


Tutorial using the  software

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# A tutorial for the R package `adegenet_1.1-0`

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# 1 Introduction

This tutorial proposes a short visit through functionalities of the **adeigenet** package for R (Ihaka & Gentleman, 1996; R Development Core Team, 2008). The purpose of this package is to facilitate the multivariate analysis of molecular marker data, especially using the **ade4** package (Chessel *et al.*, 2004). Data can be imported from popular softwares like GENETIX, or converted from simple data frame of genotypes. **adeigenet** also aims at providing a platform from which to use easily methods provided by other R packages (e.g., Goudet, 2005). Indeed, if it is possible to perform various genetic data analyses using R, data formats often differ from one package to another, and conversions are sometimes far from easy and straightforward.

In this tutorial, I first present the two object classes used in **adeigenet**, namely **genind** (genotypes of individuals) and **genpop** (genotypes grouped by populations). Then, several topics will be tackled using reproducible examples.

## 2 First steps

### 2.1 Installing the package

Current version of the package is 1.1-0, and is compatible with R 2.6.2. Here the **adeigenet** package is installed along with other recommended packages.

```
> install.packages("adeigenet", dep = TRUE)
```

Then the first step is to load the package:

```
> library(adeigenet)
```

### 2.2 Object classes

Two classes of objects are defined, depending on the scale at which the genetic information is stored: **genind** is used for individual genotypes, whereas **genpop** is used for alleles numbers counted by populations. Note that the term 'population', here and later, is employed in a broad sense: it simply refers to any grouping of individuals.

#### 2.2.1 **genind** objects

These objects can be obtained by importation from foreign softwares, from a **data.frame** of genotypes, or by conversion from a table of allelic frequencies (see 'importing data').

```

> data(nancycats)
> is.genind(nancycats)

[1] TRUE

> nancycats

#####
### Genind object ###
#####
- genotypes of individuals -

S4 class:  genind
@call:  genind(tab = truenames(nancycats)$tab, pop = truenames(nancycats)$pop)

@tab:  237 x 108 matrix of genotypes

@ind.names: vector of  237 individual names
@loc.names: vector of  9 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the  108 columns of @tab
@all.names: list of  9 components yielding allele names for each locus

Optionnal contents:
@pop:  factor giving the population of each individual
@pop.names:  factor giving the population of each individual

@other: a list containing: xy

```

A **genind** object is formal S4 object with several slots, accessed using the '@' operator (see `class?genind`). Note that the '\$' was also implemented for adegenet objects, so that slots can be accessed as if they were components of a list. The main slot in **genind** is a table of allelic frequencies of individuals (in rows) for every alleles in every loci. Being frequencies, data sum to one per locus, giving the score of 1 for an homozygote and 0.5 for an heterozygote. For instance:

```

> nancycats$tab[10:18, 1:10]

      L1.01 L1.02 L1.03 L1.04 L1.05 L1.06 L1.07 L1.08 L1.09 L1.10
010      0      0      0      0      0      0.0  0.0  0.0  1.0  0.0
011      0      0      0      0      0      0.0  0.0  0.0  0.0  0.5
012      0      0      0      0      0      0.5  0.0  0.5  0.0  0.0
013      0      0      0      0      0      0.5  0.0  0.5  0.0  0.0
014      0      0      0      0      0      0.0  0.0  1.0  0.0  0.0
015      0      0      0      0      0      0.0  0.5  0.0  0.5  0.0
016      0      0      0      0      0      0.5  0.0  0.0  0.5  0.0
017      0      0      0      0      0      0.5  0.0  0.5  0.0  0.0
018      0      0      0      0      0      0.5  0.0  0.0  0.5  0.0

```

Individual '010' is an homozygote for the allele 09 at locus 1, while '018' is an heterozygote with alleles 06 and 09. As user-defined labels are not always valid (for instance, there can be duplicated), generic labels are used for individuals, markers, alleles and eventually population. The true names are stored in the object (components `$[...].names` where ... can be 'ind', 'loc', 'all' or 'pop'). For instance :

```
> nancycats$loc.names
```

```
      L1      L2      L3      L4      L5      L6      L7      L8      L9
"fca8" "fca23" "fca43" "fca45" "fca77" "fca78" "fca90" "fca96" "fca37"
```

gives the true marker names, and

```
> nancycats$all.names[[3]]
```

```
      01      02      03      04      05      06      07      08      09      10
"133" "135" "137" "139" "141" "143" "145" "147" "149" "157"
```

gives the allele names for marker 3.

Optional components are also allowed. The slot `@other` is a list that can include any additional information. The optional slot `@pop` (a factor giving a grouping of individuals) is particular in that the behaviour of many functions will check automatically for it and behave accordingly. In fact, each time an argument 'pop' is required by a function, it is first sought in `@pop`. For instance, using the function `genind2genpop` to convert `nancycats` to a `genpop` object, there is no need to give a 'pop' argument as it exists in the `genind` object:

```
> table(nancycats$pop)
```

```
P01 P02 P03 P04 P05 P06 P07 P08 P09 P10 P11 P12 P13 P14 P15 P16 P17
 10  22  12  23  15  11  14  10   9  11  20  14  13  17  11  12  13
```

```
> catpop <- genind2genpop(nancycats)
```

```
Converting data from a genind to a genpop object...
```

```
...done.
```

```
> catpop
```

```
#####
### Genpop object ###
#####
- Alleles counts for populations -

S4 class:   genpop
@call:   genind2genpop(x = nancycats)

@tab:   17 x 108 matrix of alleles counts

@pop.names: vector of 17 population names
@loc.names: vector of 9 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the 108 columns of @tab
@all.names: list of 9 components yielding allele names for each locus

@other: a list containing: xy
```

Other additional components can be stored (like here, spatial coordinates of populations in \$xy) but will not be passed during any conversion (`catpop` has no \$other\$xy).

Finally, a `genind` object generally contains its matched call, *i.e.* the instruction that created itself. This is not the case, however, for objects loaded using `data`. When call is available, it can be used to regenerate an object.

```
> obj <- read.genetix(system.file("files/nancycats.gtx", package = "adegenet"))
```

```
Converting data from GENETIX to a genind object...
```

```
...done.
```

```
> obj$call
```

```
read.genetix(file = system.file("files/nancycats.gtx", package = "adegenet"))
```

```
> toto <- eval(obj$call)
```

```
Converting data from GENETIX to a genind object...
```

```
...done.
```

```
> identical(obj, toto)
```

```
[1] TRUE
```

### 2.2.2 genpop objects

We use the previously built `genpop` object:

```
> catpop
```

```
#####
### Genpop object ###
#####
- Alleles counts for populations -

S4 class:   genpop
@call:   genind2genpop(x = nancycats)

@tab:   17 x 108 matrix of alleles counts

@pop.names: vector of 17 population names
@loc.names: vector of 9 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the 108 columns of @tab
@all.names: list of 9 components yielding allele names for each locus

@other: a list containing: xy
```

```
> is.genpop(catpop)
```

```
[1] TRUE
```

```
> catpop$tab[1:5, 1:10]
```

	L1.01	L1.02	L1.03	L1.04	L1.05	L1.06	L1.07	L1.08	L1.09	L1.10
P01	0	0	0	0	0	0	0	2	9	1
P02	0	0	0	0	0	10	9	8	14	2
P03	0	0	0	4	0	0	0	0	1	10
P04	0	0	0	3	0	0	0	1	7	17
P05	0	0	0	1	0	0	0	0	7	10

The matrix \$tab contains alleles counts per population (here, cat colonies). These objects are otherwise very similar to **genind** in their structure, and possess generic names, true names, the matched call and an **@other** slot.

