Practicalities

Using genetic associations with the environment to infer positive selection across genomes

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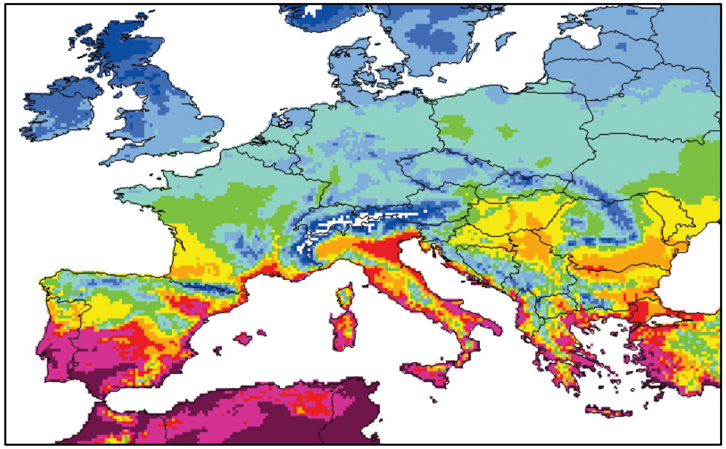
Max Planck Institute for Plant Breeding Research

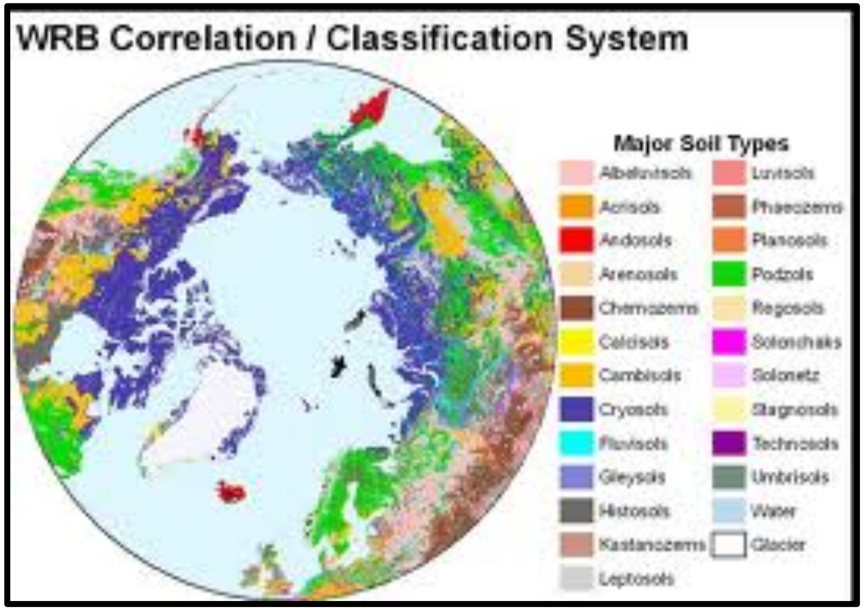
MAX-PLANCK-GESELLSCHAFT

Practical Matters

- Environmental Data Sets
- Methods
  - SAM
  - Dealing with confounding due to population structure
  - BayEnv
  - LFMM
  - Other Mixed model methods
- Simulation-based comparisons of methods



ISRIC – world soil information database



http://www.isric.org/

Climatic Research Unit University of East Anglia

Datasets are available in the following categories:

- Temperature (5°×5° gridded versions)
- Precipitation (5°×5° and 2.5°×3.75° gridded versions)
- Pressure and Circulation Indices
- UK Climate Indices
- Mediterranean climate
- Alpine climate data
- High-resolution gridded datasets
- NCEP/NCAR Reanalysis data - May 2011: updated for 2010
- Paleoclimate
- Drought indices

http://www.cru.uea.ac.uk/data

FAO GeoNetwork

- Agriculture and Livestock
- Applied Ecology
- Base Maps, Remote Sensing
- Biological and Ecological Resources
- Climate
- Fisheries and Aquaculture
- Forestry
- Human Health
- Hydrology and Water Resources
- Infrastructures
- Land Cover and Land Use
- Population and Socio-Economic Indicators
- Soils and Soil Resources
- Topography

http://www.fao.org/geonetwork/srv/en/main.home

WORLDCLIM Project provides variables at several resolutions

variable	10 minutes	5 minutes	2.5 minutes	30 seconds
minimum temperature (°C)	tmin 10m	tmin 5m	tmin 2.5m	tmin 30s
maximum temperature (°C)	tmax 10m	tmax 5m	tmax 2.5m	tmax 30s
average temperature (°C)	tavg 10m	tavg 5m	tavg 2.5m	tavg 30s
precipitation (mm)	prec 10m	prec 5m	prec 2.5m	prec 30s
solar radiation (kJ m^{-2} day^{-1})	srad 10m	srad 5m	srad 2.5m	srad 30s
wind speed (m s^{-1})	wind 10m	wind 5m	wind 2.5m	wind 30s
water vapor pressure (kPa)	vapr 10m	vapr 5m	vapr 2.5m	vapr 30s

Fick and Hijmans, 2017
www.worldclim.org

Bioclim variables are derived from monthly WORLDCLIM data to create meaningful variables

BIO1 = Annual Mean Temperature
BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3 = Isothermality (BIO2/BIO7) (* 100)
BIO4 = Temperature Seasonality (standard deviation *100)
BIO5 = Max Temperature of Warmest Month
BIO6 = Min Temperature of Coldest Month
BIO7 = Temperature Annual Range (BIO5-BIO6)
BIO8 = Mean Temperature of Wettest Quarter
BIO9 = Mean Temperature of Driest Quarter
BIO10 = Mean Temperature of Warmest Quarter
BIO11 = Mean Temperature of Coldest Quarter
BIO12 = Annual Precipitation
BIO13 = Precipitation of Wettest Month
BIO14 = Precipitation of Driest Month
BIO15 = Precipitation Seasonality (Coefficient of Variation)
BIO16 = Precipitation of Wettest Quarter
BIO17 = Precipitation of Driest Quarter
BIO18 = Precipitation of Warmest Quarter
BIO19 = Precipitation of Coldest Quarter

An early method: SAM (spatial analysis method)

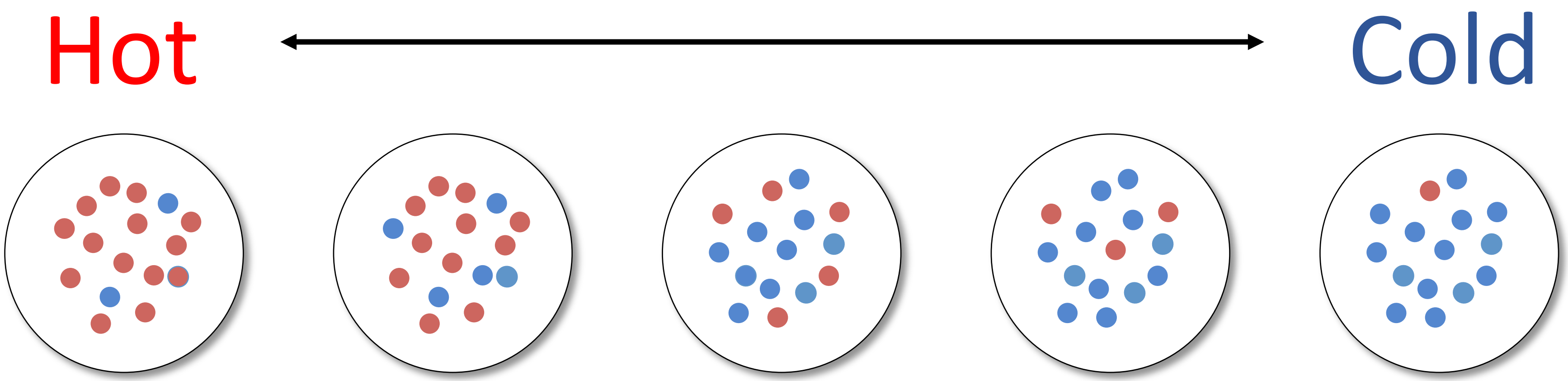
- Simple linear model method
- Use geo-referenced environmental data and marker data with a focus on microsatellite data (for each possible state, set to 0 or 1)
- Test association between each allele and environmental variable using logistic regression
- Assess significance using two methods:
  - Likelihood ratio test
  - Wald test

$$G = -2ln\frac{L}{L'}$$

$$W = \frac{\beta_i}{\sigma(\beta_i)}$$

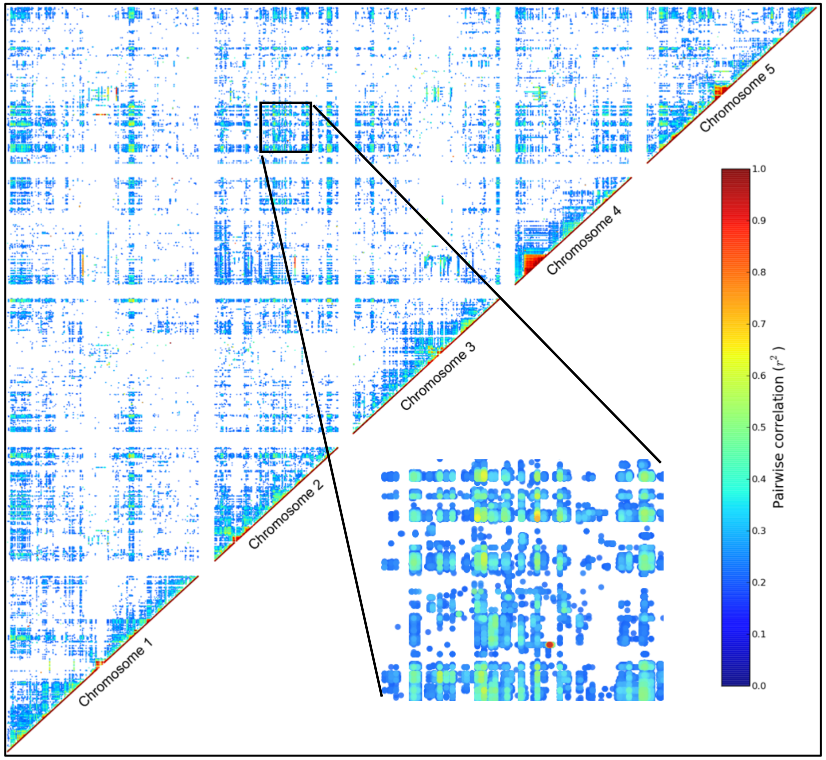
Joost et al., 2007, 2008, 2009

But confounding due to population structure may arise if structure correlates with the environmental variable



…even when the SNP has no functional effect

Population Structure causes correlations across the genome



Controlling for population structure can provide power to separate the signal from noise

Some methods to deal with Population Structure

- Genomic control: Scale down the test-statistic so that its median becomes the expected median.

