breedR : An open statistical package to analyse genetic data

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breedR

An open statistical package to analyse genetic data (WP6)

http://famuvie.github.io/breedR/

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Outline

1. Introduction
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3. Environmental effects
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5. Longitudinal data
6. Genomic data
7. Multi-environment trials
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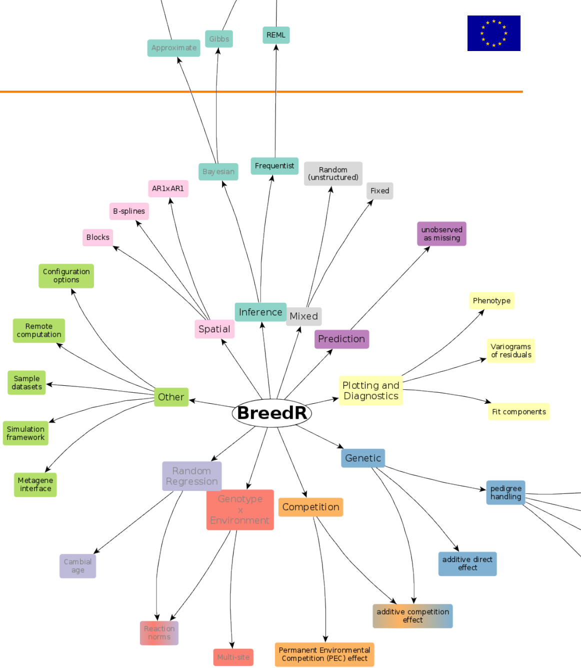
10. Remote computing



1 Introduction

What is breedR

- R-package implementing **statistical models** specifically tailored to the analysis of forest genetic resources
- A inference tool for Linear Mixed Models, with facilities for typical needs
- **breedR** acts as an **interface** providing the means to:
  1. **Combine** any number of prefabricated model **components** into a larger model
  2. Compute automatically **incidence** and **covariance matrices** from a few input parameters
  3. **Fit** the model
  4. Plot data and results, and perform **model diagnostics**

Installation

2 alternatives:

- Project web page `http://famuvie.github.io/breedR/`
  - Set up this URL as a package repository in `.Rprofile` (detailed instructions on the web)
  - `install.packages('breedR')`
  - Not possible to use `CRAN` (or yes?) due to closed-source `BLUPF90` programs
  - Stable version, with automatic updates
- GitHub dev-site `https://github.com/famuvie/breedR`
  - `if( !require(devtools) ) install.packages('devtools')`
  - `devtools::install_github('famuvie/breedR')`
  - Development version, latest features, more inestable, manual updates

Getting started

- These slides show **WHAT** can be done with breedR
- For **HOW** to perform these analyses, refer to the website:

`http://famuvie.github.io/breedR/`

Where to find help

- Package's help: `help(package = breedR)`
  - Help pages `?remlf90`
  - Code demos `demo(topic, package = 'breedR')` (omit `topic` for a list)
  - Vignettes `vignette(package = 'breedR')` (pkg and wiki)
- Wiki pages
  - Guides, tutorials, FAQ
- Mailing list `http://groups.google.com/group/breedr`
  - Questions and debates about usage and interface
- Issues page
  - Bug reports
  - Feature requests

License

Figure 1: GPL-3

- **breedR** is FOSS. Licensed GPL-3
  - `RShowDoc('LICENSE', package = 'breedR')`
- You can **use** and **distribute breedR** for any purpose
- You can **modify** it to suit your needs
  - we encourage to!
  - please consider contributing your improvements
  - you can **distribute** your modified version under the GPL
- However, **breedR** makes (intensive) use of the `BLUPF90` suite of Fortran programs, which are for *free* but not **free** (remember CRAN?)

Fitting Linear Mixed Models

$$
\begin{aligned}
y =& \mathrm{X}\beta + \mathrm{Z}u + \varepsilon \\
u \sim& \mathcal{N}(0, \mathrm{G}) \\
\varepsilon \sim& \mathcal{N}(0, \mathrm{R})
\end{aligned}
$$

- A quantitative variable y is modelled as a linear function of **fixed effects** β and **random effects** u, with unaccounted residuals ε
- The function `remlf90()` yields a **REML fit** of a model to a dataset
- Additional functions (*e.g.* `summary()`, `fixef()`, `ranef()`, `plot()`, etc.) extract and present specific results

I/O example

```r
ped   <- globulus[,1:3]

res <- remlf90(
  fixed   = phe_X ~ gg,
  genetic = list(
    model = 'add_animal',
    pedig = ped,
    id    = 'self'),
  data = globulus)

summary(res)
```

```
## Linear Mixed Model with pedigree and spatial
## effects fit by AI-REMLF90 ver. 1.122
##    Data: globulus
##   AIC  BIC logLik
##  5799 5809  -2898
##
## Variance components:
##          Estimated variances  S.E.
## genetic                3.397 1.595
## Residual              14.453 1.529
##
##              Estimate    S.E.
## Heritability   0.1887 0.08705
##
## Fixed effects:
##        value   s.e.
## gg.1  13.591 0.5014
## gg.2  14.085 0.7984
## ...
```

2 Additive-genetic effects

What is an additive genetic effect

- Random effect at **individual level**
- Based on a **pedigree** (determining the *relationship* matrix A)

$$Zu, \quad u \sim \mathcal{N}(0, \sigma_a^2 A)$$

- BLUP of **Breeding Values** from own and relatives' phenotypes
- Represents the **additive component** of the genetic value
- More **general**:
  - family effect is a particular case
  - accounts for more than one generation
  - mixed relationships
- More **flexible**: allows to select individuals within families
- More **accurate**: direct inference over the **additive-genetic** variance of the base population

