Amsterdam UMC
Universitair Medische Centra

National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Unraveling microbial ecosystems to direct prophylactic and probiotic interventions

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joint BMS-ANed and PSDM meeting | November 23, 2018

Disclosure belangen spreker

(potentiële) belangenverstrengeling	**Geen**
Voor bijeenkomst mogelijk relevante relaties met bedrijven	**Niet van toepassing**
• Sponsoring of onderzoeksgeld • Honorarium of andere (financiële) vergoeding • Aandeelhouder • Andere relatie, namelijk …	• • • •

About me

Hans Bogaards

MSc theoretical biology & bioinformatics (UU)
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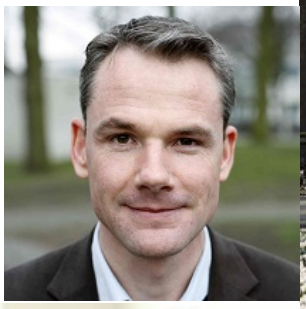
Prof.dr. Jacco Wallinga



Amsterdam UMC–VU
Prof.dr. Hans Berkhof

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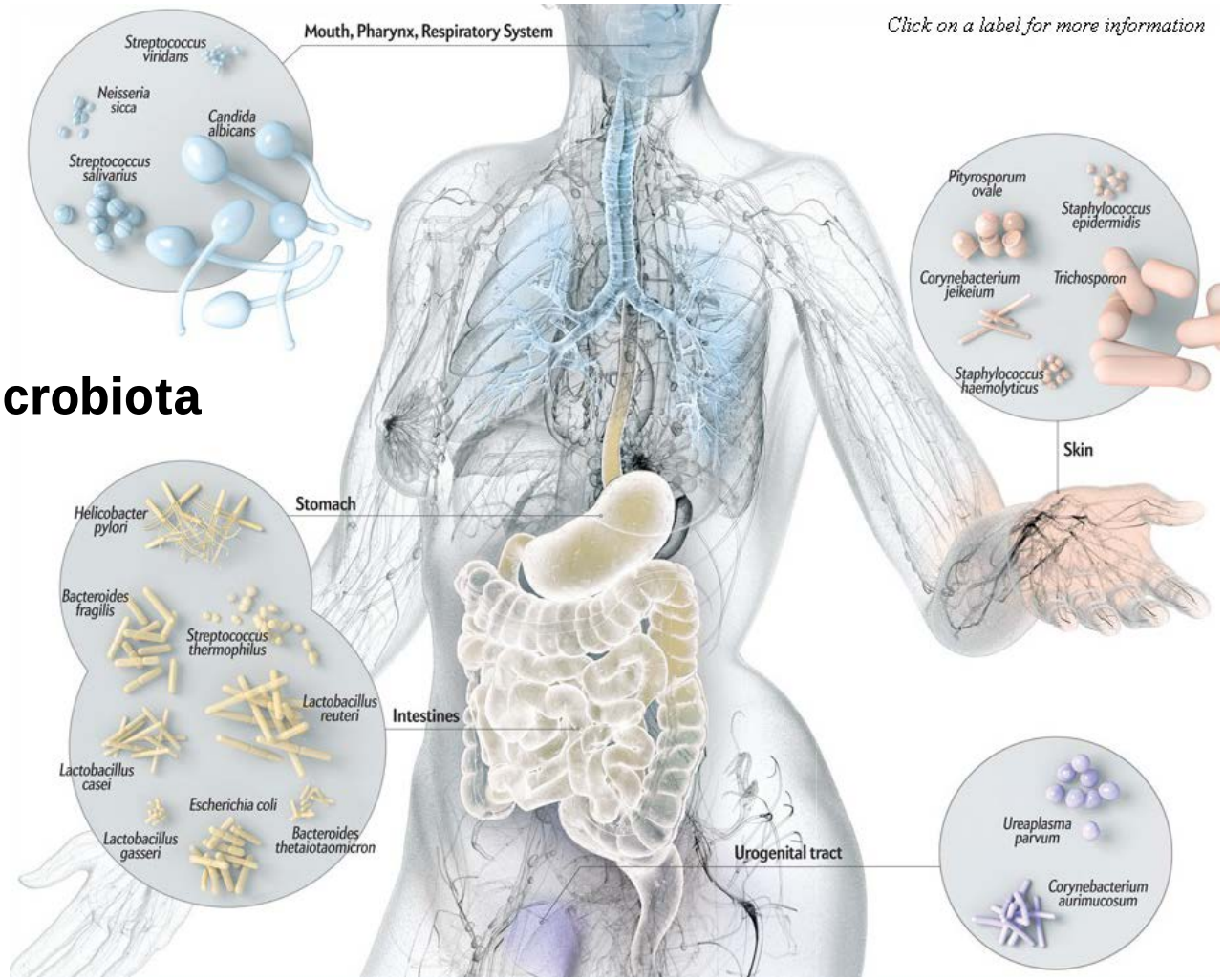
Outline

1. The human microbiome
2. Ecology meets human health
3. A case in point: vaccinating against multi-strain pathogens
4. Concluding remarks

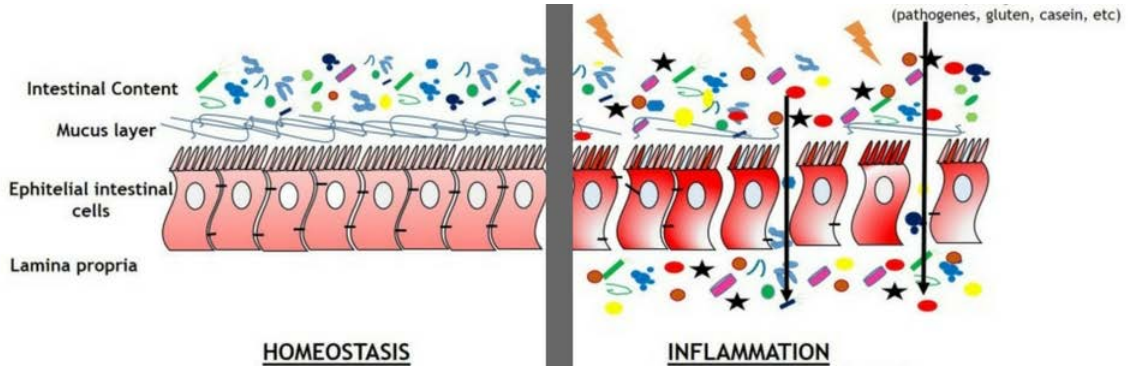
The human microbiota



Metagenomics allows investigation at unprecedented scale and detail

Human health and microbiota

- Human health is profoundly impacted by microbiota and their interactions with the host and its environment
- Inflammation and invasion of epithelial tissues are associated with an imbalance (dysbiosis) in microbial ecosystems
- Understanding the ecology of microbial communities is crucial for capturing transitions between health states

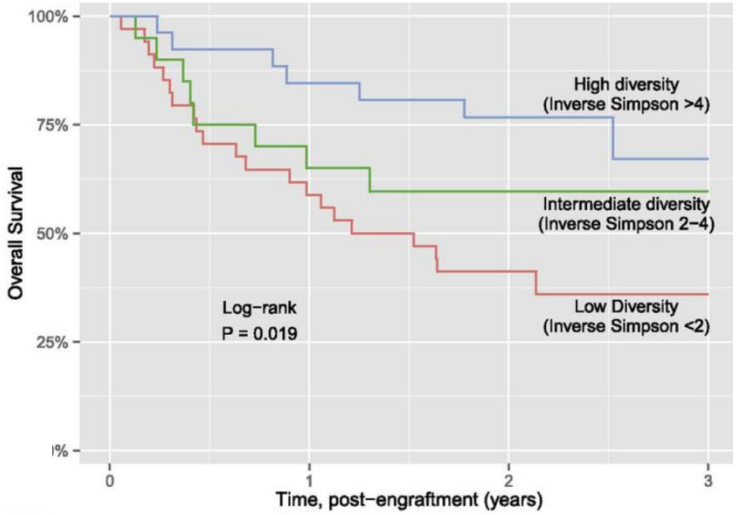


Antibiotics, microbiota and infection

- Paradoxically, antibiotic treatment can deplete the commensal bacteria of a patient's microbiota and increase their risk of subsequent infections

- During allogeneic hematopoietic stem cell transplantation (allo-HSCT), the diversity and stability of intestinal flora are disrupted in microbial ecosystems

- Assessment of fecal microbiota identifies patients at highest risk for bloodstream infection during allo-HCST



Taur et al. (*Clin Infect Dis* 2012), "Intestinal domination and the risk of bacteremia in patients undergoing allo-HCST"

Microbiome engineering?

- Approaches to re-establish depleted commensal bacteria are being developed…

REPORT | GUT MICROBIOTA

Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant

Ying Taur[1], Katharine Coyte[1,2,3], Jonas Schluter[1], Elizabeth Robilotti[1], Cesar Figueroa[1], Mergim Gjonbalaj[1], Eric R. Littmann…
+ See all authors and affiliations

Science Translational Medicine 26 Sep 2018:
Vol. 10, Issue 460, eaap9489
DOI: 10.1126/scitranslmed.aap9489

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 31, 2013 VOL. 368 NO. 5

Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*

Els van Nood, M.D., Anne Vrieze, M.D., Max Nieuwdorp, M.D., Ph.D., Susana Fuentes, Ph.D., Erwin G. Zoetendal, Ph.D., Willem M. de Vos, Ph.D., Caroline E. Visser, M.D., Ph.D., Ed J. Kuijper, M.D., Ph.D., Joep F.W.M. Bartelsman, M.D., Jan G.P. Tijssen, Ph.D., Peter Speelman, M.D., Ph.D., Marcel G.W. Dijkgraaf, Ph.D., and Josbert J. Keller, M.D., Ph.D.

SHARE

Report Slams Deadly Dutch Probiotic Study

Dec. 18, 2009 , 12:16 PM

Most of the controversy over probiotic therapies, in which live "beneficial" bacteria or other microbes are administered to treat or prevent disease, has centered on their effectiveness, not their safety. That's why it was such **a shock early last year** when during a Dutch probiotic study for acute pancreatitis, significantly more patients died in the treatment arm than in the placebo arm. Yesterday, the Dutch Health Care Inspectorate (IGZ) published a critical review of the so-called Propatria study.

- … but we need to better understand how microbes fare when transplanted;
  "Ecology matters"

MARKET INSIDER with PATTI DOMM

Gut check: Seres Therapeutics shares plunge a biome drug fails in tri

Berkeley Lovelace Jr. | @BerkeleyJr

Published 10:39 AM ET Fri, 29 July 2016 | U

CNBC

SCIENCE

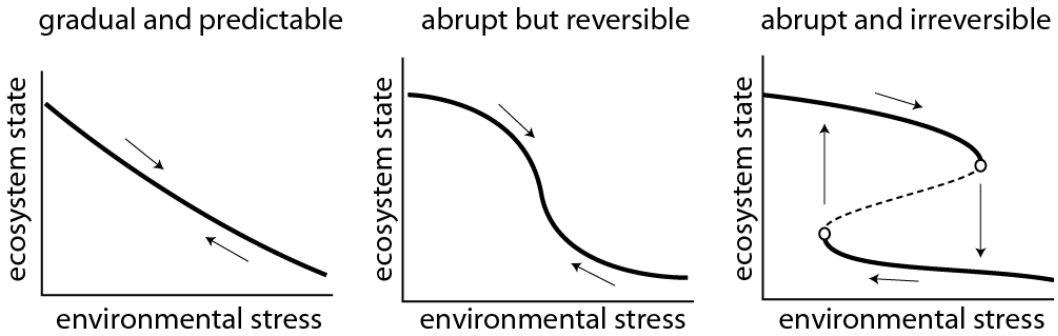
Sham Poo Washes Out

A bacterial pill that tried to duplicate the benefits of a fecal transplant has failed a clinical trial. What does that mean for the microbiome field?