- Use the first **n** principle components of the genotype matrix (Price *et al.*, 2006).

- Model the genotype effect as a random term in a mixed model, by explicitly describing the covariance structure between the individuals.

BayEnv: a linear mixed model method to assess evidence for correlations with environment

- Models the joint distribution of allele frequencies across populations for a variant as a function of
  - Population ‘history’ (null model)
  - Population ‘history’ + environment (alternative model)
- Then asks whether there is evidence a variant is an adaptation to a particular climate variable by comparing these two models in a Bayesian framework

Population history

- Demographic history is included in the model via a covariance matrix of populations
  - This is different from the assumption of quantitative trait mapping approaches, which include the kinship matrix to control for other loci that contribute to the trait (infinitesimal model)!
- The covariance structure is modeled under the assumption that transformed population allele frequencies have a multivariate normal distribution

Bayenv method

$$H_0:\ y = \beta_0 + \mu + \varepsilon$$

$$H_1:\ y = \beta_0 + \beta_1 x + \mu + \varepsilon$$

$$BF = \frac{Pr(D|M_1)}{Pr(D|M_0)}$$

where y is the vector of allele frequencies,

β_0 is the intercept,

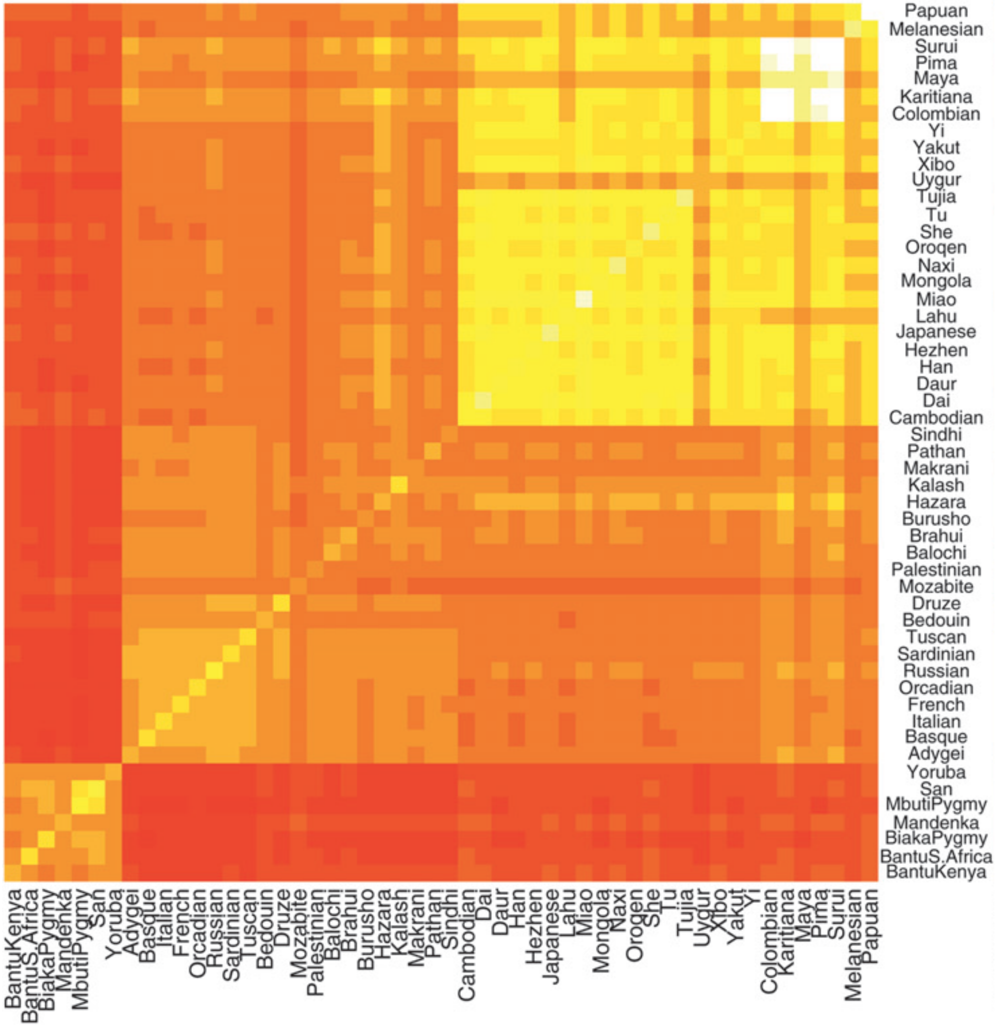
μ is the random effect term due to population history, and

ε is the random error

x is the environmental variable,

β_1 is the effect size of environmental variable on allele frequencies,

Bayenv uses the (predicted) variance/covariance matrix to control for population structure



Generating the kinship matrix

Since the population allele frequency is drawn from a normal distribution, it could be <0 or >1, which doesn't make sense, therefore, a simple transformation is used:

$$x_{kl} = g(\theta_{kl}) = \begin{cases} 0 & \text{if} \theta_{kl} < 0 \\ \theta_{kl} & 0 \leq \theta_{kl} \leq 1 \\ 1 & \theta_{kl} > 1. \end{cases}$$

Population allele frequency variable, not constrained to be between 0 and 1

Generating the kinship matrix

Joint posterior over all loci

$$P(\Omega, \theta_1, \ldots, \theta_L, \varepsilon_1, \ldots, \varepsilon_L | \mathrm{n}_1, \mathrm{m}_1, \ldots, \mathrm{n}_L, \mathrm{m}_L) \propto$$

Prior on the allele counts

Prior on the vector of a.f.s

Prior on the covariance matrix

$$\left\{ \prod_{l=1}^{l=L} P(\mathrm{n}_l, \mathrm{m}_l | \mathrm{x}_l = g(\theta_l)) P(\theta_l | \Omega, \varepsilon_l) P(\varepsilon_l) \right\} P(\Omega).$$

Prior on the ancestral frequency at a locus

- MCMC to explore the sample space and sequentially update parameters
- Decide whether to accept θ'_l based on the ratio of the alternative to the null posterior

The Bayes factor

$$BF = \frac{Pr(D|M_1)}{Pr(D|M_2)}$$

Interpreting the Bayes factor

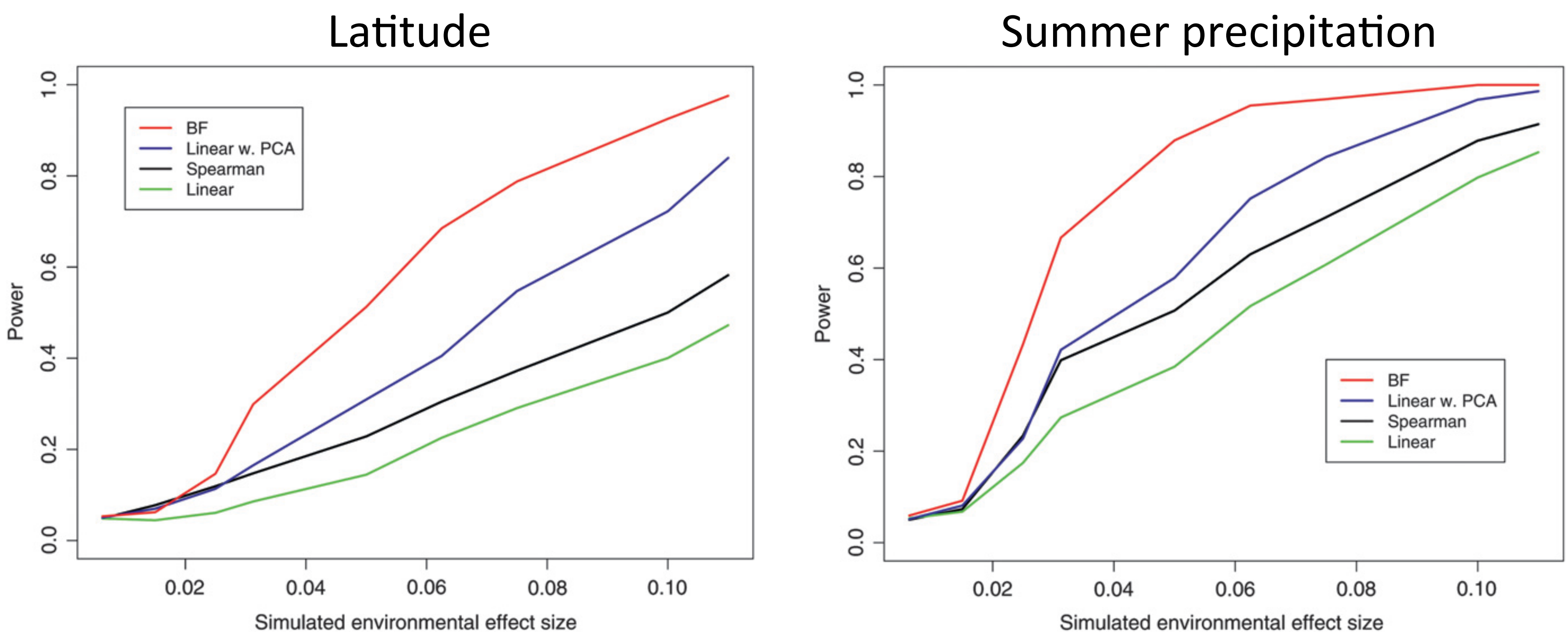
K	dB	bits	Strength of evidence
< 1:1	< 0		Negative (supports M_2)
1:1 to 3:1	0 to 5	0 to 1.6	Barely worth mentioning
3:1 to 10:1	5 to 10	1.6 to 3.3	Substantial
10:1 to 30:1	10 to 15	3.3 to 5.0	Strong
30:1 to 100:1	15 to 20	5.0 to 6.6	Very strong
> 100:1	> 20	> 6.6	Decisive