## 3 Various topics

### 3.1 Importing data

Data can be read from the softwares GENETIX (.gtx), STRUCTURE (.str or .stru), FSTAT (.dat) and Genepop (.gen) files, using the corresponding **read** function: **read.genetix**, **read.structure**, **read.fstat**, and **read.genepop**. In all cases, the **genind** will be produced. Alternatively, one can use the function **import2genind** which detects a file format from its extension and uses the appropriate routine. For instance:

```
> obj1 <- read.genetix(system.file("files/nancycats.gtx", package = "adegenet"))
```

```
Converting data from GENETIX to a genind object...
```

```
...done.
```

```
> obj2 <- import2genind(system.file("files/nancycats.gtx", package = "adegenet"))
```

```
Converting data from GENETIX to a genind object...
```

```
...done.
```

```
> all.equal(obj1, obj2)
```

```
[1] "Attributes: < Component 2: target, current do not match when deparsed >"
```

The only difference between `obj1` and `obj2` is their call (which is normal as they were obtained from different command lines). However, it happens that data are available in other formats. In all cases, it should be possible to store data in an individuals x markers table where each element is a character string coding 2 alleles. Such data are interpretable when all strings contain 2,4 or 6 characters. For instance, "11" will be an homozygote 1/1, "1209" will be an heterozygote 12/09. The function `df2genind` converts such data to a `genind`. One has to read data into R, using for instance `read.fstat`, and then use `df2genind`. Here I provide an example using a data set from the library `hierfstat`.

```
> library(hierfstat)
> toto <- read.fstat.data(paste(.path.package("hierfstat"), "/data/diploid.dat",
+   sep = "", collapse = ""), nloc = 5)
> head(toto)
```

	Pop	loc-1	loc-2	loc-3	loc-4	loc-5
1	1	44	43	43	33	44
2	1	44	44	43	33	44
3	1	44	44	43	43	44
4	1	44	44	NA	33	44
5	1	44	44	24	34	44
6	1	44	44	NA	43	44

`toto` is a data frame containing genotypes and a population factor.

```
> obj <- df2genind(X = toto[, -1], pop = toto[, 1])
> obj
```

```
#####
### Genind object ###
#####
- genotypes of individuals -

S4 class:   genind
@call: df2genind(X = toto[, -1], pop = toto[, 1])

@tab:   44 x 11 matrix of genotypes

@ind.names: vector of   44 individual names
@loc.names: vector of    5 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the  11 columns of @tab
@all.names: list of    5 components yielding allele names for each locus

Optionnal contents:
@pop:   factor giving the population of each individual
@pop.names: factor giving the population of each individual

@other: - empty -
```

Lastly, `genind` or `genpop` objects can be obtained from a data matrix similar to the `$tab` component (respectively, alleles frequencies and alleles counts). Such action is achieved by the constructors `genind` (or `as.genind`) and `genpop` (or `as.genpop`). The table passed as argument to these constructors must have correct names: rownames identify the genotypes/populations, while colnames have the form '[marker].[allele]' Here is an example for `genpop` using dataset from `ade4`:

```
> library(ade4)
> data(microsatt)
> microsatt$tab[10:15, 12:15]
```

	INRA32.168	INRA32.170	INRA32.174	INRA32.176
Mtbeliard	0	0	0	1
NDama	0	0	0	12
Normand	1	0	0	2
Parthenais	8	5	0	3
Somba	0	0	0	20
Vosgienne	2	0	0	0

`microsatt$tab` contains alleles counts, and can therefore be used to make a **genpop** object.

```
> toto <- genpop(microsatt$tab)
> toto
```

```
#####
### Genpop object ###
#####
- Alleles counts for populations -

S4 class:   genpop
@call:   genpop(tab = microsatt$tab)

@tab:   18 x 112 matrix of alleles counts

@pop.names: vector of 18 population names
@loc.names: vector of 9 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the 112 columns of @tab
@all.names: list of 9 components yielding allele names for each locus

@other: - empty -
```

## 3.2 Exporting data

Genotypes in **genind** format can be exported to the R packages *genetics* (using **genind2genotype**) and *hierfstat* (using **genind2hierfstat**). The package *genetics* is now deprecated, but the implemented class **genotype** is still used by various packages. The package *hierfstat* does not define a class, but requires data to be formatted in a particular way. Here are examples of how to use these functions:

```
> obj <- genind2genotype(nancycats)
> class(obj)
```

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

```
> obj[1:4, 1:5]
```



```

      fca8    fca23    fca43    fca45    fca77
N215    <NA> 136/146 139/139 120/116 156/156
N216    <NA> 146/146 139/145 126/120 156/156
N217 135/143 136/146 141/141 116/116 156/152
N218 135/133 138/138 139/141 126/116 150/150

```

```
> class(obj$fca8)
```

```
[1] "genotype" "factor"
```

```
> obj <- genind2hierfstat(nancycats)
> class(obj)
```

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

```
> obj[1:4, 1:5]
```

```

      pop    fca8    fca23    fca43    fca45
N215    1      NA 136146 139139 116120
N216    1      NA 146146 139145 120126
N217    1 135143 136146 141141 116116
N218    1 133135 138138 139141 116126

```

Now we can use the function `varcomp.glob` from *hierfstat* to compute 'variance' components:

```
> varcomp.glob(obj$pop, obj[, -1])
```

```

$loc
      [,1]      [,2]      [,3]
fca8 0.08867161 0.116693199 0.6682028
fca23 0.05384247 0.077539920 0.6666667
fca43 0.05518935 0.066055996 0.6793249
fca45 0.05861271 -0.001026783 0.7083333
fca77 0.08810966 0.156863586 0.6329114
fca78 0.04869695 0.079006911 0.5654008
fca90 0.07540329 0.097194716 0.6497890
fca96 0.07538325 -0.005902071 0.7543860
fca37 0.04264094 0.116318729 0.4514768

```

```

$overall
      Pop      Ind      Error
0.5865502 0.7027442 5.7764917

```

```

$F
      Pop      Ind
Total 0.08301274 0.1824701
Pop    0.00000000 0.1084610

```

A more generic way to export data is to produce a data.frame of genotypes coded by character strings. This is done by `genind2df`:

```
> obj <- genind2df(nancycats)
> obj[1:5, 1:5]
```

```
      fca8  fca23  fca43  fca45  fca77
N215  <NA> 136146 139139 116120 156156
N216  <NA> 146146 139145 120126 156156
N217 135143 136146 141141 116116 152156
N218 133135 138138 139141 116126 150150
N219 133135 140146 141145 126126 152152
```

However, some softwares will require alleles to be separated. The argument `sep` allows one to specify any separator. For instance:

```
> genind2df(nancycats, sep = "|")[1:5, 1:5]
```

```
      fca8  fca23  fca43  fca45  fca77
N215  <NA> 136|146 139|139 116|120 156|156
N216  <NA> 146|146 139|145 120|126 156|156
N217 135|143 136|146 141|141 116|116 152|156
N218 133|135 138|138 139|141 116|126 150|150
N219 133|135 140|146 141|145 126|126 152|152
```

Note that tabulations can be obtained as follows using `'\t'` character.

### 3.3 Manipulating data

Data manipulation is meant to be easy in **adegenet** (if it is not, complain!). First, as **genind** and **genpop** objects are basically formed by a data matrix (the **@tab** slot), it is natural to subset these objects like it is done with a matrix. The `[` operator does this, forming a new object with the retained genotypes/populations and alleles:

```
> titi <- toto[1:3, ]
> toto$pop.names
```

```
      P01      P02      P03      P04      P05      P06
"Baoule"  "Borgou"  "BPN"  "Charolais"  "Holstein"  "Jersey"
      P07      P08      P09      P10      P11      P12
"Lagunaire"  "Limousin"  "MaineAnjou"  "Mtbeliard"  "NDama"  "Normand"
      P13      P14      P15      P16      P17      P18
"Parthenais"  "Somba"  "Vosgienne"  "ZChoa"  "ZMbororo"  "Zpeul"
```

```
> titi
```

```
#####
### Genpop object ###
#####
- Alleles counts for populations -

S4 class:  genpop
@call: toto[i = 1:3]

@tab:  3 x 112 matrix of alleles counts

@pop.names: vector of  3 population names
@loc.names: vector of  9 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the  112 columns of @tab
@all.names: list of  9 components yielding allele names for each locus

@other: - empty -

> titi$pop.names
```

```
      P1      P2      P3
"Baoule" "Borgou" "BPN"
```

The object `toto` has been subsetted, keeping only the first three populations. Of course, any subsetting available for a matrix can be used with `genind` and `genpop` objects. For instance, we can subset `titi` to keep only the third marker:

```
> titi <- titi[, titi$loc.fac == "L3"]
> titi

#####
### Genpop object ###
#####
- Alleles counts for populations -

S4 class:  genpop
@call: titi[j = titi$loc.fac == "L3"]

@tab:  3 x 11 matrix of alleles counts

@pop.names: vector of  3 population names
@loc.names: vector of  1 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the  11 columns of @tab
@all.names: list of  1 components yielding allele names for each locus

@other: - empty -
```

Now, `titi` only contains the 11 alleles of the third marker of `toto`.

