# IEA

## LEA Overview

- LEA is an R package
- population structure
- genome-wide tests
- identifying genetic polymorphisms

## lea tutorial

```
# make directory
getwd()
setwd("G:/R CLASS practice/lea2")
#first install LEA and call it
install.packages("LEA")
library(LEA)
```

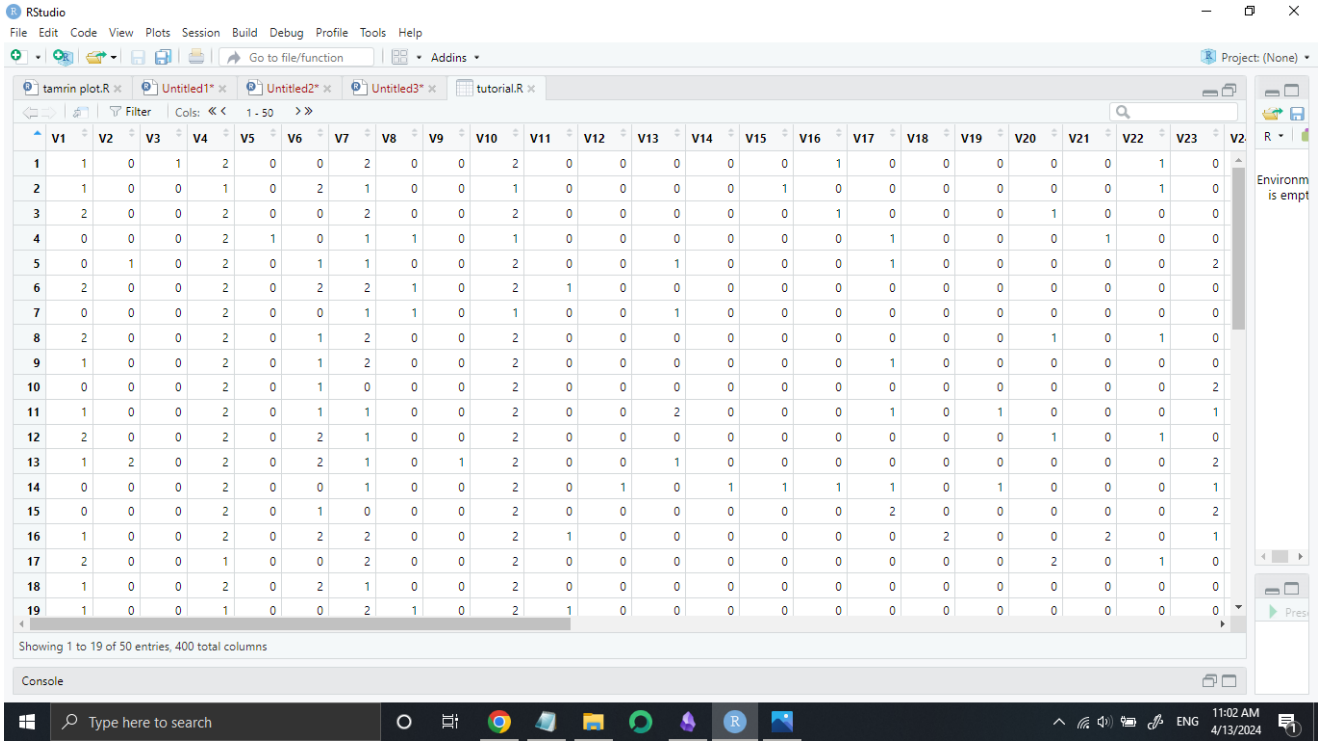
## tutorial data

LEA have a small tutorial dataset consisting of 400 SNPs genotyped for 50 diploid individuals in side the the package.

```
data("tutorial")
#type of file you need
write.lfmm(tutorial.R, "genotypes.lfmm")
write.geno(tutorial.R, "genotypes.geno")

write.env(tutorial.C, "gradients.env")
# creation of an environment gradient file:
```

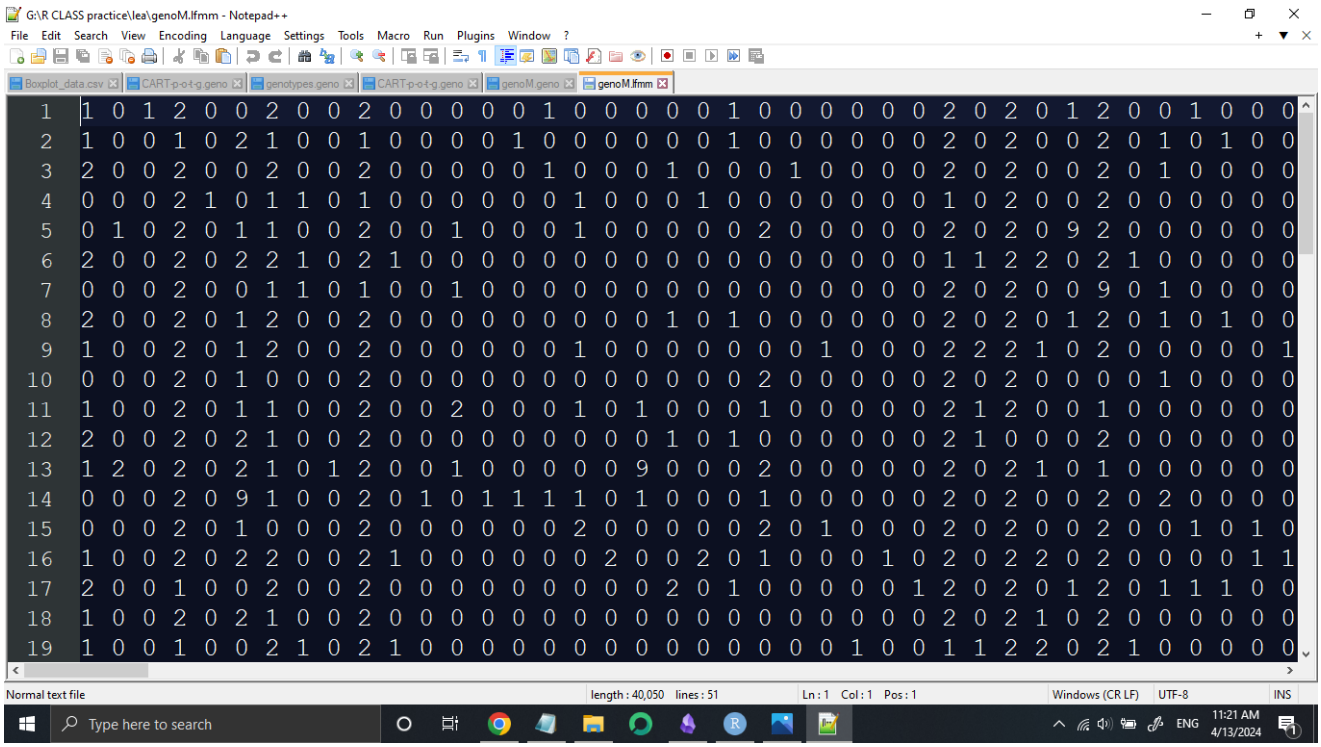
```
gradient.env. # The .env file contains a single ecological variable
```