Jeffreys 1961

In practice, BayEnv authors recommend using a ranking approach rather than trusting the BFs are well-calibrated

Comparison of Bayenv to other methods

Power to detect a correlation between allele frequency and climate



Bayenv2

- Allows calculation of a standardized set of allele frequencies by removing the covariance among populations and making the residuals available for further analyses.

- Use these to:
  - Conduct non-model based tests of population differentiation
  - Non-parametric tests of correlation (e.g., Spearman's rho)

Latent factor mixed model approach (LFMM)

- Similar to BayEnv, but uses ***factors derived from*** the covariance matrix to model population history
- Individual-based rather than population-based
- ***Simultaneously models correlation with population structure and environment***, so could gain some power when structure is correlated with the environment

Frichot et al., 2013

LFMM: The Model

$$G_{il} = \mu_l + \beta_l^T X_i + U_i^T V_l + \varepsilon_{il},$$

where

G is a response variable in a Bayesian regression model

Gaussian prior distributions on μ and β_l

U_i and V_l are scalar vectors with Gaussian priors

B_l is a vector of regression coefficients

- Use Gibbs sampler to move through sample space
- Use a stochastic algorithm to compute standard deviations and **z**-scores for the environmental effects.
- Compare each locus to the genomic background and retained loci with **z**-scores exhibiting the highest absolute values

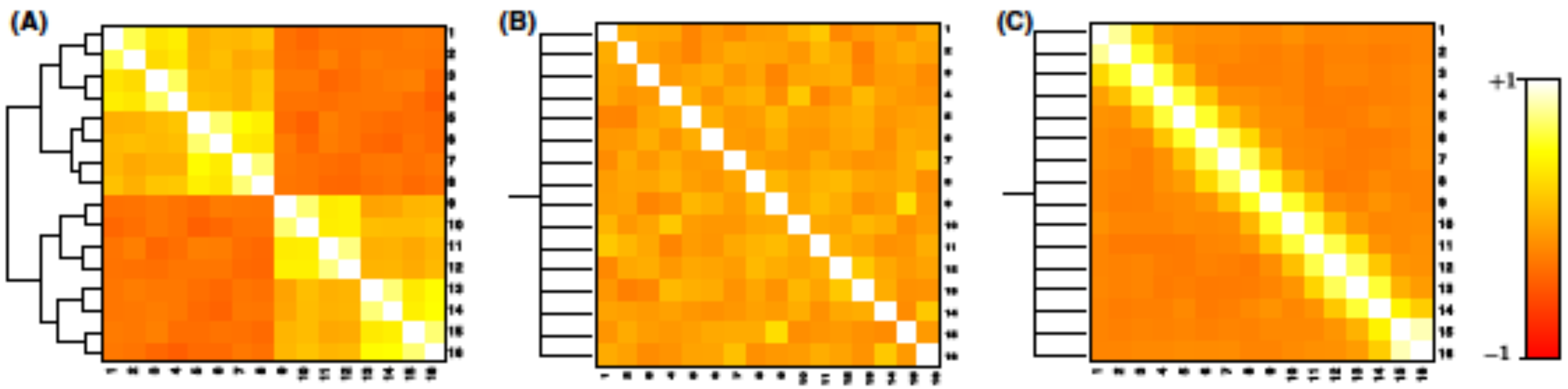
Comparison among methods

Simulated genetic data under different models:

Highly Structured Isolation with Migration

Isolation Migration Model

Stepping Stone Model



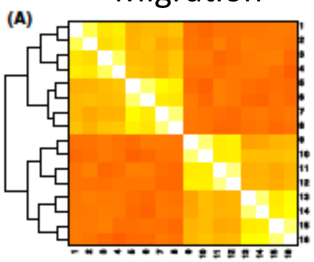
Used 4 approaches:

Population Differentiation (Bayescan)
Naive regression
LFMM
Bayenv

De Villemereuil et al., 2014

FDR vs. Significance

correlated
Highly Structured Isolation with Migration



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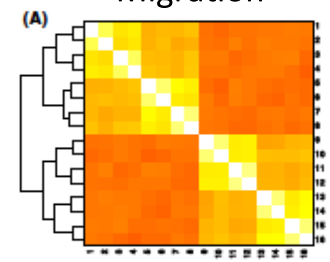
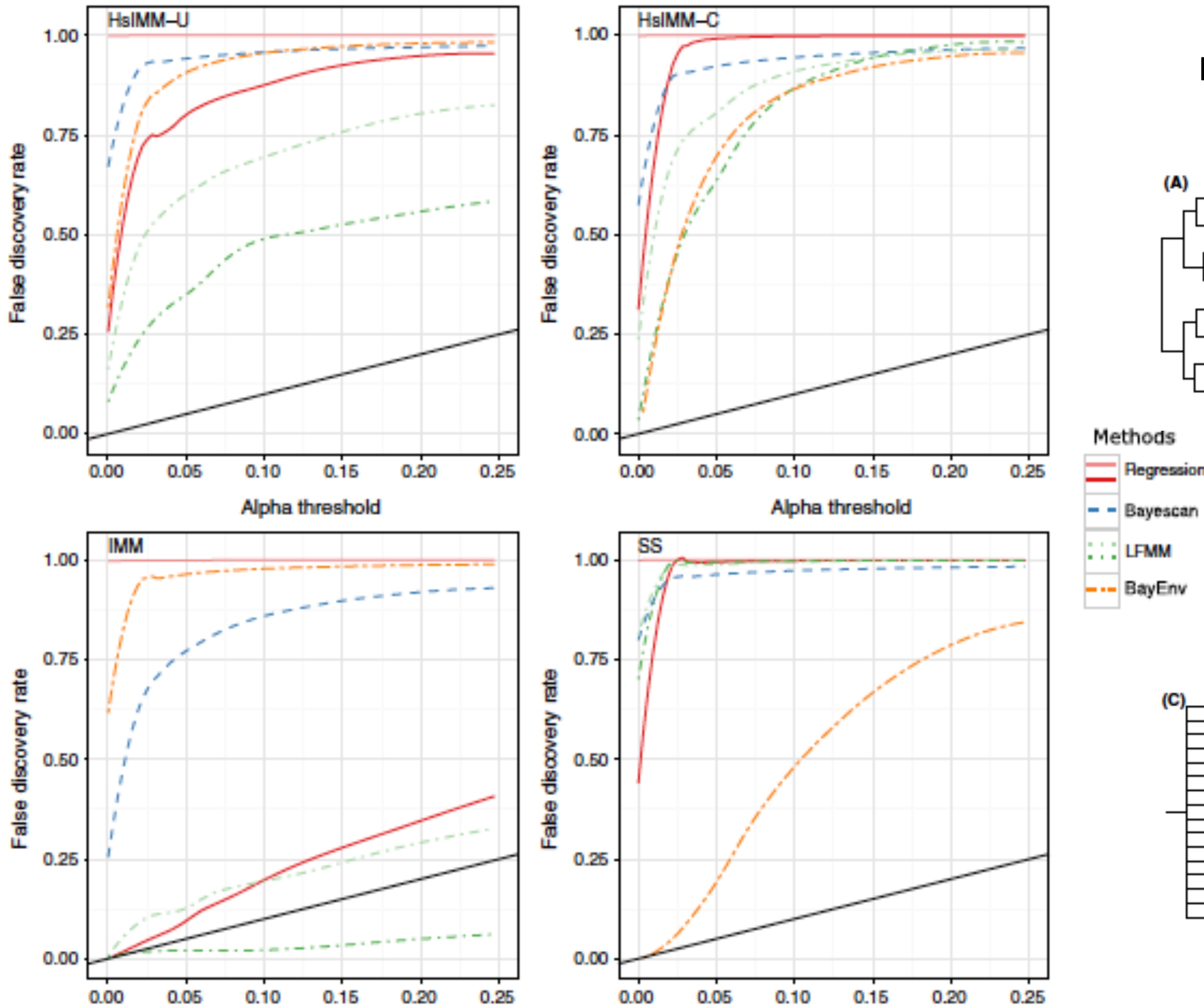
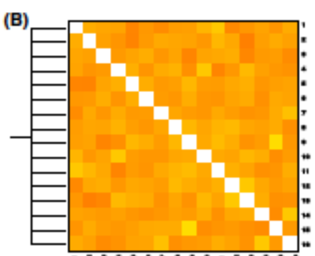
Highly Structured Isolation with Migration

