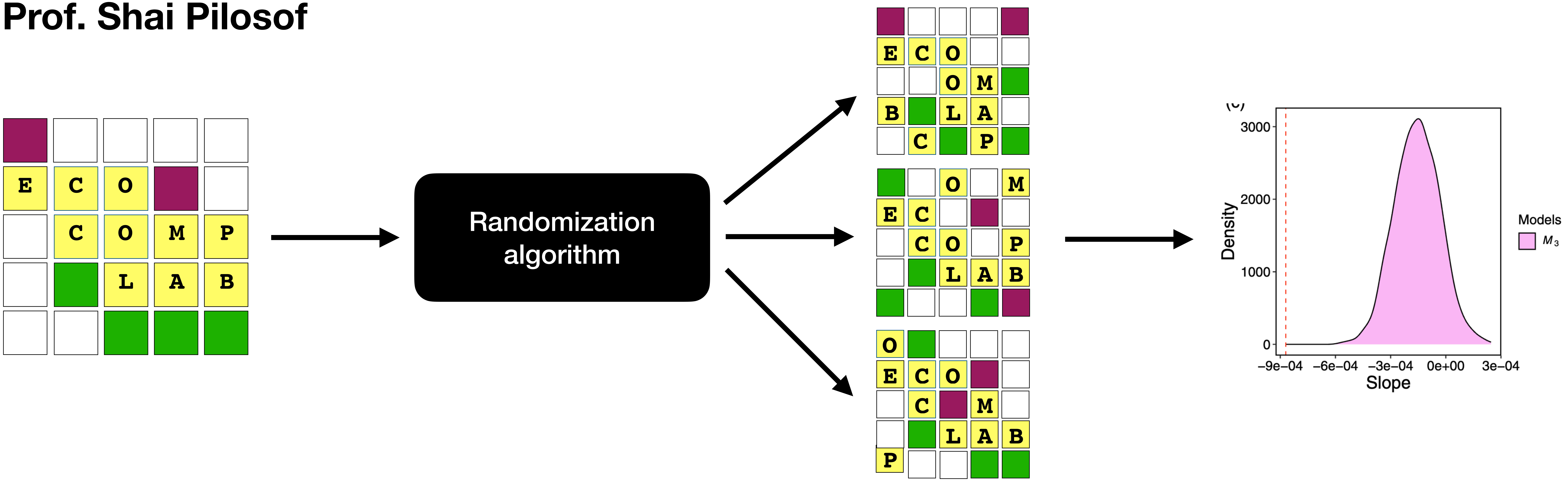
Hypothesis testing

Analysis of Ecological - Biological Networks 2026

Prof. Shai Pilosof



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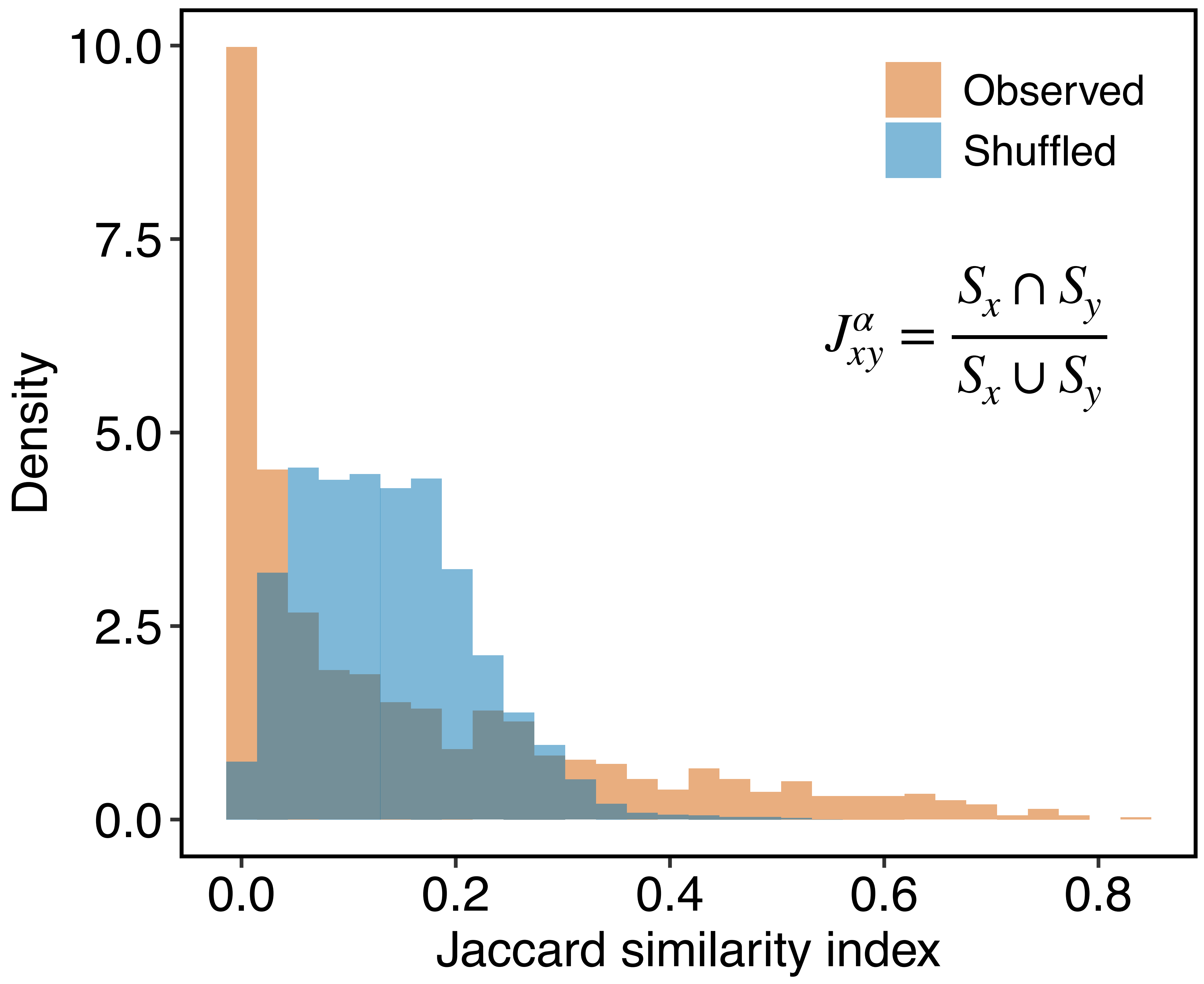
Why do we need a reference baseline?

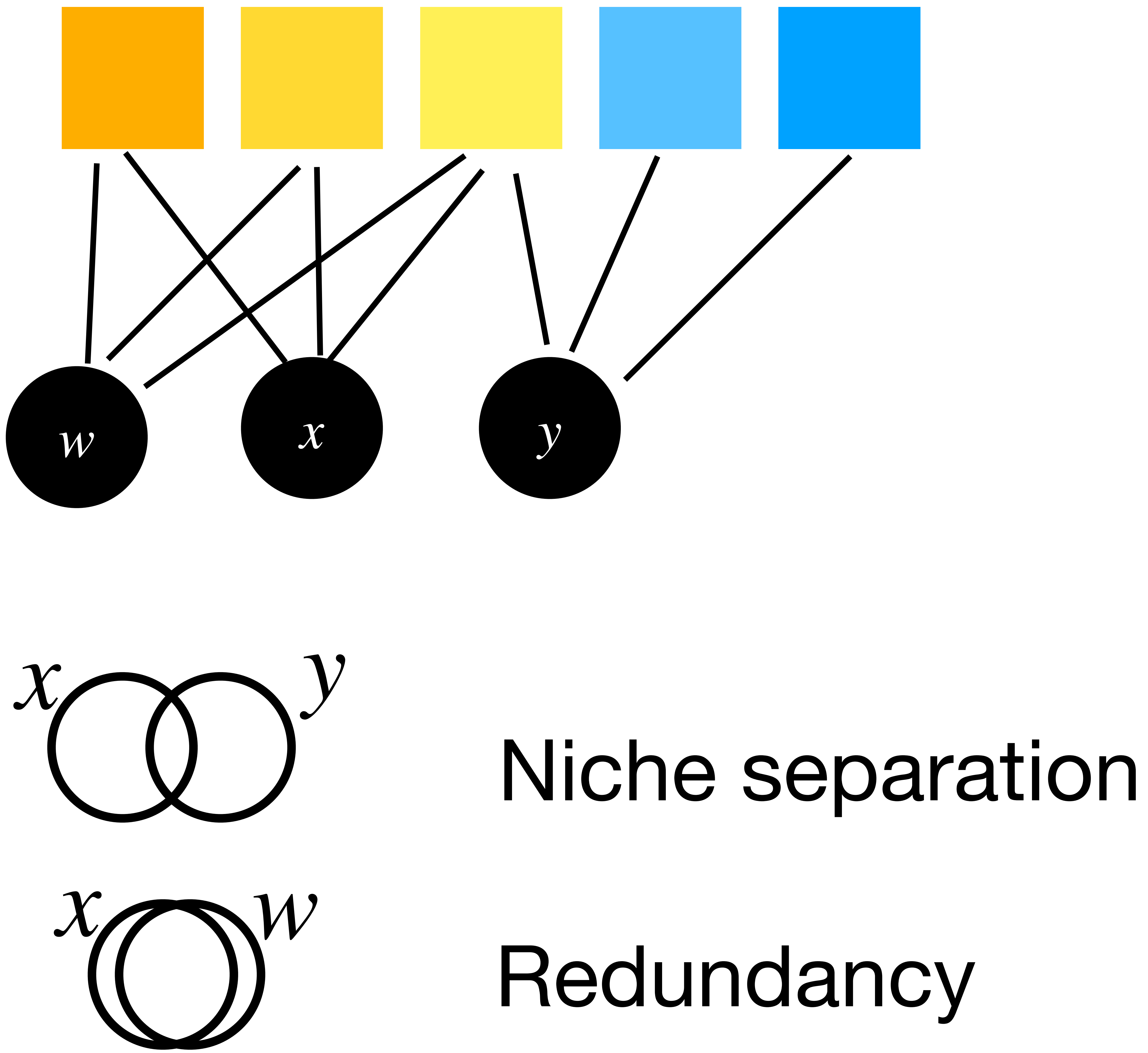
- Network metrics (nestedness, modularity, motifs) are meaningless without a reference point.

- Distinguishing patterns driven by biological mechanisms (e.g., resource selection) from neutral or random effects (e.g., relative abundance or connectance).

A pattern-generating algorithm that randomizes certain components of the data while keeping others fixed.

Chaperones have strong niche differentiation but some redundancy





Galai, He, Rotblat, Pilosof, Nature Communications 2023

Categories for a reference baseline

Fully-specified distributions (e.g., ANOVA)

Require a fully-specified null distribution (which we usually don't have)

focus on rejecting a point hypothesis (e.g., the mean Modularity is 0.4)

Non-parametric / Resampling Methods.