To simplify the task of separating data by marker, the function `seploc` can be used. It returns a list of objects (optionnally, of data matrices), each corresponding to a marker:

```

> sepCats <- seploc(nancycats)
> class(sepCats)

[1] "list"

> names(sepCats)

[1] "fca8" "fca23" "fca43" "fca45" "fca77" "fca78" "fca90" "fca96" "fca37"

> sepCats$fca45

#####
### Genind object ###
#####
- genotypes of individuals -

S4 class: genind
@call: .local(x = x)

@tab: 237 x 9 matrix of genotypes

@ind.names: vector of 237 individual names
@loc.names: vector of 1 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the 9 columns of @tab
@all.names: list of 1 components yielding allele names for each locus

Optionnal contents:
@pop: factor giving the population of each individual
@pop.names: factor giving the population of each individual
@other: a list containing: xy

```

The object `sepCats$fca45` only contains data of the marker fca45.

Following the same idea, `seppop` allows one to separate genotypes in a `genind` object by population. For instance, we can separate genotype of cattles in the dataset `microbov` by breed:

```

> data(microbov)
> obj <- seppop(microbov)
> class(obj)

[1] "list"

> names(obj)

```

[1] "Borgou"	"Zebu"	"Lagunaire"	"NDama"
[5] "Somba"	"Aubrac"	"Bazadais"	"BlondeAquitaine"
[9] "BretPieNoire"	"Charolais"	"Gascon"	"Limousin"
[13] "MaineAnjou"	"Montbeliard"	"Salers"	

```
> obj$Borgou
```

```
#####
### Genind object ###
#####
- genotypes of individuals -

S4 class: genind
@call: x[i = pop == lev]

@tab: 50 x 373 matrix of genotypes

@ind.names: vector of 50 individual names
@loc.names: vector of 30 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the 373 columns of @tab
@all.names: list of 30 components yielding allele names for each locus

Optionnal contents:
@pop: factor giving the population of each individual
@pop.names: factor giving the population of each individual

@other: - empty -
```

The returned object `obj` is a list of `genind` objects each containing genotypes of a given breed.

A last, rather vicious trick is to separate data by population and by marker. This is easy using `lapply`; one can first separate population then markers, or the contrary. Here, we separate markers inside each breed in `obj`

```
> obj <- lapply(obj, seploc)
> names(obj)
```

[1] "Borgou"	"Zebu"	"Lagunaire"	"NDama"
[5] "Somba"	"Aubrac"	"Bazadais"	"BlondeAquitaine"
[9] "BretPieNoire"	"Charolais"	"Gascon"	"Limousin"
[13] "MaineAnjou"	"Montbeliard"	"Salers"	

```
> class(obj$Borgou)
```

```
[1] "list"
```

```
> names(obj$Borgou)
```

```
[1] "INRA63" "INRA5" "ETH225" "ILSTS5" "HEL5" "HEL1" "INRA35"
[8] "ETH152" "INRA23" "ETH10" "HEL9" "CSSM66" "INRA32" "ETH3"
[15] "BM2113" "BM1824" "HEL13" "INRA37" "BM1818" "ILSTS6" "MM12"
[22] "CSRM60" "ETH185" "HAUT24" "HAUT27" "TGLA227" "TGLA126" "TGLA122"
[29] "TGLA53" "SPS115"
```

```
> obj$Borgou$INRA63
```

```
#####
### Genind object ###
#####
- genotypes of individuals -

S4 class:   genind
@call:    .local(x = x)

@tab:    50 x 9 matrix of genotypes

@ind.names: vector of 50 individual names
@loc.names: vector of 1 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the 9 columns of @tab
@all.names: list of 1 components yielding allele names for each locus

Optionnal contents:
@pop: factor giving the population of each individual
@pop.names: factor giving the population of each individual

@other: - empty -
```

For instance, `obj$Borgou$INRA63` contains genotypes of the breed Borgou for the marker INRA63.

Lastly, one may want to pool genotypes in different datasets, but having the same markers, into a single dataset. This is more than just merging the `@tab` components of all datasets, because alleles can differ (they almost always do) and markers are not necessarily sorted the same way. The function `repool` is designed to avoid these problems. It can merge any `genind` provided as arguments as soon as the same markers are used. For instance, it can be used after a `seppop` to retain only some populations:

```
> obj <- seppop(microbov)
> names(obj)
```

```
[1] "Borgou" "Zebu" "Lagunaire" "NDama"
[5] "Somba" "Aubrac" "Bazadais" "BlondeAquitaine"
[9] "BretPieNoire" "Charolais" "Gascon" "Limousin"
[13] "MaineAnjou" "Montbeliard" "Salers"
```

```
> newObj <- repool(obj$Borgou, obj$Charolais)
> newObj
```

```
#####
### Genind object ###
#####
- genotypes of individuals -

S4 class: genind
@call: repool(obj$Borgou, obj$Charolais)

@tab: 105 x 295 matrix of genotypes

@ind.names: vector of 105 individual names
@loc.names: vector of 30 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the 295 columns of @tab
@all.names: list of 30 components yielding allele names for each locus

Optionnal contents:
@pop: factor giving the population of each individual
@pop.names: factor giving the population of each individual

@other: - empty -
```

```
> newObj$pop.names
```

```
      P1      P2
"Borgou" "Charolais"
```

Done !

### 3.4 Using summaries

Both **genind** and **genpop** objects have a summary providing basic information about data. Informations are both printed and invisibly returned as a list.

```
> toto <- summary(nancycats)

# Total number of genotypes: 237

# Population sample sizes:
P01 P02 P03 P04 P05 P06 P07 P08 P09 P10 P11 P12 P13 P14 P15 P16 P17
10 22 12 23 15 11 14 10 9 11 20 14 13 17 11 12 13

# Number of alleles per locus:
L1 L2 L3 L4 L5 L6 L7 L8 L9
16 11 10 9 12 8 12 12 18

# Number of alleles per population:
P01 P02 P03 P04 P05 P06 P07 P08 P09 P10 P11 P12 P13 P14 P15 P16 P17
36 53 50 67 48 56 42 54 43 46 70 52 44 61 42 40 35

# Percentage of missing data:
[1] 2.410533

# Observed heterozygosity:
      L1      L2      L3      L4      L5      L6      L7      L8
```