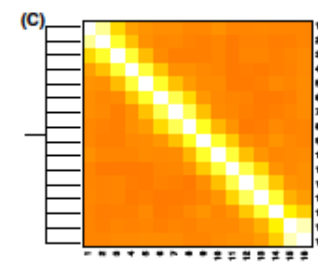




Isolation Migration Model



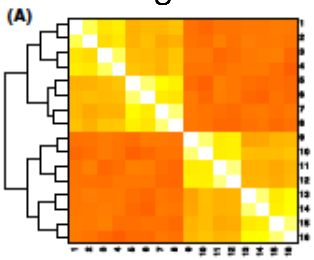
Stepping Stone Model

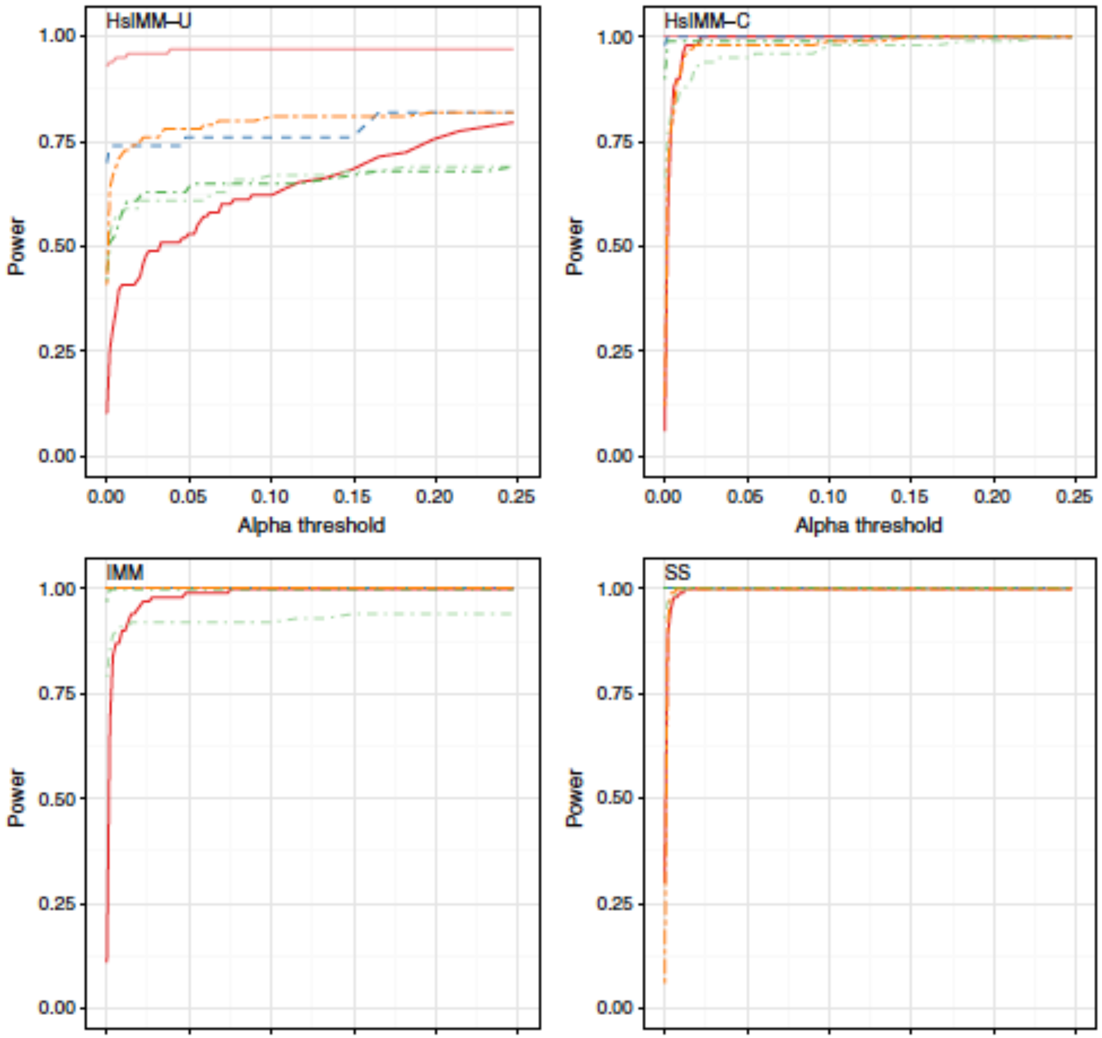


Statistical Power vs. Significance

correlated

Highly Structured Isolation with Migration





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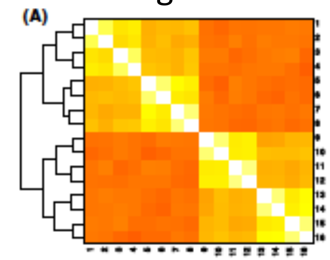
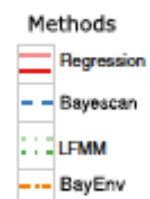
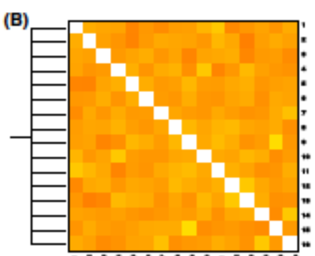
Highly Structured Isolation with Migration





Isolation Migration Model



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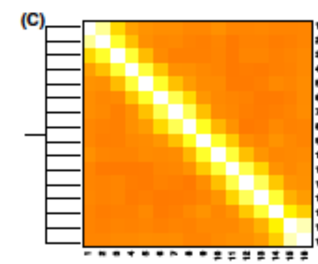
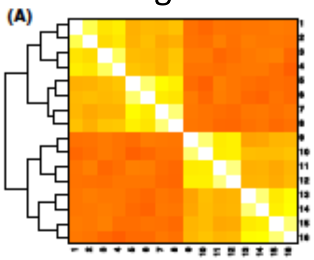


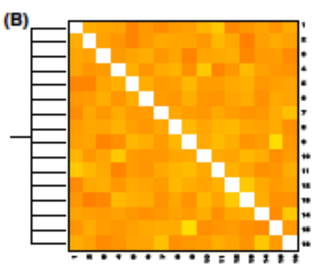
FDR vs. Significance (polygenic case)

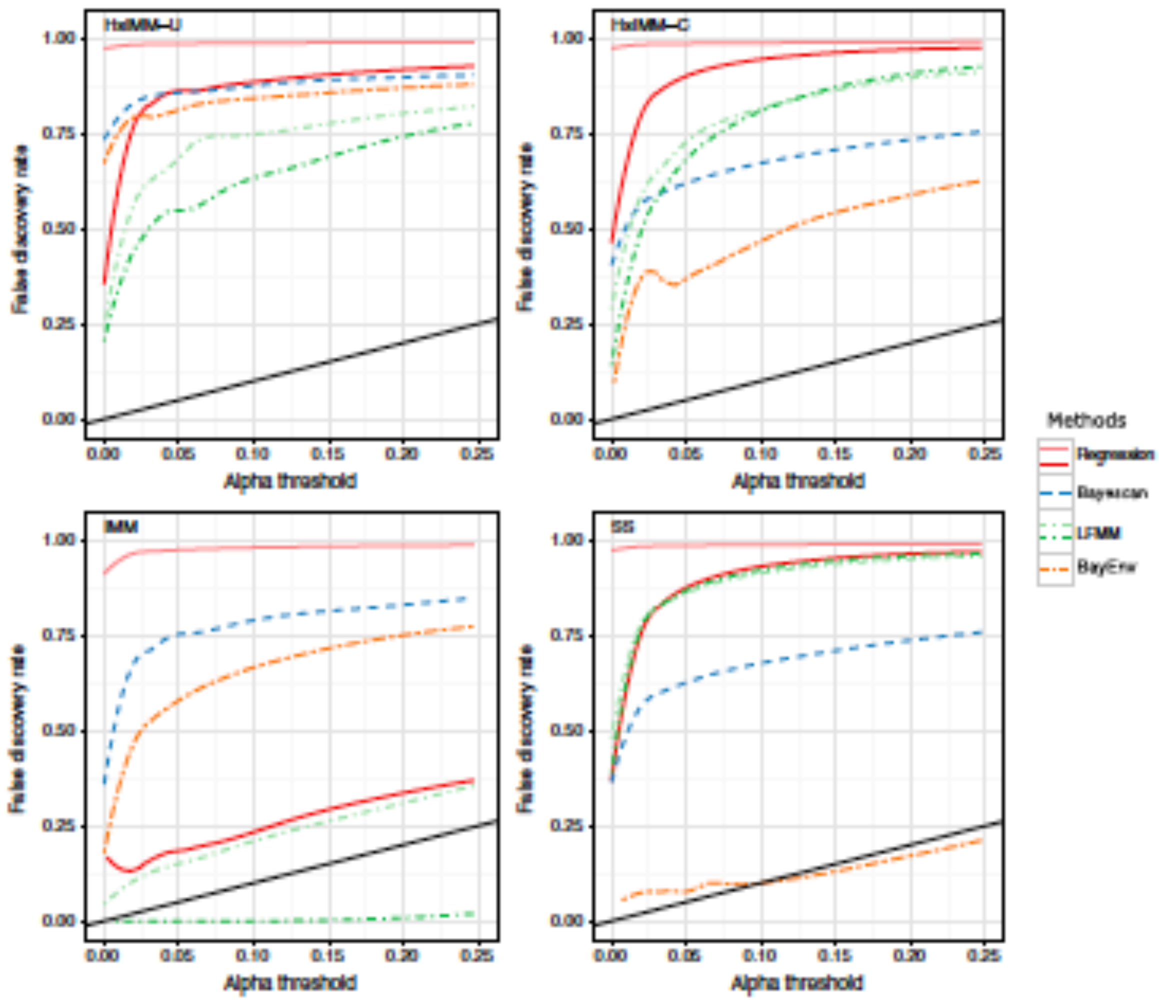
correlated

Highly Structured Isolation with Migration



Isolation Migration Model





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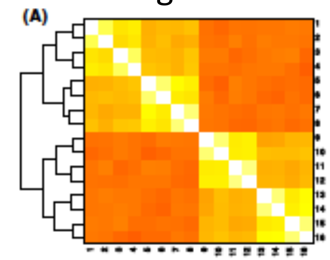
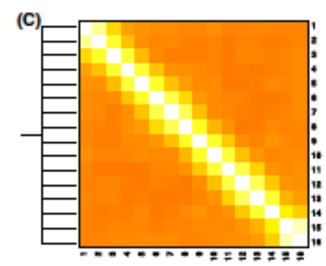
Highly Structured Isolation with Migration