ED YONG AUG 1, 2016

Ecology and the microbiome

- How do microbes compete and cooperate?
- What makes a stable, diverse microbiome?
- How does our microbiome initially assemble?
- How does it respond to environmental stress?

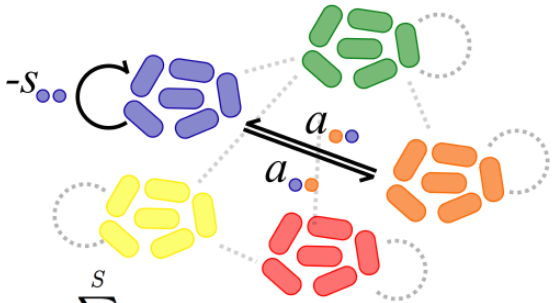


"Ecology meets human health" [NWO Complexity in Health & Nutrition]

- Aim: Characterize the dynamical properties of microbiota and their association with disease using theoretical and quantitative ecological network approaches

- Ecological network models describe interdependencies between network components in terms of resource use and environmental response

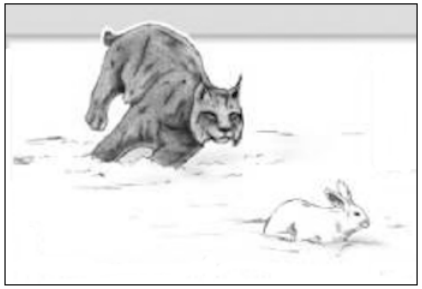
- Generalized Lotka-Volterra models have become popular tools for dynamic modeling of human microbiota…

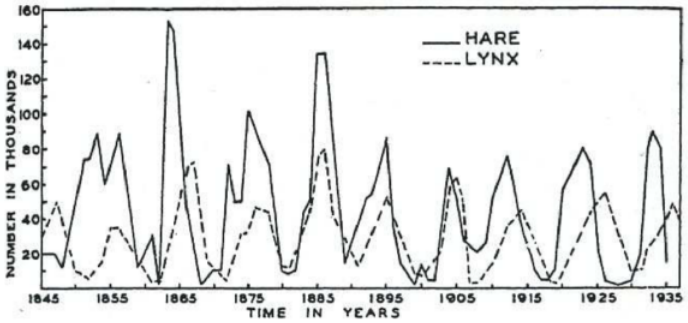


$$\frac{dX_i}{dt} = X_i(r_i - s_i X_i + \sum_{j=1, j\neq i}^{S} a_{ij} X_j)$$

Coyte et al. (*Science* 2015), "The ecology of the microbiome: Networks, competition, and stability"

Lotka – Volterra predator-prey model





Evolution equations

$$\frac{dX}{dt} = aX - bXY$$
$$\frac{dY}{dt} = dXY - cY$$

Steady states (= "equilibrium")

$$\frac{dX}{dt} = 0$$
$$\frac{dY}{dt} = 0$$

$$aX_s - bX_sY_s = 0$$
$$dX_sY_s - cY_s = 0$$

$$(0,0) \qquad \left(\frac{c}{d}, \frac{a}{b}\right)$$

Variables:
X = prey
Y = predator

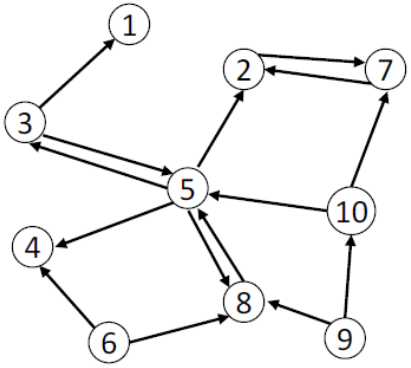
Lotka (1925), "Elements of Mathematical Biology"
Volterra (1931), "Leçons sur la théorie mathématique de la lutte pour la vie"

Multi-species Lotka-Volterra model

$$\frac{dN_i}{dt} = N_i \left(b_i + \sum_{j=1}^{S} a_{ij} N_j \right)$$

where:
N = species abundances
S = number of species
b = growth rates
a = interaction matrix

Interaction matrix

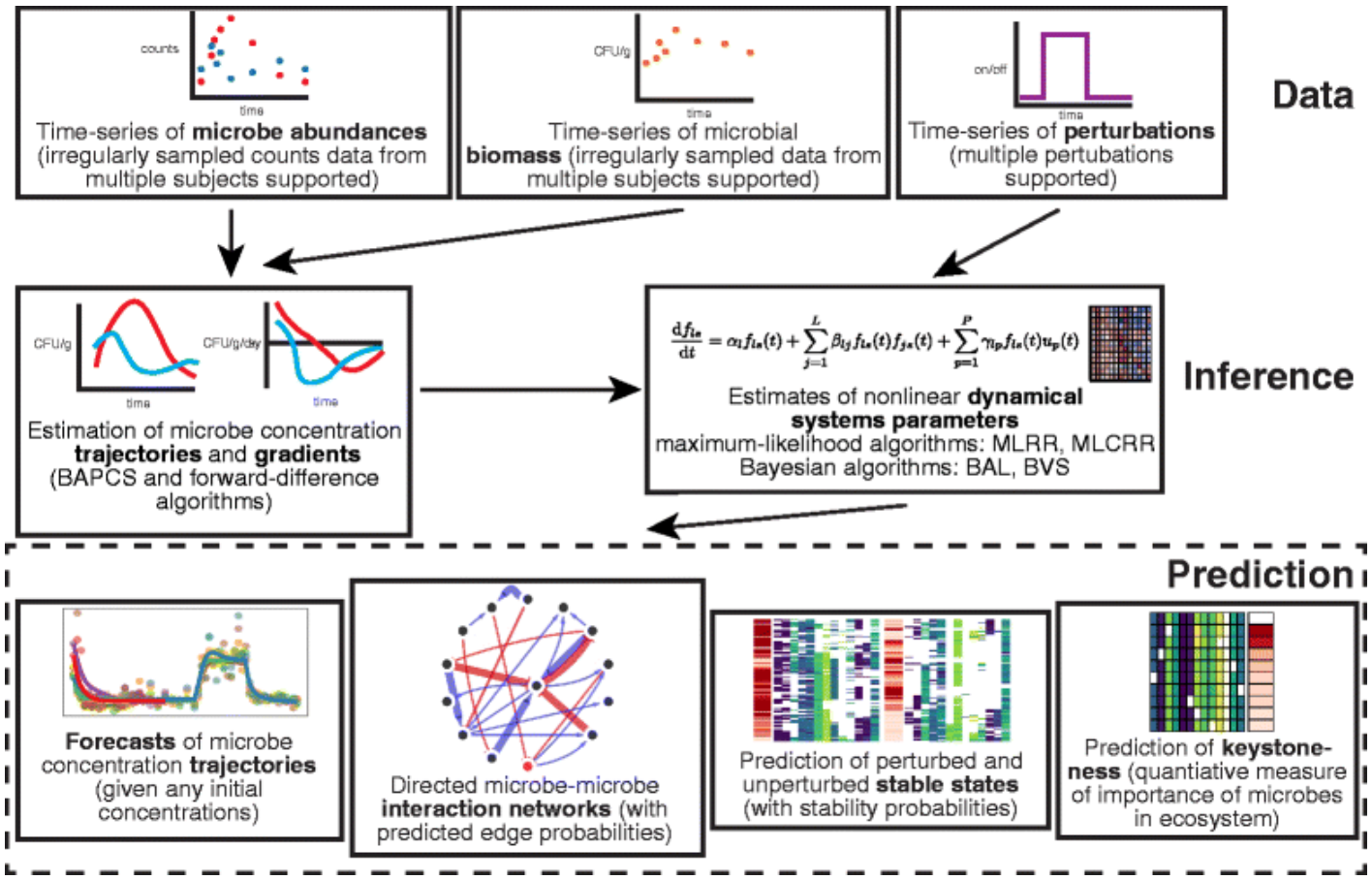


"Causal" network

(directed gaph)

NB: Detecting causal relationships is highly tricky

May (1973), "Stability and Complexity in Model Ecosystems"



Bucci et al. (*Genome Biol* 2016), "MDSINE: Microbial Dynamical Systems INference Engine for microbiome time-series analyses"

Caveat emptor

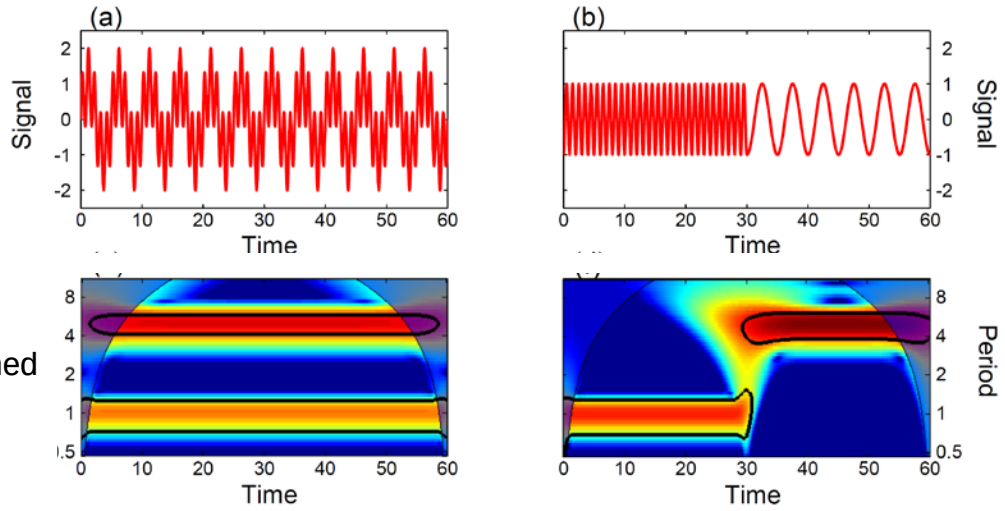
- … but these models have largely been abandoned by ecologists, because
  - Linear unbounded growth rates (type I functional response)
  - No higher-order interactions
  - No stable limit cycles
  - No bistability
- However, relatively easy to analyze, simulate and fit to data
  - … but is it really like that?