# raw data



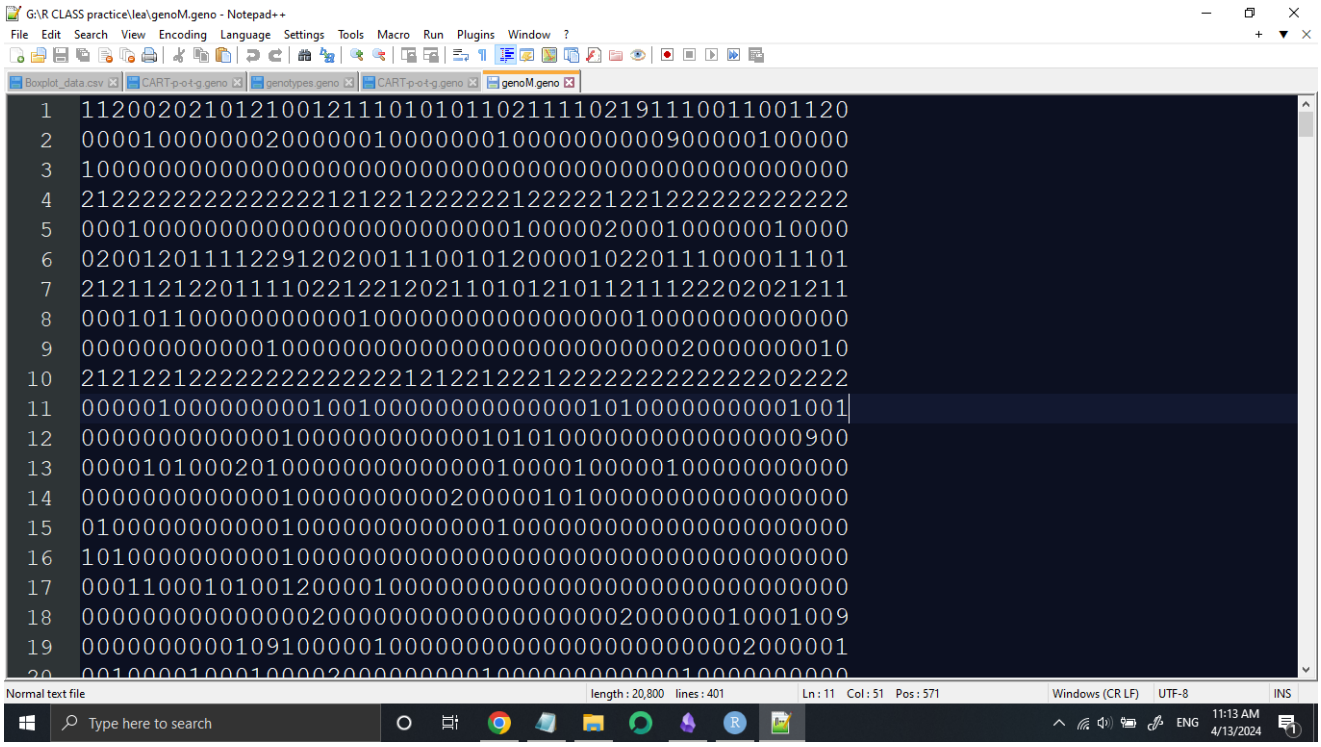
| | V1 | V2 | V3 | V4 | V5 | V6 | V7 | V8 | V9 | V10 | V11 | V12 | V13 | V14 | V15 | V16 | V17 | V18 | V19 | V20 | V21 | V22 | V23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 0 | 1 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 2 | 1 | 0 | 0 | 1 | 0 | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 3 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 2 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| 5 | 0 | 1 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| 6 | 2 | 0 | 0 | 2 | 0 | 2 | 2 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 2 | 0 | 0 | 2 | 0 | 1 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| 9 | 1 | 0 | 0 | 2 | 0 | 1 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 11 | 1 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| 12 | 2 | 0 | 0 | 2 | 0 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| 13 | 1 | 2 | 0 | 2 | 0 | 2 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 14 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| 15 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 16 | 1 | 0 | 0 | 2 | 0 | 2 | 2 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 1 |
| 17 | 2 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 |
| 18 | 1 | 0 | 0 | 2 | 0 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