A "cloud" of possible worlds that preserve specific biological constraints.

Focus on resulting patterns, not mechanism

Test a specific null hypothesis

Generative Models

Attempt to recreate the exact mechanism (e.g., species only eat things smaller than them)

Typically require many parameters

Test if a specific mechanism is necessary to explain the network

Types of reference baselines

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Test a specific null hypothesis

Commonly known as **null models**

Generative Models

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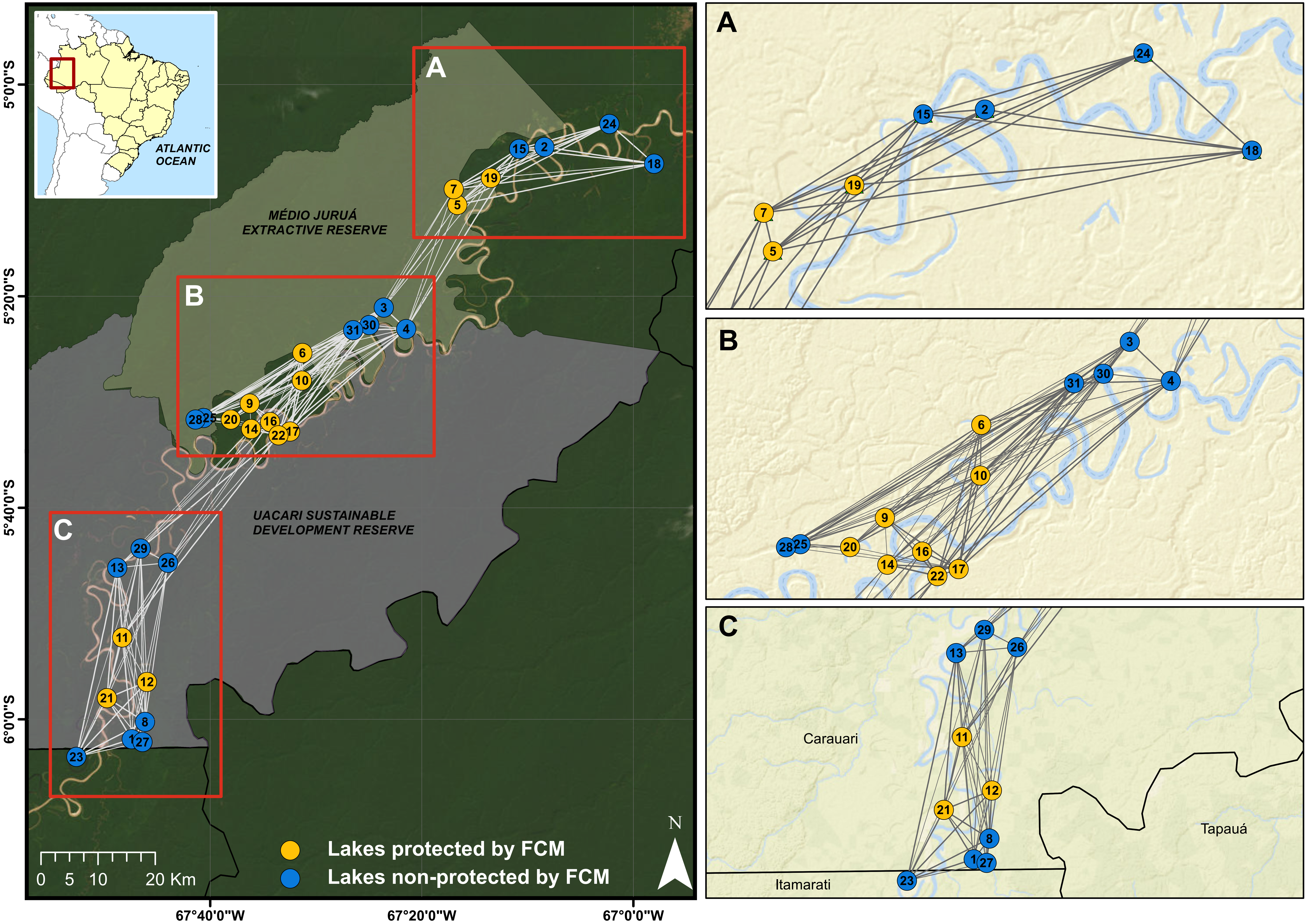
Typically require many parameters

Test if a specific mechanism is necessary to explain the network

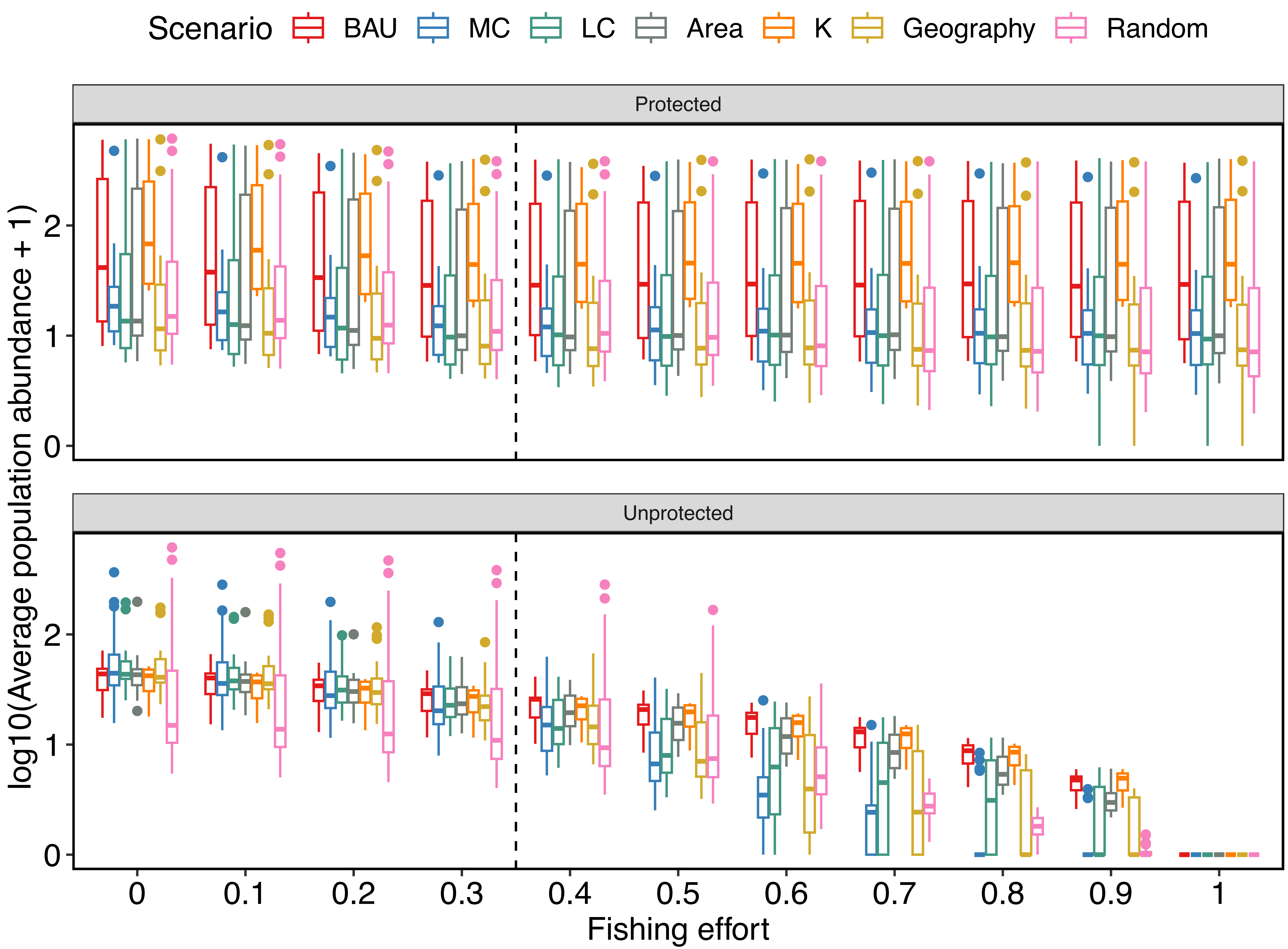
Null models - Rationale

The question: Is the observed property (e.g., modularity) a result of biological selection, or is it an inevitable byproduct of simpler constraints like species richness or link density?

The logic: If we can build a "random" version of the network that keeps the basic properties (the constraints) but shuffles the structure, we can see if the observed pattern is likely due to a biological process.