Challenges and clues in microbiome modeling

- How to infer ecological networks from data?
  - Explore links between graphical models and system dynamics
  - Look at cross-predictive ability (Granger causality)
  - Use spectral properties of time series data

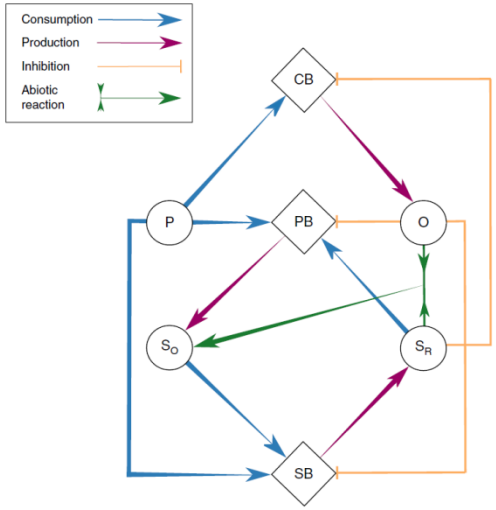
Benincà et al. (*Ecol Lett* 2009), "Coupled predator-prey oscillations in a chaotic food web"

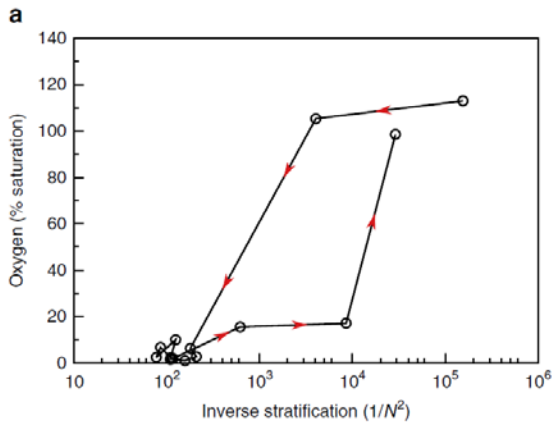
Benincà et al. (*PNAS* 2015), "Species fluctuations sustained by a cyclic succession at the edge of chaos"

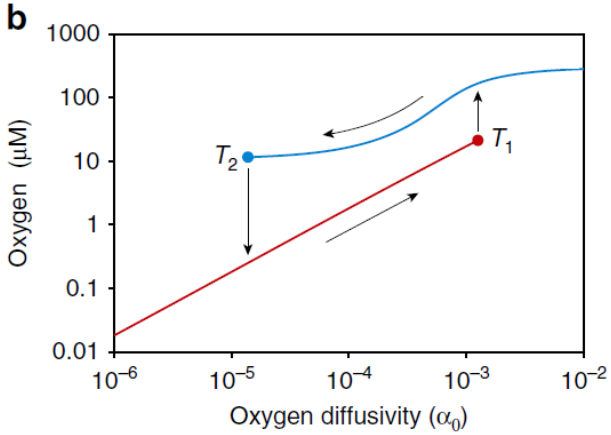


Challenges and clues in microbiome modeling

- How to model large-scale microbial communities?
  - Don't exaggerate
  - Regime shifts predictable in low-dimensional systems
  - Search for keystone species or functional sub-communities



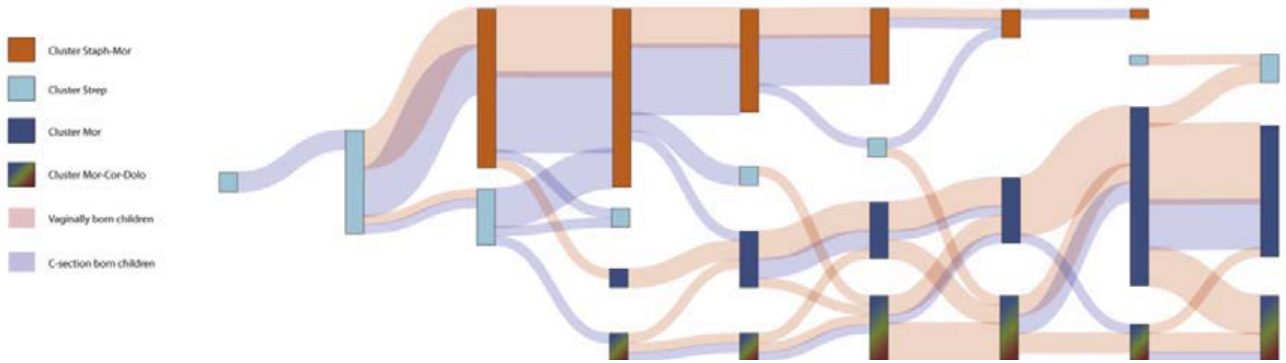




Bush et al. (*Nat Commun* 2017), "Oxic-anoxic regime shifts mediated by feedbacks between biogeochemical processes and microbial community dynamics"

Challenges and clues in microbiome modeling

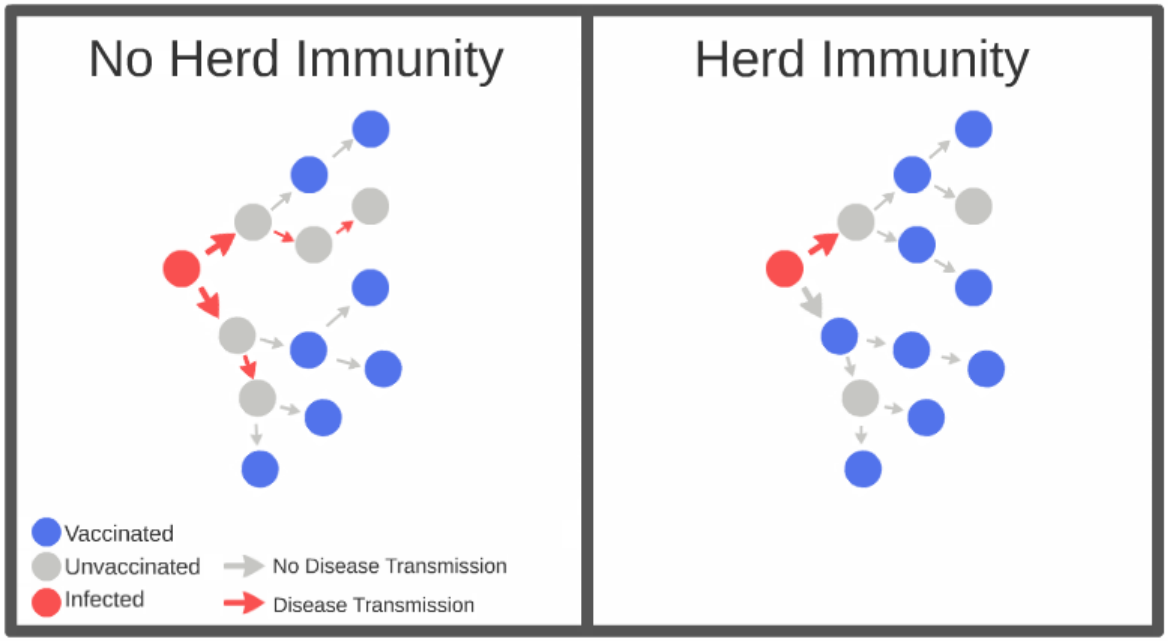
- How to link microbial dynamics to transitions between health states?
  - Focus prognostic performance, not on etiology
  - Resembles dynamic prediction in event history analysis
  - "Microbiome-age" holds potential for predicting respiratory tract infections



Bosch et al. (*EBioMedicine* 2016), "Development of upper respiratory tract microbiota in infancy is affected by mode of delivery"

A case in point: vaccinating against multi-strain pathogens

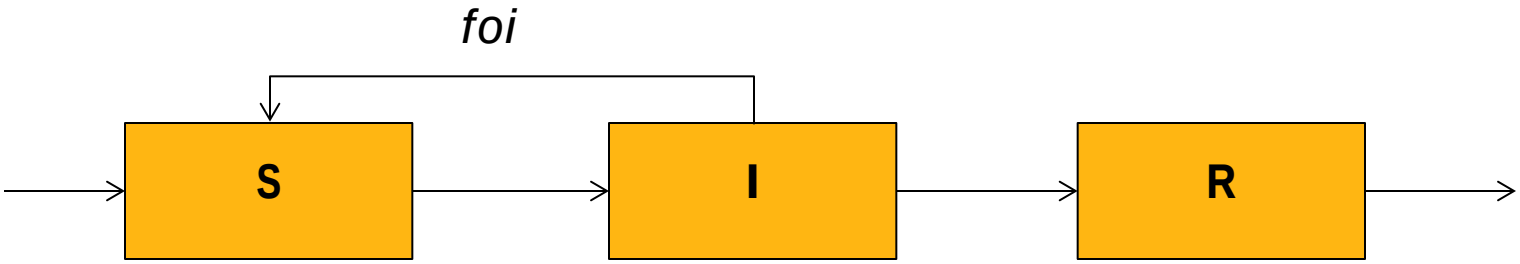
- Effects of vaccination on transmission typically not measured in RCTs
- Example: manifestation of herd immunity in partially vaccinated populations



Polyvalent vaccination: opening a can of worms?

- Novel vaccines are often directed against a subset of pathogenic strains
  - HPV, pneumococcus, meningococcus, rotavirus, influenza…
- By intervening against one strain we might affect the fate of others
  - "Ecology matters"
- Side-effects may be positive or negative (perverse outcome)
  - Intricate balance in a network of multiple interacting types
  - Ecological & evolutionary dynamics
- Projecting the population effects of polyvalent vaccination is complicated
  - Standard approaches assume that pathogen strains do not interact