# genotypes in the lfmm format

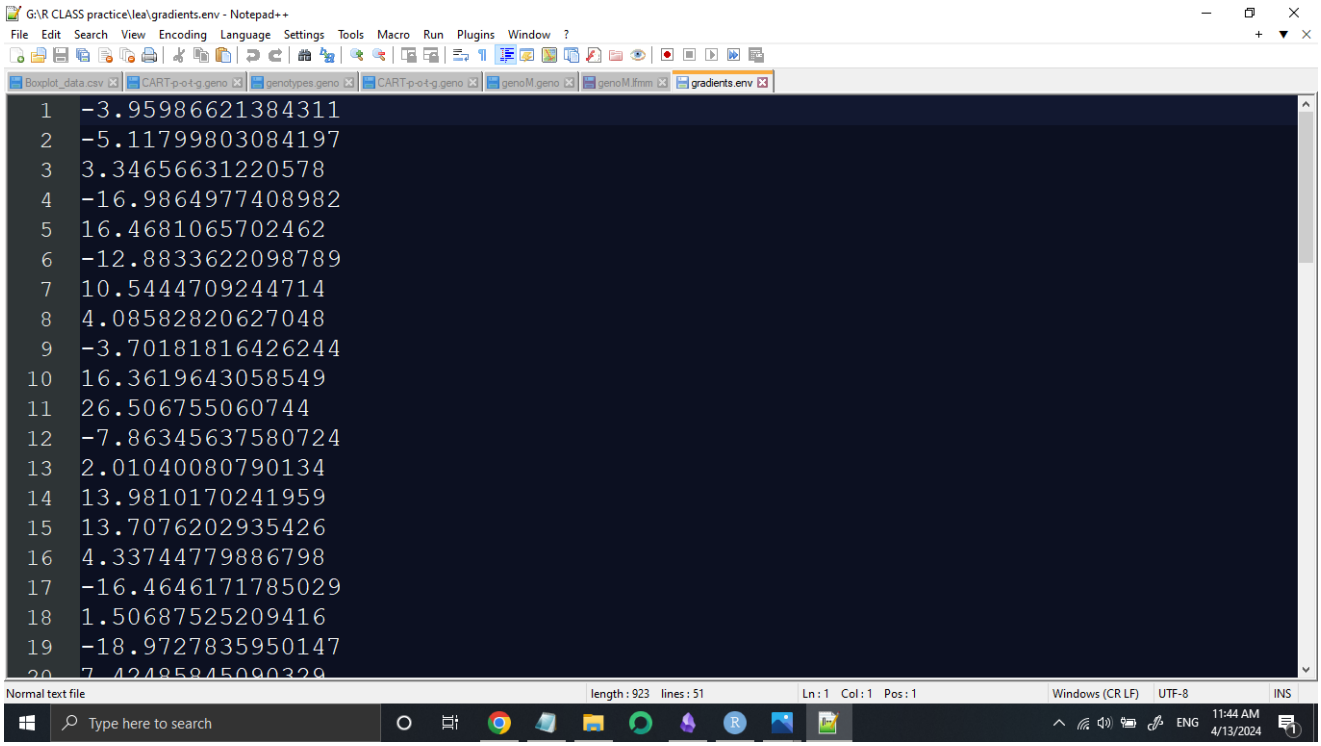


| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 0 | 1 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 |
| 2 | 1 | 0 | 0 | 1 | 0 | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 0 | 0 |
| 3 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 2 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 1 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 9 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 2 | 0 | 0 | 2 | 0 | 2 | 2 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 2 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 9 | 0 | 1 | 0 | 0 | 0 | 0 |
| 8 | 2 | 0 | 0 | 2 | 0 | 1 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 1 | 2 | 0 | 1 | 0 | 1 | 0 | 0 |
| 9 | 1 | 0 | 0 | 2 | 0 | 1 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 2 | 2 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 |
| 10 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 11 | 1 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 2 | 0 | 0 | 2 | 0 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 1 | 2 | 0 | 2 | 0 | 2 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 2 | 0 | 9 | 1 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 0 |
| 16 | 1 | 0 | 0 | 2 | 0 | 2 | 2 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| 17 | 2 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 2 | 0 | 1 | 2 | 0 | 1 | 1 | 1 | 0 | 0 |
| 18 | 1 | 0 | 0 | 2 | 0 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 2 | 2 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 |

# genotypes in in the geno format




The `geno` format has one row for each SNP. Each row contains 1 character for each individual: 0 means zero copy of the reference allele. 1 means one copy of the reference allele. 2 means two copies of the reference allele. 9 means missing data.




# Analysis of population structure

The R package LEA implements two classical approaches for the estimation of population genetic structure: principal component analysis (pca) and admixture analysis using sparse nonnegative matrix factorization (snmf)

# Principal Component Analysis

```
pc = pca("genotypes.lfmm", scale = TRUE)
tw = tracy.widom(pc)
# Available options, K (the number of PCs),
# center and scale.
# Create files: genotypes.eigenvalues - eigenvalues,
# genotypes.eigenvectors - eigenvectors,
# genotypes.sdev - standard deviations,
# genotypes.projections - projections,
# Create a pcaProject object: pc.
```

## Result

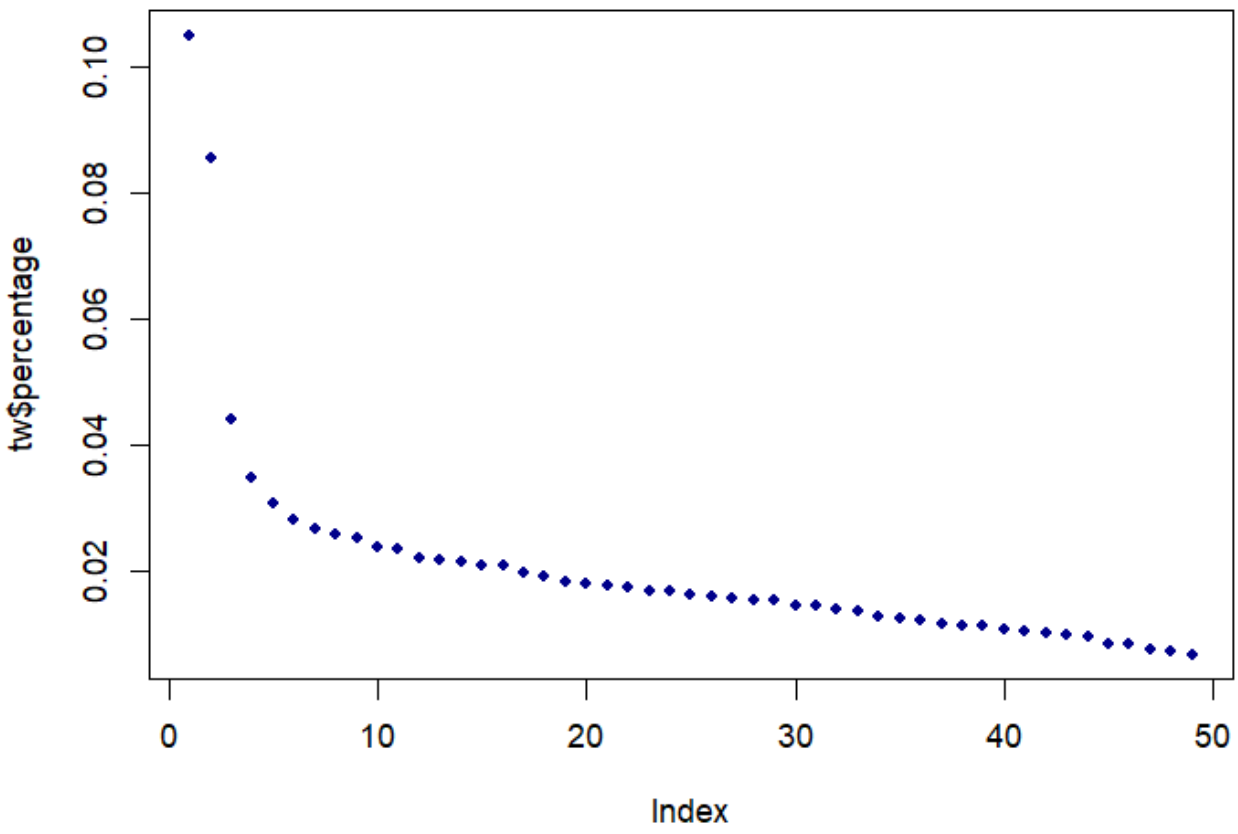
```
      -n (number of individuals)          50
        -L (number of loci)                 400
        -K (number of principal components) 50
        -x (genotype file)                  G:\R CLASS
practice\lea\genotypes.lfmm
        -a (eigenvalue file)                G:\R CLASS
practice\lea\genotypes.pca/genotypes.eigenvalues
        -e (eigenvector file)               G:\R CLASS
practice\lea\genotypes.pca/genotypes.eigenvectors
        -d (standard deviation file)        G:\R CLASS
practice\lea\genotypes.pca/genotypes.sdev
        -p (projection file)                G:\R CLASS
```

```
practice\lea\genotypes.pca/genotypes.projections
        -s data centered and scaled
```

