, Nepal's (α_{ij}) matrix is slightly more anti-symmetric, while Brazil's matrix is slightly more symmetric. Regarding the symmetry levels of the Λ fitness matrix, in the Order 1 panel, except for Iran and Nepal, where the μ values are small enough and data observations are regarded as stable equilibrium, the other three countries—Brazil, Denmark, and Mozambique—display nearly anti-symmetric fitness matrices, which are associated with oscillatory long-term behavior. In contrast, in Order 2, all five countries have random or nearly symmetric Λ fitness matrices, consistent with the convergence to stable states in their dynamics.

Site-specific predictions

Finally, we verified that in those countries where we expected oscillatory dynamics, via μ arguments [Gjini and Madec, 2021], we obtained oscillatory dynamics in simulations starting from equal relative abundances between serotypes at some point in the past (Figure 9). This was true for the Dyn countries in Method 1 (Brazil, Denmark, Mozambique, Nepal-case2). We also verified that in Iran and Nepal-case 1 (method 1), where we imposed a stable equilibrium within the fitting procedure (SS), the estimated alphas, and then lambdas, generated a dynamics converging to that stable equilibrium when starting close enough (naturally, all eigenvalues had negative real part). The question of multi-stability was addressed by computing all possible equilibria for that lambda matrix. We found that in Iran there was only one more stable equilibrium for that lambda matrix (Order 1 estimation) but this was an exclusion equilibrium with only strain 1. In Nepal, we found that the stable equilibrium fitted and found for the corresponding lambda matrix is a unique stable equilibrium, so we expect that from any initial conditions, the system would converge to this final steady state.

For the fitting procedure in Order 2, Nepal estimates are consistent with a picture of monostable coexistence steady state of 69 serotypes under that lambda matrix. Brazil, Denmark, Mozambique observations, even though were not fitted as a steady state, emerged to also be

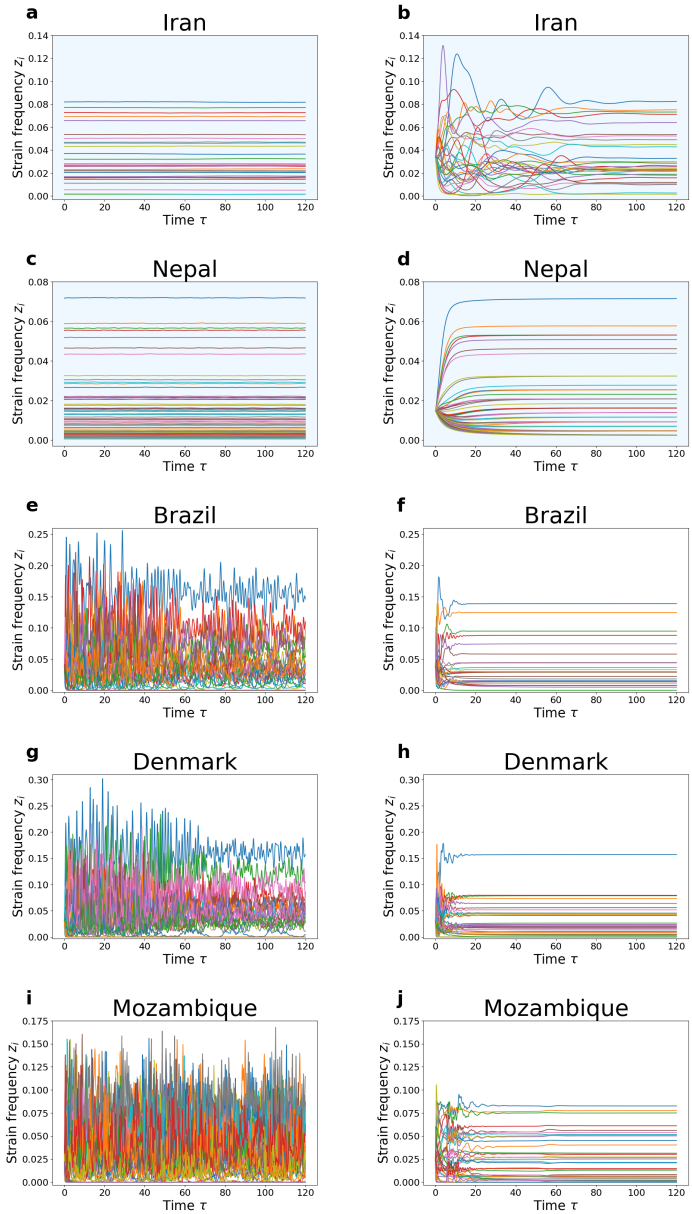

Figure 9: **Model predictions for temporal dynamics of multiple strain frequencies.** This figure illustrates the model-predicted dynamics of serotypes in each geographic site, using the estimated co-colonization and invasion fitness parameters obtained from Order 1 fitting (first column) and Order 2 fitting (second column). The light-blue shaded background denotes cases where the data are fitted to a strict stable equilibrium (Figures a & c in Order 1, Figures b & d in Order 2), while the white background indicates cases where data observations are considered as snapshots at $\tau = 50$ of the dynamics. For scenarios where data are fitted as snapshots in both orders, initial conditions assume equal frequencies for all observed serotypes in each setting (Figures e, f, g, h, i, j). In Order 1, where data are fitted to a strict stable equilibrium (Figures a & c) with a narrow basin of attraction, the initial point is set within a small vicinity of the stable state. Conversely, in Order 2 (Figures b & d), the basin of attraction is sufficiently large, allowing the initial conditions to be set at equal frequencies for all observed serotypes while still converging to equilibrium. In this visualization, we assume that the speed of dynamics of all figures are all equal to 1 i.e. $\Theta = 1$.