The central tenet of transmission modelling

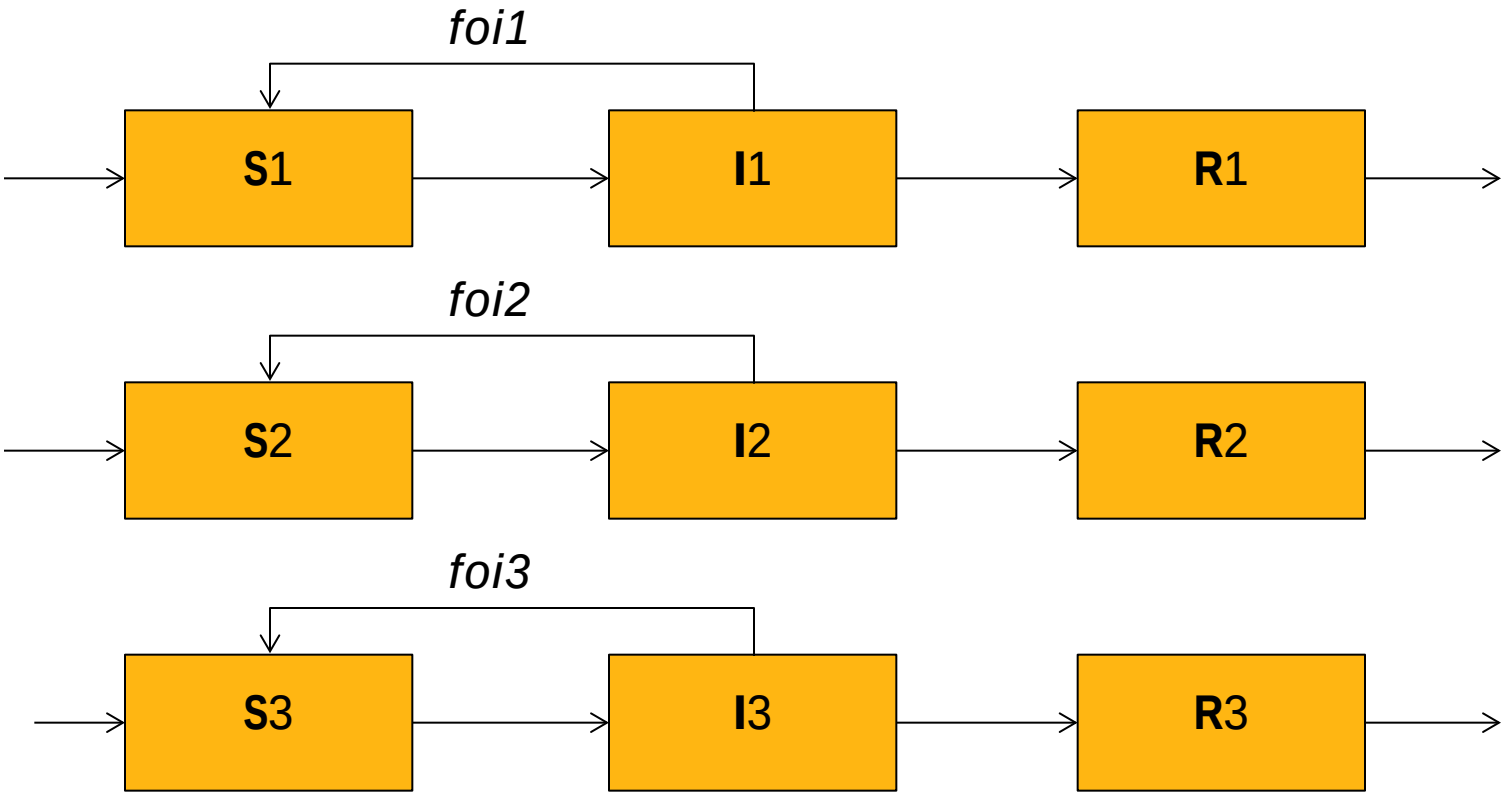


Force of infection *(foi)* depends on product **S · I**

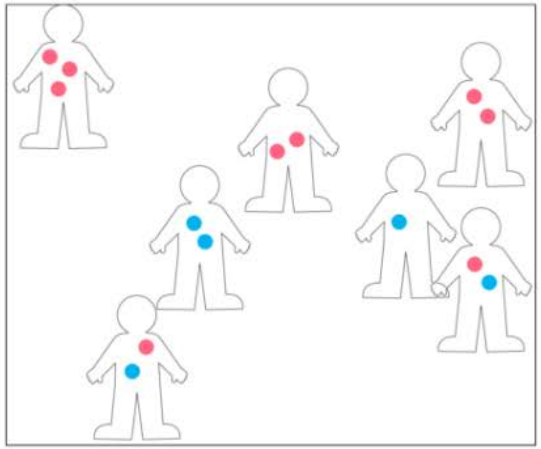
> This creates a non-linear (dynamical) system

> Non-linearity and high-dimensionality

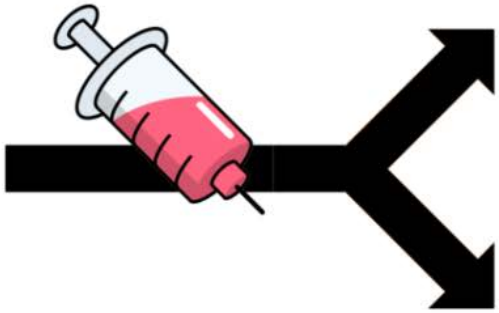
Extension to multiple pathogen strains?



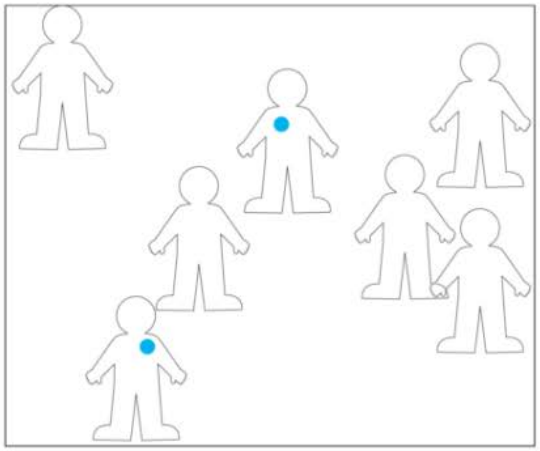
… but what if types do interact?

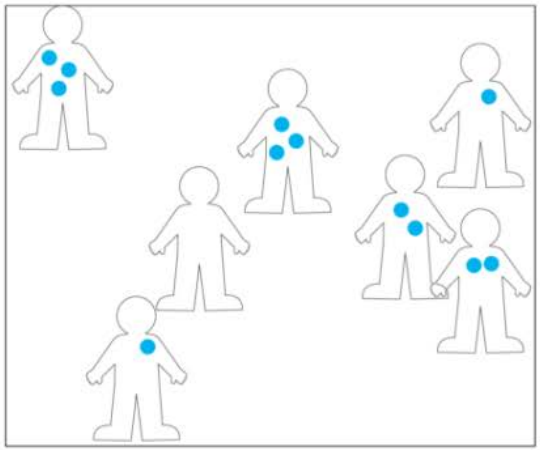


Synergy between vaccine and non-vaccine types

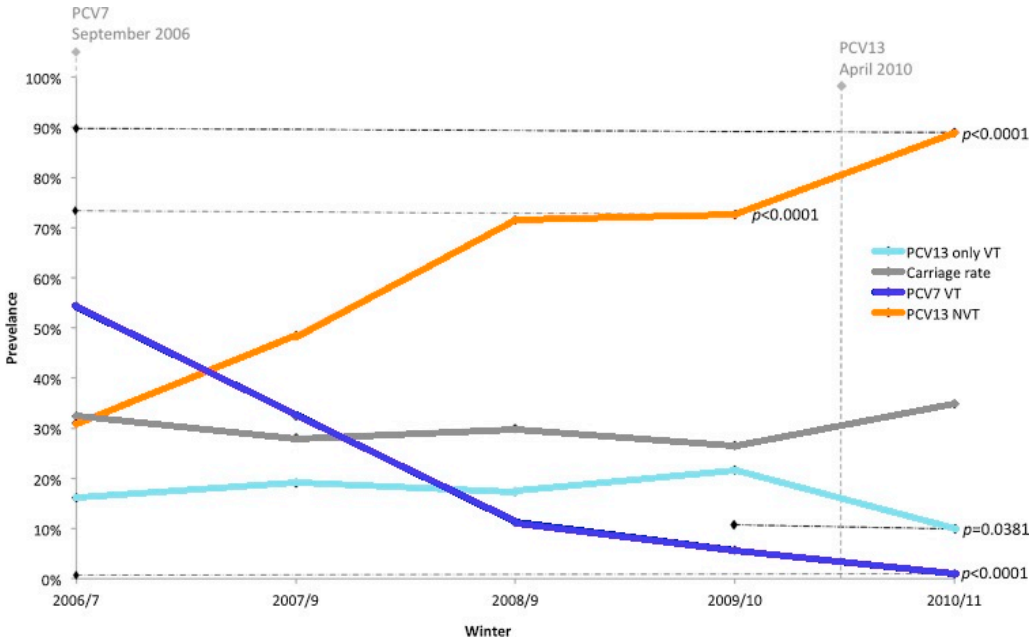


Competition between vaccine and non-vaccine types

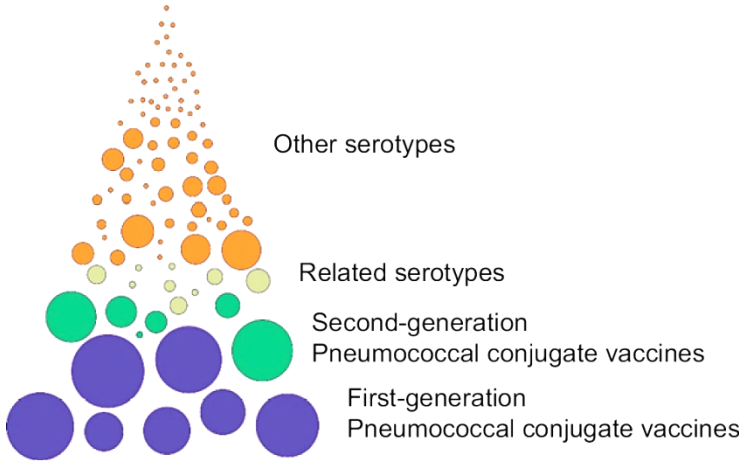




Type-replacement after pneumococcal vaccination



Gladstone et al. (*Vaccine* 2015)

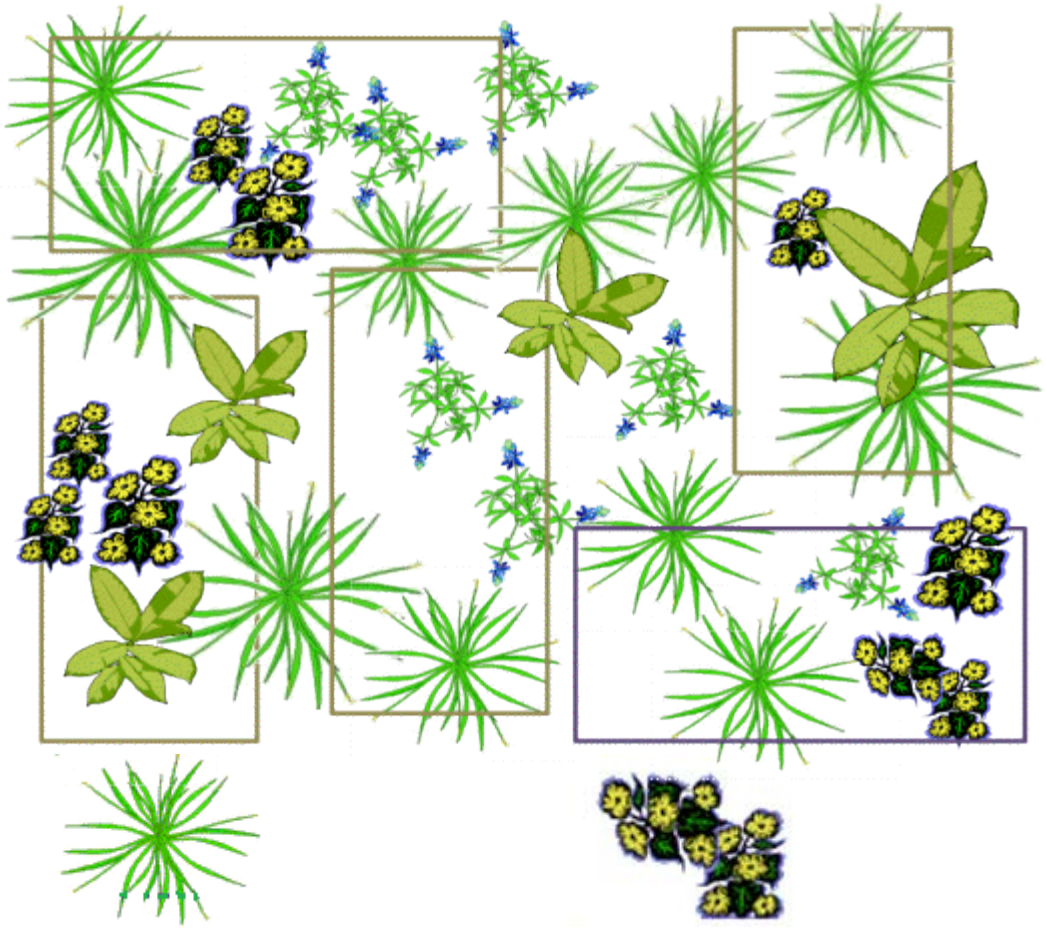


Rijkers et al. (*Risk Manag Healthc Policy* 2018)

Cobey & Lipsitch (*Science* 2012),
"Niche and neutral effects of acquired immunity permit coexistence of pneumococcal serotypes"

George Bouverie Goddard (1879), "The Struggle for Existence"

“Now that's a warm hug! Emperor penguins get so hot in their huddles they EAT fresh snow to cool down”
dailymail.co.uk



Independence: species A has no significant effect on species B (once conditioned on common causes)

biologyforlife.com

HPV types: conditionally independent (co)occurrence?

