Analysis Pipeline for Genomic Prediction Data using R and synbreed Package

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Outline

Day 1

- Introduction to the `synbreed` package
- Working with data class `gpData`
- Current development status of `synbreed` package

Day 2

- Writing `R` extensions
- `R-Forge` and SVN
- Extending the `synbreed` package, common standards
- Discussion and future work

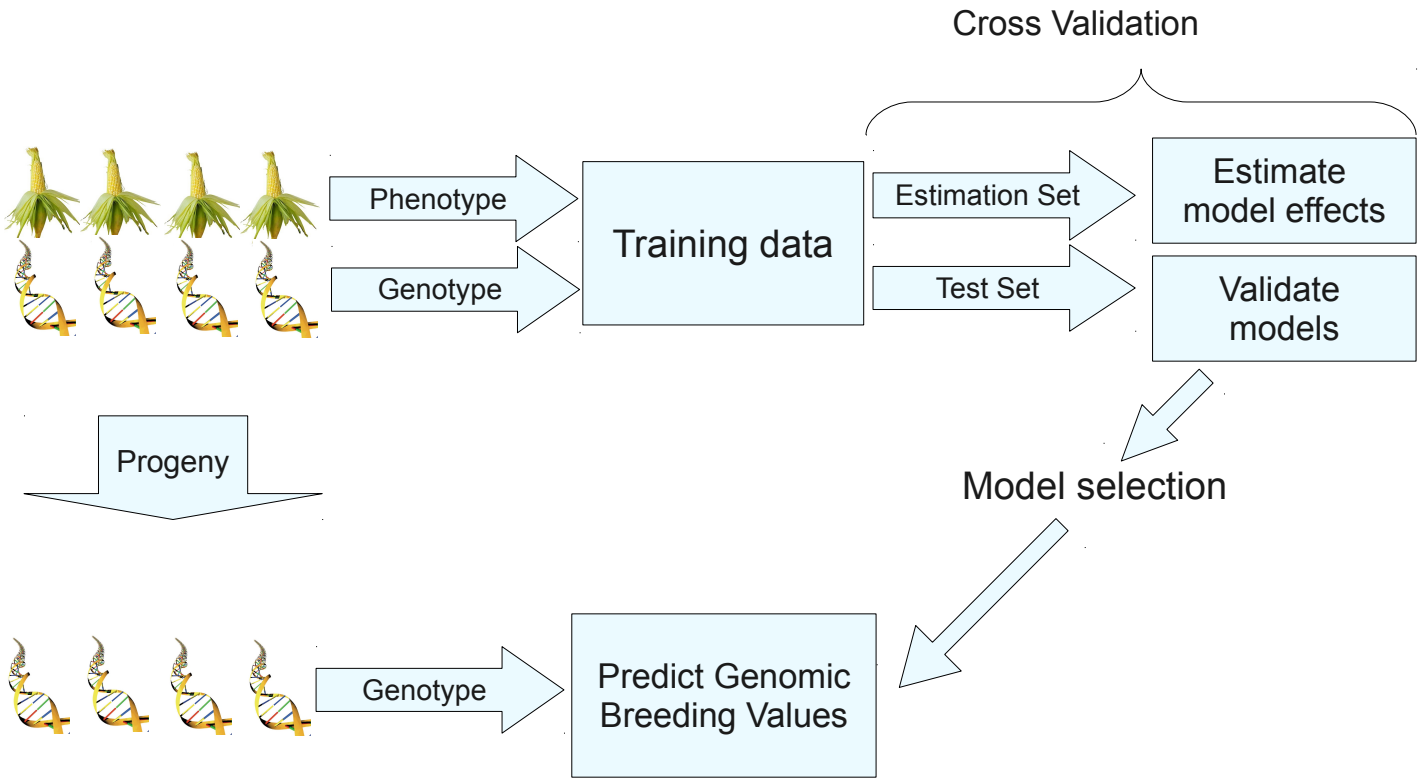
Summary - synbreed package

- Add-on for the open source environment for statistical computing R (R Development Core Team 2010)
- Title: Framework for the anaylsis of genomic prediction data using R
- Version: 0.5-0
- S3 class system (Chambers and Hastie 1992)
- Hosted on R-Forge: https://r-forge.r-project.org/projects/synbreed/
- SVN repository
- Audience: Scientists and professionals
- Package description in preparation for JSS