compatible with a stable steady state, as confirmed by computing the eigenvalues of the Jacobian at that steady state under the corresponding lambda matrices. When examining the existence of alternative stable steady states for Iran, we also retrieved a unique steady state under the corresponding lambda, obtained under Order 2 estimation.

4 Discussion

Understanding the mode and strength of between-strain interactions in any polymorphic infectious agent is important for interpreting past dynamic trends and for predicting potential ecological and evolutionary changes in the face of vaccination, antibiotics or other pressures [Wikramaratna et al., 2015, Gjini et al., 2016, Alizon et al., 2013, Auranen et al., 2010].

In general, there are 4 types of top-down data-driven approaches for estimating interactions: 1) using longitudinal data in the same host population over time (mostly addressed with Markov models) 2) using cross-sectional data in one host population over time (more easily addressed with dynamic epidemiological models over time), comparable to dynamic gLV models applied to microbiota time-series, 3) fitting one cross-sectional snapshot in one host population (inevitably needs some steady-state assumption for parameter estimation) 4) integrating cross-sectional snapshots in multiple host populations (the most challenging case, addressed here), making use of the largest amount of data and heterogeneities. While the first three above approaches are more represented in the literature, the fourth approach is more rarely found - because of all the intricate assumptions for parameter correlations across host/environmental settings that need to be clarified.

On estimation assumptions and heuristics

Here we believe to have made a significant step in a challenging methodological direction. Our main development is to propose a framework by which multiple strain frequency cross-sectional data from different sites can be integrated under the same model.

We have highlighted that a key assumption is the order of nesting of parameters in different sites, and this can produce very different parameter estimates, while preserving a very good quality of fit (Figure 4). We find that the way parameters are constrained from the start has a strong bearing on the subsequent quantification of interactions between new serotype pairs. In particular, if we impose the stable coexistence criterion among a large proportion of serotypes from the global pool, this implies strong stability constraints also on the other portion of data fitted in subsequent steps. In our case, this order, stability first, led to better quality of fit of the data, with a global data mean RMSE of 0.0025 (Table S2), while the order 1, in increasing number of serotypes, led to a slightly worse fit, with a global data mean RMSE of 0.0046 (Table S1).

In our fitting procedure, fitting a stable equilibrium with fewer degrees of freedom and more strains is time-consuming (eigenvalues must be computed with negative real parts, and equilibrium must align with observation data). In contrast, fitting dynamic snapshots is easier than a stable equilibrium. There are more degrees of freedom when fitting snapshots. We used a model-informed heuristic for deciding which sites to fit as stable steady states and which sites to fit as dynamic snapshots [Gjini and Madec, 2021]. The ratio of single to co-colonization,μ, was used to decide whether we fit a "snapshot" (μ relatively large) or a "steady-state" (μ relatively small), but here we also imposed on the estimation procedure a strong enforcement of stability.

In nesting the data according to Order 1, we prioritized accuracy over stability for Iran and Nepal, where achieving stable equilibrium required considerable effort, especially Nepal with a large number of strains, although eventually we were able to fit it quite well at the end. In the nesting of multi-site data according to Order 2, with uncertainty around Nepal's data being a snapshot or stable equilibrium, we decided to emphasize stability. Given Nepal's high number of serotypes, we fitted it first to ensure a stable coexistence equilibrium of all its serotypes (with very little effort). Brazil followed Nepal because fitting any other country before Brazil would have reduced our degrees of freedom to zero, making it impossible to fit Brazil later. Iran, having unique strains, was placed at the end to avoid increasing estimation errors for other oscillating-dynamics countries (Order 2).

A key assumption in our approach was the μ-dependence in the pairwise invasion fitnesses, but keeping the essence of each strain pair in terms of fixed α_{ij}. When we repeated the estimation procedure, assuming the same approximate $\mu \approx 6$ everywhere [Dekaj and Gjini, 2022], and a dynamic snapshot in each site, the fit of the serotype data was slightly worse but the estimated global α matrix turned out to be very similar to the one obtained under order 1, with the same pattern resulting pairwise invasion edges of predator-prey types (Table S3).