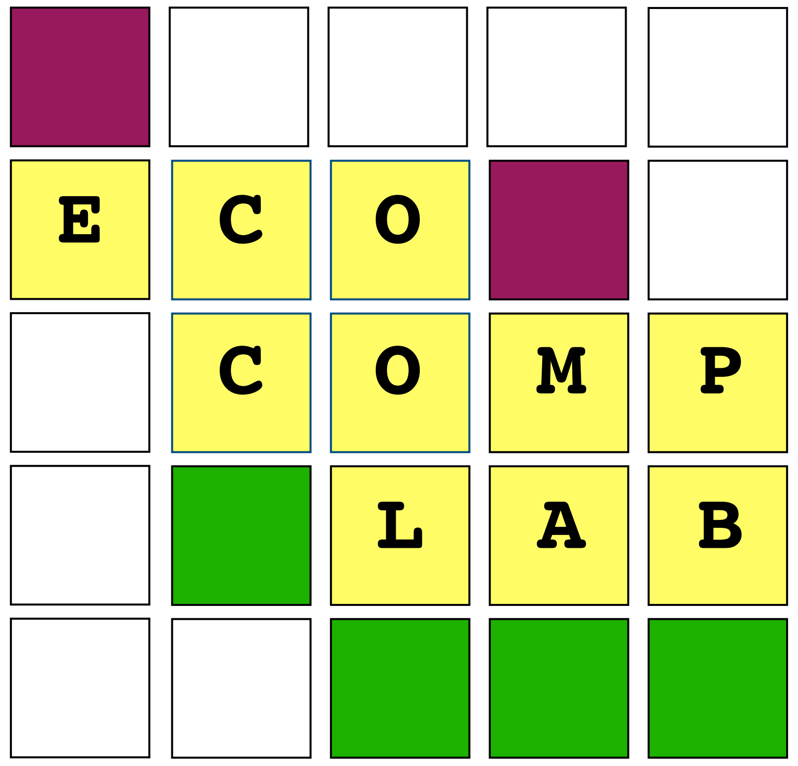


Emer et al, 2026, J Applied Ecology



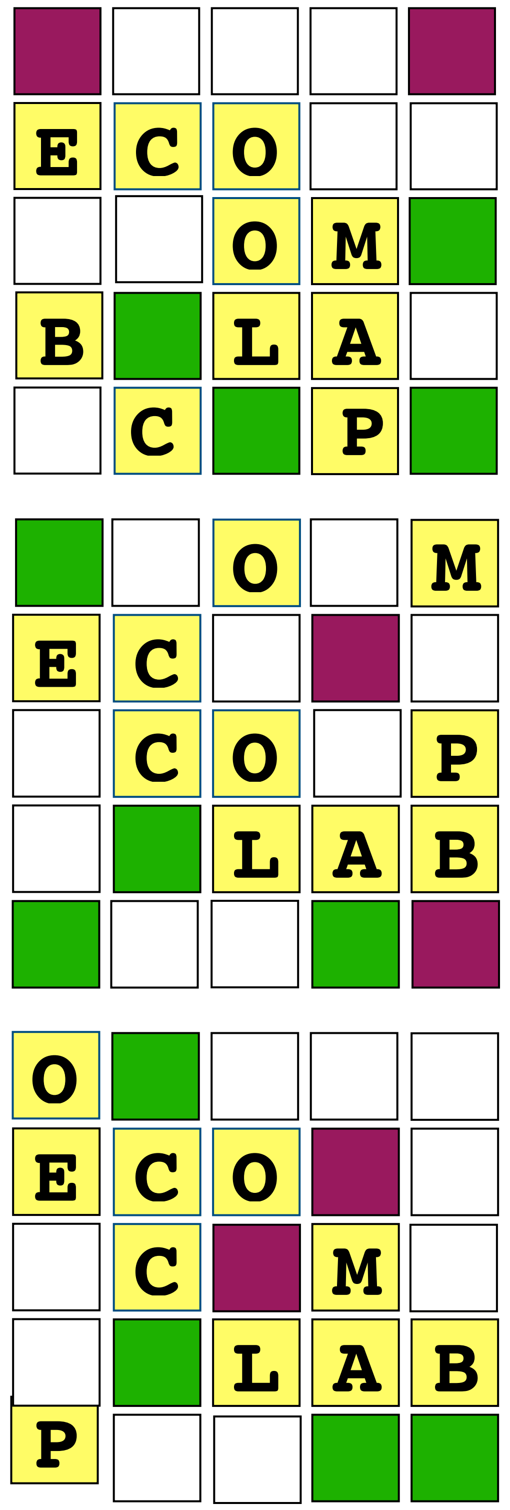
Emer et al, 2026, J Applied Ecology

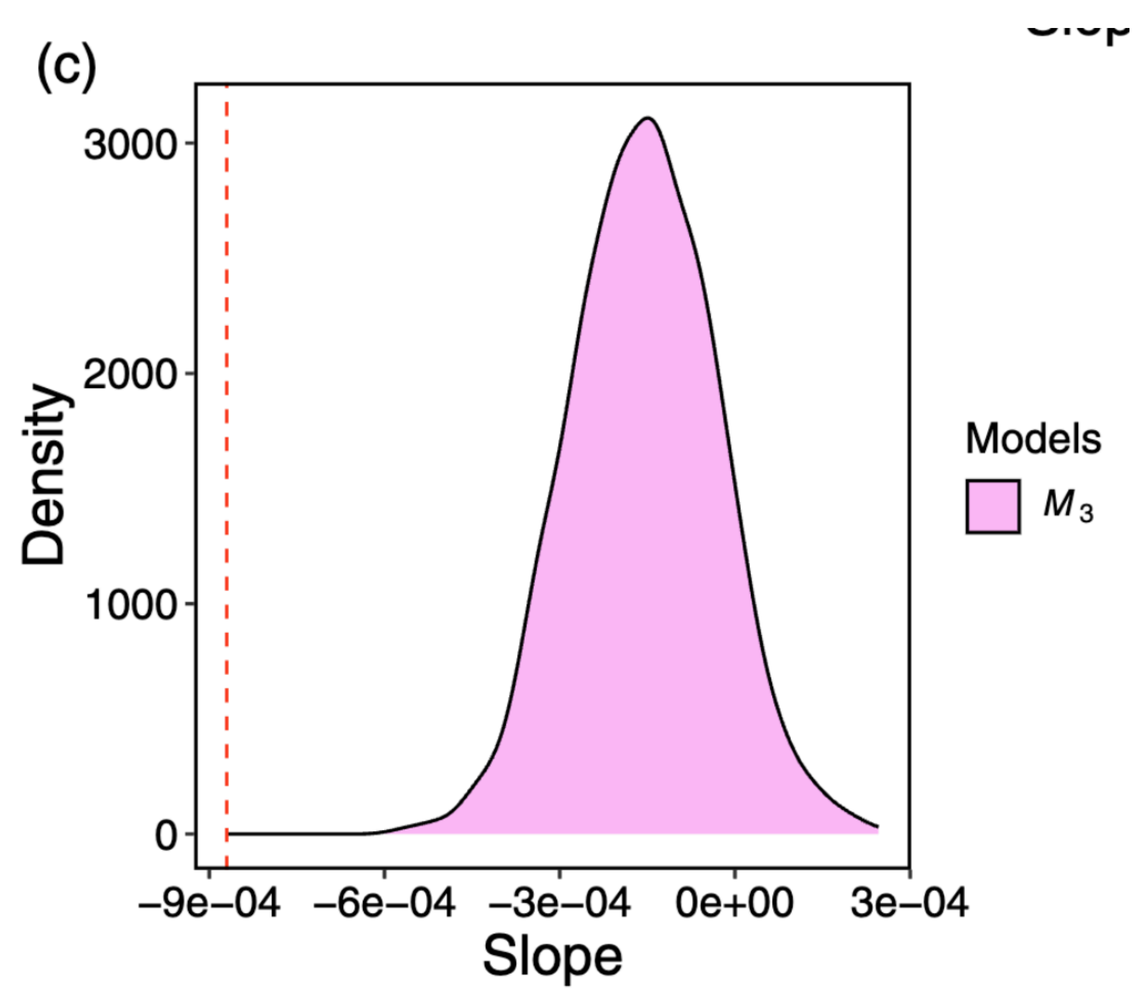
Null models - workflow



Randomization algorithm

Better to have more than one





Better to have more than one

Null models - workflow

1. **Define constraints:** Decide what to keep fixed from the original data (e.g., total links, the exact number of links for every species).

2. **Randomize**: Use a randomization procedure to generate a large "cloud" of randomized matrices (usually 1,000 or more).

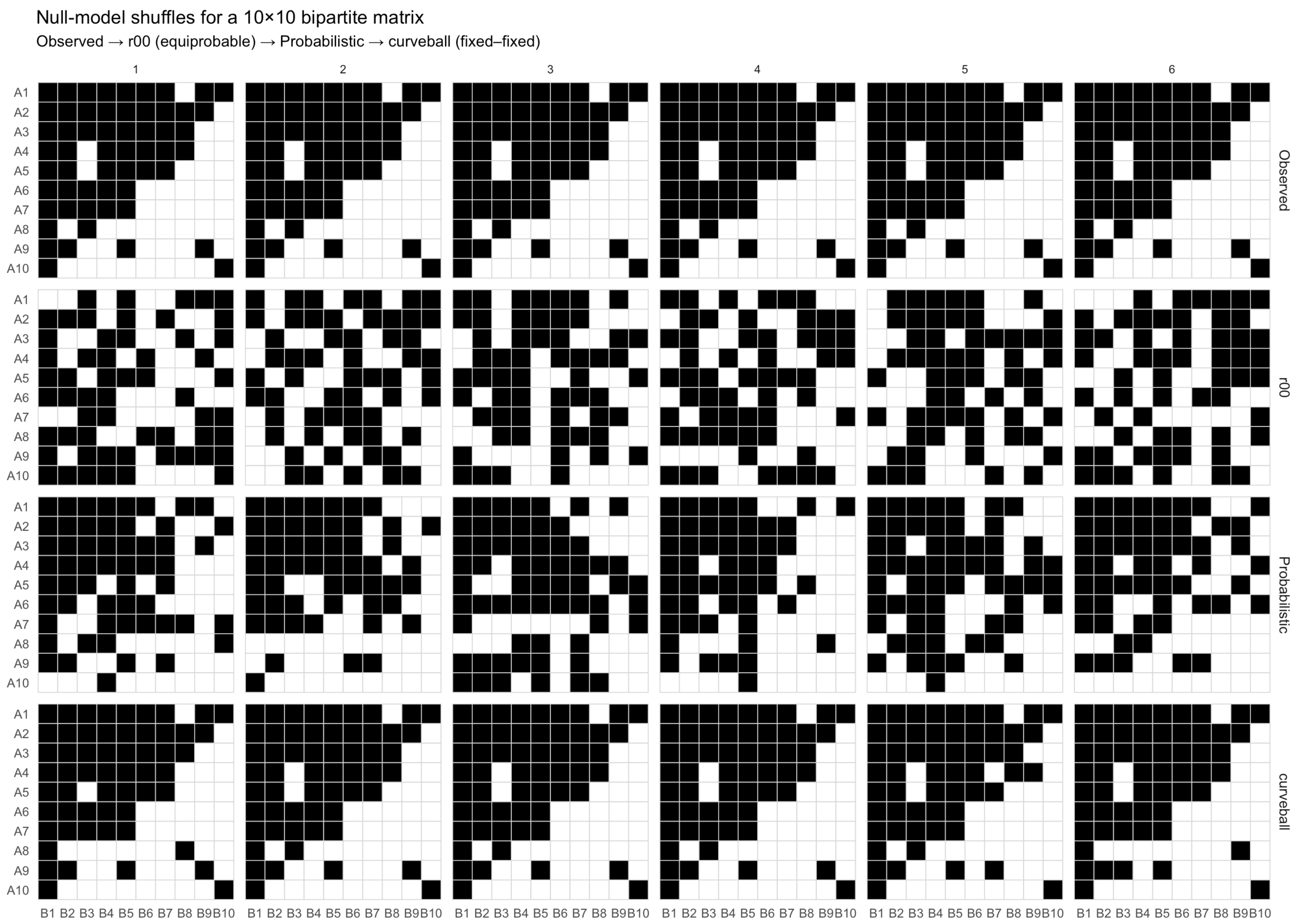
3. **Compare**: Calculate your metric (Nestedness, Modularity, etc.) for the observed network and for every single randomized network. Then compare observed to randomized.

Null models - main classes

What do we constrain in each model?