Objectives

1. Provide algorithms for methods required in the analysis of genomic prediction data
2. Create a framework for the analysis using a unified data object resembling the structure for a wide range of studies such as GS, GWAS or QTL mapping
3. Collection of methods within one open-source software package
4. Implementation flexible with respect to data structure, suitable for plant and animal breeding
5. Gateway to other R packages with models for genomic prediction

Genomic selection



Genomic selection

- Introduced by Meuwissen *et al.* (2001)
- In a recent review, Heffner *et al.* (2009, p.9) state

> *"While statistical methods of prediction must be continually advanced, an integral part of their performance will be the software packages used to implement them. In conjunction with this software, robust databases that can efficiently link breeding lines, testing environments, genotypic data, phenotypic data, and breeding programs will need to be developed to simplify flow and use of information."*

- The `synbreed` package aims to provide tools, to bring genomic selection from theory to praxis: "Analysis pipeline for genomic selection"

Starting with the package

- After installation, load package simply by

  ```
  R> library(synbreed)
  ```

- Package version and further information

  ```
  R> help(package = synbreed)
  ```

- Package vignette

  ```
  R> vignette("synbreed")
  ```

- Help on functions, e.g.

  ```
  R> help(codeGeno)
  ```

- Visualize source code

  ```
  R> fix(codeGeno)
  ```

Data structure

- All data for genomic selection is combined in a single data object

class gpData

- `pheno` : `data.frame` with phenotypes
- `geno` : `matrix` with genotypes (markers)
- `map` : `data.frame` with marker map (chr + position)
- `pedigree` : class "pedigree"
- `covar` : `data.frame` with additional covariate information, e.g. family or sex

- To create an object, use function `create.gpData`
- To assess structure, use

```
R> str(gpDataObj)
R> summary(gpDataObj)
```

Data structure

Advantages of a unified data object

- Common names for individuals and markers
- Clear data queries and merges
- Challenges: unphenotyped or ungenotyped individuals, markers without position, additional individuals in pedigree
- Only define data structure in the beginning, reuse for further analysis
- Save all data in one `Rdata` object, considerably reduced storage requirement
- All R scripts are based on the same data object (avoid missmatches)

Example data sets

```
R> data(maize)
```

Maize data

- Simulated maize breeding program using DH technology
- 1250 DH lines phenotyped for one quantitative trait and 1117 SNP markers
- Pedigree for 15 generations

```
R> data(mice)
```

Mice data (Valdar *et al.* 2006)

- Heterogeneous stock mice population analyzed in the literature
- Publicly available from `http://gscan.well.ox.ac.uk`
- 2527 individuals with 2 phenotypes (weight [g] at 6 weeks age and growth slope between 6 and 10 week (g/day))
- 1940 individuals with 12545 SNP markers

Summary method for class gpData

```
R> summary(mice)

object of class 'gpData'
covar
 No. of individuals 2527
         phenotyped 2527
          genotyped 1940
pheno
 No. of traits 2

     weight        growth.slope
 Min.   :11.90   Min.   :-0.08889
 1st Qu.:17.80   1st Qu.: 0.04556
 Median :19.90   Median : 0.08024
 Mean   :20.30   Mean   : 0.08659
 3rd Qu.:22.60   3rd Qu.: 0.12569
 Max.   :30.20   Max.   : 0.26408
 NA's   :16.00   NA's   :53.00000

geno
 No. of markers 12545
 genotypes A/G G/G A/A C/C C/A A/T T/T
 frequencies 0.15 0.277 0.311 0.081 0.
 NA's 0.444 %
map
 No. of mapped markers  12545
 No. of chromosomes     20
 markers per chromosome 1044 948 857 7
pedigree
NULL
```

Read in own data

- Simulated data from XII QTL-MAS Workshop 2008, Uppsala
- Available from
  `http://www.computationalgenetics.se/QTLMAS08/QTLMAS/DATA.html`

QTLMAS data

- 50 simulated QTLs (explained variance 0 - 5 %)
- 5865 individuals (2778 males, 3087 females)
- 6000 markers on 6 chromosomes (each of length 100cM)

```
R> qtlMASdata <- create.gpData(pheno = pheno, geno = geno2,
+       map = map, pedigree = ped, covar = covar, map.unit = "cM")
R> save("qtlMASdata", file = "qtlMASdata.Rdata")
```

Working with gpData objects

- Adding individuals

```
R> add.individuals(gpData, pheno = NULL, geno = NULL, pedigree = NULL,
+       covar = NULL)
```

