Tutorial using the  software

A tutorial for the R package `adegenet_1.2-0`

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Contents

1 Introduction

This tutorial proposes a short visit through functionalities of the adegenet package for R (??). The purpose of this package is to facilitate the multivariate analysis of molecular marker data, especially using the ade4 package (?). Data can be imported from popular softwares like GENETIX, or converted from simple data frame of genotypes. adegenet also aims at providing a platform from which to use easily methods provided by other R packages (e.g., ?). 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 adegenet, namely genind (genotypes of individuals) and genpop (genotypes grouped by populations). Then, several topics will be tackled using reproductible 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 adegenet package is installed along with other recommended packages.

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

Then the first step is to load the package:

```
> library(adegenet)
```

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
@ploidy:  2

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 additionnal 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 seeked 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, a `genind` object 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. Most of these can be read using df2genind, which transform at data.frame (imported in R using read.table, for instance) into a genind. The data.frame must contain genotypes in rows, markers in column, and each of its terms must be a character string coding the alleles. There is no restriction about the ploidy of the data. Missing data can be series of '0' or NAs. Since version 1.2-0 of adegenet, df2genind handles two different cases:

- ⋆ a separator is used between the alleles (argument 'sep')
- ⋆ no separator is used; in this case, all alleles should be coded by the same number of characters. For instance, if some alleles are coded by two characters, then there should be no '1','2','3'... but only '01', '02', and '03'.

Here, I provide an example of using df2genind to read a data set from the hierfstat package. Data are diploid, alleles are coded by one character, and there are no separator between alleles.

```
> 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
```

```
@ploidy:  2

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

@other: - empty -

> head(genind2df(obj))

  pop loc-1 loc-2 loc-3 loc-4 loc-5
1   1    44    34    34    33    44
2   1    44    44    34    33    44
3   1    44    44    34    34    44
4   1    44    44  <NA>    33    44
5   1    44    44    24    34    44
6   1    44    44  <NA>    34    44
```

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 formated 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 ues 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]
```

```
     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
N219   1 133135 140146 141145 126126
```

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]
```

```
     pop    fca8   fca23   fca43   fca45
N215   1    <NA> 136|146 139|139 116|120
N216   1    <NA> 146|146 139|145 120|126
N217   1 135|143 136|146 141|141 116|116
N218   1 133|135 138|138 139|141 116|126
N219   1 133|135 140|146 141|145 126|126
```

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: .local(x = x, i = i, j = j, drop = drop)

@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: a list containing: elements without names


> 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: .local(x = x, i = i, j = j, drop = drop)

@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: a list containing: elements without names
```

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 (optionnaly, 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
@ploidy:  2

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: .local(x = x, i = i, j = j, drop = drop)

@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
@ploidy:  2

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

@other: a list containing: coun  breed  spe
```

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
@ploidy:  2

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

@other: a list containing: coun  breed  spe
```

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
@ploidy:  2

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"
```

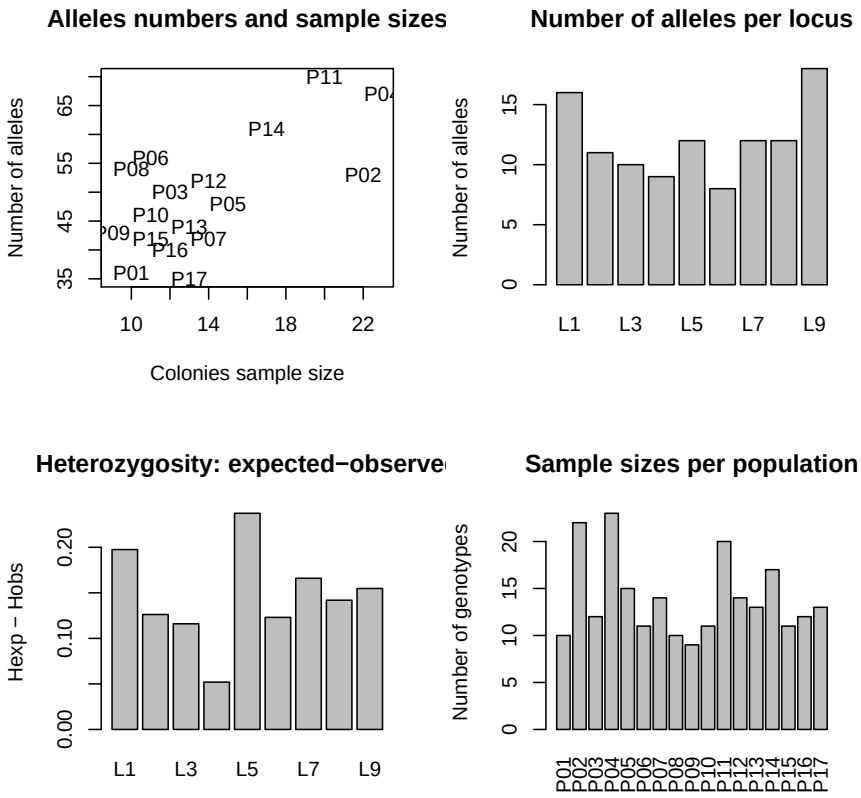
Done !