Pedigrees

- A 3-column `data.frame` or `matrix` with the codes for each individual and its parents
- A **family** effect is easily translated into a pedigree:
  - use the **family code** as the identification of a fictitious **mother**
  - use `0` or `NA` as codes for the **unknown fathers**

self	dad	mum
69	0	64
70	0	41
71	0	56
72	0	55
73	0	22
74	0	50

Predicted Breeding Values

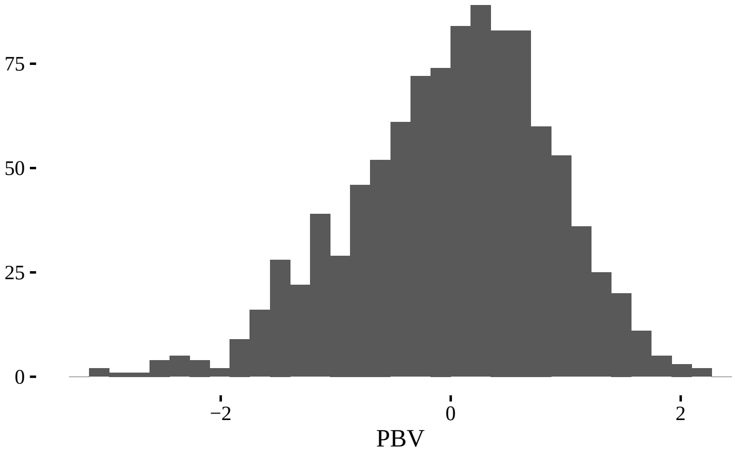


Figure 2

Predicted Breeding Values by Family

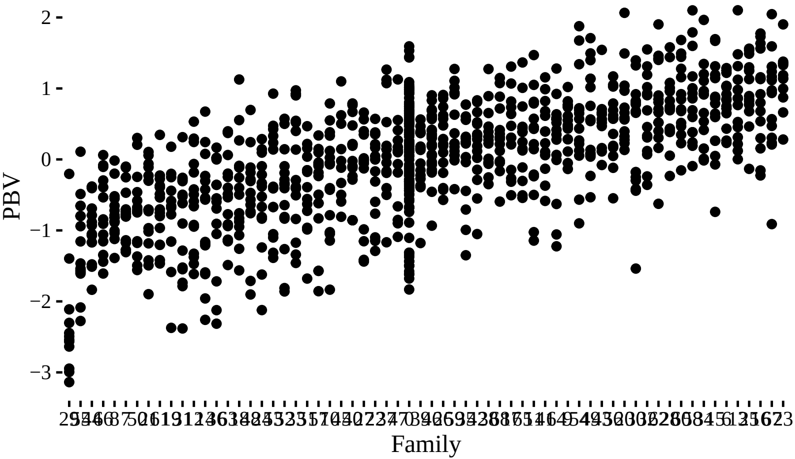


Figure 3

3 Environmental effects

What is spatial autocorrelation

- The **residuals** of any LMM must be **noise**
- However, most times there are **environmental factors** that affect the response
- This causes that observations that are close to each other **tend** to be more similar that observations that are far away
- This is called **spatial autocorrelation**
- It may affect both the estimations and their accuracy
- This is why experiments are randomized into spatial **blocks**

- You can `plot()` the spatial arrangement of various model components (e.g. residuals)
- Look like **independent** gaussian observations (i.e. noise)?
- Do you see any **signal** in the background?

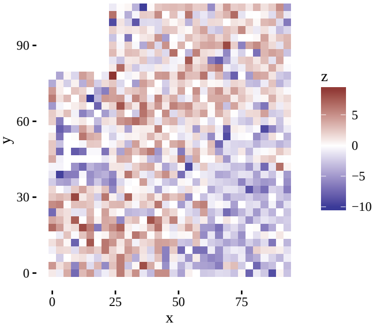


Figure 4

Plot the **variogram of residuals** with `variogram()`

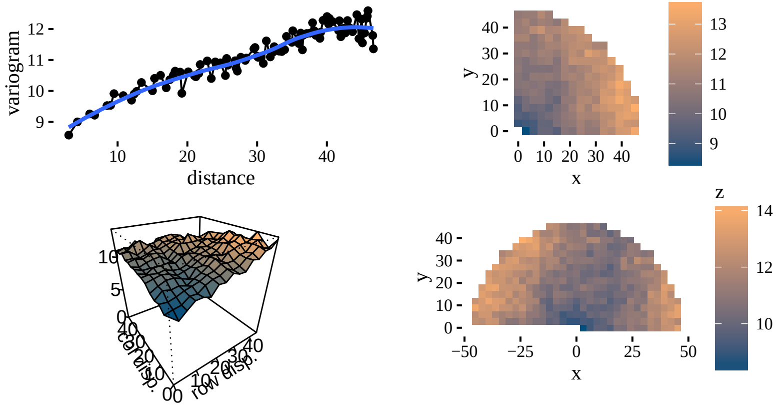
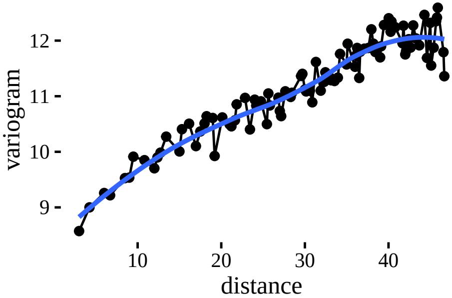


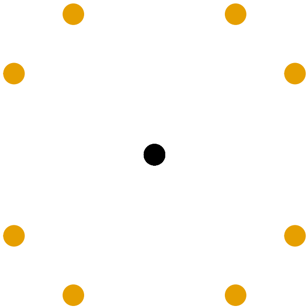
Figure 5

Isotropic variogram

$$\gamma(h) = \frac{1}{2} V[Z(\mathbf{u}) - Z(\mathbf{v})], \quad \text{dist}(\mathbf{u}, \mathbf{v}) = h$$

The **empirical** isotropic variogram is built by aggregating **all the pairs** of points separated by h, **no matter the direction**.

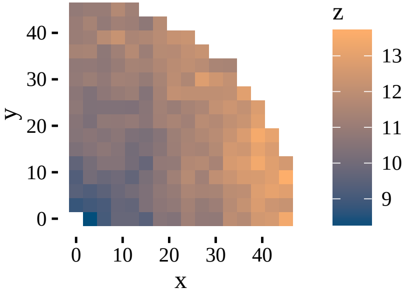


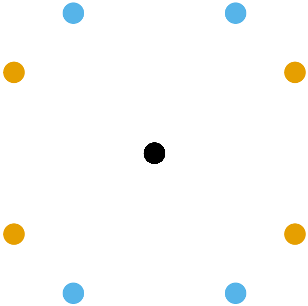


Interpreting the variograms
Row/Column variogram

$$\gamma(x, y) = \frac{1}{2} V[Z(\mathbf{u}) - Z(\mathbf{v})], \quad \text{dist}(\mathbf{u}, \mathbf{v}) = (x, y)$$

The **empirical** row/col variogram is built by aggregating **all the pairs** of points separated by exactly x rows and y columns.