	Equiprobable	Proportional	Fixed
Equiprobable	$P(a_{ij}) = 1/RC$	$P(a_{ij}) = \dfrac{\Sigma_j^C}{NR}$	$P(a_{ij}) = 1/R$
Proportional	$P(a_{ij}) = \dfrac{\Sigma_i^R}{NC}$	$P(a_{ij}) = \dfrac{\Sigma_i^R \cdot \Sigma_j^C}{N^2}$	$P(a_{ij}) = \dfrac{\Sigma_i^R}{N}$
Fixed	$P(a_{ij}) = 1/C$	$P(a_{ij}) = \dfrac{\Sigma_j^C}{N}$	$P(a_{ij}) = 1/R$

Gotelli 2000, Ecology


Null-model shuffles for a 10×10 bipartite matrix
Observed → r00 (equiprobable) → Probabilistic → curveball (fixed–fixed)
1
2
3
4
5
6
Observed
r00
Probabilistic
curveball
A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
B1 B2 B3 B4 B5 B6 B7 B8 B9 B10

Null models - comparing observed to shuffled

1. **P-value:** The proportion of random networks that have a metric value more/less extreme than your observation.

$$P_{upper} = \frac{1}{N}\sum_{i=1}^{N}[X_{null,i} \geq X_{obs}],\ P_{lower} = \frac{1}{N}\sum_{i=1}^{N}[X_{null,i} \leq X_{obs}]$$

$$P_{2-tailed} = 2 \times \min(P_{upper}, P_{lower})$$

2. **Z-score:** Measures how many standard deviations the observed value is from the null mean.

   $Z = (Observed - Mean_{null})/SD_{null}$. A score $> |1.96|$ is usually significant.

Null models - comparing observed to shuffled

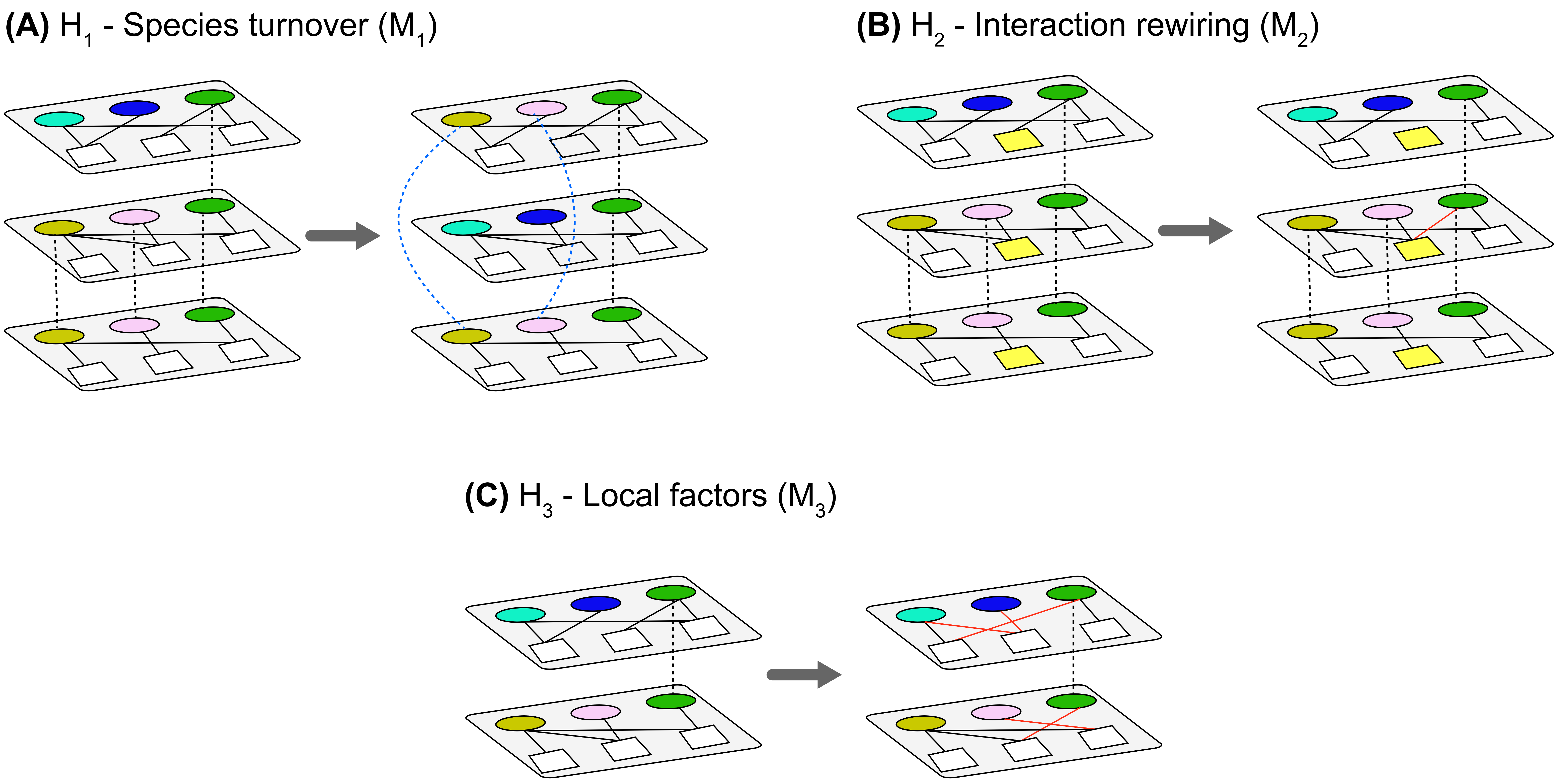
Consider:

- **Model constraints** and Type I (FP) and Type II (FN) errors
- **Z-scores can be misleading** if the maximum possible value of a metric (like NODF) changes with network size. If two networks have different "ceilings," their Z-scores aren't truly comparable. (*Song et al 2019*)
- **Non-Normality:** P-values are safer than Z-scores if the distribution of shuffled networks is skewed (not a bell curve). Always look at the histogram!

Null models - extensions

- Abundance-based models
- Weighted networks
- Phylogenetic constraints
- Metaweb-scale: Compare local networks to random draws from a regional pool of species.
- Time-shuffling: For networks sampled over seasons, shuffle interactions within time steps or across time steps to test if the "stability" or "rewiring" of the network is biologically driven.

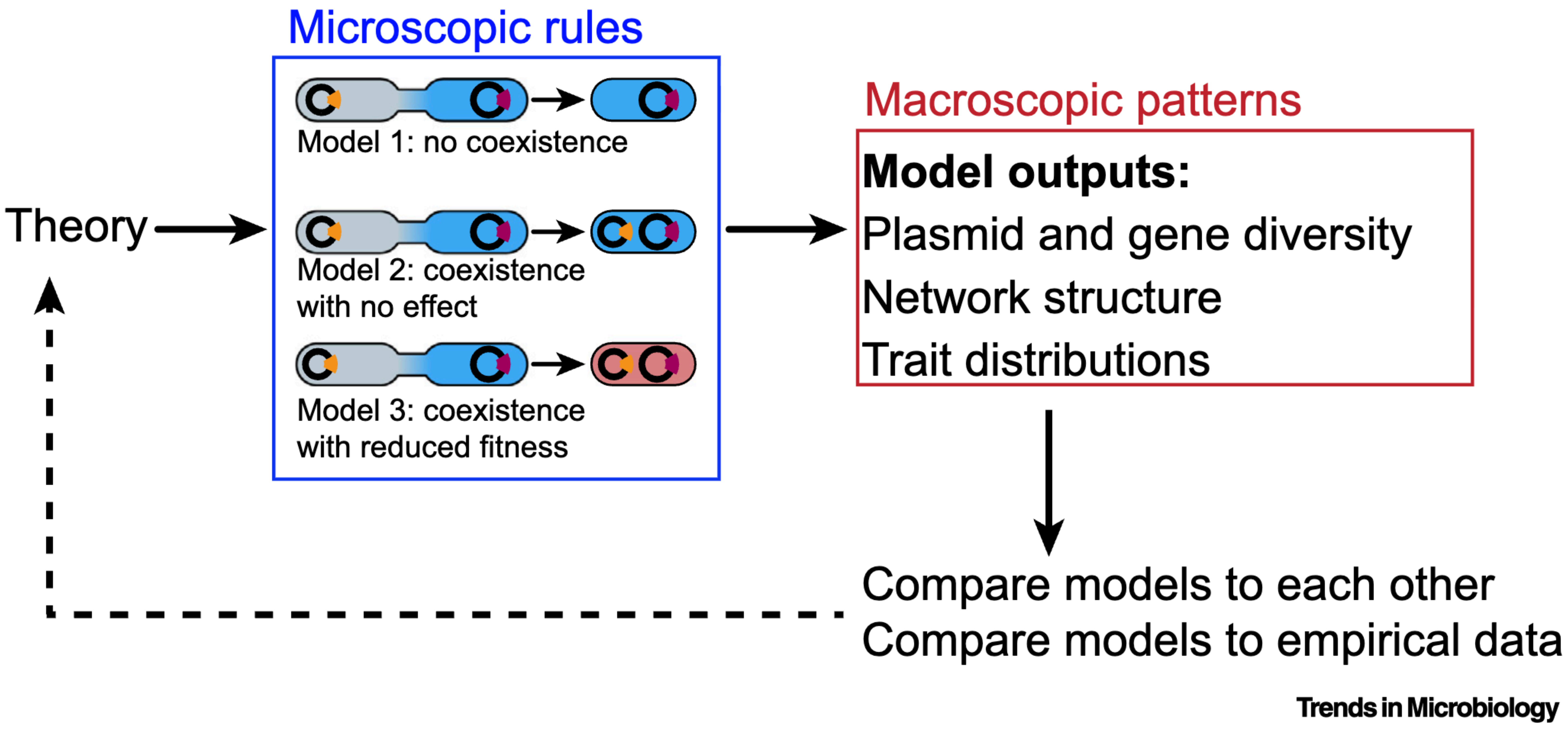
Spatial structure



Vitali et al, 2024

Generative models

Can we find a “simple” model capturing underlying processes behind repeatable patterns?



Pilosof, 2023

The Erdos Reyni model

Algorithmic Steps

1. Start with N nodes with no connections.
2. Define Probability p, which represents the likelihood of any given pair of nodes being connected by an edge.
3. Link Nodes Randomly: For each possible pair of nodes, generate a random number. If this number is less than p create an edge between the pair. Repeat for all node pairs.