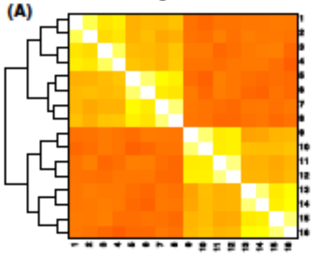
Stepping Stone Model



Power vs. Significance (polygenic case)

correlated

Highly Structured Isolation with Migration



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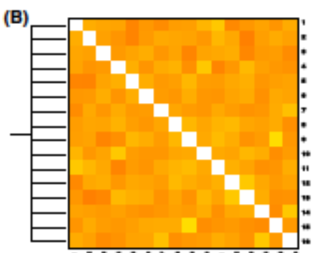

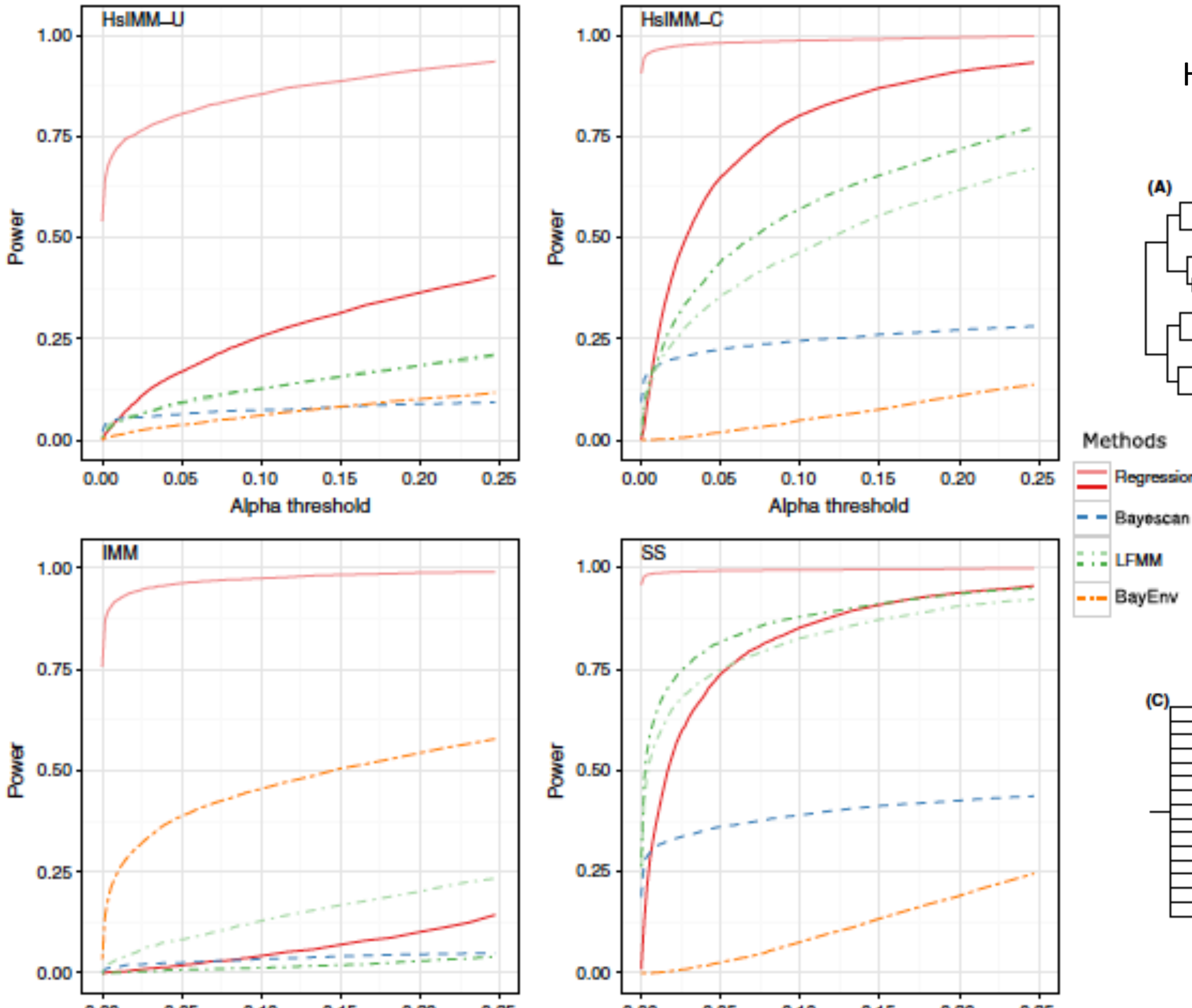

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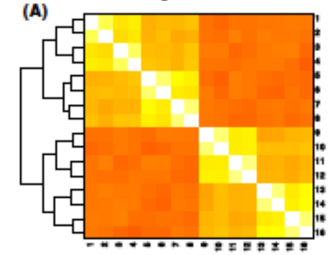
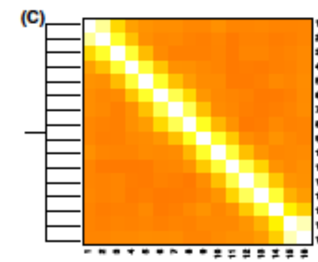
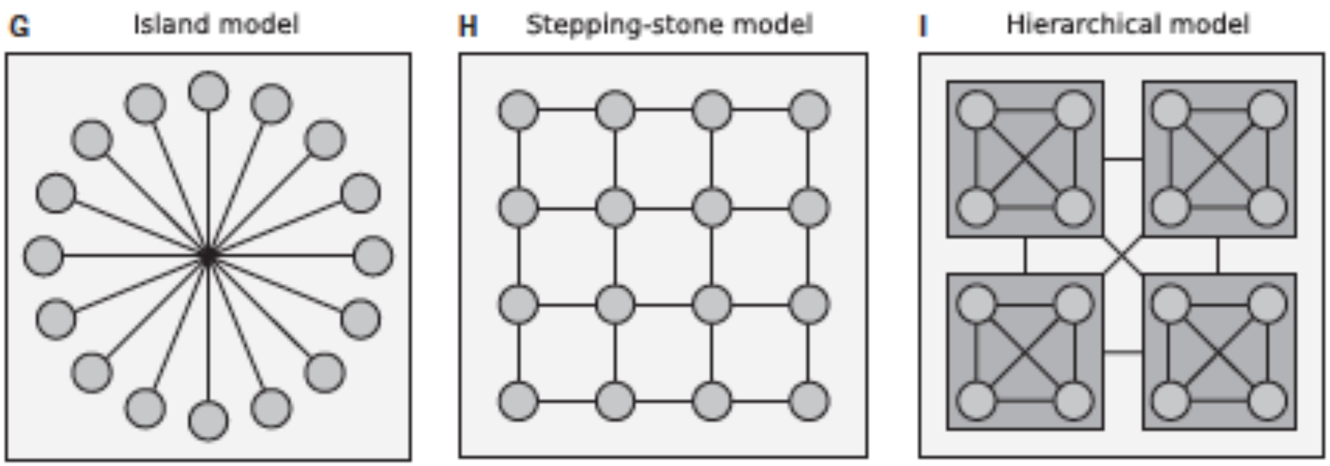
Highly Structured Isolation with Migration