- Removing individuals

```
R> discard.individuals(gpData, which)
```

- Adding markers

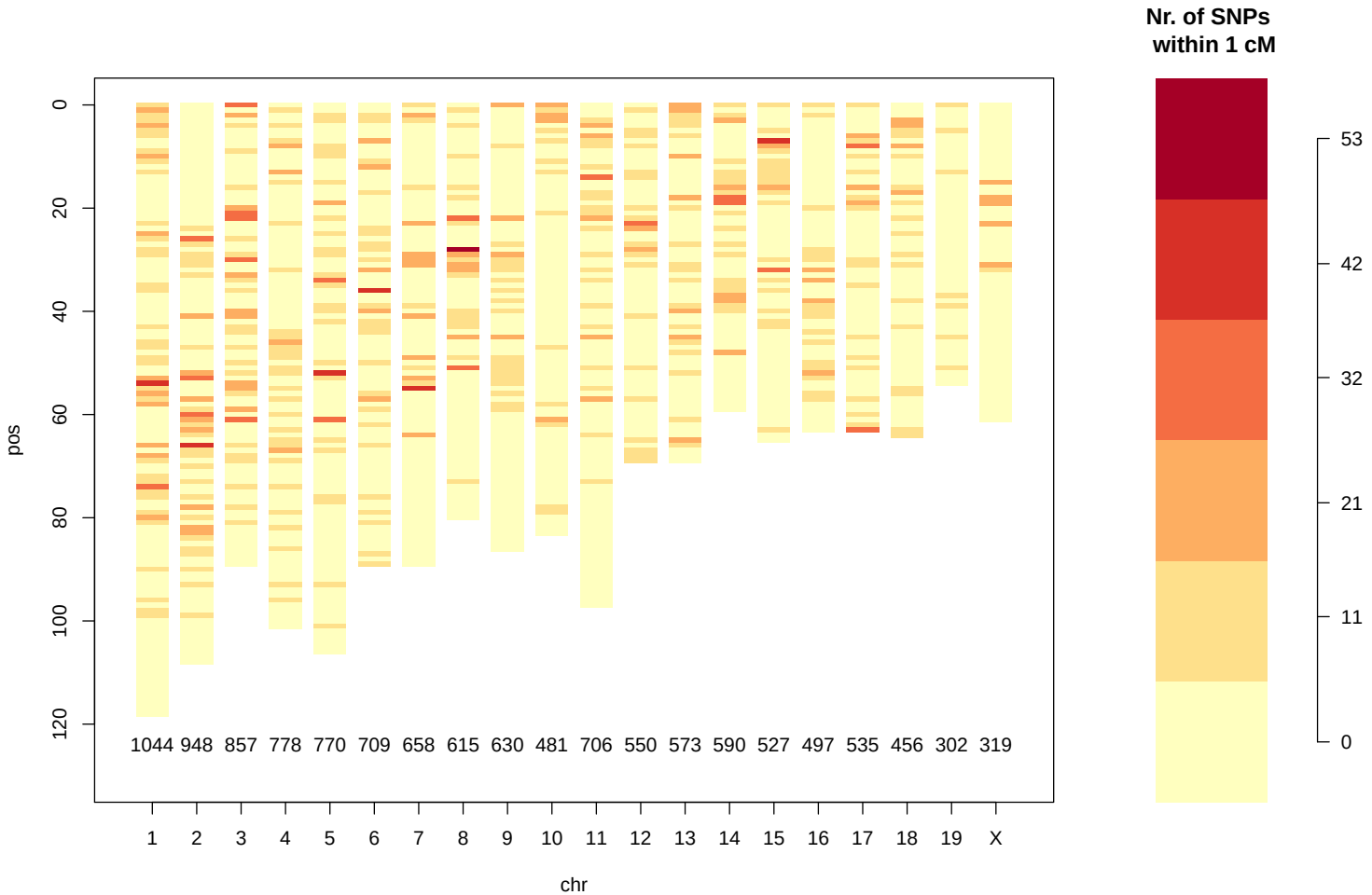
```
R> add.markers(gpData, geno, map = NULL)
```