## percentage of variance

```
#plot the percentage of variance explained by each
component plot(tw$percentage, pch = 19, col =
"darkblue", cex = .8)
```

## percentage of variance

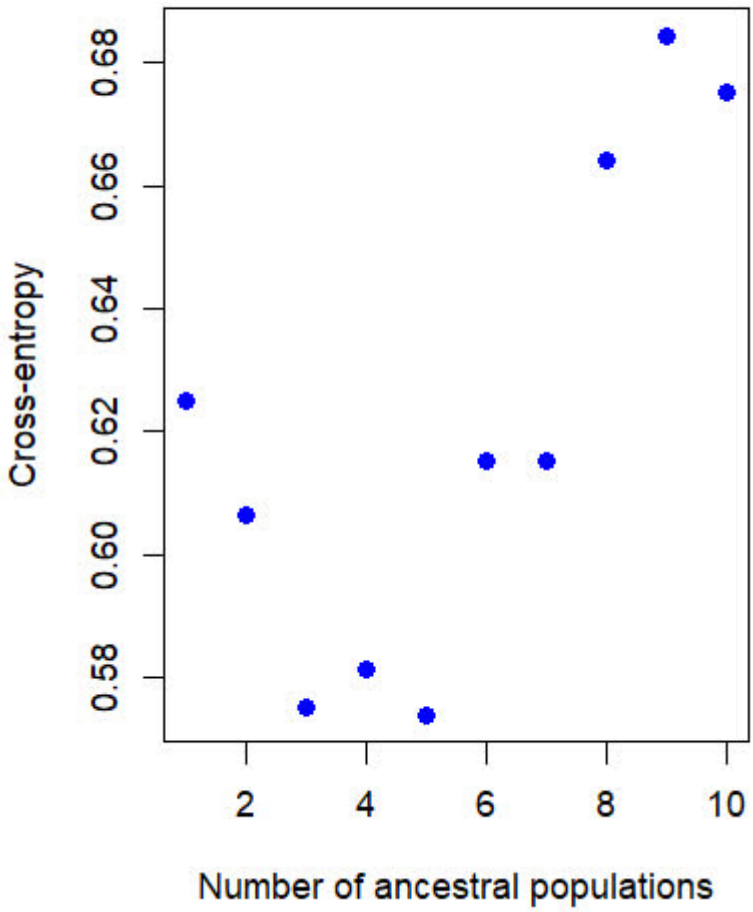


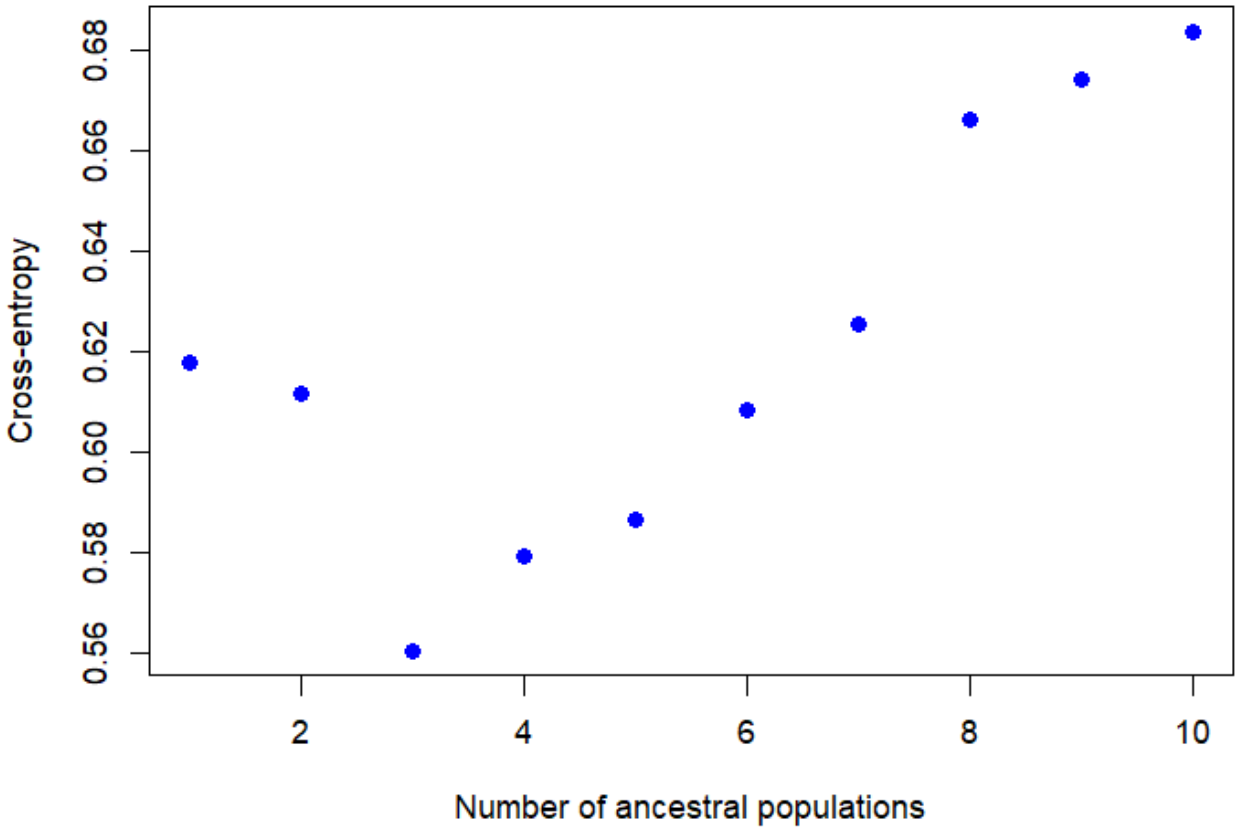

# STRUCTURE

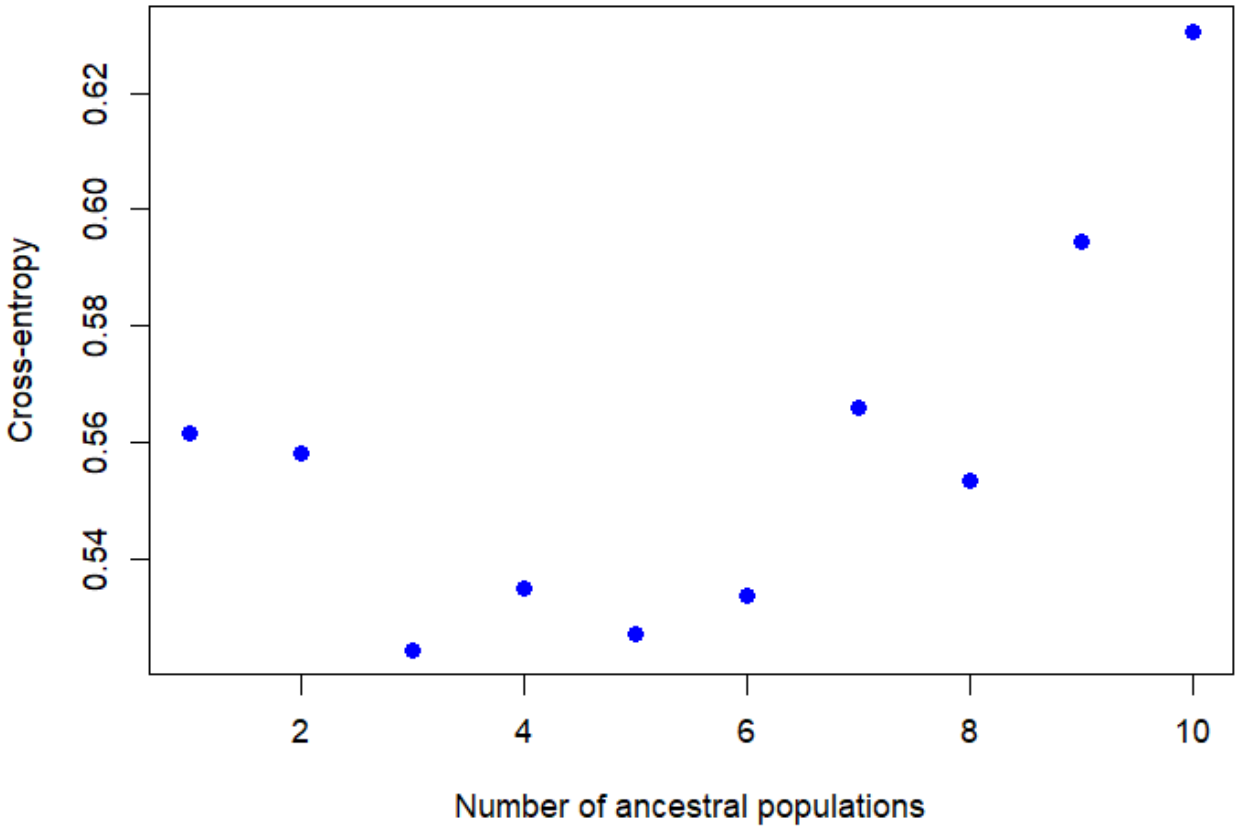
```
# main options
# K = number of ancestral populations
```

```r
# entropy = TRUE computes the cross entropy criterion,
# CPU = 4 is the number of CPU used (hidden input)
project = NULL
project = snmf("genotypes.geno", K = 1:10, entropy =
TRUE, repetitions =     10,project = "new")
plot(project, col = "blue", pch = 19, cex = 1.2)
```