Stepping Stone Model

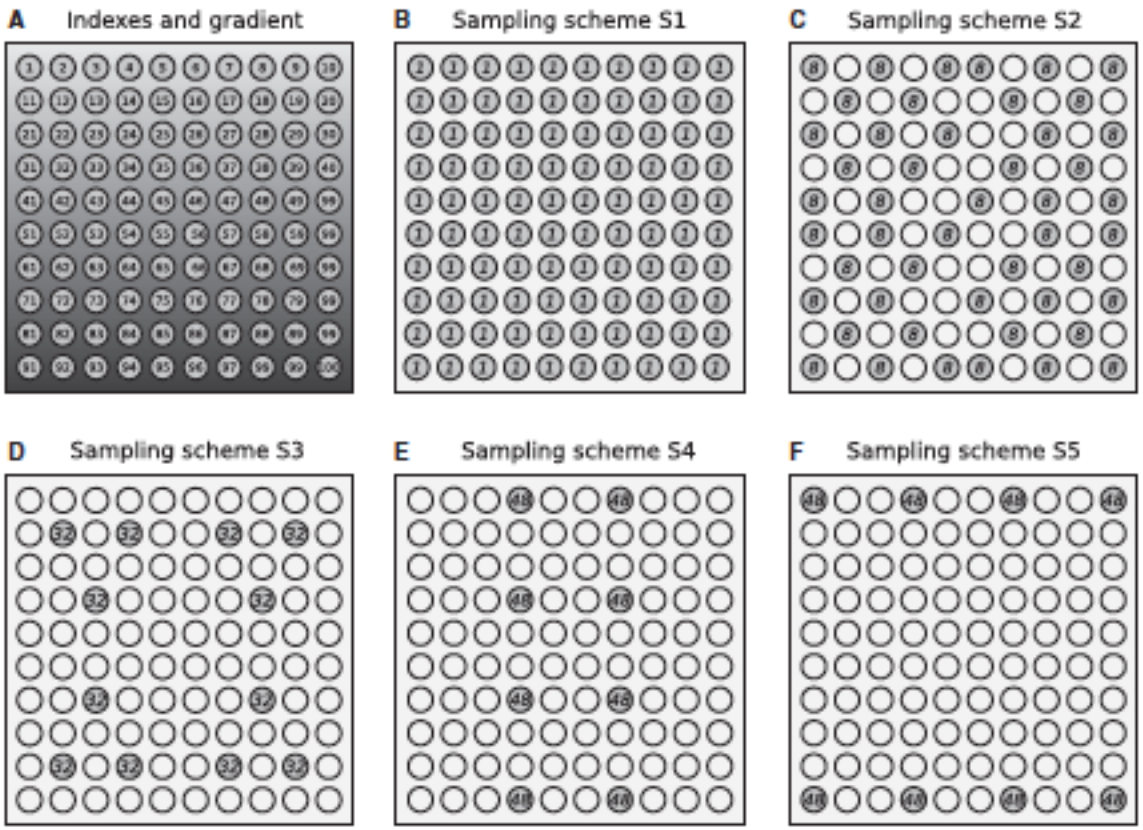


Simulation-based comparison of methods under different migration models and selfing vs outcrossing



De Mita et al., 2013

Included several sampling schemes across a grid



Diverse methods included in analysis, but useful to see how BayEnv (CWDRP) compares to others

Table 1 List of methods

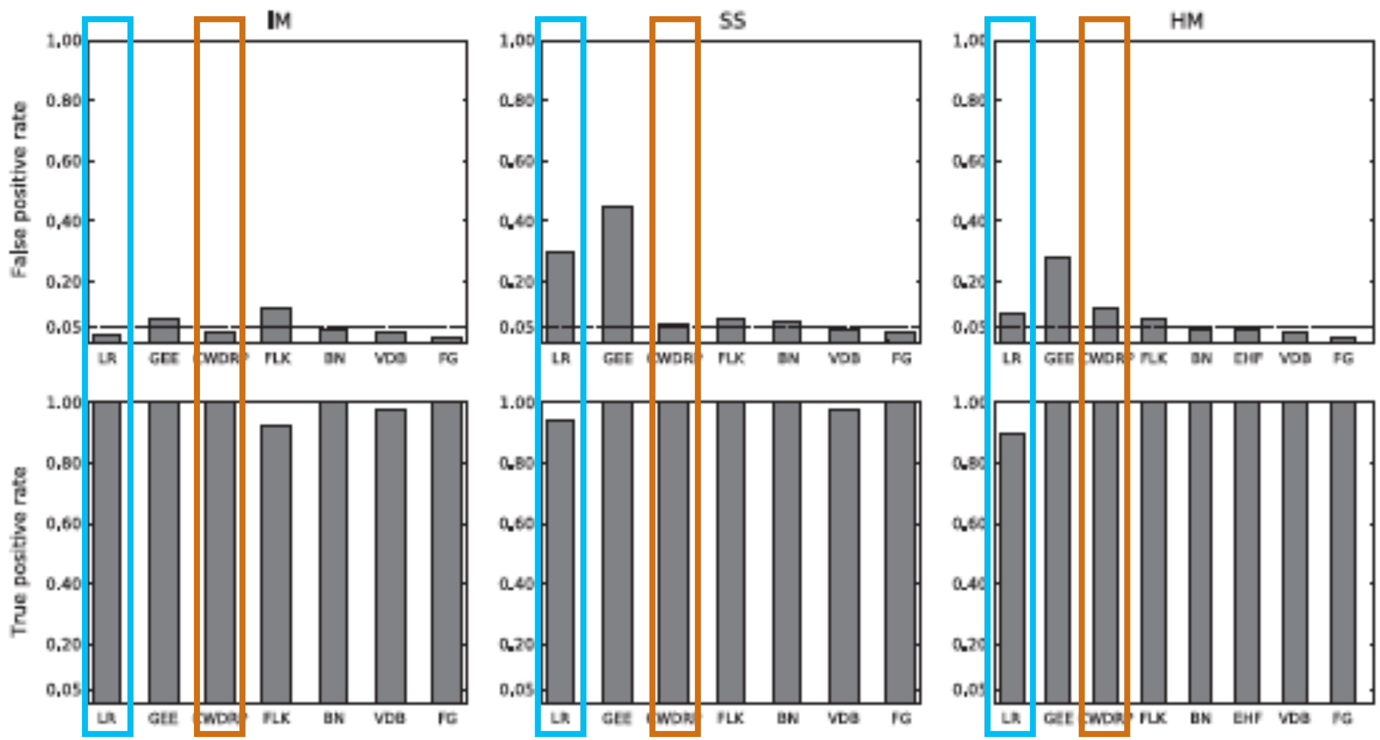
Method and reference	Technique	Underlying model	Env. variable	Control loci	Sampling	S1	S2	S3	S4	S5
LR Joost *et al.* (2007)	GLM	Independence of observations	Yes	No	Individuals	+	+	+	+	+
GEE Poncet *et al.* (2010)	GEE	Independence of clusters	Yes	No	Several individuals per population	−	+	+	+	+
CWDRP Coop *et al.* (2010)	MCMC	Island model	Yes	Yes	Frequencies	−	+	+	+	+
FLK Bonhomme *et al.* (2010)	Forward simulations	Multiple divergence model	No	Yes	Frequencies	−	+	+	+	+
BN Beaumont & Nichols (1996)	Coalescent simulations	Island model	No	Yes	Frequencies	−	+	+	+	+
EHF Excoffier *et al.* (2009)	Coalescent simulations	Hierarchical island model	No	Yes	Frequencies	−	+	+	+	+
VDB Vitalis *et al.* (2001)	Coalescent simulations	Pairwise divergence model	No	Yes	Frequencies	−	−	A pair of populations of 24 individuals		
FG Foll & Gaggiotti (2008)	RJ-MCMC	Island model	No	No	Frequencies	−	+	+	+	+

De Mita et al., 2013

There is some variation in the performance of different methods across demographic models