- Removing markers

```
R> discard.markers(gpData, which)
```

Visualization of marker map

```
R> plotGenMap(mice, dense = TRUE)
```



Summary of marker map

```
R> summaryGenMap(maize)
```

	noM	range	avDist	maxDist	minDist
1	76	157.52	2.100267	11.08	0.10
2	96	151.38	1.593474	6.81	0.03
3	99	157.44	1.606531	13.11	0.02
4	122	154.34	1.275537	13.11	0.04
5	85	155.13	1.846786	11.67	0.01
6	106	157.70	1.501905	12.46	0.02
7	154	158.98	1.039085	6.48	0.02
8	130	156.62	1.214109	7.03	0.05
9	121	157.27	1.310583	14.21	0.06
10	128	153.92	1.211969	15.19	0.08

Pedigree

- Class `pedigree` for pedigree objects
- `data.frame` with 4 (5) columns

ID	Par1	Par2	gener	sex
A	-	-	0	
B	-	-	0	
C	A	B	1	
D	A	C	2	
E	D	B	3	

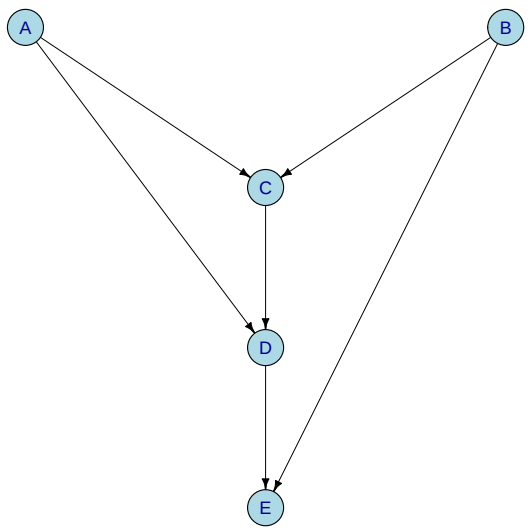
- first generation = 0
- Create pedigree object

```
R> id <- c("A", "B", "C", "D", "E")
R> par1 <- c(0, 0, "A", "A", "D")
R> par2 <- c(0, 0, "B", "C", "B")
R> ped <- create.pedigree(id, par1, par2)
```

Pedigree

Visualization of pedigree structure

```
R> plot(ped)
```



Summary of pedigree structure

```
R> summary(ped)
```

```
Number of
 individuals  5
 Par 1        2
 Par 2        2
 generations  4
```

Estimation of relatedness

- Pedigree based (expected) and realized kinship coefficients: function `kin`
  - additive numerator relationship matrix **A** (default)

    ```
    R> kin(gpData, ret = "add")
    ```
  - dominance relationship matrix **D**

    ```
    R> kin(gpData, ret = "dom")
    ```
  - kinship matrix $\mathbf{K} = \frac{1}{2}\mathbf{A}$

    ```
    R> kin(gpData, ret = "kin")
    ```
  - gametic relationship matrix (dimension $2n \times 2n$)

    ```
    R> kin(gpData, ret = "gam")
    ```
- object `gpData` with filled slot `pedigree`

Estimation of relatedness

- Relationship matrix for `maize` data (fully homozygous inbred lines with inbreeding coefficient $F=1$)

```
R> A <- kin(maize, DH = maize$covar$DH)
```

- Object of class `relationshipMatrix`

```
R> class(A)

[1] "relationshipMatrix"
```

- Row names = col names = names of individuals
- S3 summary method

```
R> summary(A)

dimension                      1610 x 1610
rank                           1460
range of off-diagonal values   0 -- 1.757812
number of unique values        1435
range of diagonal values       1 -- 2
```

Processing marker data

- Raw marker data could by coded by alleles or by genotypes
- `synbreed` algorithms only for biallelic markers
- Data processing algorithms collected in function `codeGeno`

Features of `codeGeno`

- Recode data as number of copies of the minor allele, i.e. 0, 1, and 2
- Preselect markers (MAF, missing values, LD)
- Impute missing genotypes, either by
  - Random imputation by marginal allele distribution
  - Imputation by full-sib family information (only for homozygous inbred lines)
  - Beagle (Browning and Browning 2009)
  - Beagle after family
  - A fixed value

Algorithm of codeGeno

```
R> codeGeno(gpData, impute = FALSE, impute.type = c("fix",
+       "random", "family"), replace.value = NULL, maf = NULL,
+       nmiss = NULL, label.heter = "AB", keep.identical = TRUE,
+       verbose = FALSE)
```