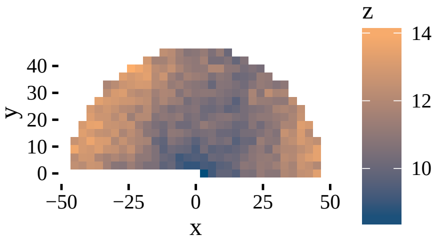


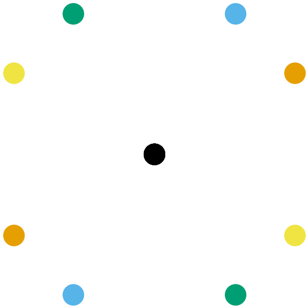


Interpreting the variograms
Anisotropic variogram

$$\gamma(\mathbf{x}) = \frac{1}{2} V[Z(\mathbf{u}) - Z(\mathbf{v})], \quad \mathbf{u} = \mathbf{v} \pm \mathbf{x}$$

The **empirical** anisotropic variogram is built by aggregating **all the pairs** of points **in the same direction** separated by $|\mathbf{x}|$.





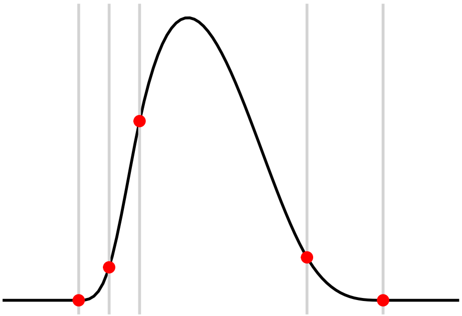
Accounting for spatial autocorrelation

- Include an explicit **spatial effect** in the model
- I.e., a **random effect** with a specific covariance structure that reflects the spatial relationship between individuals

$$Zu, \quad u \sim \mathcal{N}(0, \sigma_s^2 I)$$

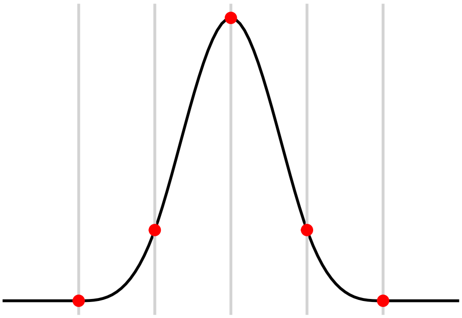
- u is the vector of random effects for the blocks
- Z is an indicator matrix such that $Z[i,j] = 1$ if the observation i belongs to block j
- σ_s^2 is the spatial variance parameter
- The **block** effect, is a very particular case of spatial effect:
  - It is designed from the begining, possibly using prior knowledge
  - Can account for non-spatial effects (e.g. operator)
  - Introduces **independent** effects between blocks
  - Most neighbours are within the same block (i.e. share the same effect)

A **cubic B-spline** $B(x)$:



- **Piecewise** curve defined in the intervals determined by 5 **knots**
- Each *piece* is a polynomial of 3rd degree

A **cubic B-spline** $B(x)$ with regularly spaced knots:



- The curve is constrained for C^2 **continuity** at each knot
- Only 1 degree of freedom controls the **scale**

A number of overlapping curves form a **base** of B-splines $\{B_j(x)\}$

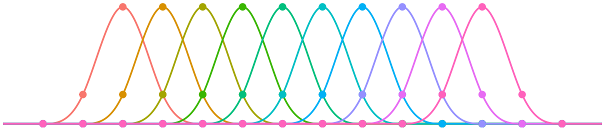


Figure 10

Spatial modelling
Bidimensional Splines

Each, can be **scaled** using a coefficient $\{u_j B_j(x)\}$

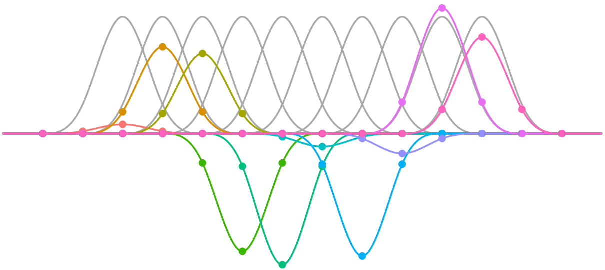


Figure 11

And **summed** to a **linear combination** $f(x) = \sum_j u_j B_j(x)$

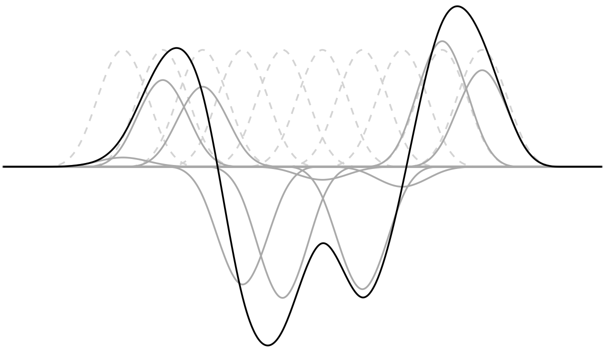


Figure 12

Bidimensional Splines in a Mixed Model

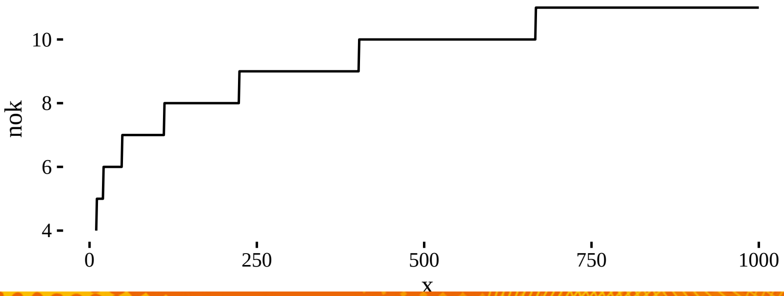
- $f(x) = \sum_j u_j B_j(x)$ provides a **spline representation** of a wide family of curves, in terms of a vector of coefficients u
- For any set of points $x = \{x_i\}$, the vector of values $f(x_i)$ can be written as a matrix operation $f = [B_j(x_i)]u$
- breedR extends this to **two dimensions** and defines a random effect

$$Bu, \quad u \sim \mathcal{N}(0, \sigma_s^2 R_s)$$

  - u is the vector of spline effects
  - B is the matrix of spline bases evaluated at the observations
  - σ_s^2 is the spatial variance parameter
  - R_s imposes a fixed positive correlation between coefficients of neighbouring spline bases