The Erdos Reyni model

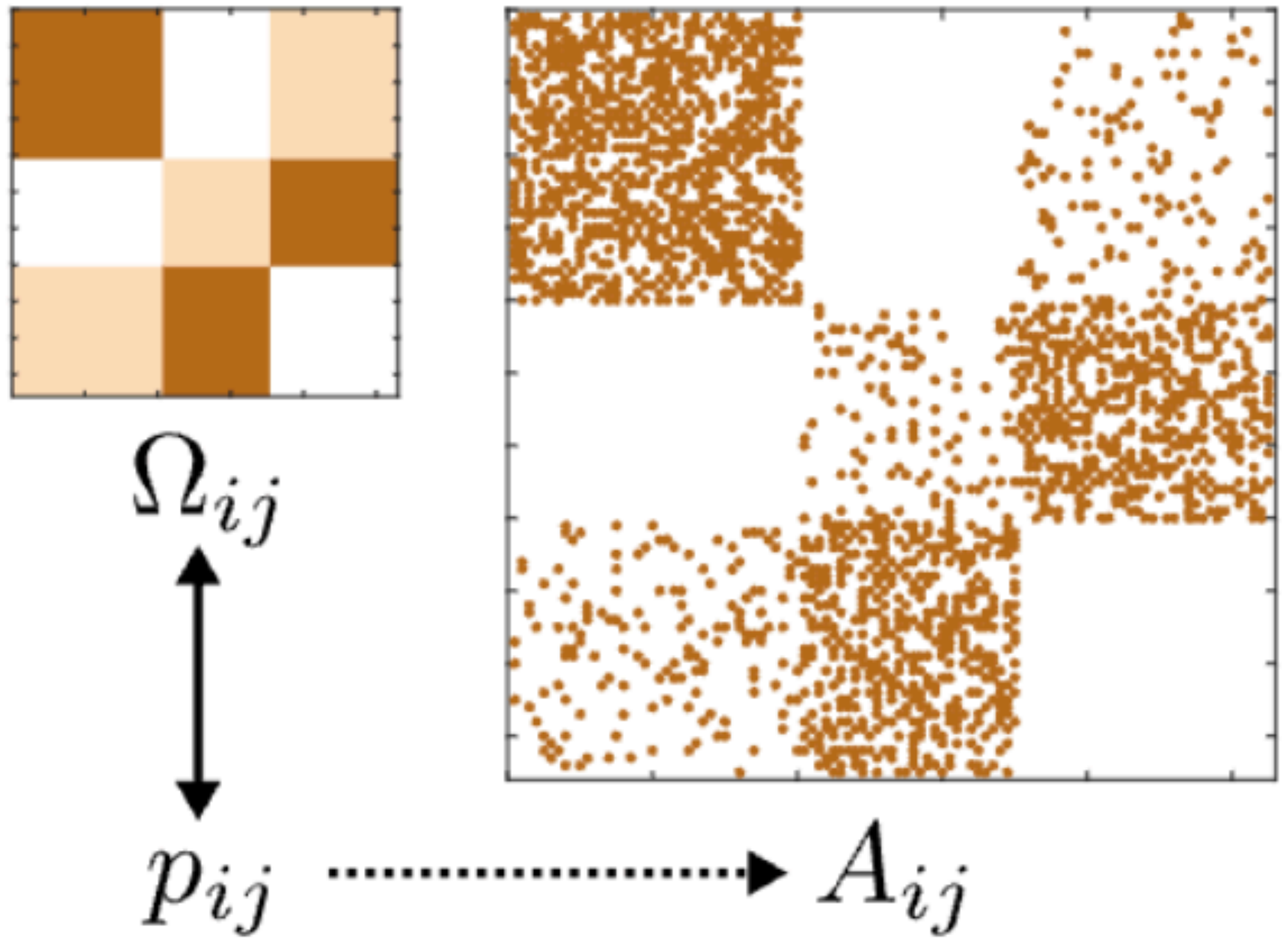
- Degree Distribution: a binomial distribution, which approximates a Poisson distribution for large N and small p.

Biological interpretation:

- Usefulness: Helpful in modeling random interactions such as random colonization of habitats or random mating.
- Limitations: Lacks structure and hierarchy typical to real biological networks like food webs or mutualistic networks.

Generating structured networks: Stochastic block models

$$P(A_{ij} = 1 \mid z_i, z_j) = \Omega_{z_i z_j}$$

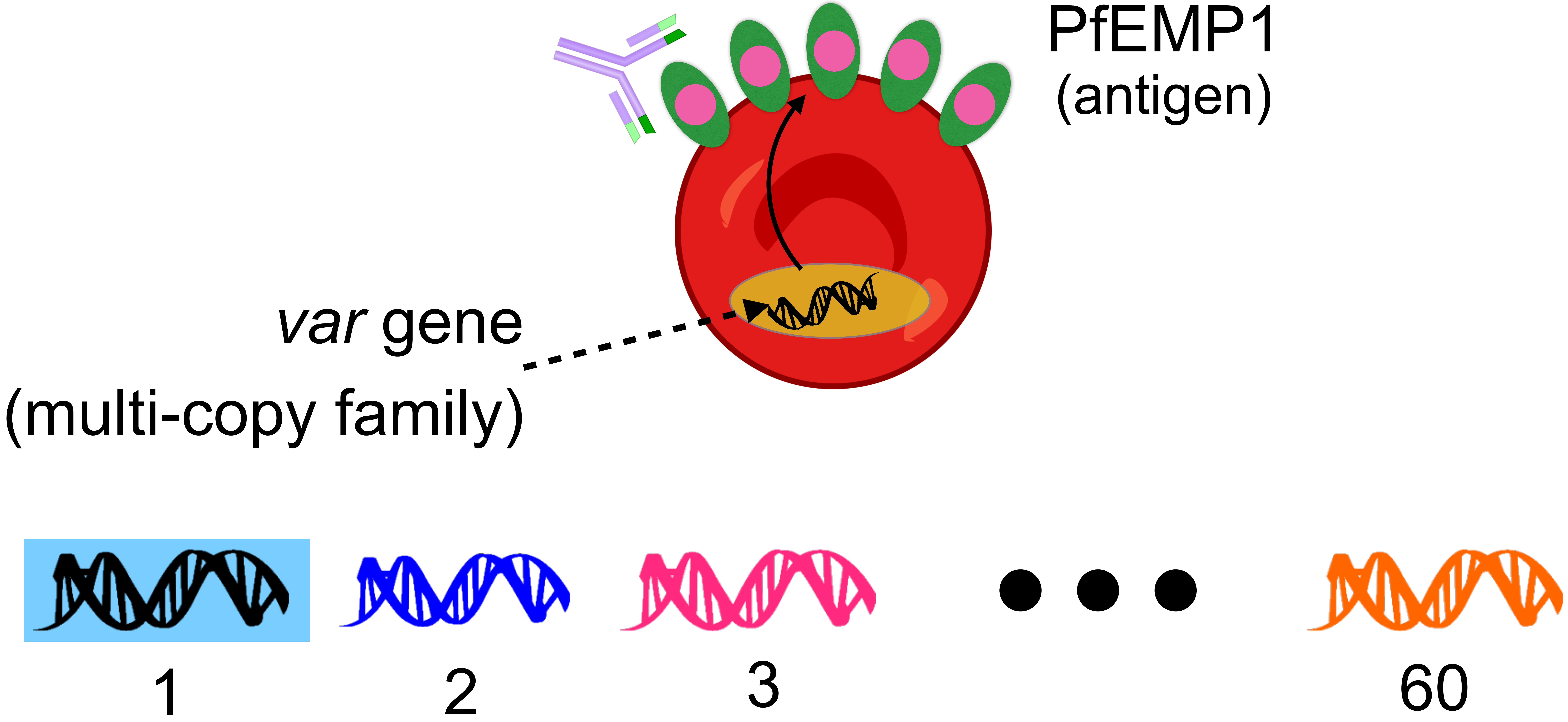


Set the probability of links within and between groups and then generate networks.

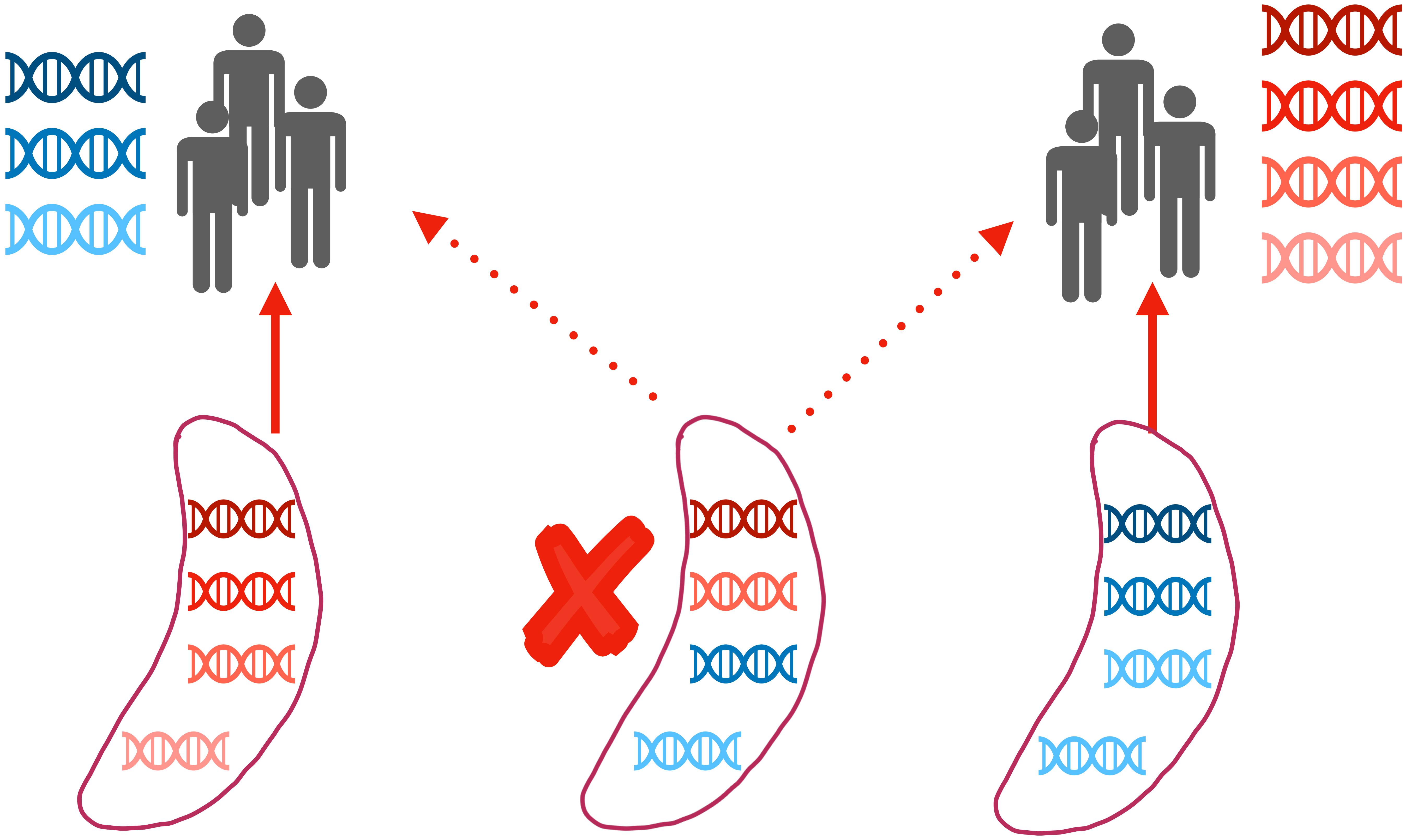
Generating other structures such as modularity and nestedness is also possible (some models exist out there).