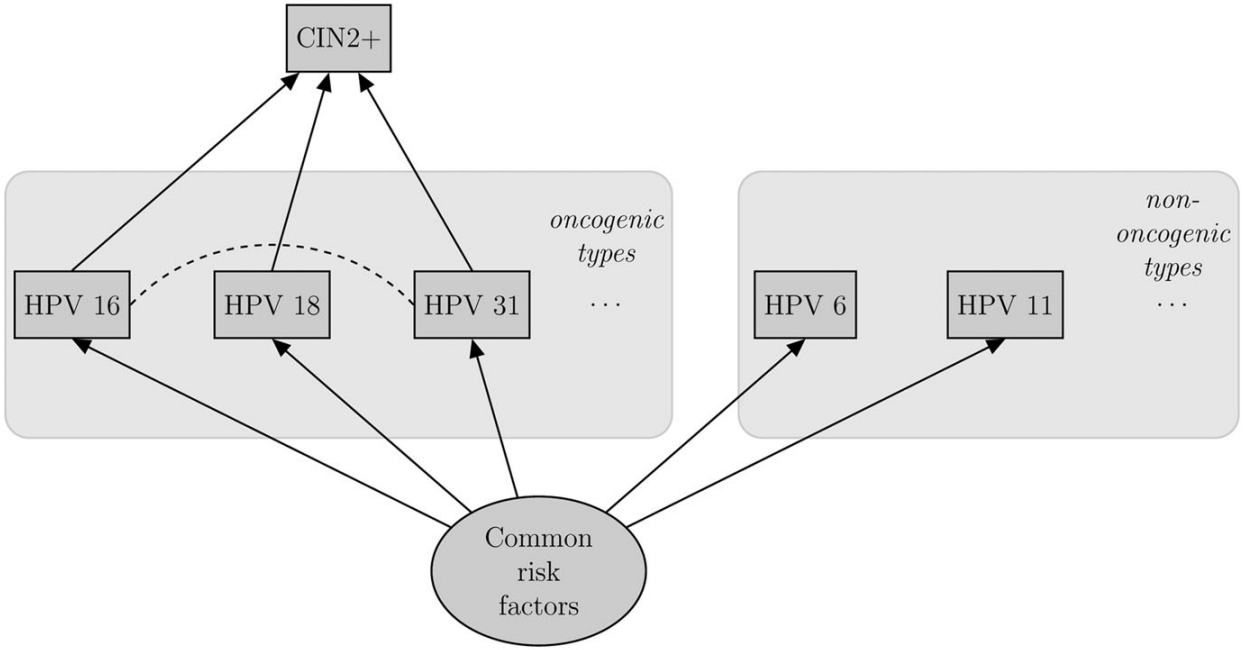


Figure 1. Conditional independent diagram for multiple human papillomavirus (HPV) infections including observed variables *(rectangular nodes)* and unobserved variables *(circular node)*. CIN2+, cervical intraepithelial neoplasia 2 or worse.

892 • JID 2011:203 (1 April) • EDITORIAL COMMENTARY

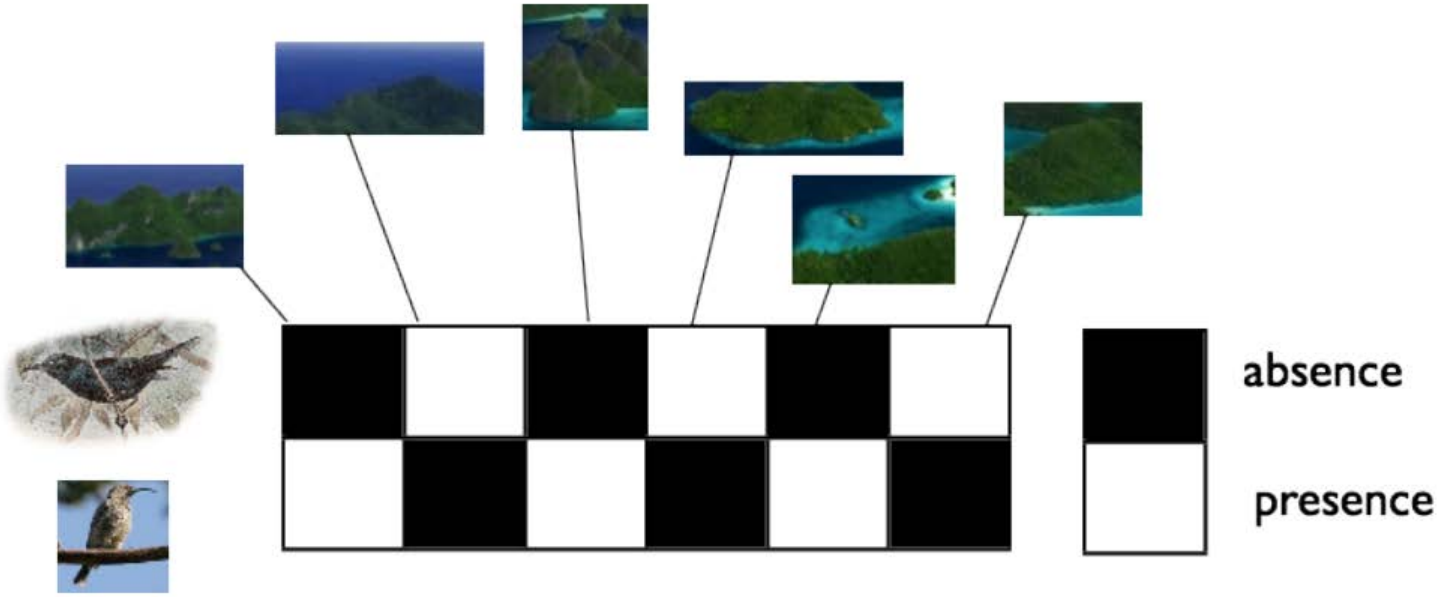
Plummer et al. (*J Infect Dis* 2011), "Multiple HPV Infections: The Exception or the Rule?"

Can we predict type replacement prior to vaccination?

- i.e., Can we infer strain interactions from epidemiological data?
  - Cross-sectional prevalence data (co-occurrence)
  - Longitudinal data on acquisition and clearance
  - Serological surveillance data (current status)
- How do heuristic approaches relate to transmission dynamics?
- How to correct for unobserved confounding?
  - Usefulness of random effect models?

Can we infer strain interactions from patterns of co-occurrence?

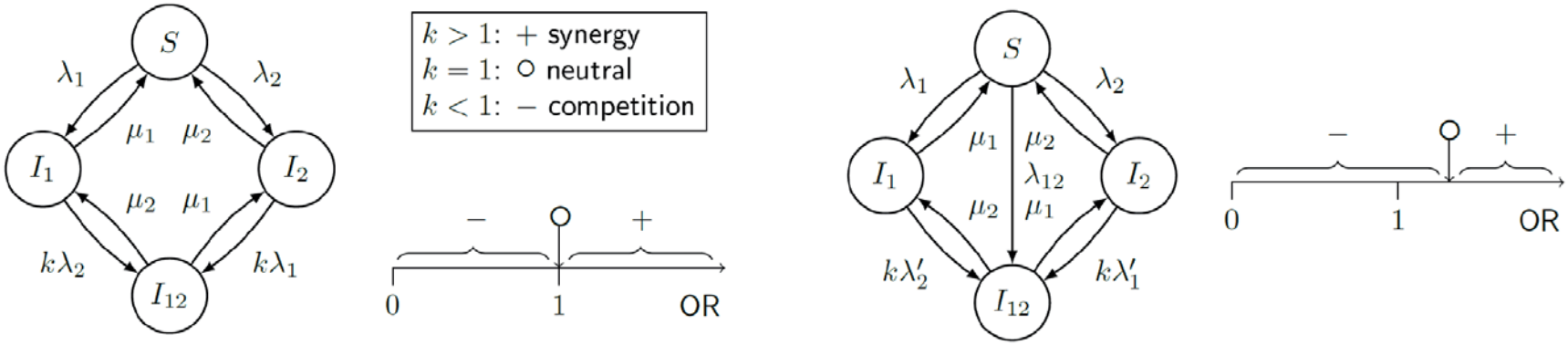
- Suggestion that competition between species could be seen from their joint presence/absence across habitats (checkerboard pattern) received heavy criticism in ecology



Diamond (1975), "Assembly of species communities“ in Cody (ed.), “Ecology and Evolution of Communities"

Yet, we can! [… under specific conditions]

- The odds ratio (OR) of coinfection is an unbiased estimator of composite interactions in acquisition and clearance in a 2-type SIS transmission model
  - Proof involves system's reversibility

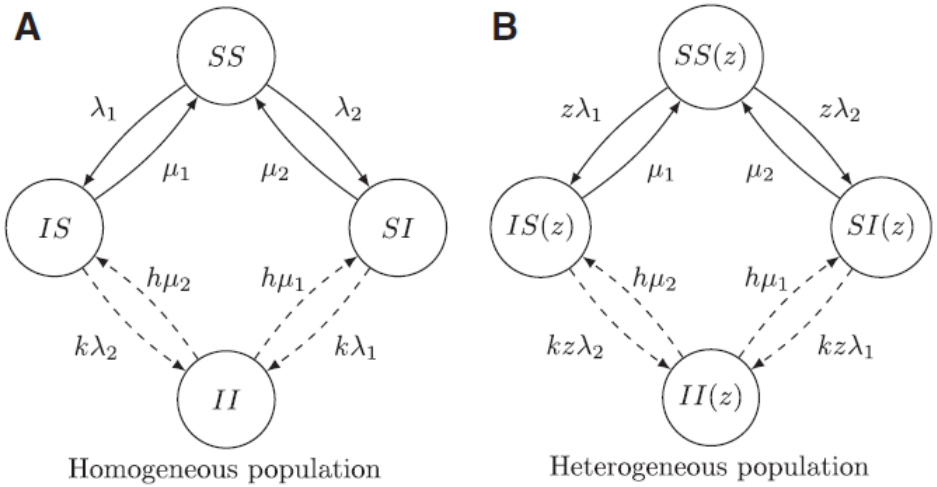


  - Left model (sequential acquisition) is reversible, right model is not

Man et al. (*Epidemiology* 2018), "Inferring pathogen type interactions using cross-sectional prevalence data: opportunities and pitfalls for predicting type replacement"

Yet, we can! [… if corrected for confounders]

- The crude OR overestimates the composite of interaction parameters if both types share common risk factors (e.g., contacts, susceptibility, …)
  - Proof involves Chebyshev's inequality