1. Discarding markers with fraction $>$ `nmiss` of missing values
2. Recoding alleles as number of the minor alleles, i.e. 0, 1 and 2
3. Replace of missing values by replace.value or impute missing values according to `impute.type`
4. Recoding of alleles after imputation, if necessary due to changes in allele frequencies by imputed alleles
5. Discarding markers with a minor allele frequency of $\leq$ `maf`
6. Discarding duplicated markers if `keep.identical=FALSE`
7. Restoring original data format (`gpData`, `matrix` or `data.frame`)

Usage of function codeGeno

```
R> which.heter <- function(x) substr(x, 1, 1) != substr(x,
+      3, 3)
R> mc <- codeGeno(mice, label.heter = which.heter, maf = 0.1,
+      nmiss = 0.2, keep.identical = FALSE, verbose = TRUE,
+      impute = TRUE, impute.type = "random")

step 1 : 64 marker(s) removed with > 20 % missing values
step 2 : Recoding alleles
step 3 : Random imputing of missing values
         approximate run time  72.27  seconds
step 4 : Recode alleles due to imputation
step 5 : 3190 marker(s) removed with maf < 0.1
step 6 : 1084 duplicated marker(s) removed
step 7 : Restoring original data format

Summary of imputation
  total number of missing values                  : 80604
  number of random imputations                    : 80604
  approximate fraction of correct imputations     : 0.685
```

Analysis of LD

- LD : non-random association between alleles at different loci
- LD is computed as coefficient of determination R^2 between markers k and l

$$LD_{kl} = \frac{\mathrm{Cov}(\mathbf{x_k}, \mathbf{x_l})^2}{\mathrm{Var}(\mathbf{x_k})\mathrm{Var}(\mathbf{x_l})} = \frac{(p_{ij} - p_i p_j)^2}{p_i(1-p_i)p_j(1-p_j)}$$

  with p_{ij} as frequency of haplotype ij and p_i and p_j the frequencies of allele i at locus k and allele j at locus l
- Computation and visualization of LD combined in functions
  - `LDDist` : LD decay as scatterplot or stacked histogram
  - `LDMap` : LD heatmap using package `LDheatmap` (Shin *et al.* 2006)
- Store LD values, separated by chromosome, e.g.

```
R> LDtable <- LDDist(maize, chr = 1)
R> LDmatrix <- LDMap(maize, chr = 1:3)
```