Rosvall et al 2020, Peixoto 2021

Case study: malaria and human immunity



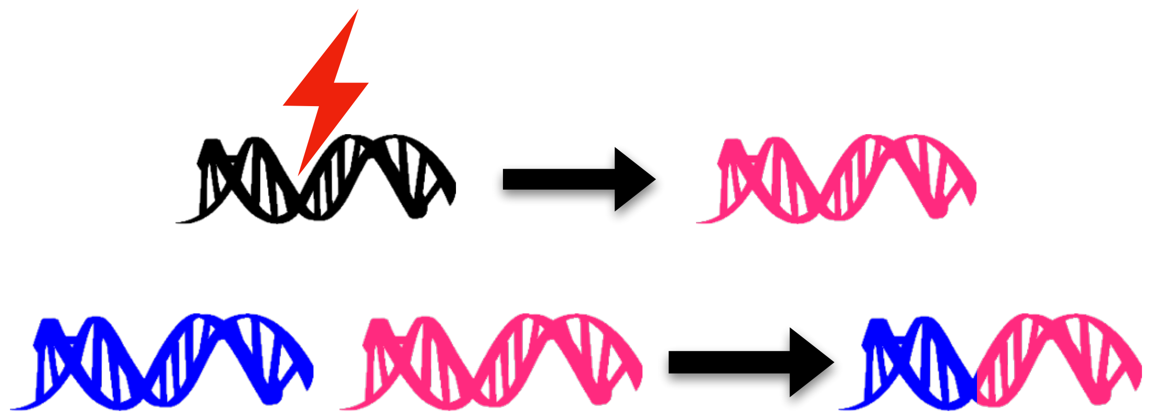
Competition mediated by immune selection results in a clustered population structure (limiting similarity)



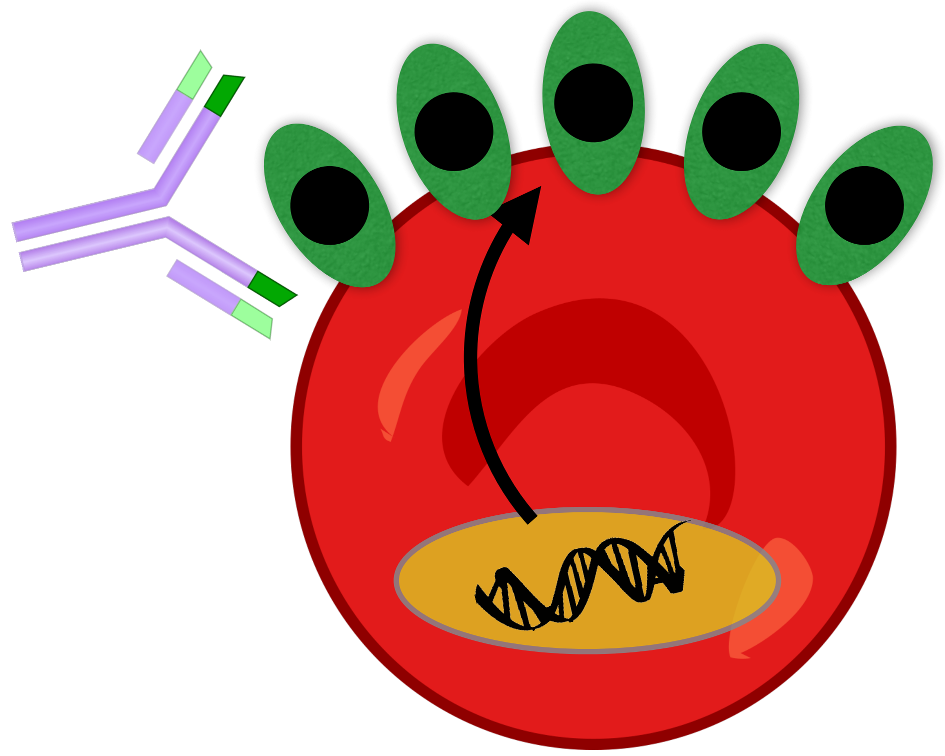
Gupta et al 1994;
Gupta & Day 1994;
Gupta et al. 1998