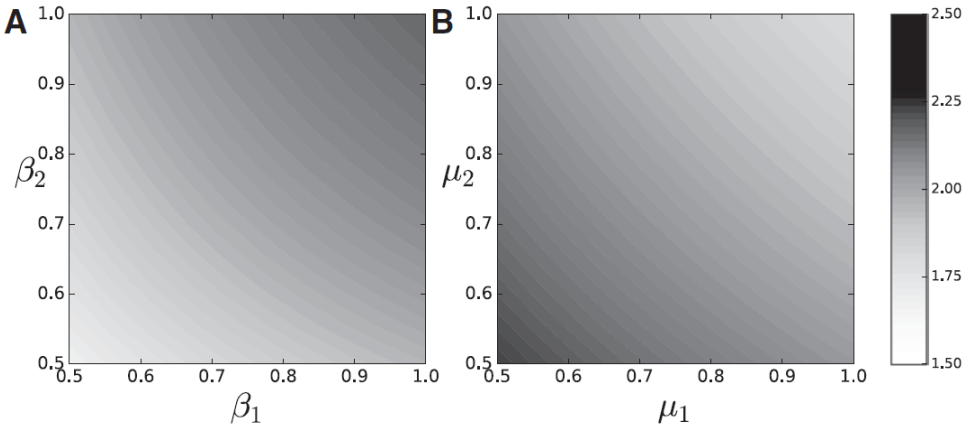


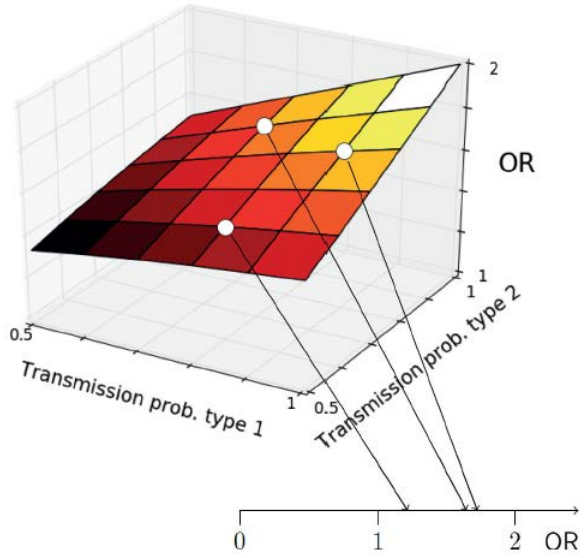
  - Risk factors are homogeneous in A and heterogeneous (with frailty *z*) in B

Man et al. (*Epidemiology* 2018), "Inferring pathogen type interactions using cross-sectional prevalence data: opportunities and pitfalls for predicting type replacement"

Yet, we cannot correct for unobserved confounding

- The positive bias depends on type-specific parameters, suggesting differential adjustments for different combinations under identical common risk factors



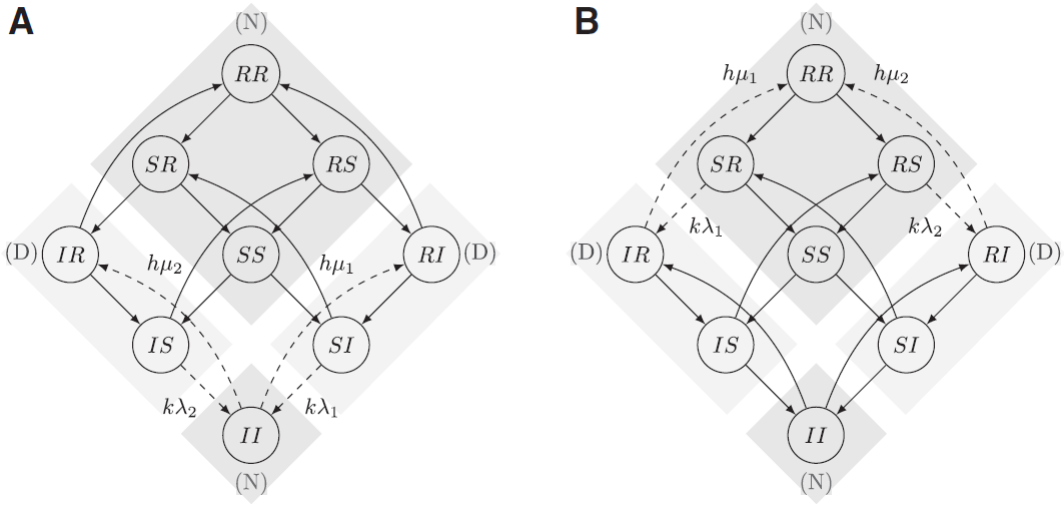


– Dependence of the crude OR on type-specific viral transmissibility and clearance

Man et al. (*Epidemiology* 2018), "Inferring pathogen type interactions using cross-sectional prevalence data: opportunities and pitfalls for predicting type replacement"

Beware: different forms of bias type-specific vs. cross-immunity

- Type-specific immunity induces shrinkage, whereas cross-immunity reverses the correspondence between the OR and the composite of interaction parameters
  - Differential effects of competition on numerator and denominator

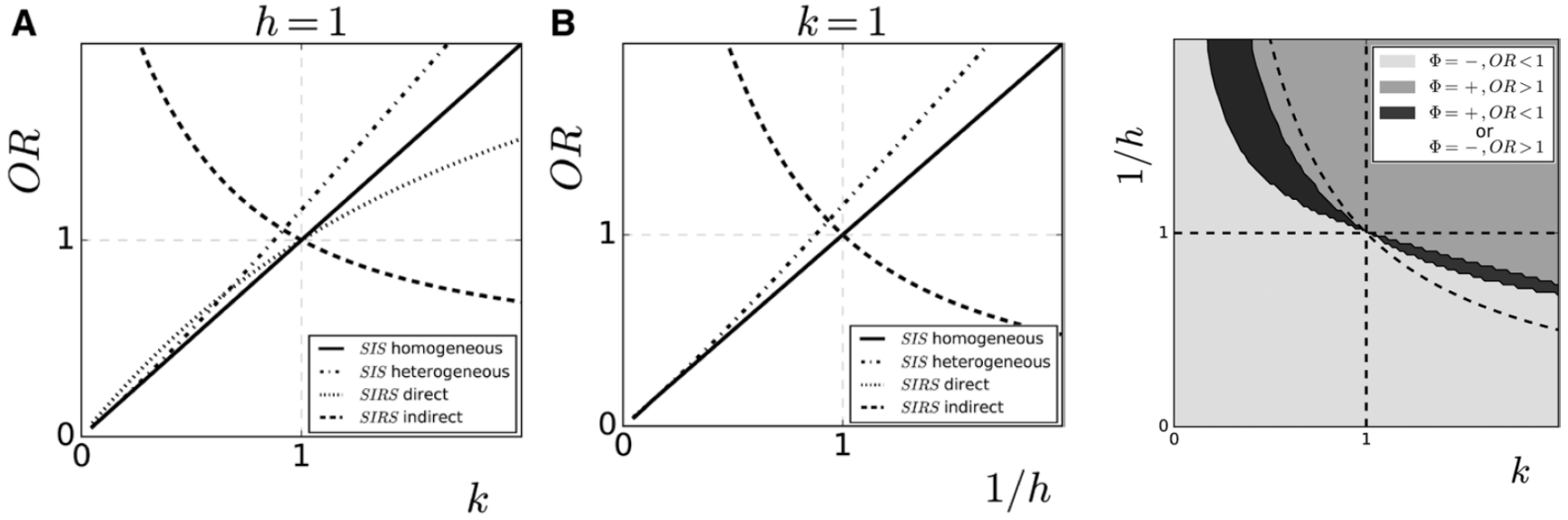


  - Interactions are only direct in A and indirect (through cross-immunity) in B

Man et al. (*Epidemiology* 2018), "Inferring pathogen type interactions using cross-sectional prevalence data: opportunities and pitfalls for predicting type replacement"

Can we predict type replacement from patterns of co-occurrence?

- Previous results suggest that type-replacement could (in principle) be predicted from cross-sectional data collected prior to vaccination



– OR and competition in acquisition (*k*) and clearance (*h*) and occurrence of type replacement (Φ)

Man et al. (*Epidemiology* 2018), "Inferring pathogen type interactions using cross-sectional prevalence data: opportunities and pitfalls for predicting type replacement"

Can we predict type replacement prior to vaccination?

- Using OR<1 to predict type replacement is justified provided that
  - Direct interactions during infection are unidirectional
  - The presence of immunity-induced synergism can be excluded
- Sensitivity of predictor depends on
  - Residual confounding
  - Existence of cross-immunity
- Accurate if SIS dynamics can be assumed and risk factors are well known

Can we extend this predictor to an arbitrary number of types?

- Yes, in case of SIS dynamics we can generalize according to

$$OR_{VT,i} = \left(\frac{\sum_{X \in \mathcal{A}_{VT,i}} I_X}{\sum_{X \in \mathcal{A}_{VT}} I_X} \right) / \left(\frac{\sum_{X \in \mathcal{A}_i} I_X}{\sum_{X \in \mathcal{A}_0} I_X} \right)$$

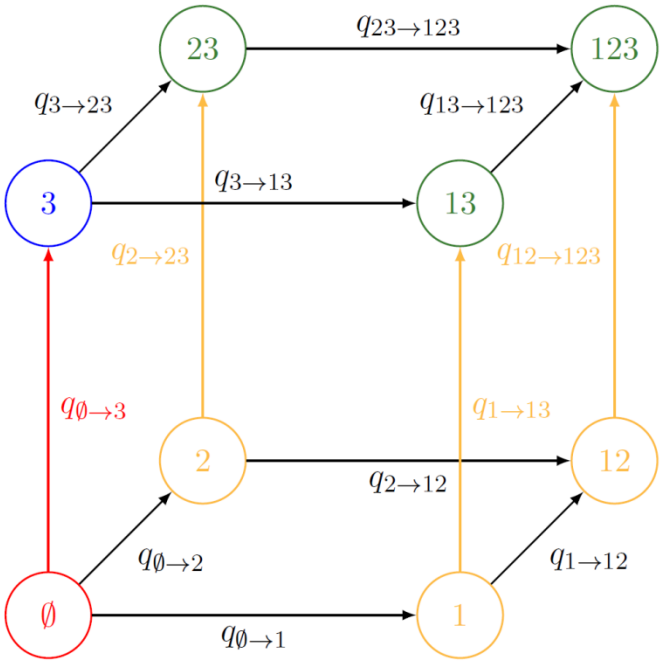
- This is the odds of infection with non-vaccine type *i* in presence vs. absence of coinfection with any of the vaccine types

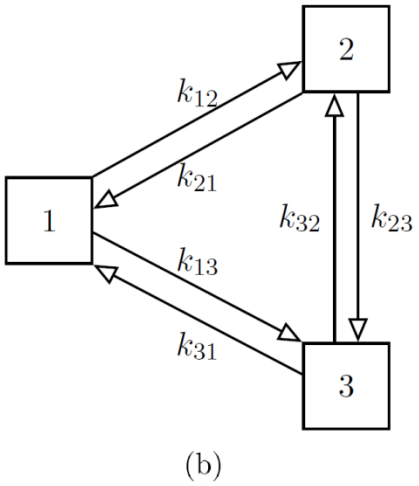
- This odds ratio approximates the more intuitive hazard ratio

$$HR_{VT,i} = \left(\frac{\sum_{X \in \mathcal{A}_{VT}} I_{X|\mathcal{A}_{VT}} q_{X \to X \cup \{i\}}}{\sum_{X \in \mathcal{A}_0} I_{X|\mathcal{A}_0} q_{X \to X \cup \{i\}}} \right) / \left(\frac{\sum_{X \in \mathcal{A}_{VT,i}} I_{X|\mathcal{A}_{VT,i}} q_{X \to X \setminus \{i\}}}{\sum_{X \in \mathcal{A}_i} I_{X|\mathcal{A}_i} q_{X \to X \setminus \{i\}}} \right)$$