## Result

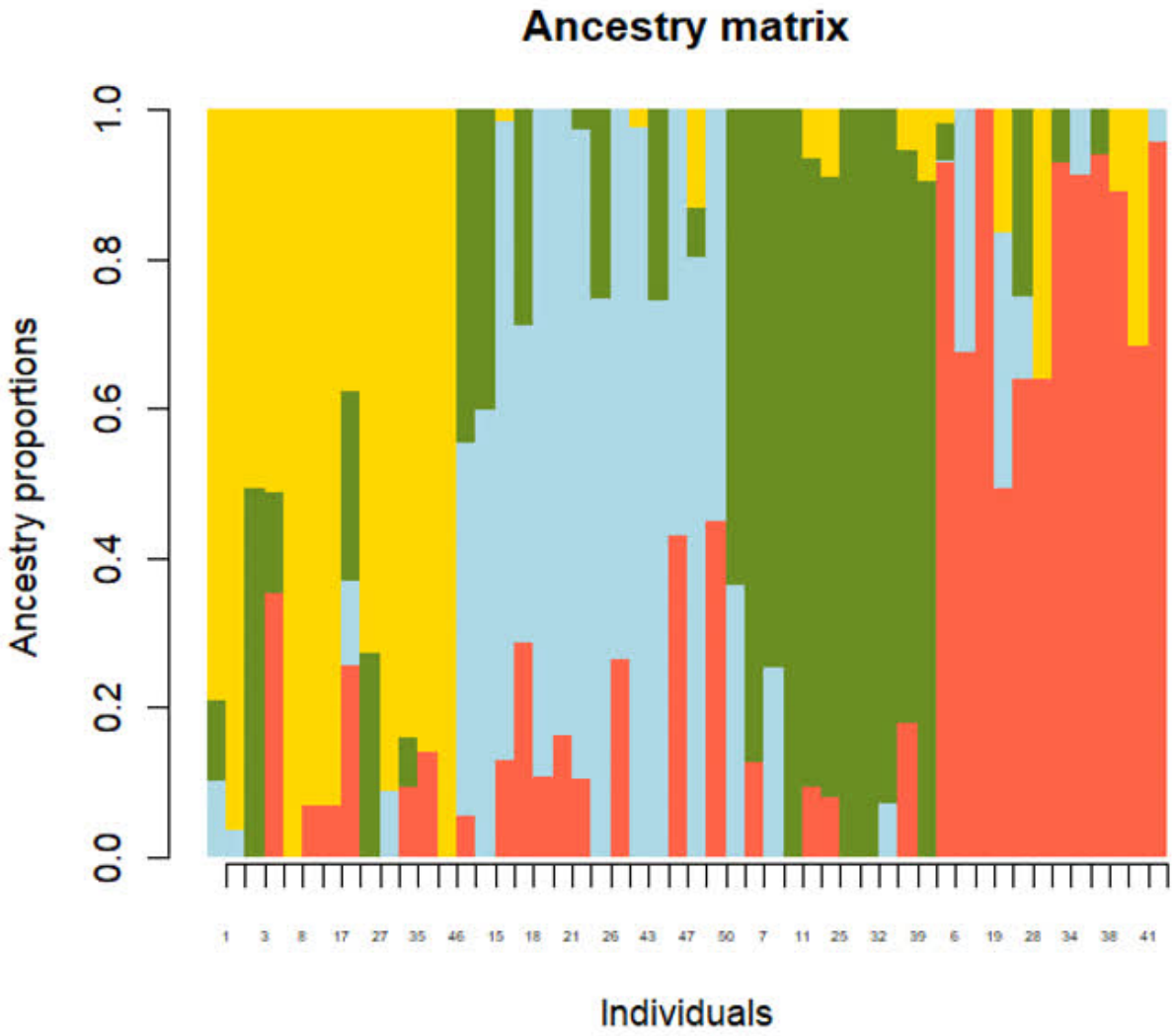

Cross-entropy
0.58
0.60
0.62
0.64
0.66
0.68
2
4
6
8
10
Number of ancestral populations


Cross-entropy
0.56
0.58
0.60
0.62
0.64
0.66
0.68
2
4
6
8
10
Number of ancestral populations

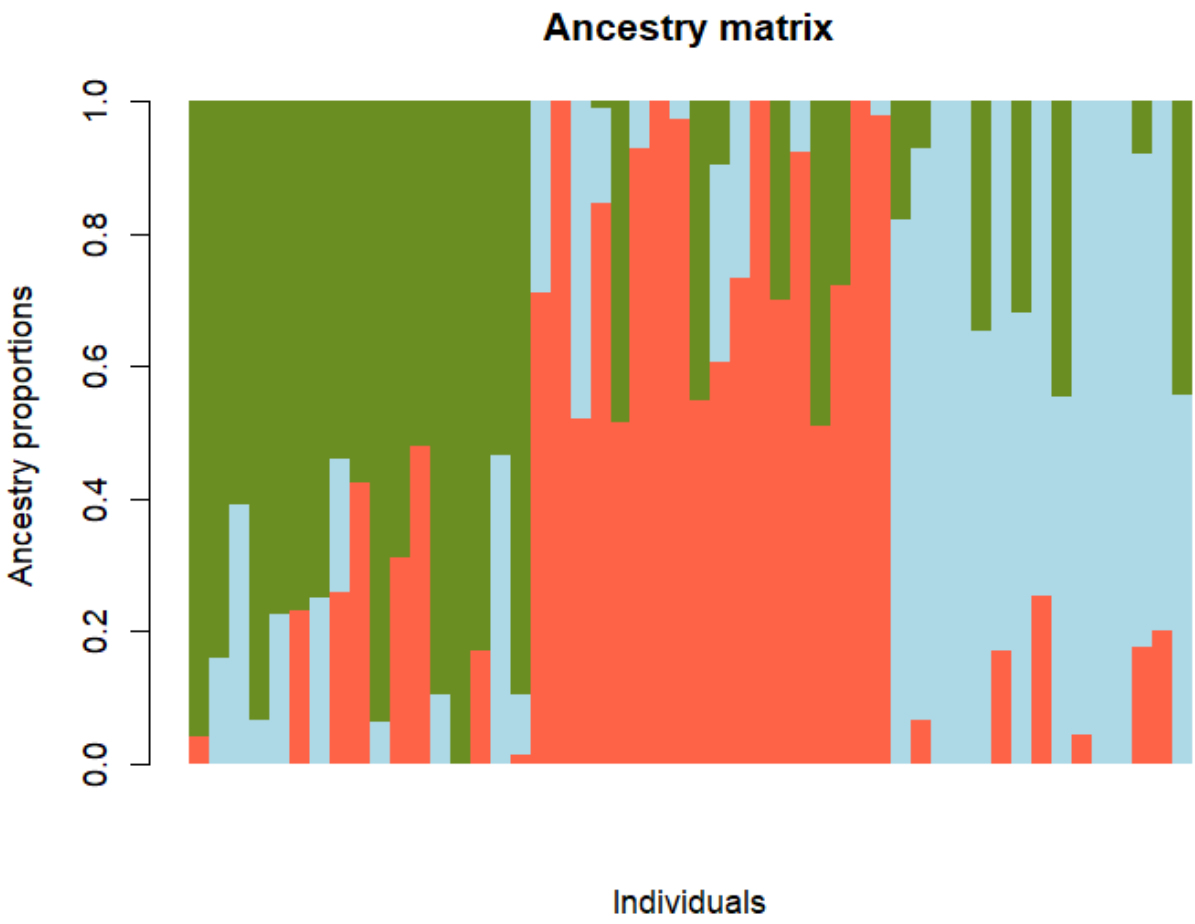

Cross-entropy
0.54
0.56
0.58
0.60
0.62
2
4
6
8
10
Number of ancestral populations

```r
best = which.min(cross.entropy(project, K = 3))
my.colors <- c("tomato", "lightblue",
               "olivedrab", "gold")
barchart(project, K = 3, run = best,
         border = NA, space = 0,
         col = my.colors,
         xlab = "Individuals",
         ylab = "Ancestry proportions",
         main = "Ancestry matrix") -> bp
axis(1, at = 1:length(bp$order),
     labels = bp$order, las=1,
     cex.axis = .4)
```