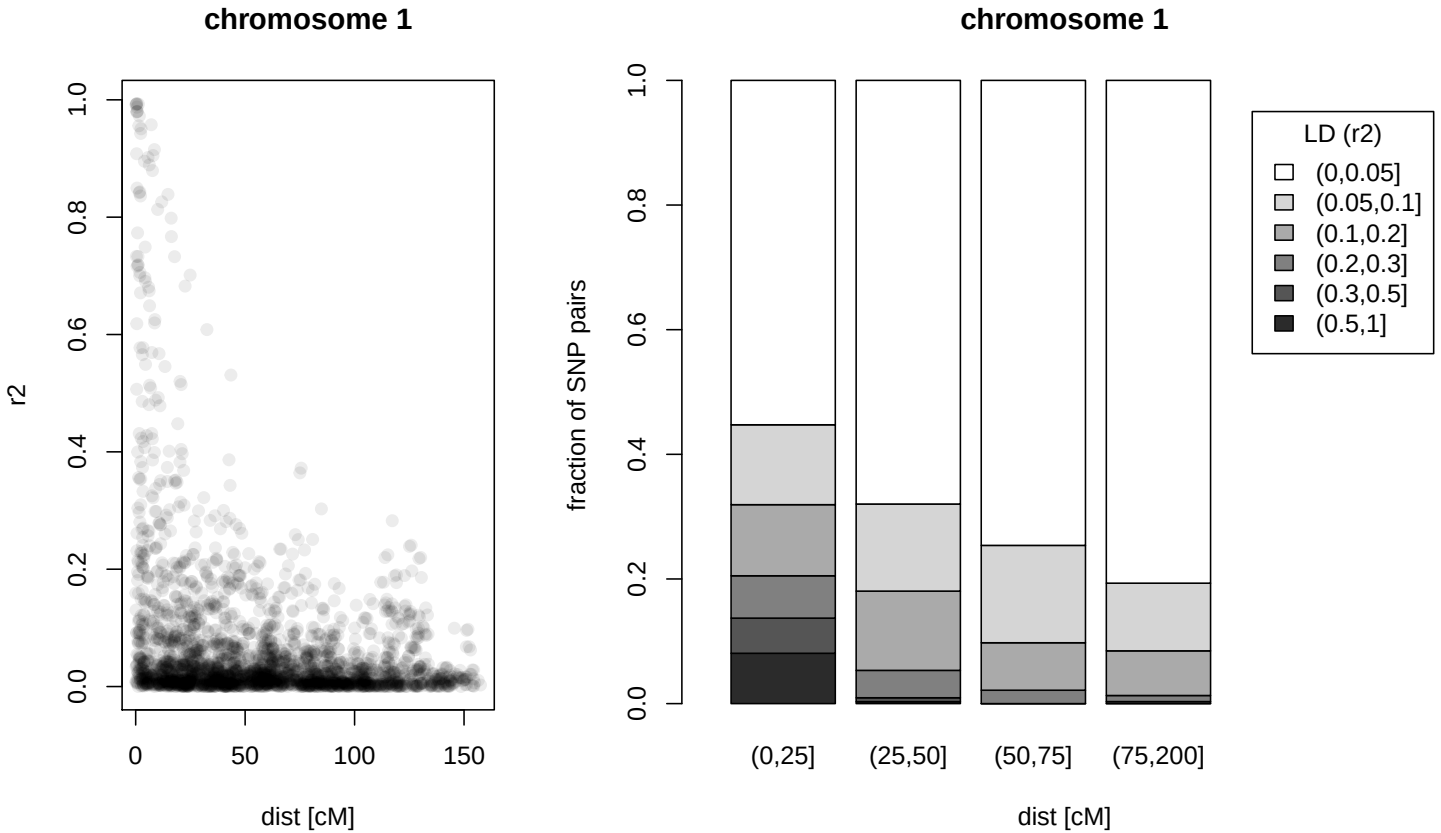


Figure: LD decay as a scatter plot (argument `type="p"`) and stacked histogram (argument `type="bars"`) for first chromosome of `maize` data.

Estimation of relatedness

- Marker based (realized) relatedness:

  realized method proposed by Habier *et al.* (2007)

$$\mathbf{U} = \frac{\mathbf{Z}\mathbf{Z}'}{2\sum_{i=1}^{p} p_i(1-p_i)}$$

  with $\mathbf{Z} = \mathbf{M} - \mathbf{P}$ and $\mathbf{M}$ is the $n \times p$ marker matrix and $\mathbf{P}$ is a $n \times p$ matrix with column wise minor allele frequencies