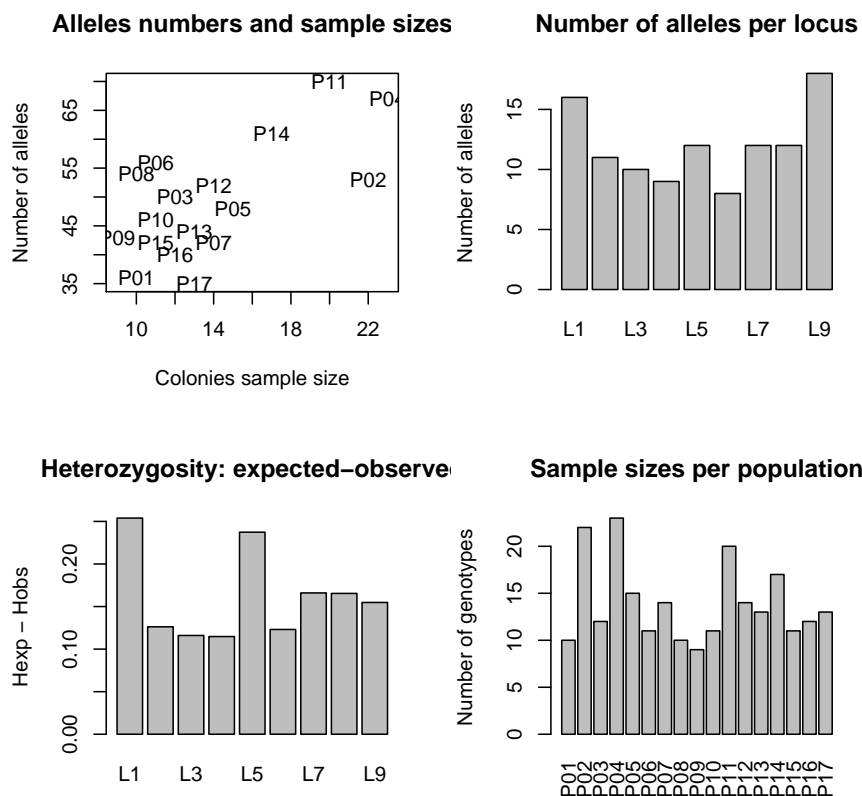
```
0.6118143 0.6666667 0.6793249 0.6455696 0.6329114 0.5654008 0.6497890 0.5949367
L9
0.4514768
```

```
# Expected heterozygosity:
L1 L2 L3 L4 L5 L6 L7 L8
0.8657224 0.7928751 0.7953319 0.7603095 0.8702576 0.6884669 0.8157881 0.7603493
L9
0.6062686
```

```
> names(toto)
```

```
[1] "N" "pop.eff" "loc.nall" "pop.nall" "NA.perc" "Hobs" "Hexp"
```

```
> par(mfrow = c(2, 2))
> plot(toto$pop.eff, toto$pop.nall, xlab = "Colonies sample size",
+      ylab = "Number of alleles", main = "Alleles numbers and sample sizes",
+      type = "n")
> text(toto$pop.eff, toto$pop.nall, lab = names(toto$pop.eff))
> barplot(toto$loc.nall, ylab = "Number of alleles", main = "Number of alleles per locus")
> barplot(toto$Hexp - toto$Hobs, main = "Heterozygosity: expected-observed",
+      ylab = "Hexp - Hobs")
> barplot(toto$pop.eff, main = "Sample sizes per population", ylab = "Number of genotypes",
+      las = 3)
```





Is mean observed H significantly lower than mean expected H ?

```
> bartlett.test(list(toto$Hexp, toto$Hobs))

Bartlett test of homogeneity of variances

data: list(toto$Hexp, toto$Hobs)
Bartlett's K-squared = 0.2556, df = 1, p-value = 0.6132

> t.test(toto$Hexp, toto$Hobs, pair = T, var.equal = TRUE, alter = "greater")

Paired t-test

data: toto$Hexp and toto$Hobs
t = 9.4044, df = 8, p-value = 6.7e-06
alternative hypothesis: true difference in means is greater than 0
95 percent confidence interval:
 0.1299211      Inf
sample estimates:
mean of the differences
      0.1619421
```

Yes, it is.

### 3.5 Testing for structuration among populations

The G-statistic test (Goudet *et al.*, 1996) is implemented for **genind** objects and produces a **randtest** object (package **ade4**). The function to use is **gstat.randtest**, and requires the package *hierfstat*:

```
> library(ade4)
> toto <- gstat.randtest(nancycats, nsim = 99)
> toto
```

```
Monte-Carlo test
Call: gstat.randtest(x = nancycats, nsim = 99)
```

```
Observation: 3416.974
```

```
Based on 99 replicates
Simulated p-value: 0.01
Alternative hypothesis: greater
```

Std.Obs	Expectation	Variance
27.41626	1762.94007	3639.75536

```
> plot(toto)
```

Now that the test is performed, one can ask for F statistics. To get these, data are first converted to be used in the *hierfstat* package:

```

> library(hierfstat)
> toto <- genind2hierfstat(nancycats)
> head(toto)

      pop  fca8 fca23 fca43 fca45 fca77 fca78 fca90 fca96 fca37
N215   1    NA 136146 139139 116120 156156 142148 199199 113113 208208
N216   1    NA 146146 139145 120126 156156 142148 185199 113113 208208
N217   1 135143 136146 141141 116116 152156 142142 197197 113113 210210
N218   1 133135 138138 139141 116126 150150 142148 199199   91105 208208
N219   1 133135 140146 141145 126126 152152 142148 193199 113113 208208
N220   1 135143 136146 145149 120126 150156 148148 193195   91113 208208

> varcomp.glob(toto$pop, toto[, -1])

$loc
      [,1]      [,2]      [,3]
fca8 0.08867161 0.116693199 0.6682028
fca23 0.05384247 0.077539920 0.6666667
fca43 0.05518935 0.066055996 0.6793249
fca45 0.05861271 -0.001026783 0.7083333
fca77 0.08810966 0.156863586 0.6329114
fca78 0.04869695 0.079006911 0.5654008
fca90 0.07540329 0.097194716 0.6497890
fca96 0.07538325 -0.005902071 0.7543860
fca37 0.04264094 0.116318729 0.4514768

$overall
      Pop      Ind      Error
0.5865502 0.7027442 5.7764917

$F
      Pop      Ind
Total 0.08301274 0.1824701
Pop   0.00000000 0.1084610

```

F statistics are provided in \$F; for instance, here,  $F_{st}$  is 0.083.

### 3.6 Testing for Hardy-Weinberg equilibrium

The Hardy-Weinberg equilibrium test is implemented for **genind** objects. The function to use is **HWE.test.genind**, and requires the package *genetics*. Here we first produce a matrix of p-values (**res="matrix"**) using parametric test. Monte Carlo procedure are more reliable but also more computer-intensive (use **permut=TRUE**).

```

> toto <- HWE.test.genind(nancycats, res = "matrix")
> dim(toto)

```

```
[1] 17 9
```

One test is performed per locus and population, *i.e.* 153 tests in this case. Thus, the first question is: which tests are highly significant?

```
> colnames(toto)

[1] "fca8" "fca23" "fca43" "fca45" "fca77" "fca78" "fca90" "fca96" "fca37"

> which(toto < 1e-04, TRUE)

      row col
P14   14   2
P02    2   7
P02    2   8
P05    5   9
```

Here only 4 tests indicate departure from HW. Rows give populations, columns give markers. Now complete tests are returned, but the significant ones are already known.

```
> toto <- HWE.test.genind(nancycats, res = "full")
> toto$fca23$P06
```

Pearson's Chi-squared test

```
data: tab
X-squared = 19.25, df = 10, p-value = 0.0372
```

```
> toto$fca90$P10
```

Pearson's Chi-squared test

```
data: tab
X-squared = 19.25, df = 10, p-value = 0.0372
```

```
> toto$fca96$P10
```

Pearson's Chi-squared test

```
data: tab
X-squared = 4.8889, df = 10, p-value = 0.8985
```

```
> toto$fca37$P13
```

Pearson's Chi-squared test

```
data: tab
X-squared = 14.8281, df = 10, p-value = 0.1385
```

### 3.7 Performing a Principal Component Analysis on **genind** objects

The tables contained in **genind** objects can be submitted to a Principal Component Analysis (PCA) to seek a typology of individuals. Such analysis is straightforward using *ade4* to prepare data and *ade4* for the analysis *per se*. One has first to replace missing data. Putting each missing observation at the mean of the concerned allele frequency seems the best choice (NA will be stuck at the origin).

```
> data(microbov)
> any(is.na(microbov$tab))
```

```
[1] TRUE
```

```
> sum(is.na(microbov$tab))
```

```
[1] 6325
```

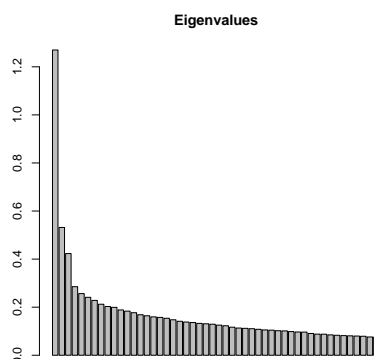
There are 6325 missing data. Assuming that these are evenly distributed (for illustration purpose only!), we replace them using **na.replace**. As we intend to use a PCA, the appropriate replacement method is to put each NA at the mean of the corresponding allele (argument 'method' set to 'mean').

```
> obj <- na.replace(microbov, method = "mean")
```

```
Replaced 6325 missing values
```