## Result


Ancestry matrix
Ancestry proportions
0.0
0.2
0.4
0.6
0.8
1.0
1 3 8 17 27 35 46 15 18 21 26 43 47 50 7 11 25 32 39 6 19 28 34 38 41
Individuals

k=5

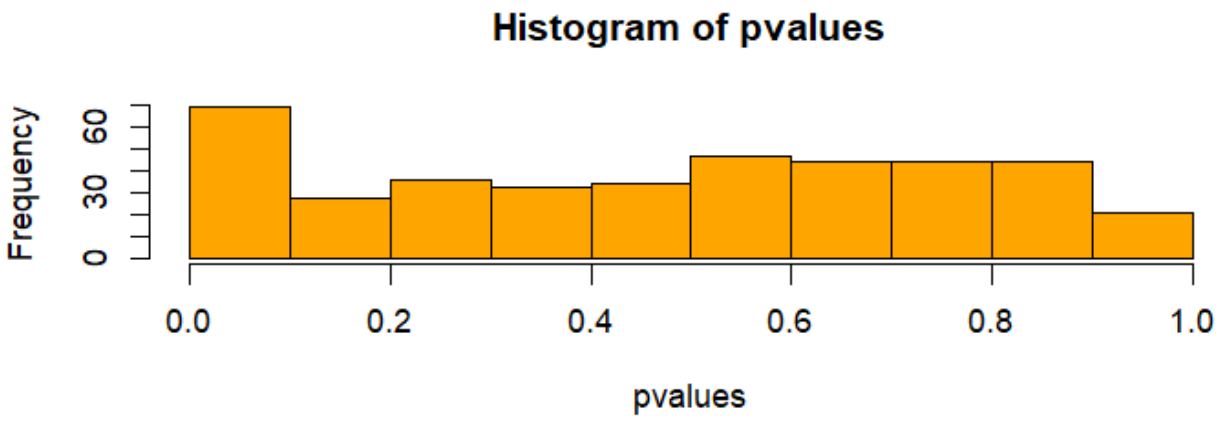


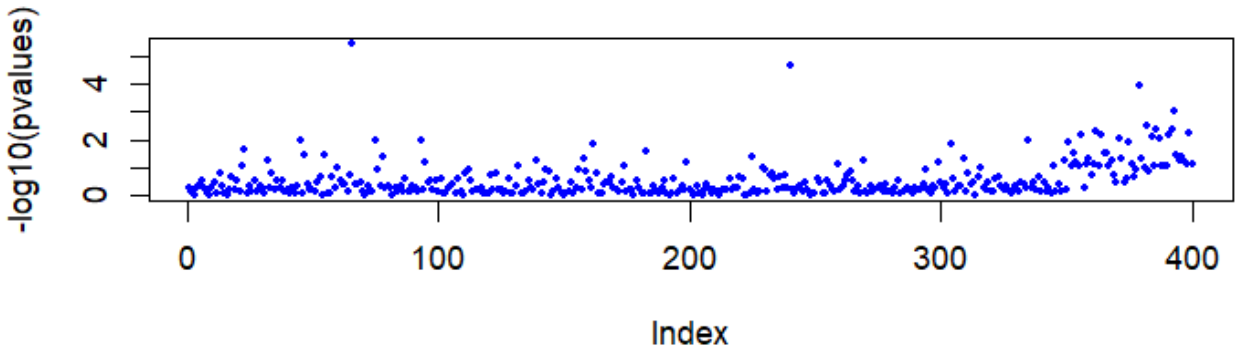

k=3

# Population differentation tests

```
p = snmf.pvalues(project,
                 entropy = TRUE,
                 ploidy = 2,
                 K = 3)
pvalues = p$pvalues
par(mfrow = c(2,1))
hist(pvalues, col = "orange")
plot(-log10(pvalues), pch = 19, col = "blue", cex = .5)
```

# Result



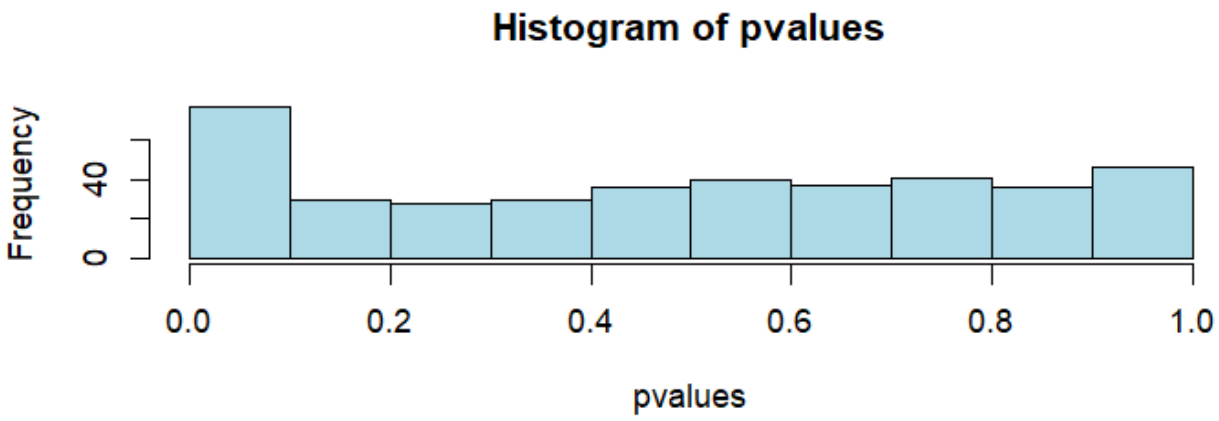

P-values for population differentiation tests with snmf()