On time scale arguments and ϵ

Our method is based on the slow-fast dynamic approximation leading to the replicator equation [Madec and Gjini, 2020]. We have chosen to fix the scale of alphas in different sites and the time-point of simulating hypothetical dynamics $\tau = 50$, starting for equal initial relative abundances. We have also taken Θ, the speed of dynamics in each country, equal to 1. The only variation we have taken into account is μ and of course k variation (Table 1). This gives freedom to then assign real time units to τ according to an appropriate scaling in each country if data from another time-point becomes available. We could indeed use the estimated matrices to make dynamic predictions for later or earlier time points, linking to the year of the data used in our estimation, for example for Denmark, connecting data from [Harboe et al., 2012] to another dataset [Auranen et al., 2010].

How can we obtain the epidemiological co-colonization coefficients k_{ij} from the α's? We need an estimate of the small parameter ϵ. One way to get a rough estimate of this parameter could be to utilize the confidence intervals for k (Table 1) and interpret them as 95%confidence intervals for the statistical mean of k_{ij}, which typically is given by $\bar{k} \pm 1.96\sigma/\sqrt{n}$ where σ is the true standard deviation and n sample size. In such approximation, σ is related to ϵ and could be used to obtain finally a plausible value for $k_{ij} = k + \epsilon\alpha_{ij}$ for each co-observed pair of serotypes in each system.

Limitations

Our method has limitations. We have not taken into account the uncertainty in μ or the frequencies of serotypes related to host sample size (Table 1). Instead we have assumed a fixed known ratio I/D in each site. We limited ourselves to considering only extreme assumptions about coexistence, either a totally arbitrary dynamics snapshot or stable coexistence fixed point. We could relax the assumption of stability in later investigations, including for example permanence of the observed species [Aubin and Sigmund, 1988] as a criterion in the estimation procedure, imposing that the mean frequency over time corresponds to the observed data. Our estimation of the 92×92 interactions matrix is not complete. We could only estimate 73% of the entries

of this matrix for the pairs of serotypes that were co-observed in the same site at least once. This poses limits to our prediction with the estimates we have got. In other settings where the serotype composition is entirely contained in the estimated portion, we can apply the model to make predictions of the dynamics. In other settings, where new serotype pairs might be observed, we will have to estimate the best estimate possible for the new pairwise interactions, keeping fixed those interactions that were already estimated here, and following the same spirit outlined here.

We also did not consider sub-serotype diversity, assuming to a first-order approximation, homogeneity within serotype in pneumococcus. This is a strong assumption, but strain-level diversity could be explored in the future. The model structure could also be altered or expanded, as we have already mentioned previously [Madec and Gjini, 2020, Le et al., 2022, 2023]. Increasing model complexity however, would possibly augment nonlinearity and computational requirements for parameter estimation. It remains yet an interesting avenue to explore under new evidence-based structural assumptions.

Due to the limited scope of our study, we also did not go to study in detail the actual identities of serotypes displaying certain interactions and certain invasion fitnesses, but the results of our matrix estimations are all public, and could be examined carefully in a second follow-up study, to elucidate serotype-specific patterns and signatures of dominance on the collective dynamics.

Towards bottom-up trait-based interactions and invasion fitness

A critical issue in modelling multi-strain systems is how to best represent the relationships between the biological entities involved. A common approach is to consider strain space as a continuous volume, surface or even a single dimension where the Euclidean distance between points serves as a metric for antigenic and/or genetic similarity [Gog and Swinton, 2002, Lin et al., 1999, Bedford et al., 2012].

We could link our estimated alpha distribution for pneumococcus serotypes to some genetic or antigenic distance between them, like it is done for influenza models or other more abstract multi-strain models, a direction worth exploring in the near future. This would mean going from *'serotype predicts prevalence'* [Weinberger et al., 2009], to *'serotype predicts interactions predicts prevalence'* paradigm. Similarly, exploring microbial ecosystem's invasion fitness in pneumococcus on the basis of trait-based interactions could be a ground for verifying theoretical expectations from other more general ecological invasibility models [Hui et al., 2021]. Such bottom-up approaches would help also to establish whether the mode of serotype coexistence is the one predicted by our estimation results of Order 1 or Order 2, suggesting principles in favor of oscillatory vs. stable coexistence, which at this point remain uncertain.

Future data and applications in other systems

We provide a framework based on coupled mechanistic invasion fitness networks, able to identify and quantify the common ecological underpinning of multiple coexistence patterns (linked via a resource gradient, here μ). We believe this is the first large-scale investigation of between-serotype interactions in pneumococcus, something that would be computationally prohibitive using the full SIS model with coinfection and multiple strains. Our spirit and results fall into a broad ecological topic increasingly interested on multi-species interactions and assembly in variable environments [Chesson, 1994, Grilli, 2020, Lewnard and Hanage, 2019]. Our estimation results on the rescaled interaction matrix are similar to the ones obtained by Tokita [2023],

applied to murine microbiota time-series using the replicator system. We believe applications of our framework to microbiota snapshots in different hosts, connected via a mechanistic gradient, as proposed in [Gjini and Madec, 2023], could be a next natural step for applications.

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Supplementary material

S1 Data files. **S2** Code files. **S3 Text** Order 1, details of parameter estimation. **S4 Text** Order 2, details of parameter estimation. **5** Extended results files. **S6** Supporting figures and tables. **S7** Estimation process with equal $\mu = 6$ across sites.

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