Modeling framework: agent-based formulation

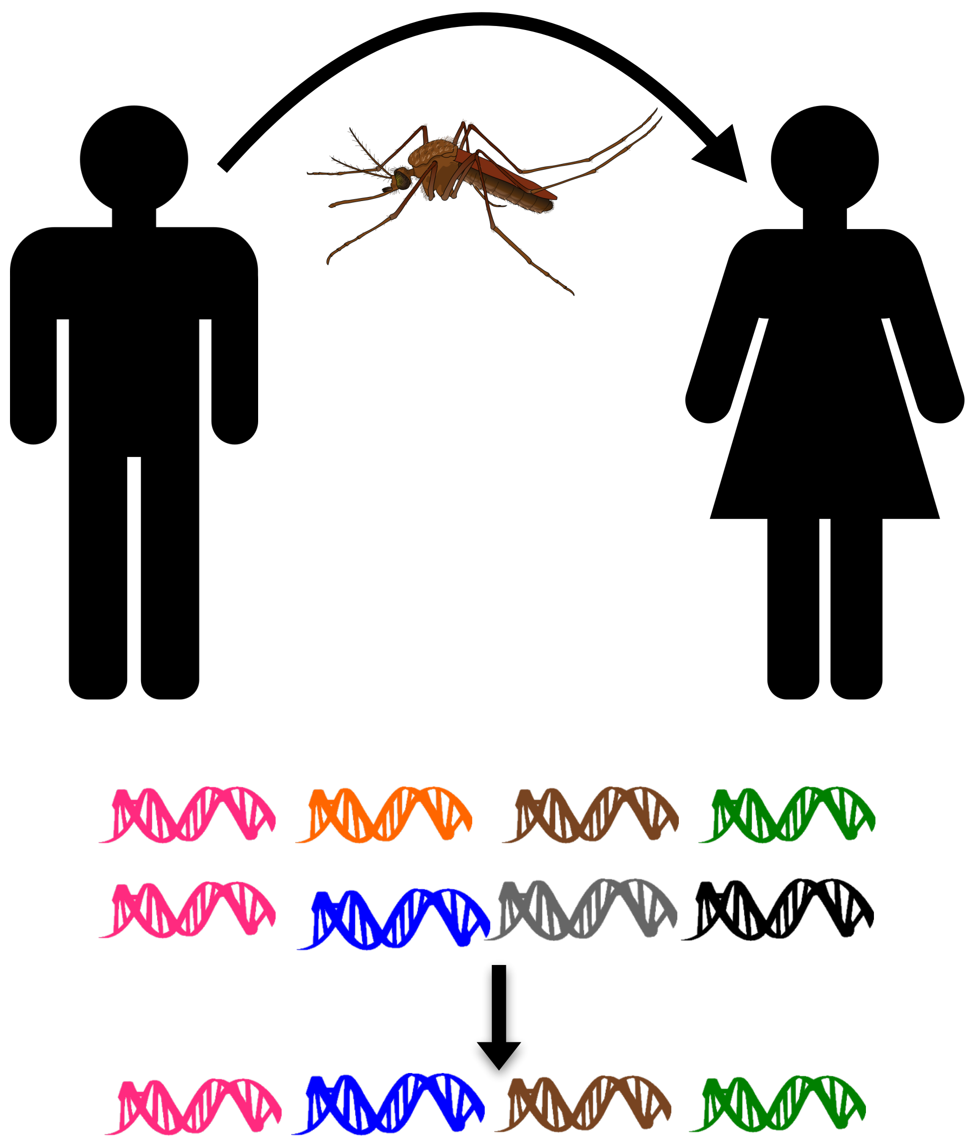
Parasite diversification



Host Memory



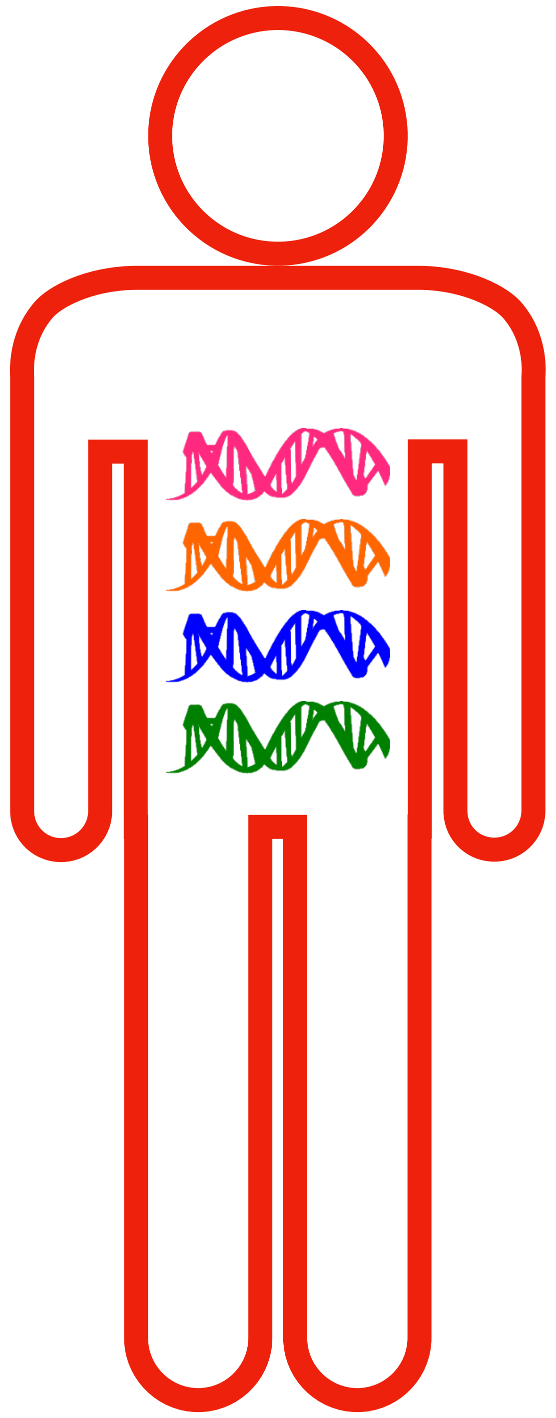
Transmission



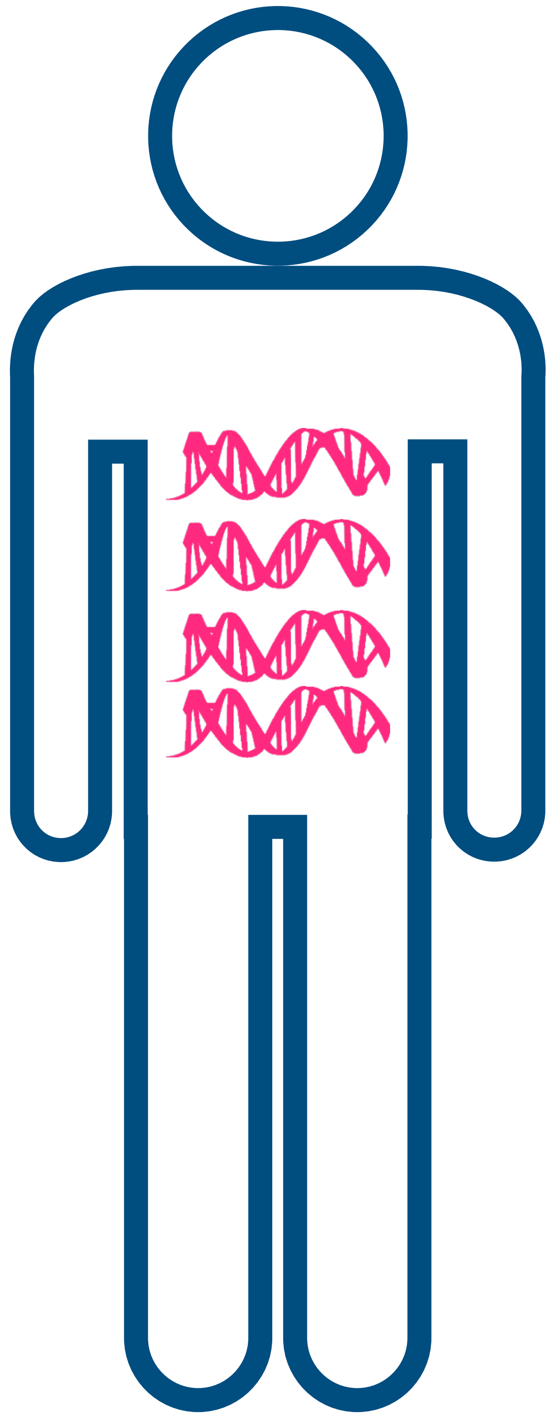
He, Pilosof et al. Nature Communications 2018

Selection + neutral models: testing hypotheses

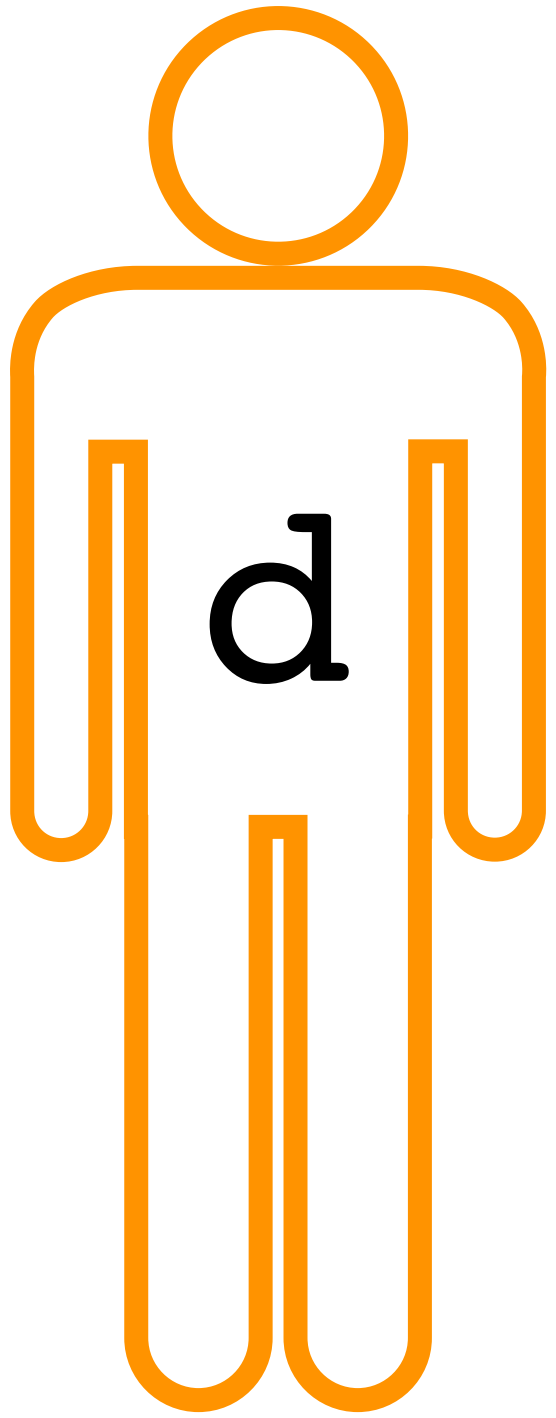
Duration of infection is the phenotypic trait under selection