Spatial modelling
Number of knots of a `splines` model

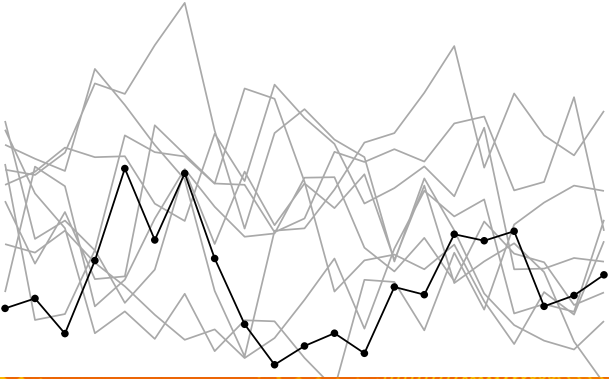
- The **smoothness** of the spatial surface can be controlled modifying the number of base functions
- This is directly determined by the **number of knots** (nok) in each dimension
- If not explicitly set, it is determined heuristically by breedR as a function of the number of observations



- An AR1(ρ) on the line is a collection of random variables $\{x_i\}$ where

$$x_t = \rho x_{t-1} + \varepsilon_t, \quad \varepsilon_t \sim \mathcal{N}(0,1), |\rho| < 1$$

- A few random simulations with $\rho = 0.5$:



breedR extends this model to the plane using and defines a component

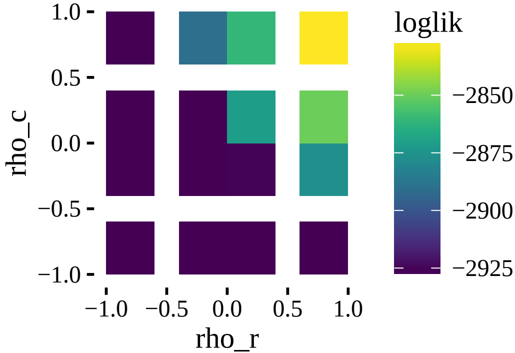
$$Zu, \quad u \sim \mathcal{N}(0, \sigma_s^2 R_{\text{AR}})$$

- u is the vector of random effects **for each individual location** on a regular grid
- Z is an **indicator matrix** such that $Z[i,j] = 1$ if the observation i is at site j
- σ_s^2 is the spatial variance parameter
- R_{AR} defines a separable correlation structure based on the kronecker product of two AR1 processes

Spatial modelling

Autoregressive parameters of a AR model

- The **smoothness** of the AR effects can be controlled by the autoregressive parameters (ρ_x, ρ_y) in each dimension
- They can be **given explicitly**
- Otherwise, breedR fits a model for each combination of parameters in a default grid and returns the most likely



Predicted spatial effects
by spatial model

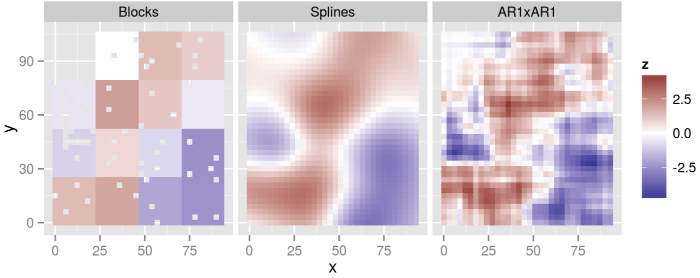


Figure 16: spatial-effects

- All capture a similar underlying **environmental pattern**
- with somewhat increasing ranges of variability

Model residuals
by spatial model

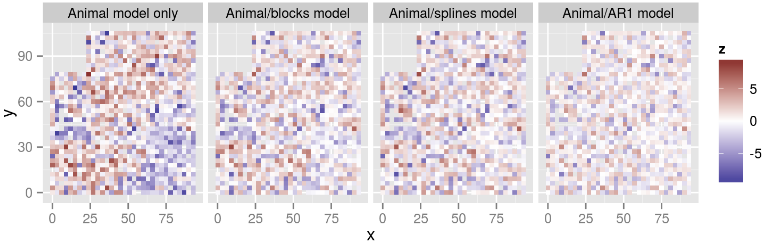


Figure 17: change residuals

- The spatial variability is taken mostly from the model residuals
- which increasingly look like pure noise

4 Competition effects

Competition model assumptions

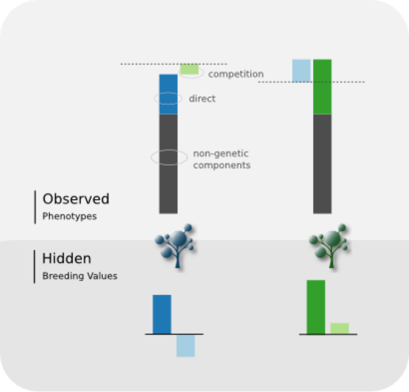


Figure 18: Competition model

- Each individual have **two** (unknown) Breeding Values (BV):
  - `direct` BV affects its **own** phenotype,
  - `competition` BV affects its **neghbours'**
- The total effect of the neighbouring `competition` BVs is given by their **distance-weighted sum**