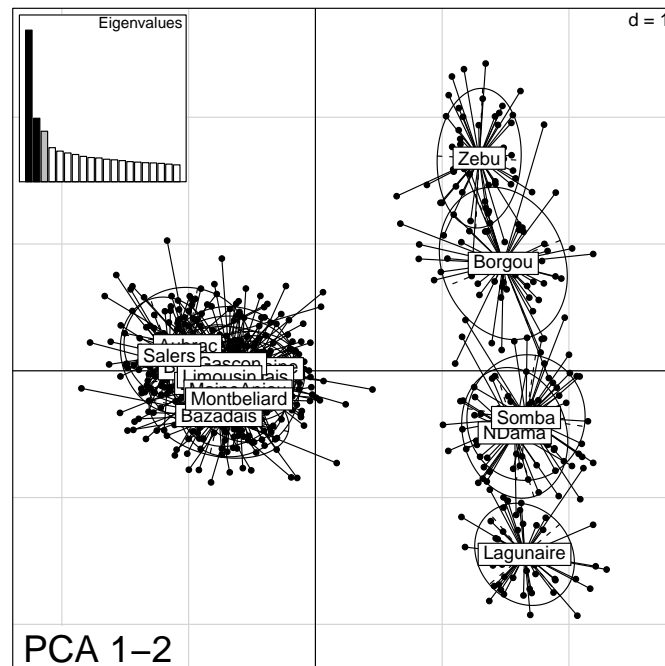
Done. Now, the analysis can be performed. Data are centred but not scaled as 'units' are the same.

```
> pca1 <- dudi.pca(obj$tab, cent = TRUE, scale = FALSE, scannf = FALSE,
+               nf = 3)
> barplot(pca1$eig[1:50], main = "Eigenvalues")
```



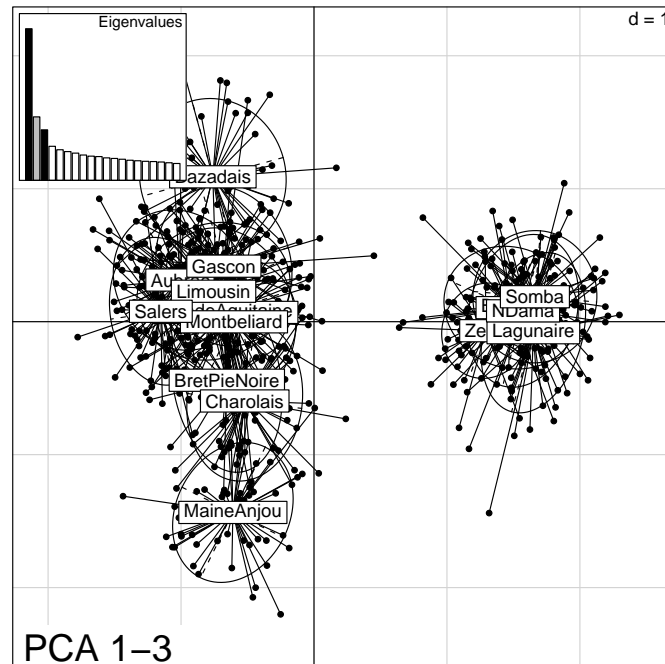
Here we represent the genotypes and 95% inertia ellipses for populations.

```
> s.class(pca1$li, obj$pop, lab = obj$pop.names, sub = "PCA 1-2",
+         csub = 2)
> add.scatter.eig(pca1$eig[1:20], nf = 3, xax = 1, yax = 2, posi = "top")
```



This plane shows that the main structuring is between African and French breeds, the second structure reflecting genetic diversity among African breeds. The third axis reflects the diversity among French breeds: Overall, all breeds seem well differentiated.

```
> s.class(pca1$li, obj$pop, xax = 1, yax = 3, lab = obj$pop.names,
+         sub = "PCA 1-3", csub = 2)
> add.scatter.eig(pca1$eig[1:20], nf = 3, xax = 1, yax = 3, posi = "top")
```



### 3.8 Performing a Correspondance Analysis on **genpop** objects

Being contingency tables, the **@tab** in **genpop** objects can be submitted to a Correspondance Analysis (CA) to seek a typology of populations. The approach is very similar to the previous one for PCA. Missing data are first replaced during conversion from **genind**, but one could create a **genpop** with NAs and then use **na.replace** to get rid of missing observations.

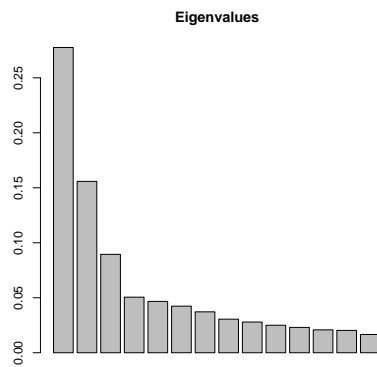
```
> data(microbov)
> obj <- genind2genpop(microbov, missing = "chi2")
```

Converting data from a **genind** to a **genpop** object...

Replaced 0 missing values

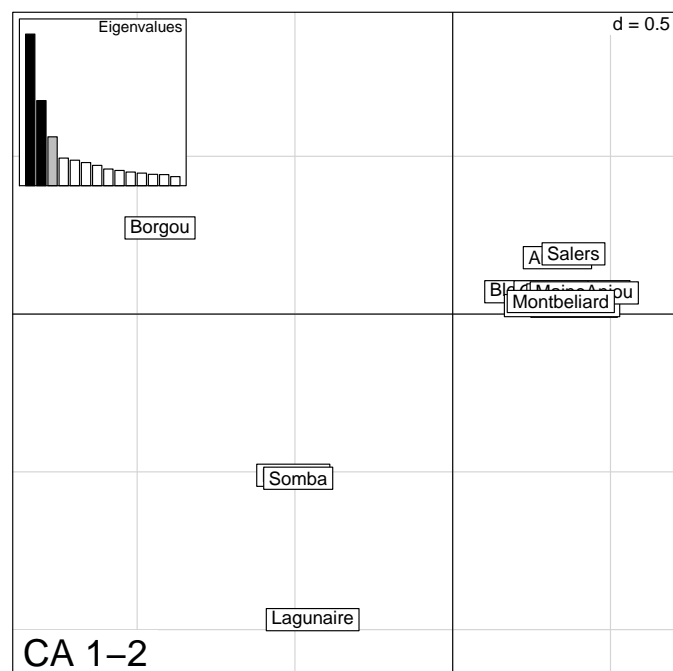
...done.

```
> ca1 <- dudi.coa(as.data.frame(obj$tab), scannf = FALSE, nf = 3)
> barplot(ca1$eig, main = "Eigenvalues")
```

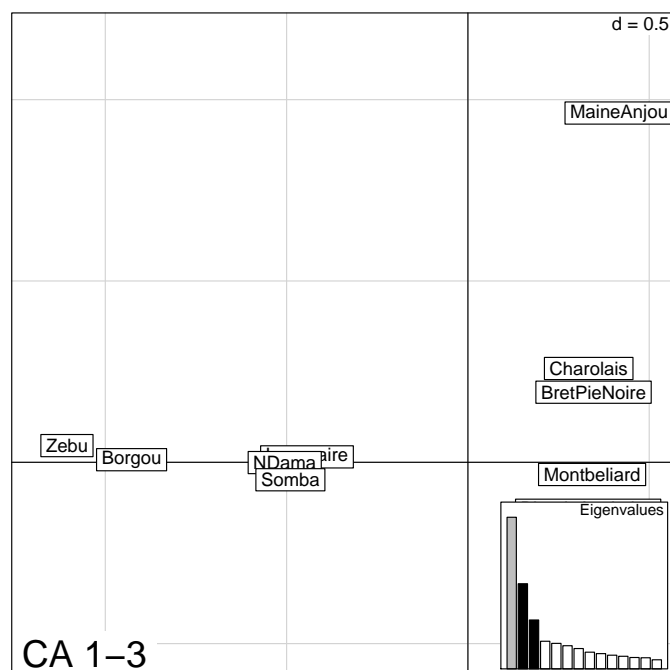


Now we display the resulting typologies:

```
> s.label(ca1$li, lab = obj$pop.names, sub = "CA 1-2", csub = 2)
> add.scatter.eig(ca1$eig, nf = 3, xax = 1, yax = 2, posi = "top")
```



```
> s.label(ca1$li, xax = 1, yax = 3, lab = obj$pop.names, sub = "CA 1-3",
+         csub = 2)
> add.scatter.eig(ca1$eig, nf = 3, xax = 2, yax = 3, posi = "bottomright")
```



Once again, axes are to be interpreted separately in terms of continental differentiation, a among-breed diversities.