Simple linear model

BayEnv

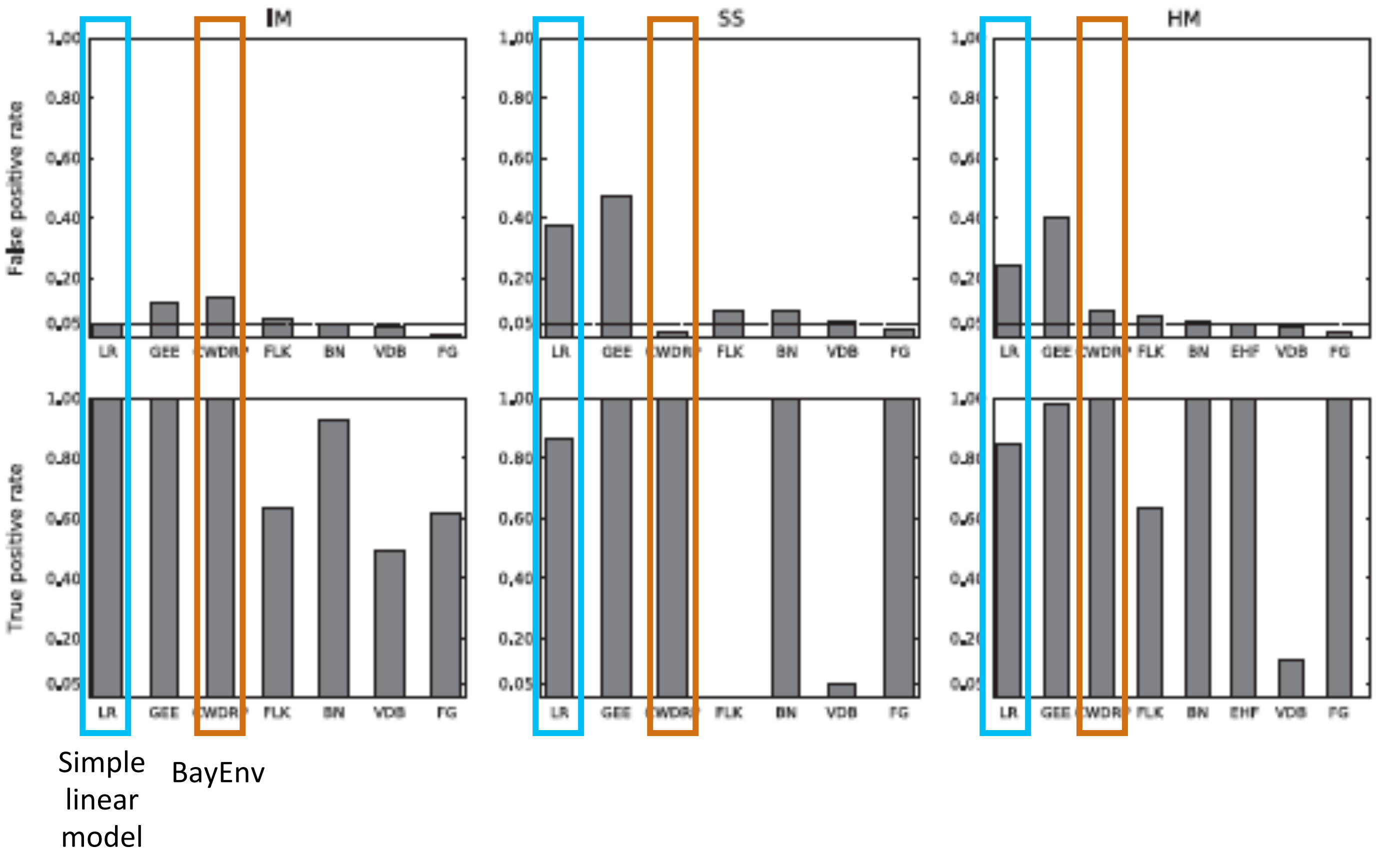


De Mita et al., 2013

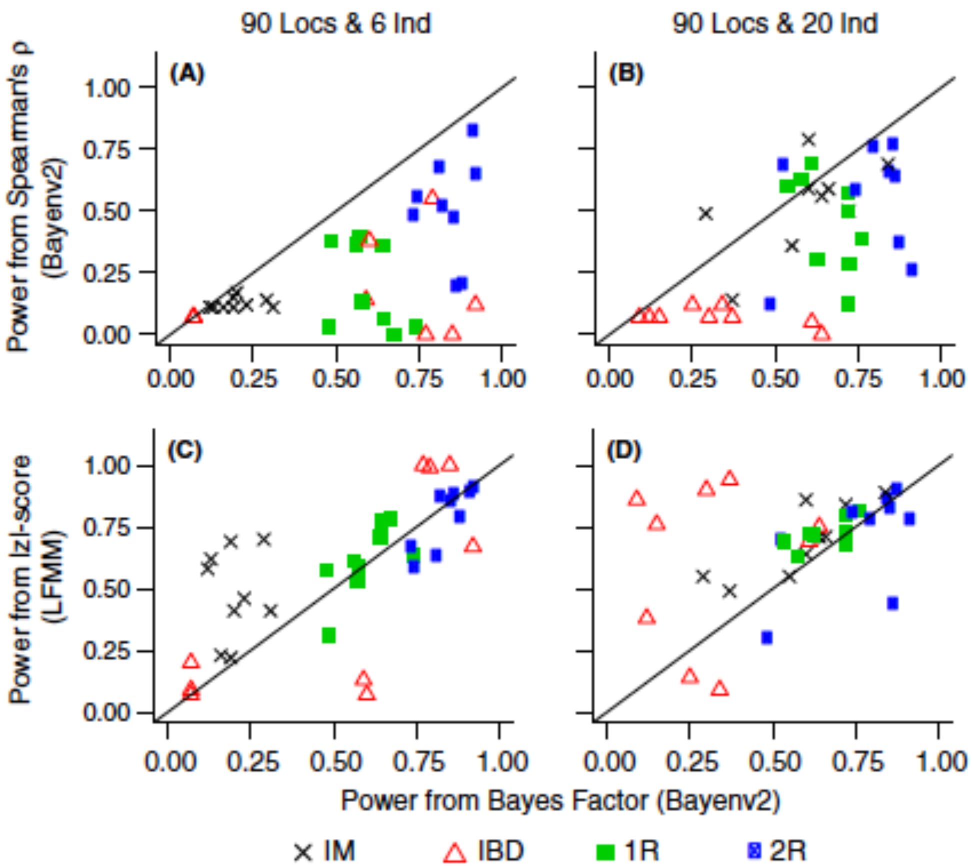
Several methods perform very poorly in models with selfing

Simple linear model

BayEnv



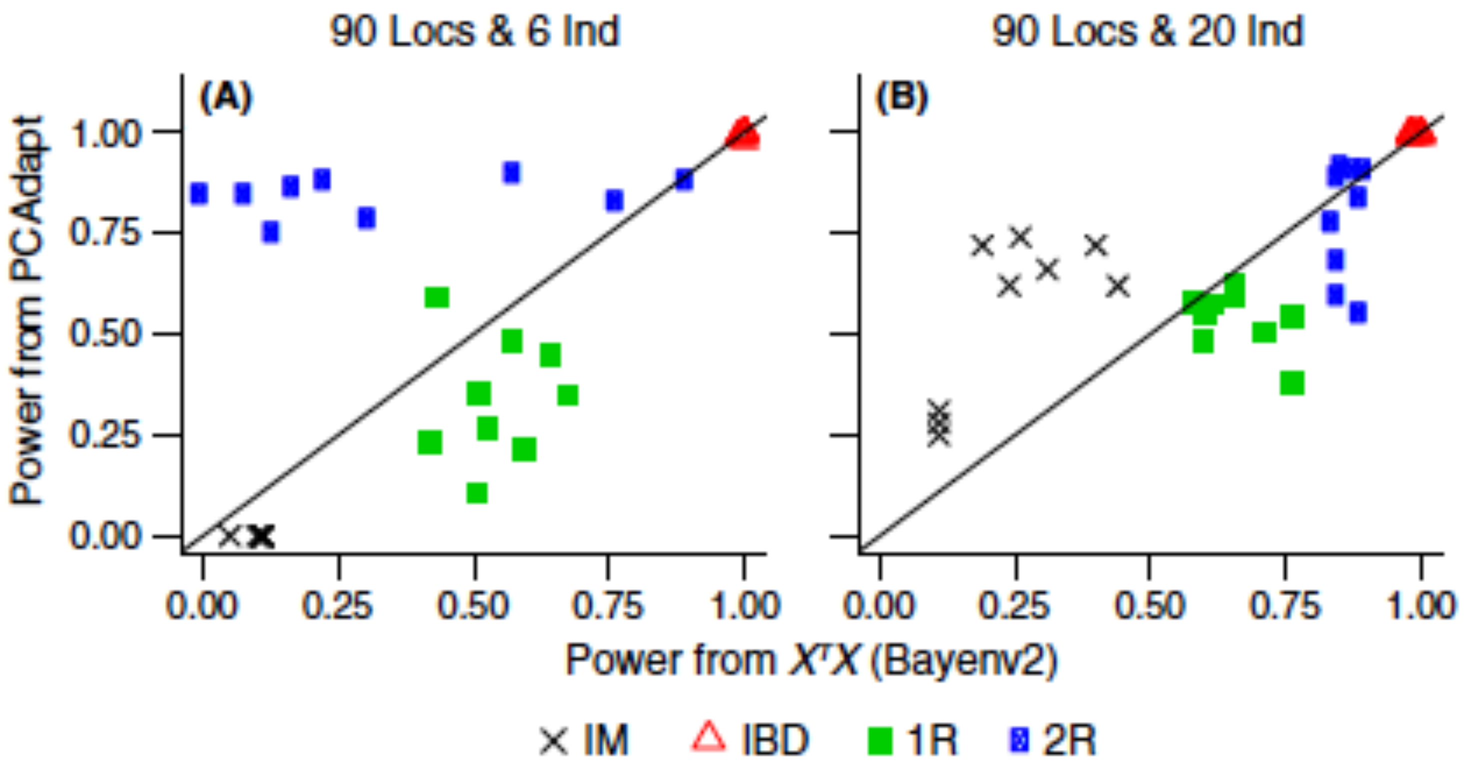
Depending on the migration model and sampling scheme, different methods perform best



IM: isolation migration
IBD: isolation by distance
1R: expansion from 1 refugium
2R: expansion from 2 refugia

Lotterhos and Whitlock 2015

PCA Adapt also performs well



Sampling and Scale

Linear model-based methods assume the residuals are normally distributed and have a constant variance

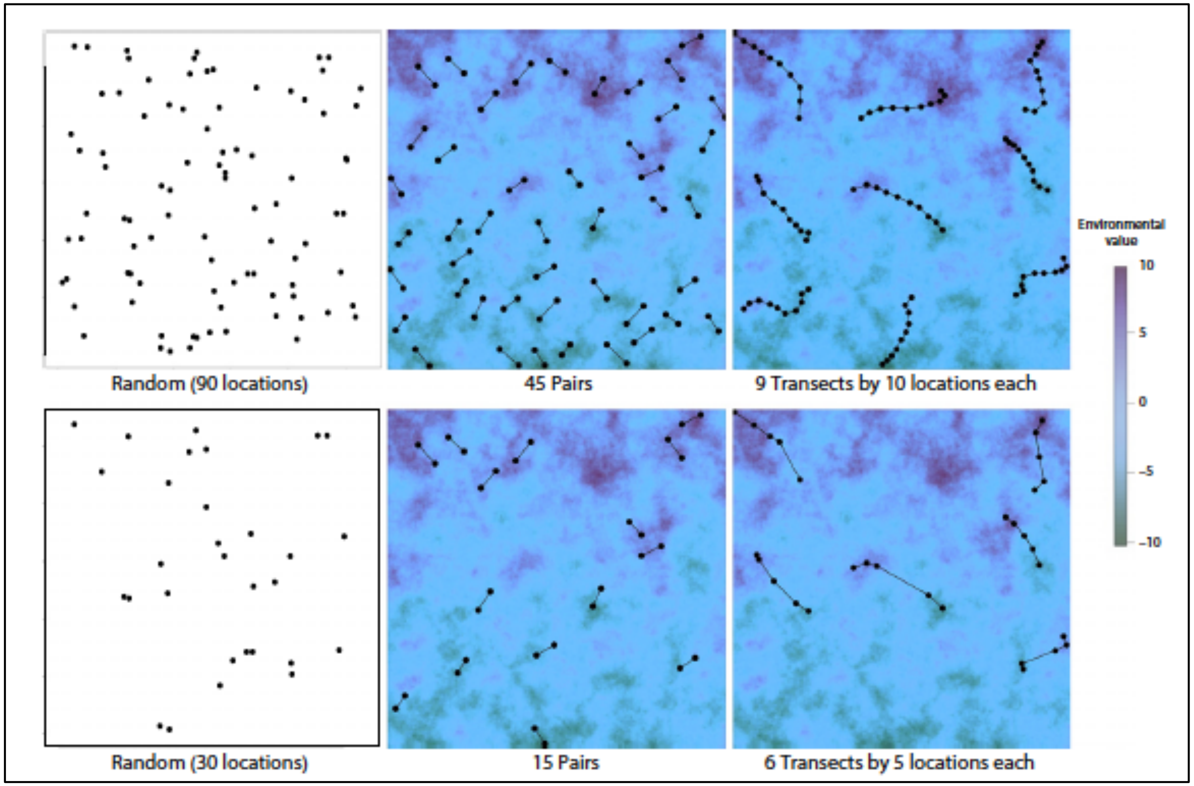
Cases where a single sample or population is divergent from the others genetically and resides in a divergent environment are especially problematic and can strongly affect the results.