Weighted neighbour competition effect

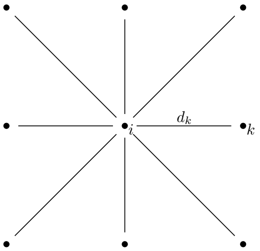


Figure 19: distance-plot

Let ∂i be the set of neighbouring locations of tree i, and $u_c = (u_{c,k})'$ the vector of `competition` BVs

$$\omega_i(\alpha) = \sum_{k \in \partial i} z_{ik}(\alpha) u_{c,k}$$

where $z_{ik}(\alpha) \propto 1/d_{ik}^{\alpha}$, such that

$$\sum_{k \in \partial i} z_{ik}(\alpha)^2 = 1.$$

This condition is **variance-estabilizer** ensuring $\forall i$:

$$\mathrm{Var}(\omega_i) = \mathrm{Var}(u_c) = \sigma_c^2$$

The decay parameter

The **decay parameter** α controls the **relative intensity of competition** of the neighbours

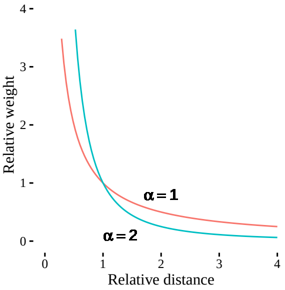


Figure 20

- The weights z_{ik} are **scale-invariant**
- *e.g.* a tree twice as far is weighted $1/2^{\alpha}$ as much
- higher values of α concentrate the weights on the closest trees

Random-effect representation

$$Z_d u_d + Z_c(\alpha) u_c, \quad \begin{pmatrix} u_d \\ u_c \end{pmatrix} \sim \mathcal{N}(0, \Sigma_a \otimes A), \quad \Sigma_a = \begin{pmatrix} \sigma_d^2 & \sigma_{dc} \\ \sigma_{dc} & \sigma_c^2 \end{pmatrix}$$

- Each set of BVs is modelled as a zero-mean **random effect** with structure matrix given by the **pedigree** and independent **variances** σ_d^2 and σ_c^2
- Both random effects are modelled jointly with **covariance** σ_{dc}
- Z_d is an indicator matrix linking observations and individuals
- $Z_c(\alpha)$ weights the competition effect of the neighbours with (fixed) **decay** parameter α

Permanent Environmental Competition Effect

$$Z_p u_p, \quad Z_p = Z_c, u \sim \mathcal{N}(0, \sigma_p^2 I)$$

- **Optional** companion effect with **environmental** (rather than genetic) basis
- Modelled as an individual **independent** random effect that affects **neighbouring** trees in the same (weighted) way

Diagnosis of competition

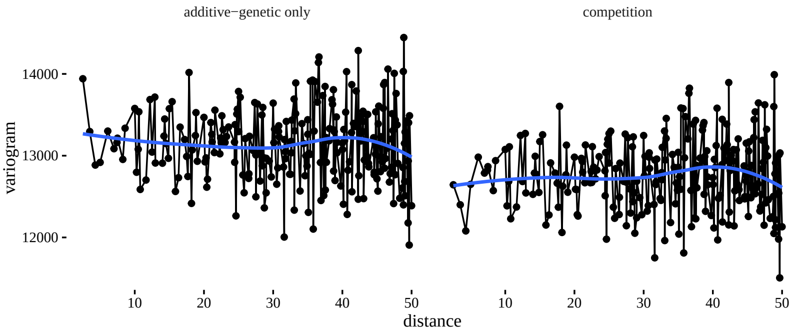


Figure 21

- Competition is often observable **in the first lag** of the variogram of residuals
  - increased antagonism between neighbouring phenotypes

Breeding for growth and collaboration

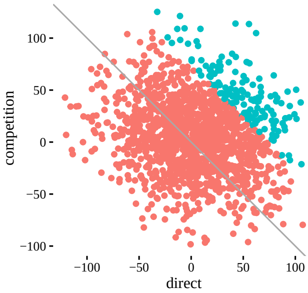


Figure 22

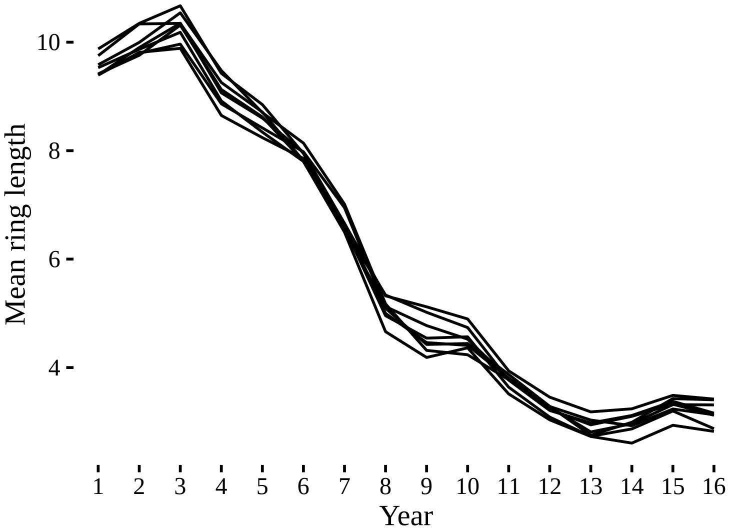
- `direct` and `competition` BV are usually **negatively correlated**
- selection based only on direct genetic merit tends to favour competitive individuals, hampering the **global performance**
- the competition model allows for selection based on a **joint assessment**

5 Longitudinal data

What is longitudinal data

- Measurements **repeated** in **time** or along some **climatic or geographical variable** (*e.g.* temperature, precipitation, latitude, altitude, ...)
- All model parameters (*e.g.* variances, random effects) can be **functions** of the longitudinal variable
- Increased complexity: from estimating **numerical values** (dimension 0) to estimating (infinte-dimensional) **functions** (with finite data)
- Strategies:
  - assume parametric shape (*e.g.* linear regression)
  - nonparametric components (*e.g.* splines, Legendre polynomials)

Repeated measurements in time
Mean response evolution by replicate - Larix dataset