### 3.9 Analyzing a single locus

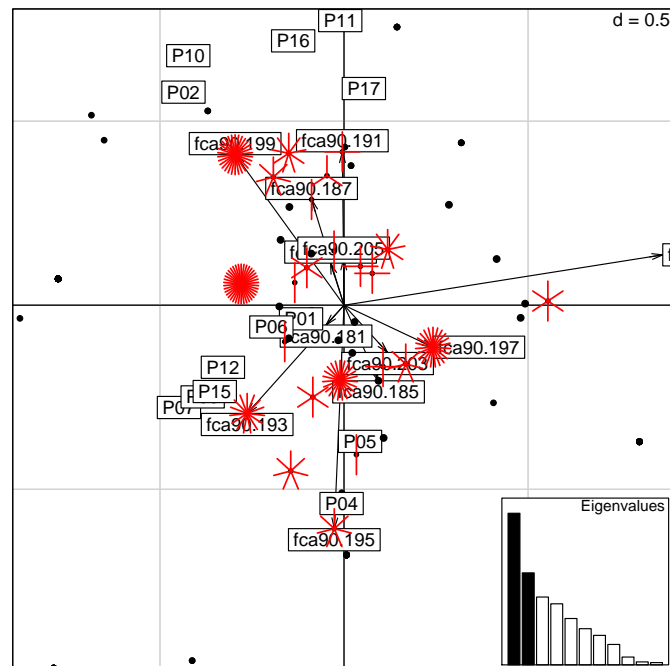
Here the emphasis is put on analyzing a single locus using different methods. Any marker can be isolated using the `seplloc` instruction.

```
> data(nancycats)
> toto <- seplloc(nancycats, truenames = TRUE, res.type = "matrix")
> X <- toto$fca90
```

`fca90.ind` is a matrix containing only genotypes for the marker `fca90`. It can be analyzed, for instance, using an inter-class PCA. This analysis provides a typology of individuals having maximal inter-colonies variance.

```
> library(ade4)
> pcaX <- dudi.pca(X, cent = T, scale = F, scannf = FALSE)
> pcabetX <- between(pcaX, nancycats$pop, scannf = FALSE)
> s.arrow(pcabetX$c1, xlim = c(-0.9, 0.9))
> s.class(pcabetX$l1, nancycats$pop, cell = 0, cstar = 0, add.p = T)
> sunflowerplot(X %*% as.matrix(pcabetX$c1), add = T)
> add.scatter.eig(pcabetX$eig, xax = 1, yax = 2, posi = "bottomright")
```





Here the differences between individuals are mainly expressed by three alleles: 199, 197 and 193. However, there is no clear structuration to be seen at an individual level. Is  $F_{st}$  significant taking only this marker into account? We perform the G-statistic test and eventually compute the corresponding F statistics. Note that we use the constructor `genind` to generate an object of this class from `X`:

```
> fca90.ind <- genind(X, pop = nancycats$pop)
> gstat.randtest(fca90.ind, nsim = 999)
```

```
Monte-Carlo test
Call: gstat.randtest(x = fca90.ind, nsim = 999)
```

```
Observation: 437.135
```

```
Based on 999 replicates
Simulated p-value: 0.001
Alternative hypothesis: greater
```

Std.Obs	Expectation	Variance
14.40010	188.08892	299.10841

```
> F <- varcomp(genind2hierfstat(fca90.ind))$F
> rownames(F) <- c("tot", "pop")
> colnames(F) <- c("pop", "ind")
> F
```

	pop	ind
tot	0.09168833	0.2098744
pop	0.00000000	0.1301162

In this case the information is best summarized by F statistics than by an ordination method. It is likely because all colonies are differentiated but none forming clusters of related colonies.

### 3.10 Testing for isolation by distance

Isolation by distance (IBD) is tested using Mantel test between a matrix of genetic distances and a matrix of geographic distances. It can be tested using individuals as well as populations. This example uses cat colonies. We use Edwards' distance *versus* Euclidean distances between colonies.

```
> data(nancycats)
> toto <- genind2genpop(nancycats, miss = "0")
```

Converting data from a genind to a genpop object...

Replaced 9 missing values

...done.

```
> Dgen <- dist.genpop(toto, method = 2)
> Dgeo <- dist(nancycats$other$xy)
> library(ade4)
> ibd <- mantel.randtest(Dgen, Dgeo)
> ibd
```

Monte-Carlo test

Call: mantel.randtest(m1 = Dgen, m2 = Dgeo)

Observation: 0.00492068

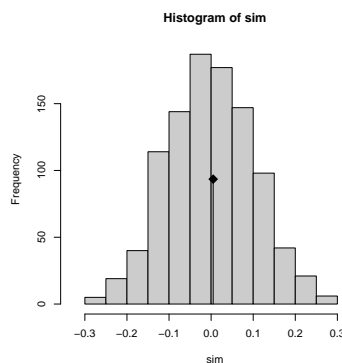
Based on 1000 replicates

Simulated p-value: 0.4825175

Alternative hypothesis: greater

	Std.Obs	Expectation	Variance
	0.012738600	0.003583708	0.011015428

```
> plot(ibd)
```



Isolation by distance is clearly not significant.

### 3.11 Using Monmonier's algorithm to define genetic boundaries

Monmonier's algorithm (Monmonier, 1973) was originally designed to find boundaries of maximum differences between contiguous polygons of a tessellation. As such, the method was basically used in geographical analysis. More recently, Manni *et al.* (2004) suggested that this algorithm could be employed to detect genetic boundaries among georeferenced genotypes (or populations). This algorithm is implemented using a more general approach than the initial one in `adeigenet`.

Instead of using Voronoi tessellation as in original version, the functions `monmonier` and `optimize.monmonier` can handle various neighbouring graphs such as Delaunay triangulation, Gabriel's graph, Relative Neighbours graph, etc. These graphs defined spatial connectivity among 'points' (genotypes or populations), any couple of points being neighbours (if connected) or not. Another information is given by a set of markers which define genetic distances among these 'points'. The aim of Monmonier's algorithm is to find the path through the strongest genetic distances between neighbours. A more complete description of the principle of this algorithm will be found in the documentation of `monmonier`. Indeed, the very purpose of this tutorial is simply to show how it can be used on genetic data.

Let's take the example from the function's manpage and detail it. The dataset used is `sim2pop`.

```
> data(sim2pop)
> sim2pop

#####
### Genind object ###
#####
- genotypes of individuals -

S4 class: genind
@call: old2new(object = sim2pop)

@tab: 130 x 241 matrix of genotypes

@ind.names: vector of 130 individual names
@loc.names: vector of 20 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the 241 columns of @tab
@all.names: list of 20 components yielding allele names for each locus

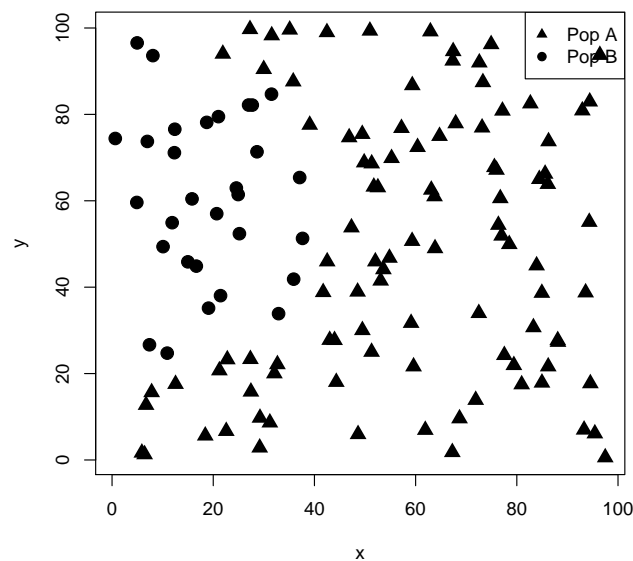
Optionnal contents:
@pop: factor giving the population of each individual
@pop.names: factor giving the population of each individual

@other: a list containing: xy

> summary(sim2pop$pop)
```

```
P1 P0
100 30
```

```
> temp <- sim2pop$pop
> levels(temp) <- c(17, 19)
> temp <- as.numeric(as.character(temp))
> plot(sim2pop$other$xy, pch = temp, cex = 1.5, xlab = "x", ylab = "y")
> legend("topright", leg = c("Pop A", "Pop B"), pch = c(17, 19))
```