  sm Simple matching coefficient for homozygous inbred lines

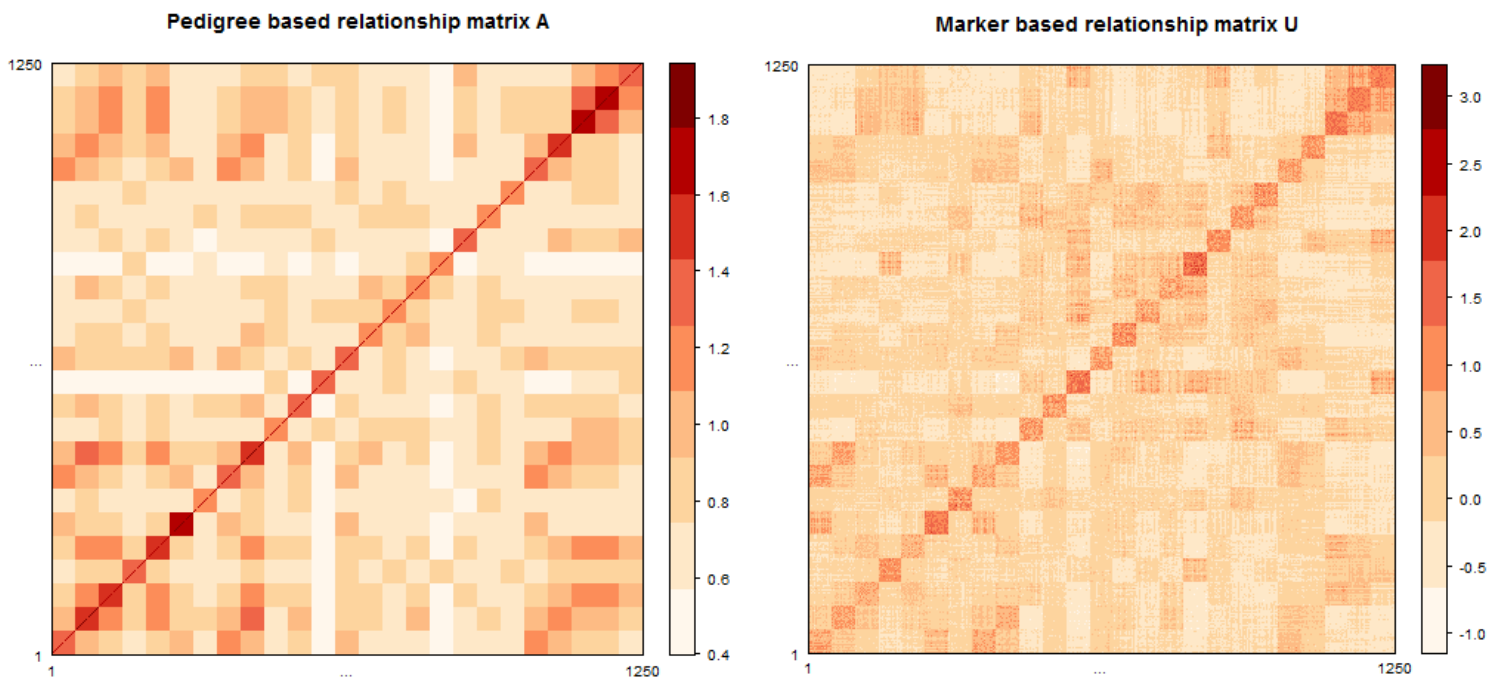
- Object `gpData` with filled slot `geno`, alleles coded as 0, 1, and 2
- Resulting object again of class `relationshipMatrix`
- For `maize` data (homozygous inbred lines): denominator $= 8\sum_{i=1}^{p} p_i(1-p_i)$

```
R> U <- kin(codeGeno(maize), ret = "realized")/4
```

Kinship coefficients as heatmaps

- S3 plot method for heatmap for class `relationshipMatrix`

```
R> plot(A[maize$covar$genotyped, maize$covar$genotyped])
R> plot(U)
```



Genomic prediction

- Use package `regress` (Clifford and McCullagh 2009) for BLUP

```
R> library(regress)
```

- Data processing

```
R> y <- maize$pheno$Trait
R> AA <- A[maize$covar$genotyped, maize$covar$genotyped]
```

- 'Animal model'

```
R> mod1 <- regress(y ~ 1, Vformula = ~AA)
```

- G-BLUP

```
R> mod2 <- regress(y ~ 1, Vformula = ~U)
```

- Reference: TBV from simulation

```
R> tbv <- maize$covar$tbv[maize$covar$genotyped]
```

Genomic prediction - Results

```
R> summary(mod1)

Maximised Residual Log Likelihood is -3349.847

Linear Coefficients:
             Estimate Std. Error
 (Intercept) 1179.535      2.883

Variance Coefficients:
             Estimate Std. Error
          AA   10.712      4.530
          In   70.269      4.182

R> cor(mod1$predicted, y)
           [,1]
[1,] 0.5770398

R> cor(mod1$predicted, tbv)
         [,1]
[1,] 0.58681
```

Genomic prediction - Results

```
R> summary(mod2)

Maximised Residual Log Likelihood is -3223.837

Linear Coefficients:
             Estimate Std. Error
 (Intercept) 1178.921      0.197

Variance Coefficients:
             Estimate Std. Error
          U   106.100     14.716
          In   48.578      2.287
R> cor(mod2$predicted, y)
           [,1]
[1,] 0.7264322
R> cor(mod2$predicted, tbv)
           [,1]
[1,] 0.8563112
```

Cross Validation

```
R> maize2 <- codeGeno(maize)
R> maize2$pheno <- data.frame(rownames(maize2$pheno), maize2$pheno[,
+     1])
R> X <- matrix(rep(1, nrow(maize2$pheno)), ncol = 1)
R> Z <- diag(nrow(maize2$pheno))
R> cv.maize <- crossVal(maize2$pheno, X, Z, cov.matrix = list(U),
+     k = 5, Rep = 2, Seed = 123, sampling = "random",
+     varComp = mod2$sigma, VC.est = "commit")
Model with  1  covariance matrix/ces
random  sampling
Replication:  1   Fold:  1
Replication:  1   Fold:  2
Replication:  1   Fold:  3
Replication:  1   Fold:  4
Replication:  1   Fold:  5
random  sampling
Replication:  2   Fold:  1
Replication:  2   Fold:  2
Replication:  2   Fold:  3
Replication:  2   Fold:  4
```