Climatic gradient

Mean ring-length by Mean Annual Precipitation

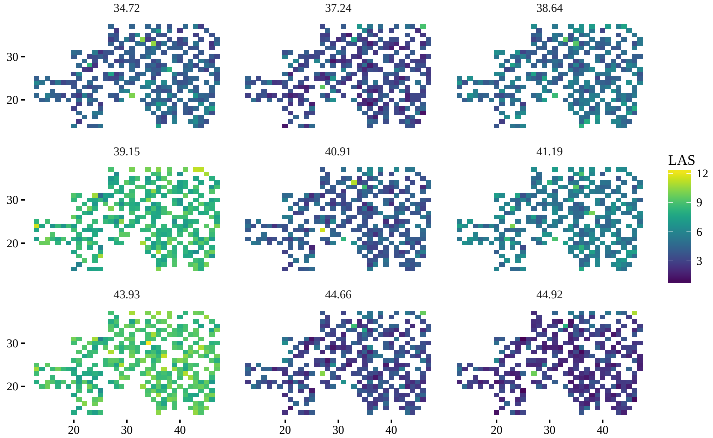
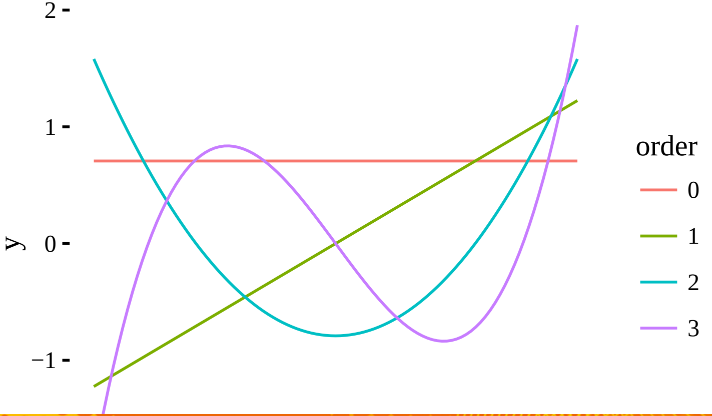


Figure 24

Legendre Polynomials

- Family of **orthogonal** polynomials **dense** in $\mathscr{L}_2$
  - Any regular curve can be approximated as much as needed by taking a linear combination of polynomials up to a sufficiently high order



Random-Regression model

For each observation of an individual i at j

$$\begin{aligned} y_{ij} =& \mathrm{X}_i\beta + \Sigma_{k=0}^{\mathrm{ord}} a_{ik}\mathcal{L}_k(j) + \varepsilon_{ij} \\ (a_0', \ldots, a_{\mathrm{ord}}')' \sim& \mathcal{N}(0, \Sigma \otimes \mathrm{A}) \\ \varepsilon \sim& \mathcal{N}(0, \sigma_e^2) \end{aligned}$$

- The Breeding Value of an individual is a **function** of an environmental variable
- This function is parameterised as a **linear combination** of Legendre orthogonal polynomials of order up to a fixed `ord`
- Each individual is described by `ord` $+ 1$ correlated **coefficients**

Example of results

- Functional Breeding Values for each individual

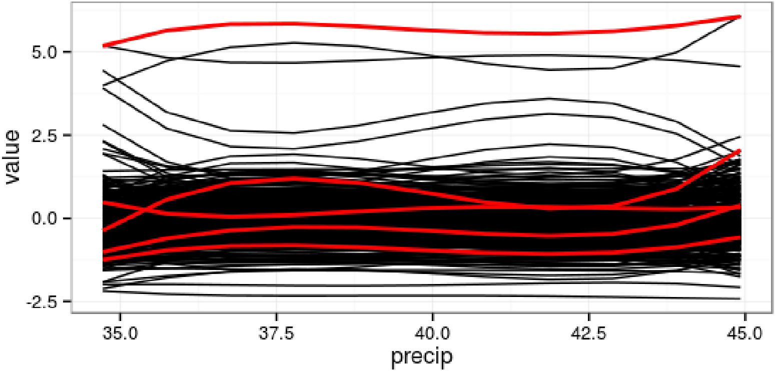


Figure 26: random-regression

6 Genomic data

Using genomic markers

- breedR allows random effects with an **arbitrary covariance** structure (`generic`)
- This can be used to leverage **genomic information** (GBLUP)

The Generic model

This additional component allows to introduce a random effect ψ with **arbitrary** incidence and covariance matrices Z and Σ:

$$Z\psi, \quad \psi \sim \mathcal{N}(0, \sigma_\psi^2 \Sigma_\psi)$$

Applications:

- include additional not-predefined components *e.g.* Dominance, Hybrid populations, Genomic evaluation, etc.

GBLUP

$$Zu, \quad u \sim \mathcal{N}(0, \sigma_G^2 G)$$

- Use markers to compute a **relationship matrix** G for individuals
  - Several methods available
  - *e.g.* VanRaden et al. 2009

$$G = XX' / \sum 2p(1-p)$$

- **Replace** the `additive-genetic` model, which uses the pedigree-based relationship matrix A with a `generic` model with a genomic relationship matrix G
- Z is an **indicator** matrix linking observations with individuals
- Predicts genetic value of **individuals**, not markers
- Improved **accuracy** wrt pedigree-pased evaluation

Relationship matrices
pedigree-based vs. genomic

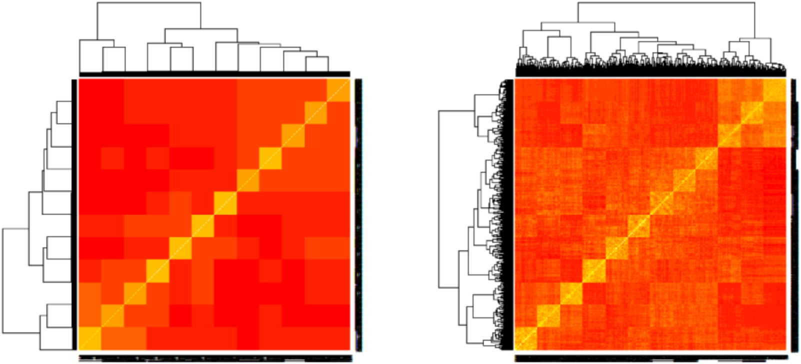


Figure 27: relationship-matrices

Note the increased level of detail in the relationship structuure

7 Multi-environment trials

Douglas dataset

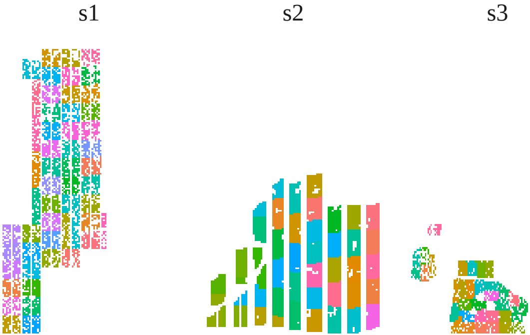


Figure 28

Particularities

Fixed effects

- some can be **transversal** accross environments
  - *e.g.* origin, provenance
- other take different values at each environment
  - *e.g.* the intercept, replicate
  - modelled as a fixed **interaction**