There are two sampled populations in this dataset, with unequal sample sizes (100 and 30). Twenty microsatellite-like loci are available for all genotypes (no missing data). So, what do **monmonier** ask for?

```
> args(monmonier)
```

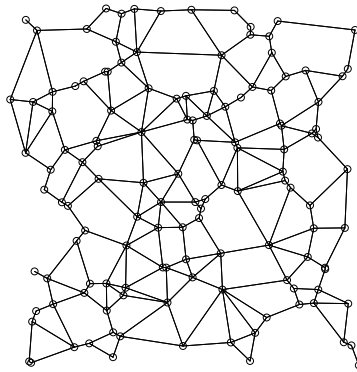
```
function (xy, dist, cn, threshold = NULL, nrun = 1, skip.local.diff = rep(0,
  nrun), scanthres = is.null(threshold))
  NULL
```

The first argument (**xy**) is a matrix of geographic coordinates, already stored in **sim2pop**. Next argument is an object of class **dist**, which is basically a distance matrix cut in half. For now, we will use the classical Euclidean distance among alleles frequencies of genotypes. This is obtained by:

```
> D <- dist(sim2pop$tab)
```

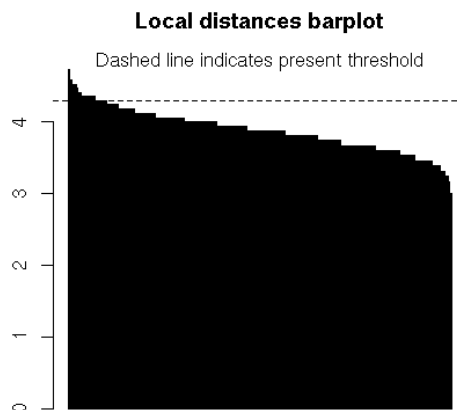
The next argument (**cn**) is a connection network. As existing routines to build such networks are spread over several packages, the function **chooseCN** will help you choose one. This is an interactive function, so difficult to demonstrate here (see **?chooseCN**). Here we ask the function not to ask for a choice (**ask=FALSE**) and select the second type of graph which is the one of Gabriel (**type=2**).

```
> gab <- chooseCN(sim2pop$other$xy, ask = FALSE, type = 2)
```



The obtained network is automatically plotted by the function. It seems we are now ready to proceed to the algorithm.

```
> mon1 <- monmonier(sim2pop$other$xy, D, gab$cn)
```



This plot shows all local differences sorted in decreasing order. The idea behind this is that a significant boundary would cause local differences to decrease abruptly after the boundary. This should be used to choose the *threshold* difference for the algorithm to stop. Here, no boundary is visible: we stop.

Why do the algorithm fail to find a boundary? Either because there is no genetic differentiation to be found, or because the signal differentiating both populations is too weak to overcome the random noise in genetic distances. What is the  $F_{st}$  between the two samples?

```
> library(hierfstat)
> temp <- genind2hierfstat(sim2pop)
> varcomp.glob(temp[, 1], temp[, -1])$F
```

	Pop	Ind
Total	0.03824374	-0.07541793
Pop	0.00000000	-0.11818137

This value is somewhat moderate ( $F_{st} = 0.038$ ). Is it significant?

```
> gtest <- gstat.randtest(sim2pop)
> gtest
```

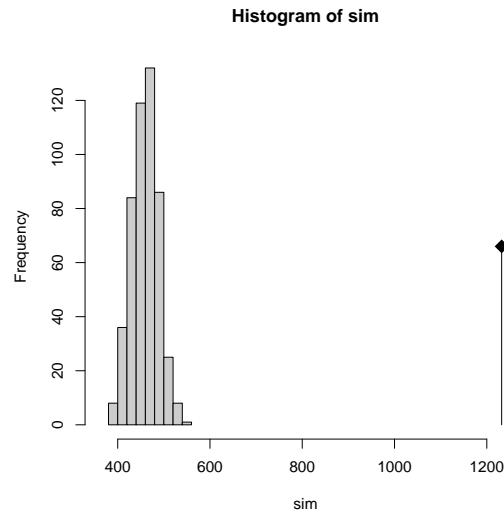
```
Monte-Carlo test
Call: gstat.randtest(x = sim2pop)

Observation: 1232.192

Based on 499 replicates
Simulated p-value: 0.002
Alternative hypothesis: greater
```

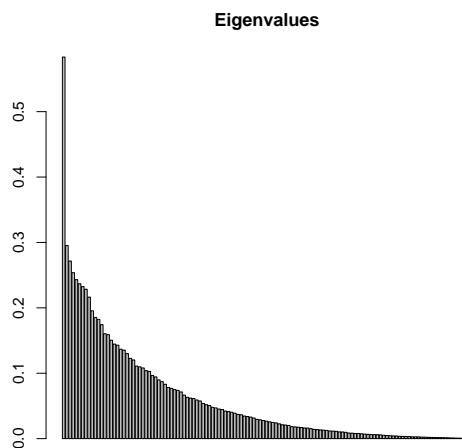
Std.Obs	Expectation	Variance
27.34202	457.22598	803.34855

```
> plot(gtest)
```



Yes, it is very significant. The two samples are indeed genetically differentiated. So, can Monmonier's algorithm find a boundary between the two populations? Yes, if we get rid of the random noise. This can be achieved using simple ordination method like Principal Coordinates Analysis.

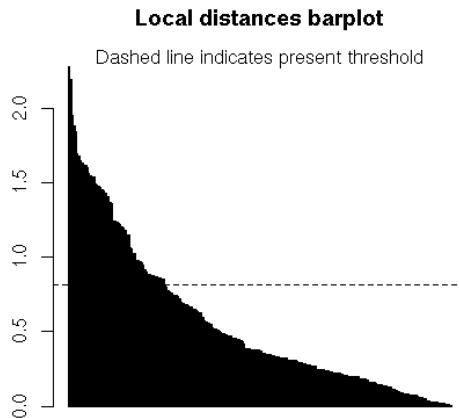
```
> library(ade4)
> pco1 <- dudi.pco(D, scannf = FALSE, nf = 1)
> barplot(pco1$eig, main = "Eigenvalues")
```



We retain only the first eigenvalue. The corresponding coordinates are used to redefine the genetic distances among genotypes. The algorithm is then rerun.

```
> D <- dist(pco1$li)
```

```
> mon1 <- monmonier(sim2pop$other$xy, D, gab$cn)
```



```
#####
# List of paths of maximum differences between neighbours #
#           Using a Monmonier based algorithm           #
#####

$call:monmonier(xy = sim2pop$other$xy, dist = D, cn = gab, scanthres = FALSE)

# Object content #
Class: monmonier
$run (number of successive runs): 1
$run1: run of the algorithm
$threshold (minimum difference between neighbours): 0.8154
$xy: spatial coordinates
$cn: connection network

# Runs content #
# Run 1
# First direction
Class: list
$path:
      x      y
Point_1 14.98299 93.81162
Point_2 30.74508 87.57724
Point_3 33.66093 86.14115
...

$values:
2.281778 1.617905 1.953220 ...
# Second direction
Class: list
$path:
      x      y
Point_1 14.98299 93.81162
```



```
$values:
2.281778
```