Cross Validation

```
R> summary(cv.maize)
Object of class 'cvData'

 5 -fold cross validation with 2 replications
Sampling:    random
Variance components:   committed
Number of random effects: 1
Number of individuals:  1250
Size of the TS:    250 -- 250

Results:
          Min   Mean +- pooled SE   Max
 Predictive ability:  0.4589   0.5287 +- 0.0079   0.5691
 Bias:          0.8747   1.0061 +- 0.0253   1.118

Seed start:  123
Seed replications:
[1] 28758 78831
```

Structure of an R package

- An R package is structured into

R	R code, `*.R`
man	documentation files, `*.Rd`
data	subdirectory for data files, `*.Rdata`
inst	citation file, subdirectory `doc`: package vignette
src	external source code, i.e. `*.c`, `*.cc` or `*.cpp`, `*.f`, `*.f90` or `*.f95`

- Every function in `R` has a documentation in `man`
- Create skeleton for a documentation

```
R> myFunc <- function(x) x^2 + 1
R> prompt(myFunc)
```

Creating R packages

- Build an R package from source code

  `R CMD build synbreed`
- This gives pre-compiled version of packages for binary distributions
- Install package from `synbreed.tar.gz`

  `R CMD INSTALL synbreed`
- CRAN check

  `R CMD check synbreed`
- Create Manual

  `R CMD Rd2pdf synbreed`
- See also 'Writing R Extensions' Manual

S3 methods

- Objects could have a class or multiple classes

```
R> class(maize)

[1] "gpData"

R> class(ped)

[1] "pedigree"   "data.frame"
```

- One could assign any class to an object without consistency checks (and get unexpected results)

```
R> class(ped) <- "character"
```

- Generic methods for objects of a certain class : `foo.cl`, function `foo` for class `cl`
- Examples

```
R> summary(ped)
R> summary.pedigree(ped)
```

- See also 'Writing R Extensions': names spaces and `S3Method` for registering S3 methods

R-Forge

- `http://r-forge.r-project.org/`
- R-Forge is a subversion system for R package developer
- Work is organized in 'Projects'
- Source code management and version control
- Authorized collaborators can 'check out' or 'update' the project file structure
- SVN keeps track of the complete repository history
- Package submission to CRAN

R-Forge

- Automatic build from daily snapshot
- Install packages from R-Forge

```
R> install.packages("synbreed", repos = "http://R-Forge.R-project.org")
```

- No anonymous access at the moment
- Roles in a project: Administrator, Senior developer, Junior developer
- Additional features: Project website, mailing lists, project categorization, news, forums
- For Synbreed: `synbreed-news@lists.r-forge.r-project.org`
- More information on R-Forge in Theußl and Zeileis (2009)

SVN

- Repository

  `svn+ssh://username@svn.r-forge.r-project.org/svnroot/synbreed`
- More information on SVN

  `http://svnbook.red-bean.com`
- SVN tool for Windows

  `http://tortoisesvn.net/downloads`

Extensions ?

- Stand-alone function for LD, additional measures, e.g. D'
- Imputation of missing genotypes: link to other software packages: `Beagle` (Browning and Browning 2009) and/or `fastPhase` (Scheet and Stephens 2006)
- Genomic prediction methods, e.g. BayesA, BayesB (use external source code?)
- Simulation methods
- Parallel computing
- ...

Extension

- Package `synbreed` as framework for the analysis of breeding data
- Promotion of own methods (citation in documentation)
- Usage of a common data class
- Guidelines for a collaborative software development
  - Common standards (S3 or S4)
  - Common example data sets
  - Standards for documentation
  - Quality checks

Dependencies for the `synbreed` package

To load the `synbreed` package, we require packages

- lattice
- igraph
- MASS
- LDheatmap
- qtl
- doBy
- BLR

Gateway from `synbreed` to package `qtl`

- Package `qtl` for QTL analysis in experimental crosses
- Main data class `cross`
- Conversion from `gpData` to `cross`

  ```
  R> gpData2cross(gpDataObj)
  ```

- Conversion from `cross` to `gpData`

  ```
  R> cross2gpData(crossObj)
  ```

Literature

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Synbreed

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