Performance under various interactions structures

- Multiplicative pairwise-symmetric, -asymmetric, or groupwise symmetric



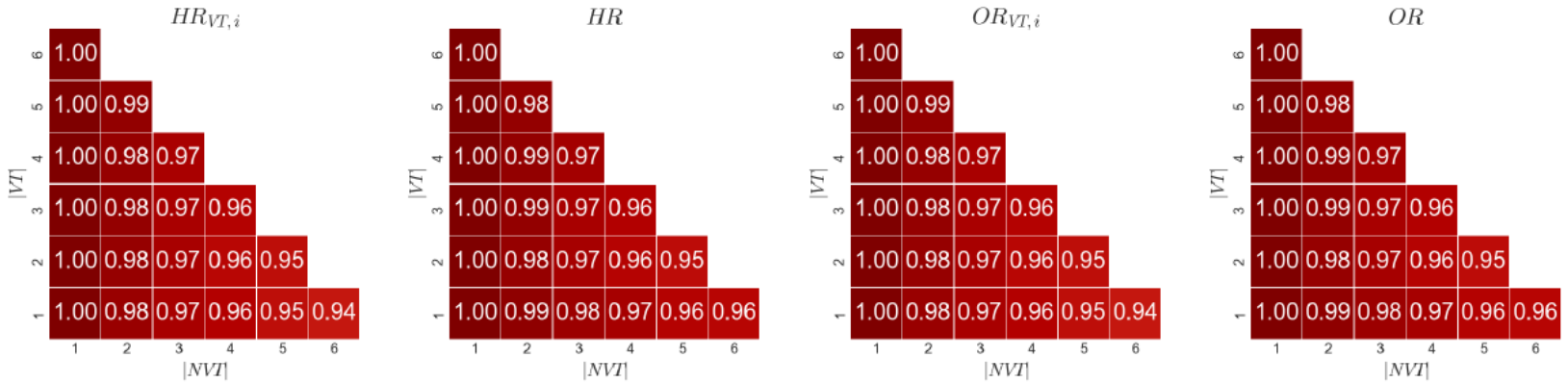


(b)

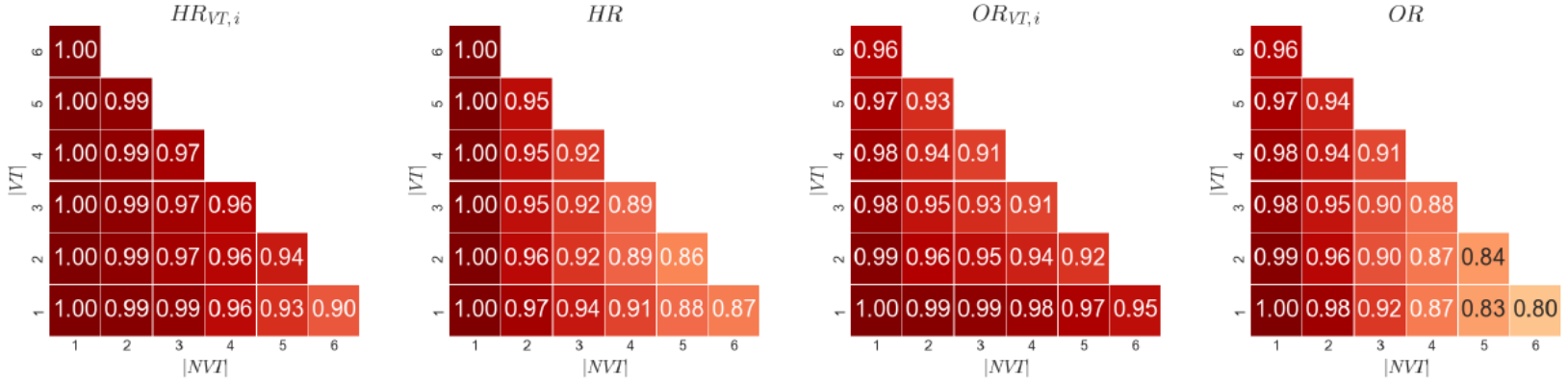
– Illustrative example of transitions and interactions (b) in a model with three types

Performance under various interactions structures

Pairwise-symmetric:



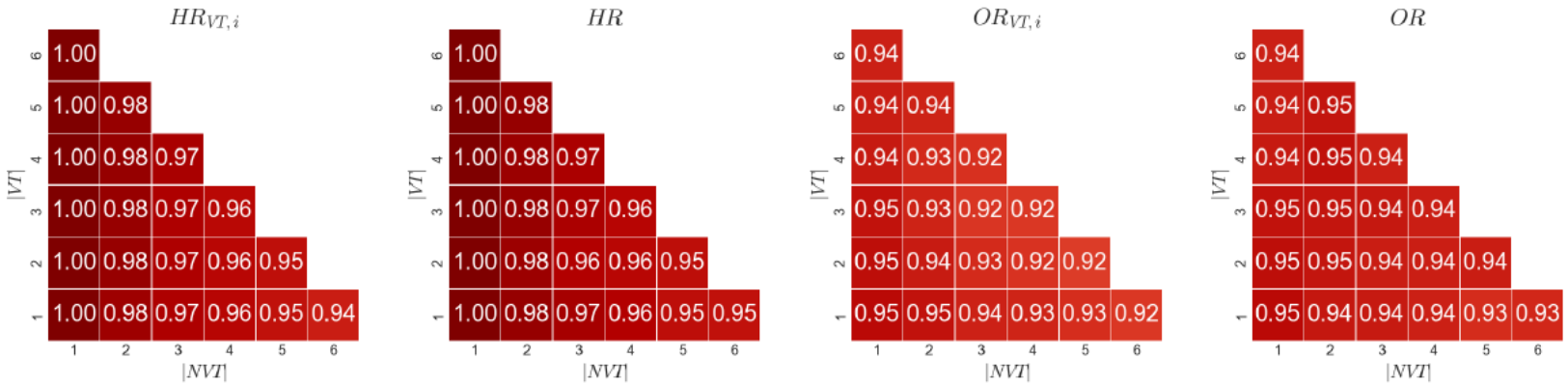
Groupwise-symmetric:



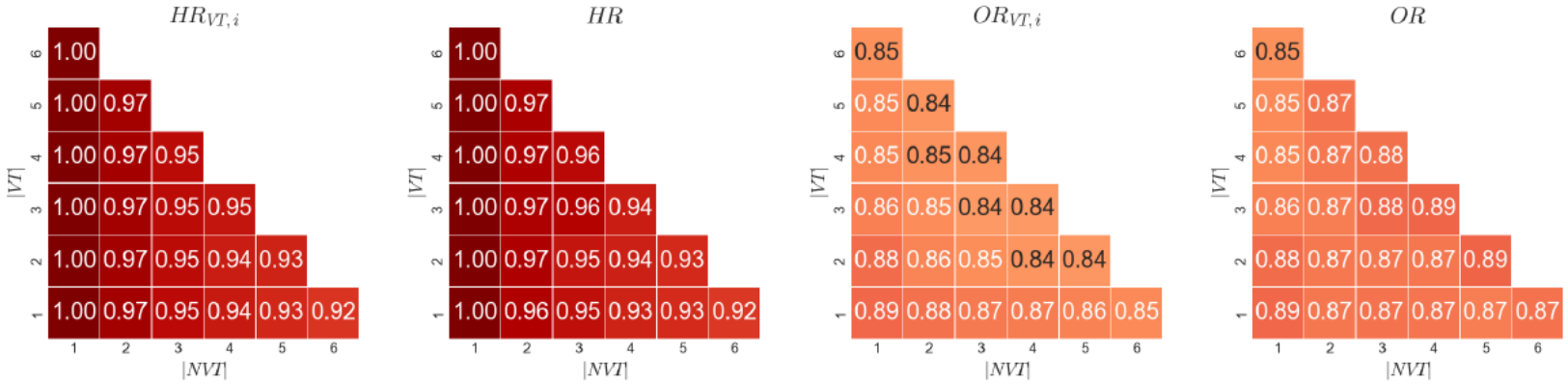
– Concurrence of pre-vaccination predictions and ‘true’ outcome of vaccination

Performance under various interactions structures

Mildly pairwise-asymmetric ($\epsilon = 0.2$):



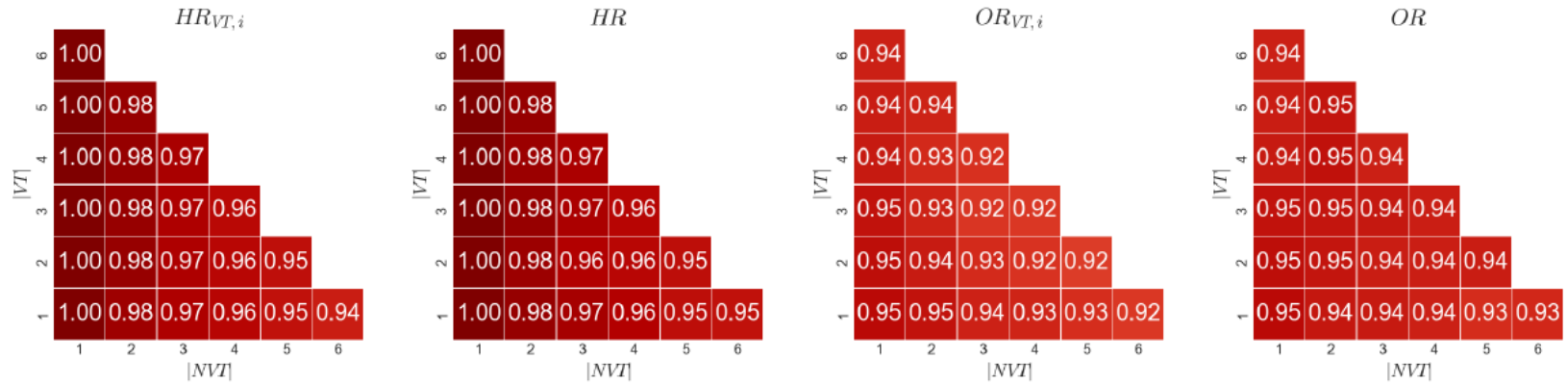
Moderately pairwise-asymmetric ($\epsilon = 0.5$):



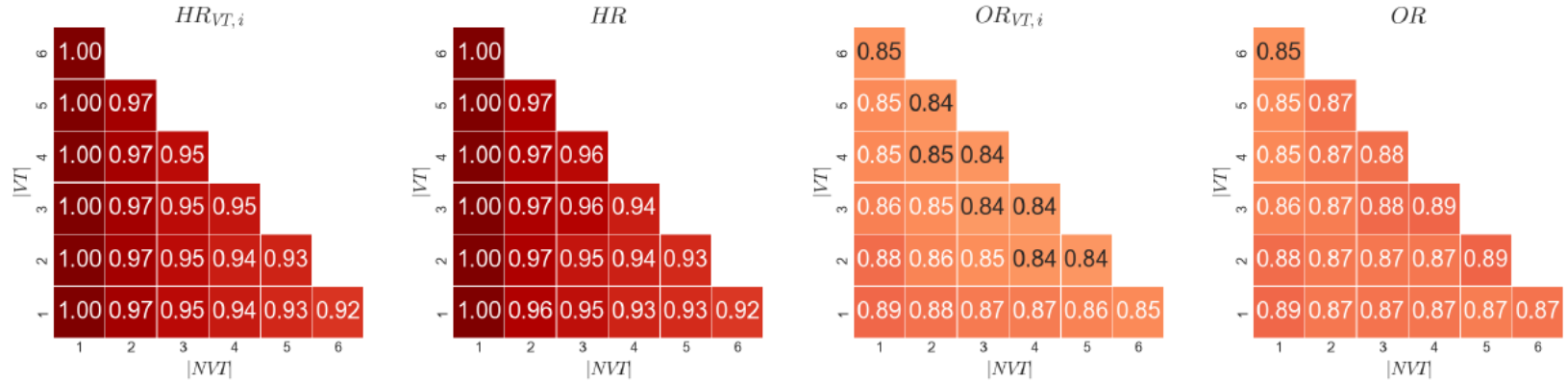
– Concurrence of pre-vaccination predictions and ‘true’ outcome of vaccination