This may take some time... but never more than five minutes on an 'ordinary' personal computer. The object `mon1` contains the whole information about the boundaries found. As several boundaries can be seeked at the same time (argument `nrun`), you have to specify about which run and which direction you want to get informations (values of differences or path coordinates). For instance:

```
> names(mon1)
```

```
[1] "run1"      "nrun"      "threshold" "xy"        "cn"        "call"
```

```
> names(mon1$run1)
```

```
[1] "dir1" "dir2"
```

```
> mon1$run1$dir1
```

```
$path
```

	x	y
Point_1	14.98299	93.81162
Point_2	30.74508	87.57724
Point_3	33.66093	86.14115
Point_4	35.28914	81.12578
Point_5	33.85756	74.45492
Point_6	38.07622	71.47532
Point_7	41.97494	70.02783
Point_8	43.45812	67.12026
Point_9	42.20206	59.59613
Point_10	42.48613	52.55145
Point_11	40.08702	48.61795
Point_12	39.20791	43.89978
Point_13	38.81236	40.34516
Point_14	37.32112	36.35265
Point_15	37.96426	30.82105
Point_16	32.79703	28.00517
Point_17	30.12832	28.60376
Point_18	20.92496	29.21211
Point_19	16.05811	22.72600
Point_20	11.72524	21.15519
Point_21	10.18696	16.61536

```
$values
```

```
[1] 2.2817775 1.6179049 1.9532197 1.6799848 1.4021738 1.4308542 1.5410382
[8] 1.6028722 1.6496059 1.4521920 1.4708930 1.6912244 1.5587670 1.2031668
[15] 0.8787830 1.3595118 1.2323711 1.8381892 1.6198537 2.1902853 0.8653928
```

It can also be useful to identify which points are crossed by the barrier; this can be done using `coords.monmonier`:

```
> coords.monmonier(mon1)

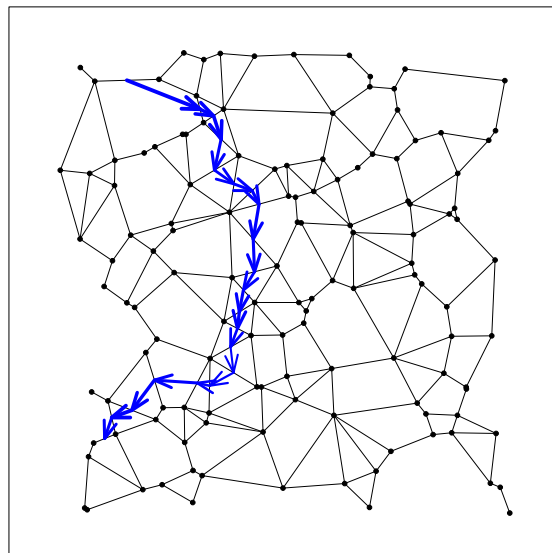
$run1
$run1$dir1
      x.hw      y.hw first second
Point_1 14.98299 93.81162   11   125
Point_2 30.74508 87.57724   44   128
Point_3 33.66093 86.14115   20   128
Point_4 35.28914 81.12578   68   128
Point_5 33.85756 74.45492   68   117
Point_6 38.07622 71.47532   68   122
Point_7 41.97494 70.02783   35   122
Point_8 43.45812 67.12026   69   122
Point_9 42.20206 59.59613   22   122
Point_10 42.48613 52.55145   22   124
Point_11 40.08702 48.61795   13   124
Point_12 39.20791 43.89978   13   127
Point_13 38.81236 40.34516   62   127
Point_14 37.32112 36.35265   62   130
Point_15 37.96426 30.82105   94   130
Point_16 32.79703 28.00517   16   130
Point_17 30.12832 28.60376   85   130
Point_18 20.92496 29.21211   63   119
Point_19 16.05811 22.72600   61   126
Point_20 11.72524 21.15519   89   126
Point_21 10.18696 16.61536   74   89

$run1$dir2
      x.hw      y.hw first second
Point_1 14.98299 93.81162   11   125
```

The returned dataframe contains, in this order, the  $x$  and  $y$  coordinates of the points of the barrier, and the identifiers of the two 'parent' points, that is, the points whose barycenter is the point of the barrier.

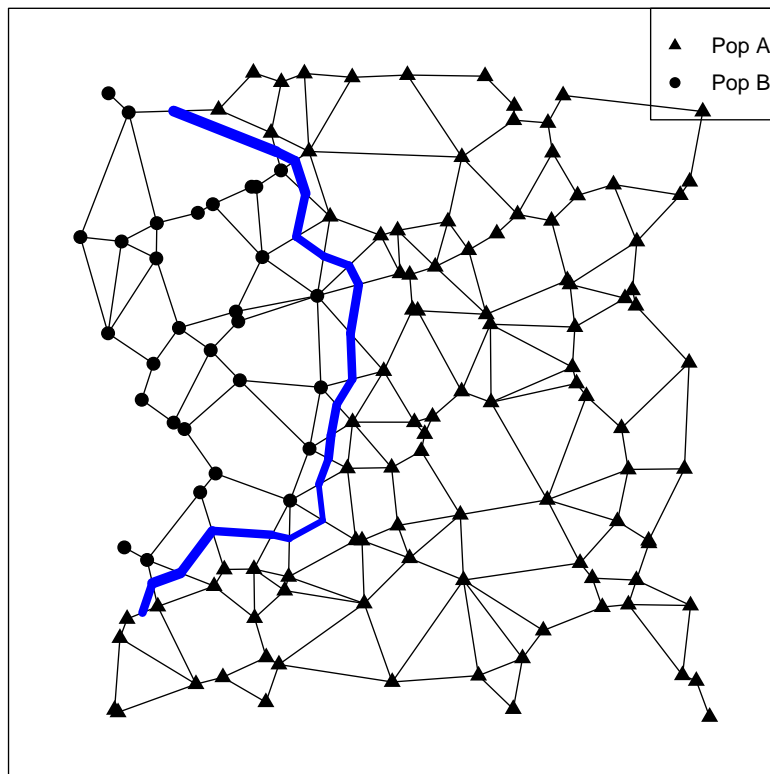
Finally, you can plot very simply the obtained boundary using the method `plot`:

```
> plot(mon1)
```



see arguments in `?plot.monmonier` to customize this representation. Last, we can compare the inferred boundary with the actual distribution of populations:

```
> plot(mon1, add.arrows = FALSE, bwd = 8)
> temp <- sim2pop$pop
> levels(temp) <- c(17, 19)
> temp <- as.numeric(as.character(temp))
> points(sim2pop$other$xy, pch = temp, cex = 1.3)
> legend("topright", leg = c("Pop A", "Pop B"), pch = c(17, 19))
```



Not too bad...

### 3.12 How to simulate hybridization?

The function `hybridize` allows to simulate hybridization between individuals from two distinct genetic pools, or more broadly between two `genind` objects. Here, we use the example from the manpage of the function, to go a little further. Please have a look at the documentation, especially at the different possible outputs (outputs for the software STRUCTURE is available!).

```
> temp <- seppop(microbov)
> names(temp)
```

```
[1] "Borgou"      "Zebu"      "Lagunaire"  "NDama"
[5] "Somba"      "Aubrac"    "Bazadais"   "BlondeAquitaine"
[9] "BretPieNoire" "Charolais" "Gascon"     "Limousin"
[13] "MaineAnjou"  "Montbeliard" "Salers"
```

```
> salers <- temp$Salers
> zebu <- temp$Zebu
> zebler <- hybridize(salers, zebu, n = 40, pop = "zebler")
```

A first generation (F1) of hybrids 'zebler' is obtained. Is it possible to perform a backcross, say, with 'salers' population? Yes, here it is:

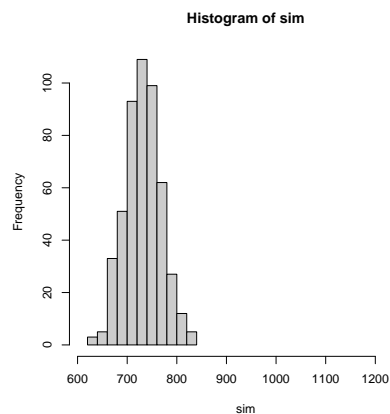
```
> F2 <- hybridize(salers, zebler, n = 40)
> F3 <- hybridize(salers, F2, n = 40)
> F4 <- hybridize(salers, F3, n = 40)
```

and so on... Are these hybrids still genetically distinct? Let's merge all hybrids in a single dataset and test for genetic differentiation:

```
> dat <- repool(zebler, F2, F3, F4)
> test <- gstat.randtest(dat)
> plot(test)
> temp <- genind2hierfstat(dat)
> varcomp.glob(temp[, 1], temp[, -1])$F
```

```

      Pop      Ind
Total 0.01791709 -0.02947454
Pop    0.00000000 -0.04825624
```



The  $F_{st}$  is not very strong (0.013) but still very significant: hybrids are still pretty well differentiated.

## References

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