Random effects

- some can be **transversal** accross environments
  - *e.g.* main effect of the genotype
- other can be **environment-specific**
  - *e.g.* blocks, GxE, residuals
  - modelled as a **group** of (correlated or not) random effects

Statistical model

$$
\begin{aligned}
y =& \mathrm{X}_0\beta_0 + \mathrm{Z}_0 u_0 + \mathrm{X}\sum_e \mathbb{1}_{\{e\}}\beta_e + \mathrm{Z}\sum_e \mathbb{1}_{\{e\}} u_e + \varepsilon \\
u_0 \sim& \mathcal{N}(0, \mathrm{G}_0) \\
(u_1, \dots)' \sim& \mathcal{N}(0, \Sigma_\mathrm{G} \otimes \mathrm{G}) \\
\varepsilon \sim& \mathcal{N}(0, \mathrm{D}_R \otimes \mathrm{I})
\end{aligned}
$$

- Particular case of the general LMM
- For each **environment** e, there is a **group** of random effects u_e, each with covariance structure G, possibly cross-correlated through Σ_G
- Independent site-specific residual variances

$$
\begin{aligned}
\text{C13} =& \text{orig} + \text{site} + \text{fam} + \sum_{e=1}^{3} f_e \mathbb{1}_e + \varepsilon \\
\text{fam} \sim& \mathcal{N}(0, \sigma_f^2 \mathrm{I}) \\
(f_1, f_2, f_3)' \sim& \mathcal{N}(0, \Sigma_{G\times E} \otimes \mathrm{I}) \\
\varepsilon \sim& \mathcal{N}(0, D_3 \otimes \mathrm{I})
\end{aligned}
$$

- One **global** family effect (fam)
- One group of three **site-specific** family effects $(f_i,\ i = 1, 2, 3)$
- Jointly, they represent the $G \times E$ **interaction** with genetic cross-covariation $\Sigma_{G\times E}$

Predicted genetic effects
by family

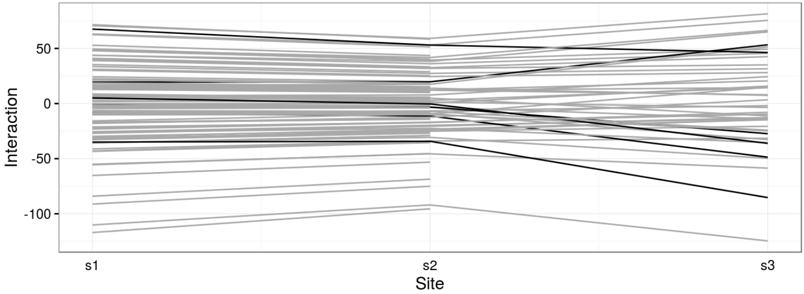


Figure 29: GxE-interaction

- Sum of main and interaction effects
- Note:
  - different **variances** per site
  - high genetic **correlation**
  - some families are more **interactive**

Genetic correlations

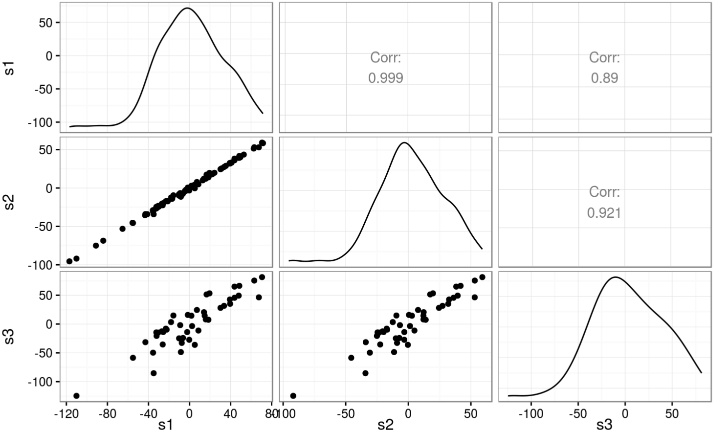


Figure 30: genetic-correlations

Ecovalence

Measure of **interactivity** of families

For each family x

$$\varphi(x) \propto \sum_{i \in x} \sum_{e} f_e^2$$

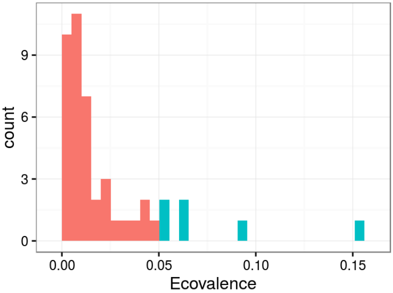


Figure 31: ecovalence

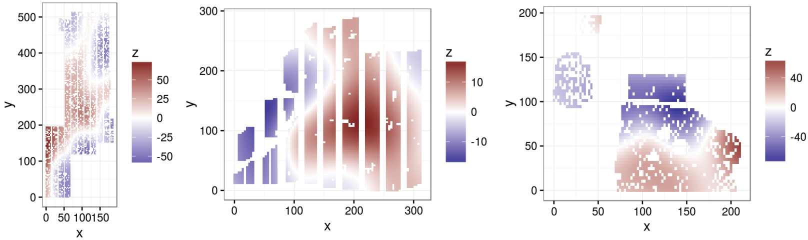


Figure 32: spatialxE

8 | Multi-trait models

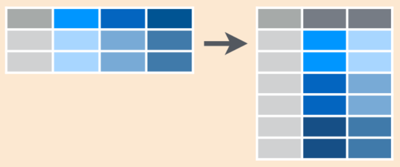
$$
\begin{aligned}
Y_1 &= X\beta_1 + Zu_1 + \varepsilon_1 \\
Y_2 &= X\beta_2 + Zu_2 + \varepsilon_2, \\
(u_1, u_2)' &\sim N(0, \Sigma_u \otimes G) \\
(\varepsilon_1, \varepsilon_2)' &\sim N(0, \Sigma \otimes I_n).
\end{aligned}
$$