Immune selection



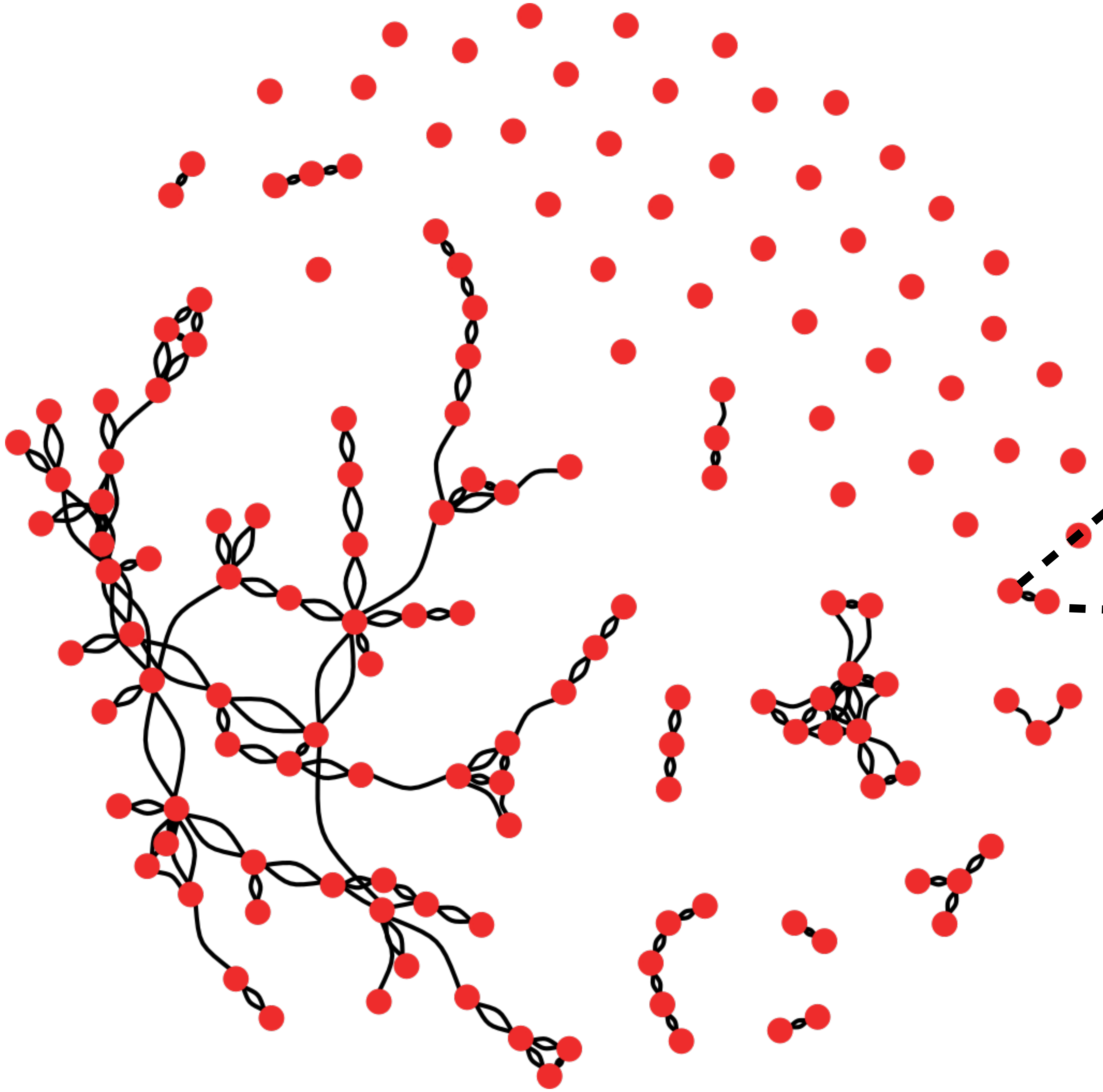
Generalized immunity

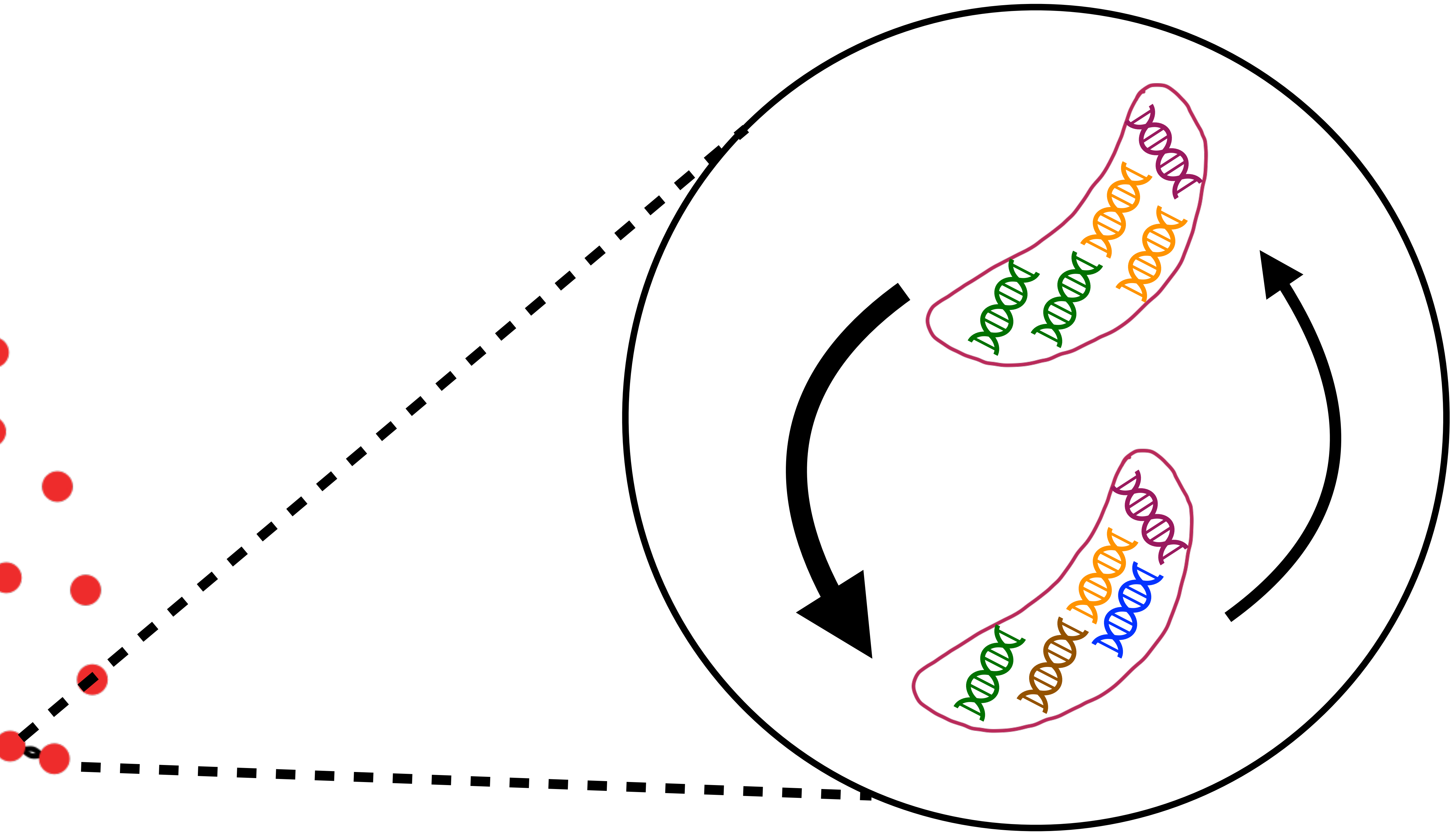


Complete neutrality

He, Pilosof et al. Nature Communications 2018

Competition (network links) is driven by immense selection

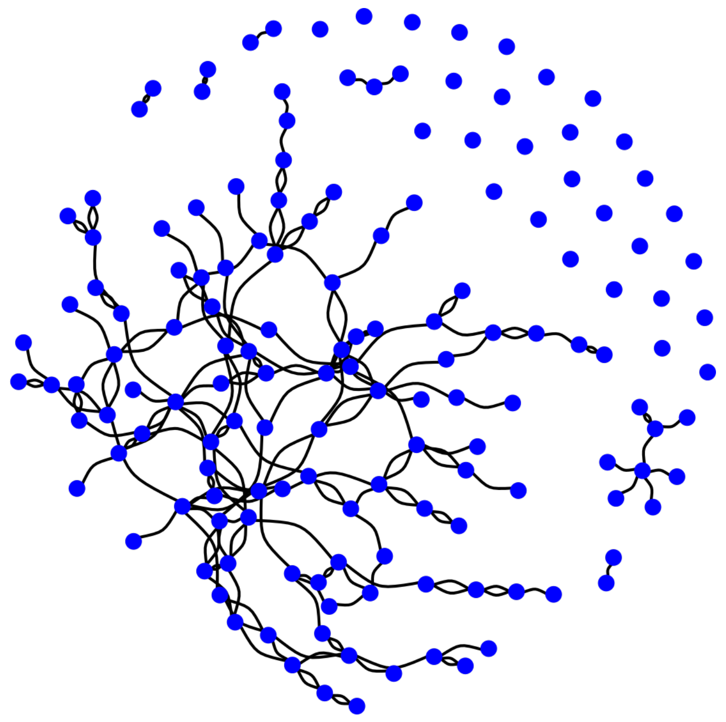




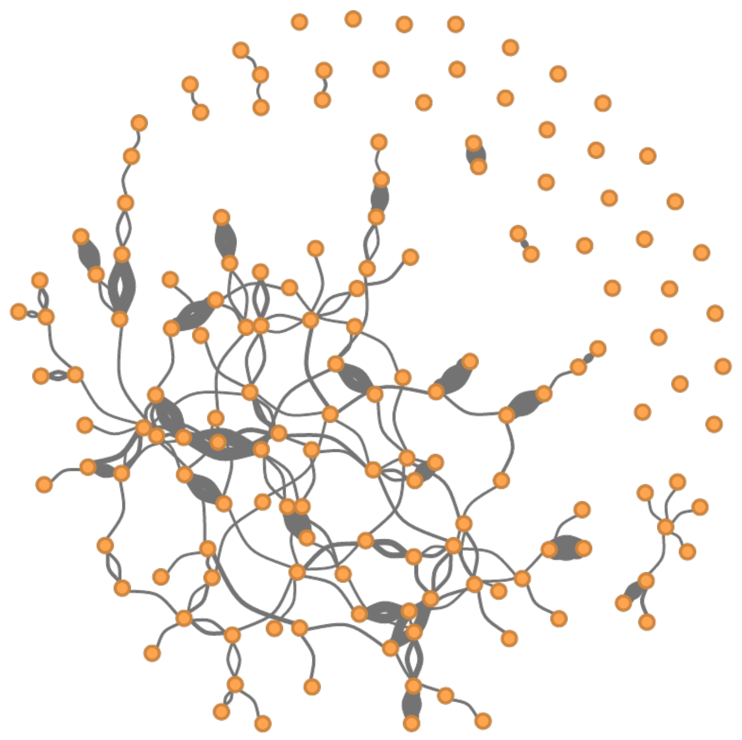
$$S_{i \to j} = \frac{n_i \cap n_j}{n_i}$$

He, Pilosof et al. Nature Communications 2018

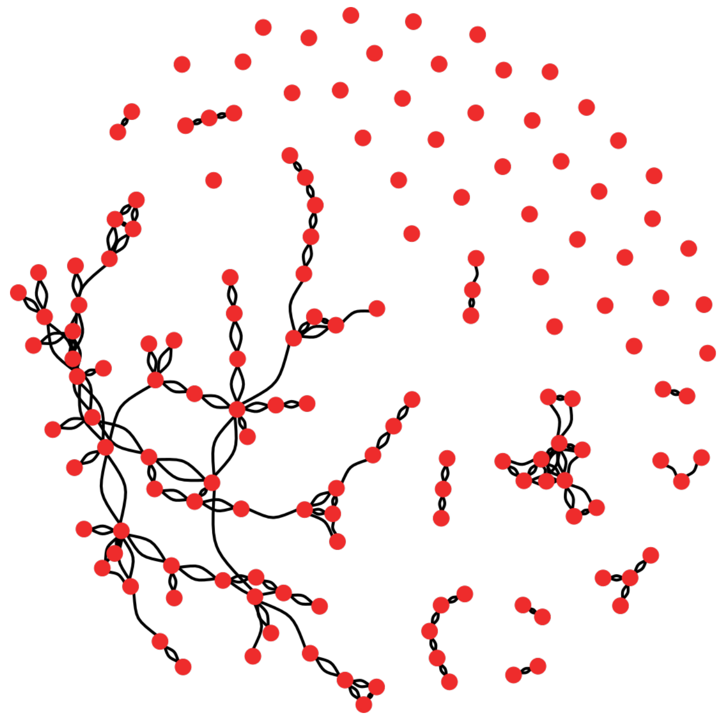
Combined approach to separate networks



Generalized immunity



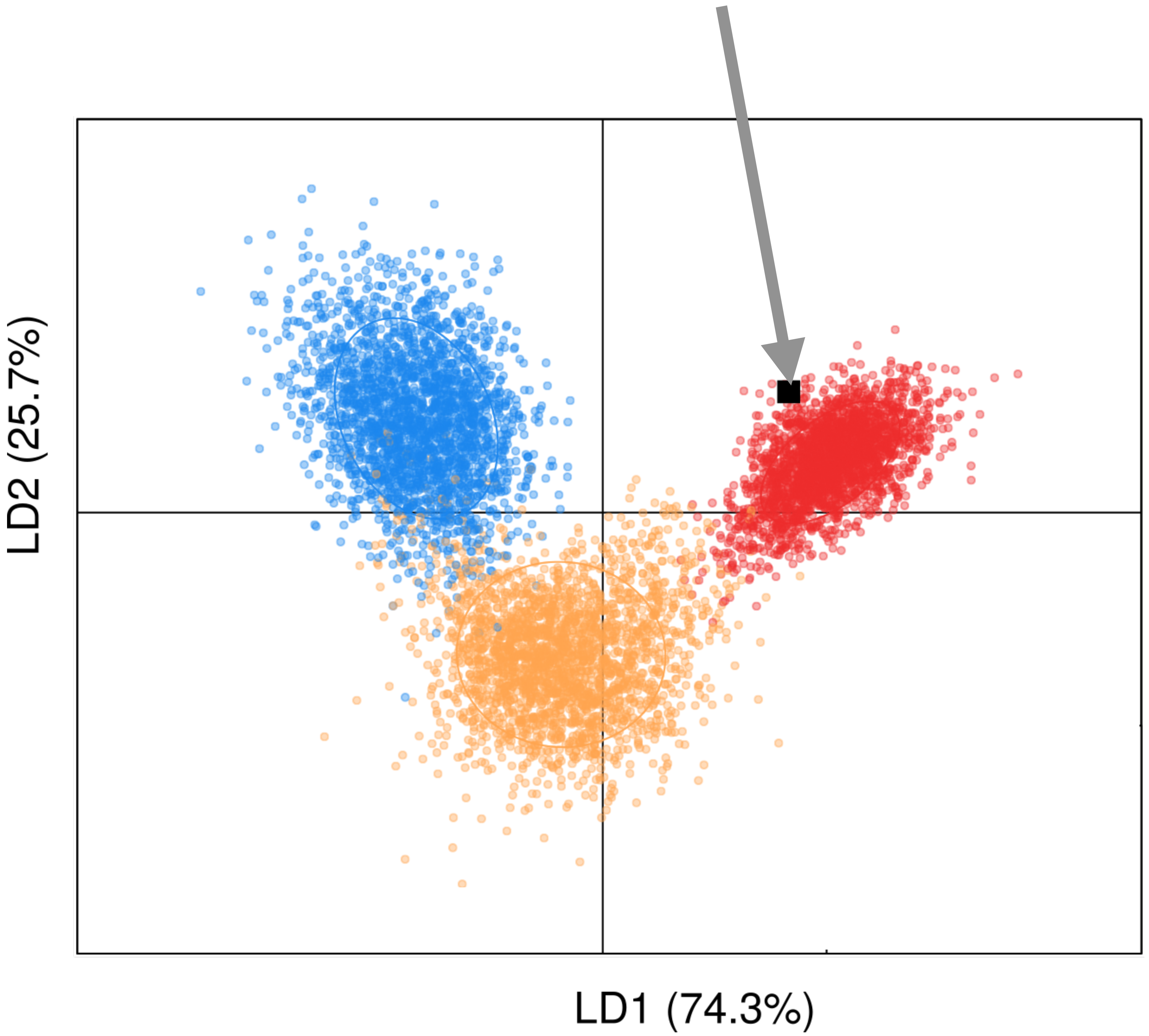
complete neutrality



Immune selection

A set of network properties + Discriminant analysis of principal components

empirical data



He, Pilosof et al. Nature Communications 2018