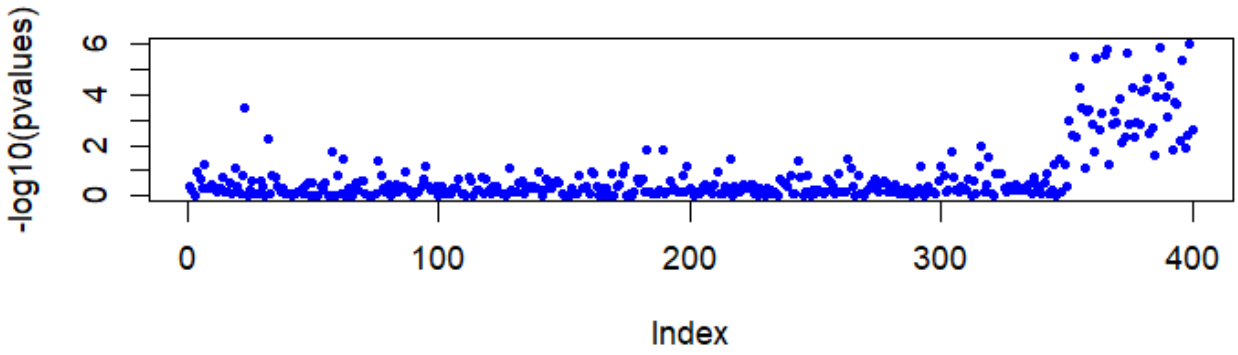
# Ecological association tests using lfmm

```
# creation of a genotype matrix with missing genotypes
dat = as.numeric(tutorial.R)
dat[sample(1:length(dat), 100)] <- 9
dat <- matrix(dat, nrow = 50, ncol = 400)
write.lfmm(dat, "genoM.lfmm")
## [1] "genoM.lfmm"
project.missing = snmf("genoM.lfmm", K = 4,
                       entropy = TRUE, repetitions = 10,
                       project = "new")

####
# select the run with the lowest cross-entropy value
best = which.min(cross.entropy(project.missing, K = 4))
```

```r
# Impute the missing genotypes
impute(project.missing, "genoM.lfmm",
       method = 'mode', K = 4, run = best)
## Missing genotype imputation for K = 4
## Missing genotype imputation for run = 1
## Results are written in the file:
genoM.lfmm_imputed.lfmm
# Proportion of correct imputation results
dat.imp = read.lfmm("genoM.lfmm_imputed.lfmm")
mean( tutorial.R[dat == 9] == dat.imp[dat == 9] )
## [1] 0.83

project = NULL
project = lfmm("genotypes.lfmm",
               "gradients.env",
               K = 6,
               repetitions = 5,
               project = "new")

p = lfmm.pvalues(project, K = 6)
pvalues = p$pvalues

par(mfrow = c(2,1))
hist(pvalues, col = "lightblue")
plot(-log10(pvalues), pch = 19, col = "blue", cex = .7)
```

## Result




```
for (alpha in c(.05,.1,.15,.2)) {
  # expected FDR
  12
  print(paste("Expected FDR:", alpha))
  L = length(pvalues)
  # return a list of candidates with expected FDR alpha.
  # Benjamini-Hochberg's algorithm:
  w = which(sort(pvalues) < alpha * (1:L) / L)
  candidates = order(pvalues)[w]
  # estimated FDR and True Positive Rate
  Lc = length(candidates)
  estimated.FDR = sum(candidates <= 350)/Lc
  print(paste("Observed FDR:",
              round(estimated.FDR, digits = 2)))
  estimated.TPR = sum(candidates > 350)/50
  print(paste("Estimated TPR:",
```

```
                round(estimated.TPR, digits = 2)))
```

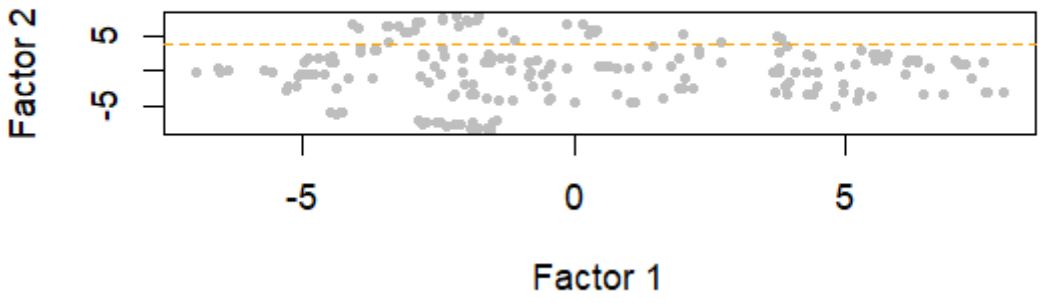
```
[1] "Expected FDR: 0.05"
[1] "Observed FDR: 0.02"
[1] "Estimated TPR: 0.84"
[1] "Expected FDR: 0.1"
[1] "Observed FDR: 0.06"
[1] "Estimated TPR: 0.88"
[1] "Expected FDR: 0.15"
[1] "Observed FDR: 0.13"
[1] "Estimated TPR: 0.94"
[1] "Expected FDR: 0.2"
[1] "Observed FDR: 0.13"
[1] "Estimated TPR: 0.96"
```

# Ecological association tests using lfmm2

```
# load simulated data
data("offset_example")
# 200 diploid individuals genotyped at 510 SNP
Y <- offset_example$geno
# 4 environmental variables
X <- offset_example$env
mod.lfmm2 <- lfmm2(input = Y, env = X, K = 2)

# Simulate non-null effect sizes for 10 target loci
#individuals
n = 100
#loci
L = 1000
# Environmental variable
X = as.matrix(rnorm(n))
```

```r
# effect sizes
B = rep(0, L)
target = sample(1:L, 10)
# GEA significance test
# showing the K = 2 estimated factors
plot(mod.lfmm2@U, col = "grey", pch = 20,
     xlab = "Factor 1",
     ylab = "Factor 2")

B[target] = runif(10, -10, 10)
# Create 3 hidden factors and their loadings
U = t(tcrossprod(as.matrix(c(-1,0.5,1.5)), X)) +
  matrix(rnorm(3*n), ncol = 3)
V <- matrix(rnorm(3*L), ncol = 3)

pv <- lfmm2.test(object = mod.lfmm2,
                 input = Y,
                 env = X,
                 full = TRUE)
plot(-log10(pv$pvalues), col = "grey", cex = .5, pch =
19)
abline(h = -log10(0.1/510), lty = 2, col = "orange")
```

## Result


Factor 2
5
-5
-5
0
5
Factor 1