Possible solutions:

- try transforming the data
- leave out outliers
- use a non-parametric method (e.g., BayEnv, Partial Mantel)

How does power compare across different sampling schemes?

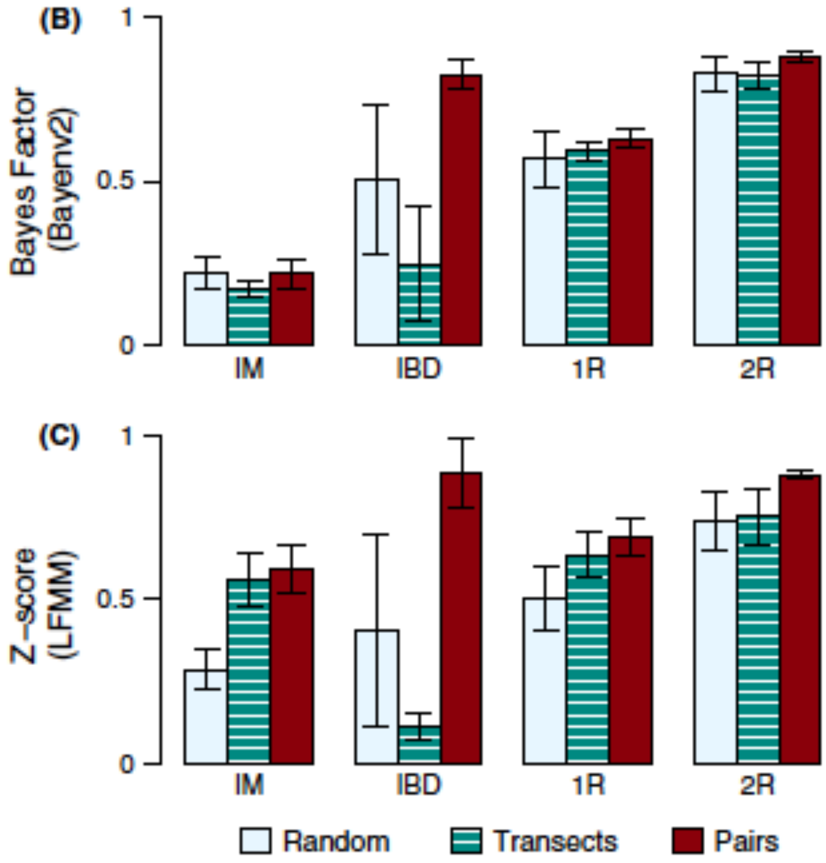
Random vs. paired vs. transects



Lotterhos and Whitlock 2015

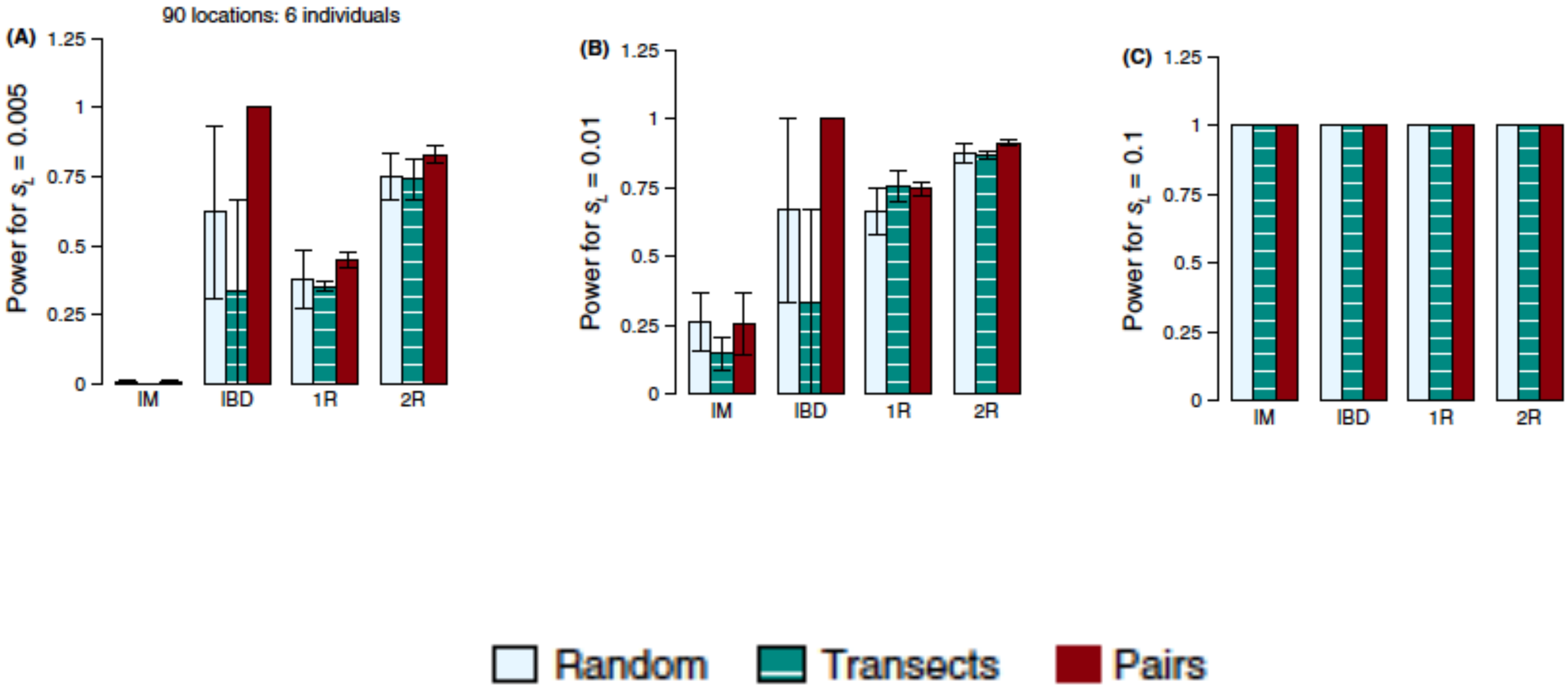
Paired > transects > random

Power



IM: isolation migration
IBD: isolation by distance
1R: expansion from 1 refugium
2R: expansion from 2 refugia

For some migration models, BayEnv has power at a low selection coefficient



Genotype-phenotype association studies ("GWAS") are similar to genotype-environment association studies

- Genotype-phenotype association:

Calculate a correlation between a SNP and a phenotype while controlling for other SNPs in the genome

- Genotype-environment association:

Calculate a correlation between a SNP and an environmental variable controlling for population structure

$$Y = X\beta + u + \epsilon,\ u \sim N(0, \sigma_g K),\ \epsilon \sim N(0, \sigma_e I)$$

Genotype-phenotype association studies ("GWAS") are similar to genotype-environment association studies

- Genotype-phenotype association:

Calculate a correlation between a SNP and a phenotype while controlling for other SNPs in the genome

Mixed model approach for genotype-phenotype mapping

$$Y = X\beta + u + \epsilon, \quad u \sim N(0, \sigma_g K), \quad \epsilon \sim N(0, \sigma_e I)$$

Phenotype SNP effect 'Error' terms Kinship matrix Other random error

Q:
But why is a covariance matrix used in G-P association mapping to represent other SNPs contributing to the phenotype**??**

A:
Fisher's infinitesimal model states that traits are shaped by many many small effect loci scattered across the genome

This means that the error term in a G-P mixed model is similar to the error terms used in G-E associations

Q:
Why is this cool?

A:
Because a lot more work has been done to speed up G-P association methods compared to G-E association methods

Using G-E methods facilitates large-scale genome-wide analyses

GEMMA

- We will use GEMMA for conducting climate correlation analyses in the tutorial
- GEMMA uses a linear mixed model approach to remove the effects of kinship before estimating the correlation between a SNP and a phenotype (here climate variable)
- GEMMA is based on the earlier EMMA software and gives equivalent results, but is much faster (linear in the number of individuals versus quadratic).
- This speed is accomplished by replacing the eigen decomposition of the K(inship) matrix with a set of recursion equations

GEMMA

- GEMMA provides an estimate of β (PVE) and can conduct several tests to assess significance for the explanatory power of the SNP:
  - LRT requires calculation of ML estimate, but is generally considered more reliable than Wald or score
  - Wald (A Wald test is conducted by comparing the coefficient's estimated value with the estimated standard error for the coefficient – assumes normality)
  - Score test (Cochran-Armitage test for trend assuming additive effect)