Performance under various interactions structures

Mildly pairwise-asymmetric ($\epsilon = 0.2$):



Moderately pairwise-asymmetric ($\epsilon = 0.5$):



– Concurrence of pre-vaccination predictions and ‘true’ outcome of vaccination

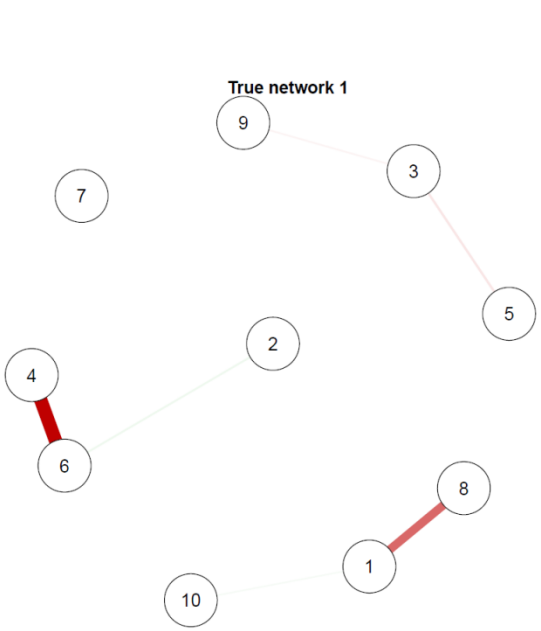
Can we infer the network of interactions from co-occurrence data?

- Yes, by modeling the joint probability density of infection by multiple strains
  - By log-linear analysis with model selection

    Agresti (2007), "An Introduction to Categorical Data Analysis (2nd ed.) "
  - Alternating logistic regressions via GEE

    Carey et al. (*Biometrika* 1993), "Modelling multivariate binary data with alternating logistic regressions"
  - Regularized Ising model

    Ravikumar et al. (*Ann Stat* 2010), "High-dimensional Ising model selection using ℓ1-regularized logistic regression"
- Approaches may behave different with limited small sample size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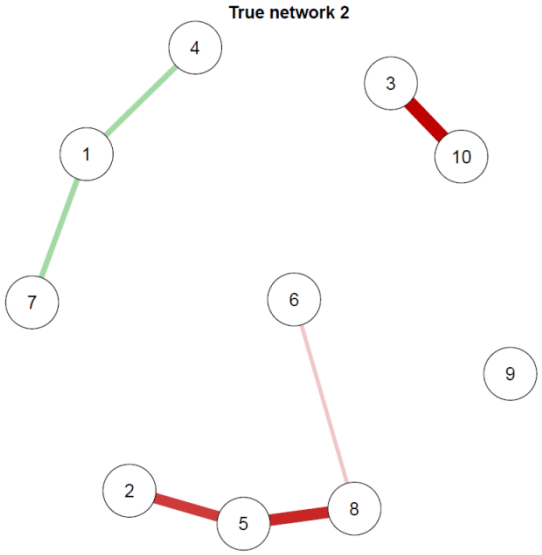
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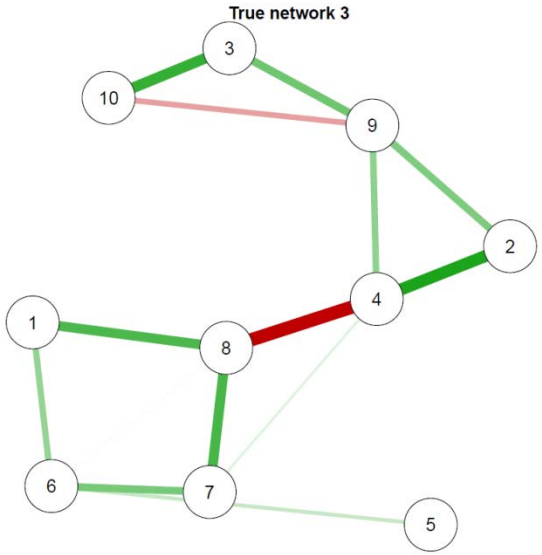
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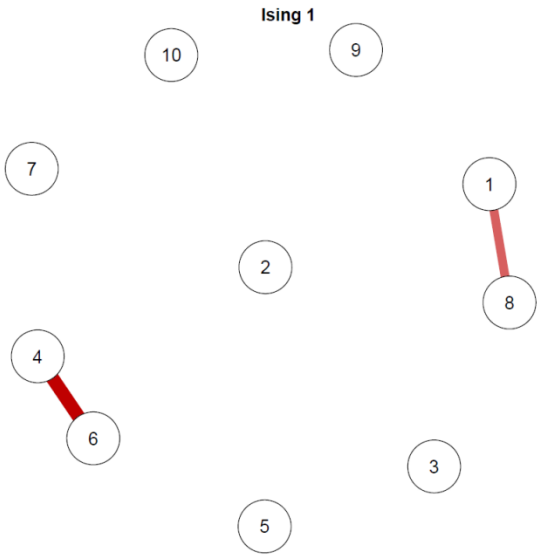
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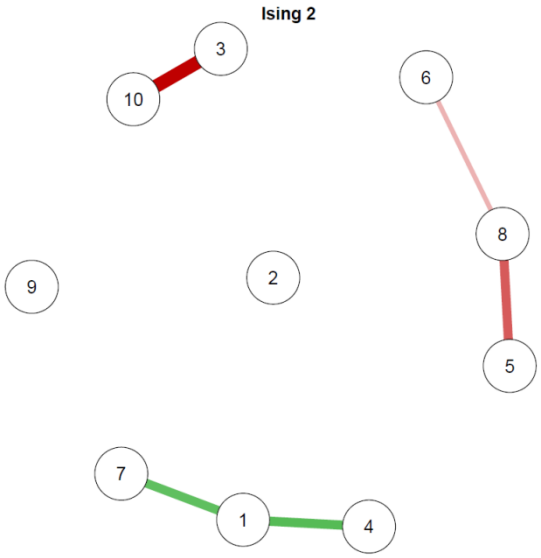
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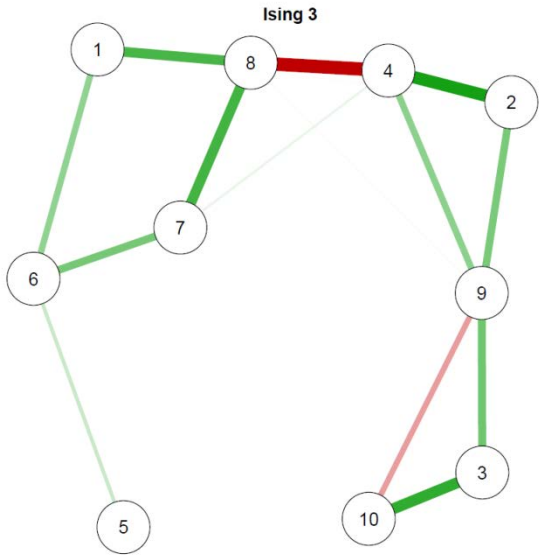
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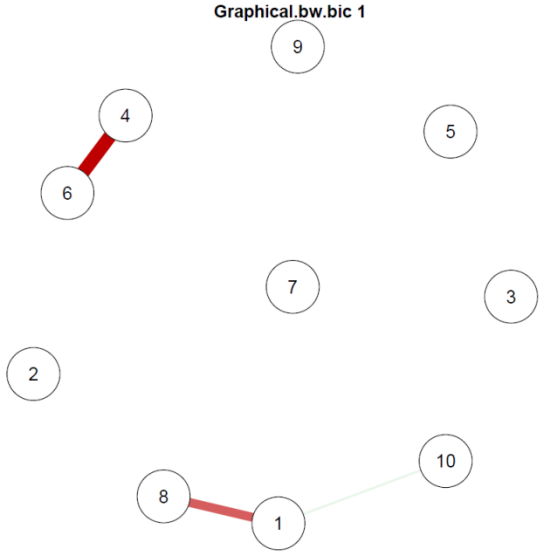
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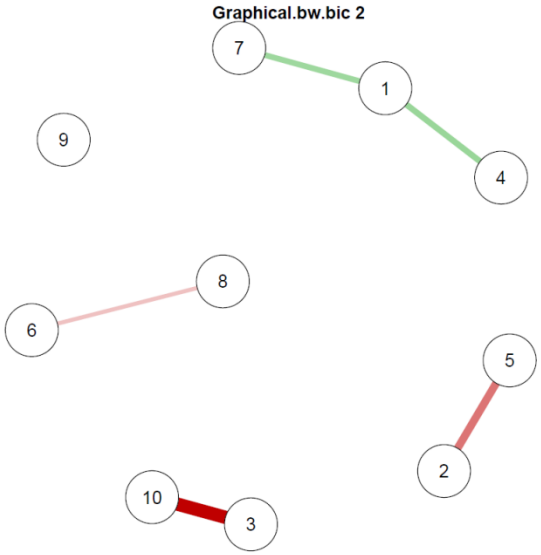
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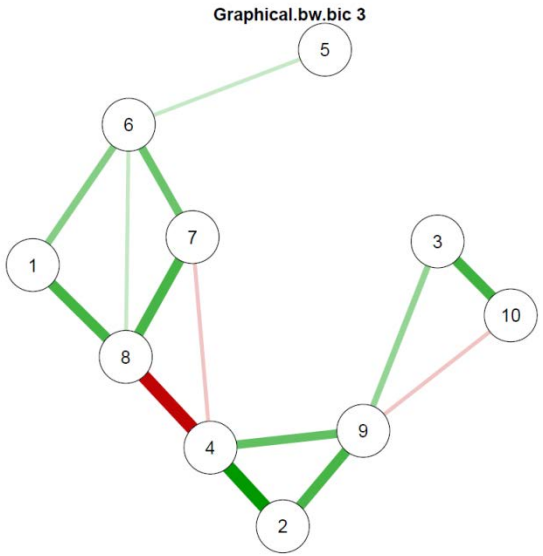
Graphical.bw.bic 3
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- **Biostatistical challenges in R&D**
  - Microbiome engineering is already a reality, not just a promise
  - Interplay between empirical and computational approaches

- **"Ecology matters!"**
  - Within-host ecology (microbiome)
  - Between-host ecology (multi-strain pathogens)

- **Novel approaches are needed to model microbial ecosystems**
  - Integration between theoretical ecology, systems biology, epidemiology, biostatistics

- **Multi-scale & multi-model approach**
  - If we are to understand a system on different levels of resolution, we should understand how different models and approached relate and when they are consistent

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“Thank you”