- Σ_u and Σ either **diagonal** or **fully-parameterized** 2×2 matrices
- Some of the fixed or random effects can affect only a **subset of the traits**
  - *e.g.* fixed effect of operator

Limitation
of breedR's implementation

- All fixed and random effects are assumed to be **trait-specific**
  - **transversal effects** not directly supported (ultimately by PROGSF90)
- Simpler covariance structures **not supported**
  - *e.g.* independent effects with shared variance, exchangeable structure
- A workaround is to **reshape the dataset** to long-layout

Multi-trait with reshaping
wide to long-layout



- Reshaping operation:
  - Stack traits into a **single variable** `value`
  - Additional variable `trait`
  - Duplicate individual information and other variables
- Use single-trait models with MET syntax
  - `trait` instead of `site`
- This overcomes the limitations breedR's multi-trait implementation
  - more complex models like multi-trait **and** multisite become cumbersome

9 Simulation framework

breedR's simulation function
Simulating data under competition

- Simulate datasets of any size, from ~~any~~ most supported models
- See `?simulation` for details on the syntax

```
Source: local data frame [500 x 14]

    self  sire   dam  beta     x     y    spatial       BV1
   (dbl) (dbl) (dbl) (dbl) (int) (int)      (dbl)     (dbl)
1     41    14    40     1     1    19 -1.0738194 -1.023654
2     42    18    33     1    18    22  1.6654315  2.477488
3     43    11    31     1    19    19  0.7047348  1.961305
4     44     5    38     1     1    23  0.4698724  1.207112
5     45    16    24     1     7    12 -0.7233131 -1.919742
6     46     9    38     1     3     5 -0.1197804  1.174054
..   ...   ...   ...   ...   ...   ...        ...       ...
Variables not shown: BV2 (dbl), wnc (dbl), pec (dbl), wnp (dbl), resid
  (dbl), phenotype (dbl)
```

Applications
a.k.a. what the heck I want a simulator for?

- check models under **ideal** scenarios
- **Bootstrapping**:
  - compute heritability (and it s.e.) for complex models
  - compute more accurate s.e. for fixed and random effects
  - inference on arbitrary hypotheses (involving any combination of model parameters)

Heritability of complex models

e.g. competition or splines models fitted by EM-REML (rather than AI)

- Thus, heritability **not available**
- Other methods (e.g. *Delta*) **not feasible**
- Even when available, is **approximate** (relies on asymptotic normality of parameters)

Example

1. Fit the model to your data
2. Write a function to **simulate data** from your fitted model parameters
3. Write a function to **fit a simulated dataset** and return realised heritability

- Repeated calls to this function yields the **sampling distribution** of heritability
- Compute **SE** and **CI** from numerical summaries

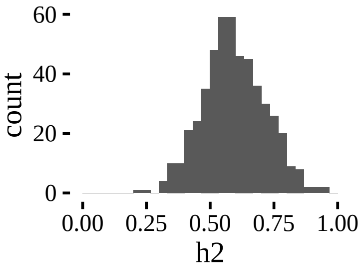


Figure 33

More accurate Standard Errors

- SE in breedR's output are **approximate**
- Rely on asymptotic normality (same as heritability)
- Same Bootstrapping procedure applies

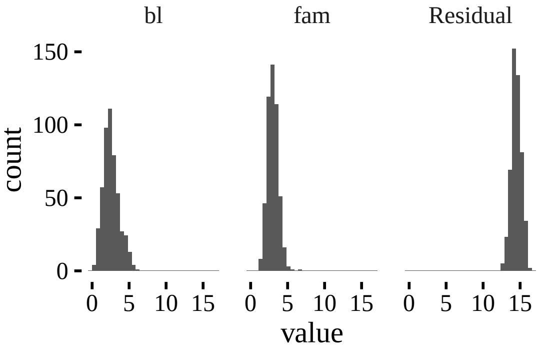


Figure 34

10 | Remote computing

Remote computation

If you have access to a **Linux** server through **SSH**, you can perform computations remotely

- Take advantage of more **memory** or **faster** processors
- **Parallelize** jobs
- Free **local resources** while fitting models
- See `?remote` for details

Configuring a server

1. Windows users: install `cygwin` with `ssh` beforehand (`http://cygwin.org/`)
2. configure the client and server machines so that passwordless SSH authentication works
3. Set breedR options `remote.host`, `remote.user`, `remote.port` and `remote.bin` (see `?breedR.setOption`)
   - Optionally, set these options permanently in `$HOME/.breedRrc`

```
writeLines(
  c("remote.host = '123.45.678.999'",
    "remote.user = 'uname'",
    "remote.bin  = 'remote/path/to/breedR/bin/linux'"),
  con = file.path(Sys.getenv('HOME'), '.breedRrc'))
```

Fitting models remotely

```
res <- remlf90(..., breedR.bin = "remote")
```

- Fit model **remotely**
- R-console stays in **stand-by** until job is finished
- When job finishes (provided that connection keeps alive), results are automatically **retrieved**

Identical in use to local computing, but without the processor/memory burden

Submitting jobs

```
res <- remlf90(..., breedR.bin = "submit")
```

- Fit model **remotely**
- Connection is **closed** in the meanwhile
- R-console is **active**
- Typing `res` **queries** the server for the job status (Running/Finished/Aborted)

Parallel computing

- After you **submit** a job, you are free to submit more (specially with multiple-processor servers)
- Query the **status** of all jobs with `breedR.qstat()`
- **Kill** some job with `breedR.qdel(res)` or all jobs with `breedR.qnuke()`

breedR

http://famuvie.github.io/breedR/