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] 2.344116

 # Observed heterozygosity:
       L1        L2        L3        L4        L5        L6        L7        L8
0.6682028 0.6666667 0.6793249 0.7083333 0.6329114 0.5654008 0.6497890 0.6184211
       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 = 0.047, df = 1, p-value = 0.8284
```

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

        Paired t-test

data:  toto$Hexp and toto$Hobs
t = 8.3294, df = 8, p-value = 1.631e-05
alternative hypothesis: true difference in means is greater than 0
95 percent confidence interval:
 0.1134779       Inf
sample estimates:
mean of the differences
              0.1460936
```

Yes, it is.

3.5 Testing for structuration among populations

The G-statistic test (**?**) 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
   29.88308  1755.36364  3091.77236


> 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 *adegenet* 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
```

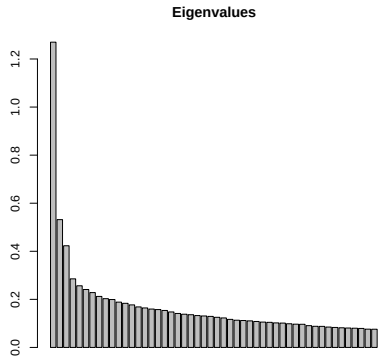
There are 6325 missing data. Assuming that these are evenly distributed (for illustration purpose only!), we replace them using na.replace. As we intend to use a PCA, the appropriate replacement method is to put each NA at the mean of the corresponding allele (argument 'method' set to 'mean').

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

```
 Replaced 6325 missing values
```

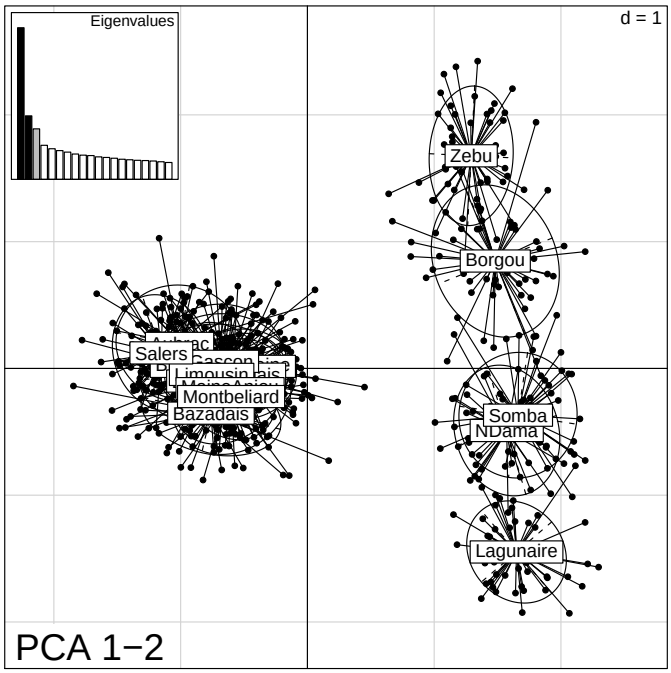
Done. Now, the analysis can be performed. Data are centred but not scaled as 'units' are the same.

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



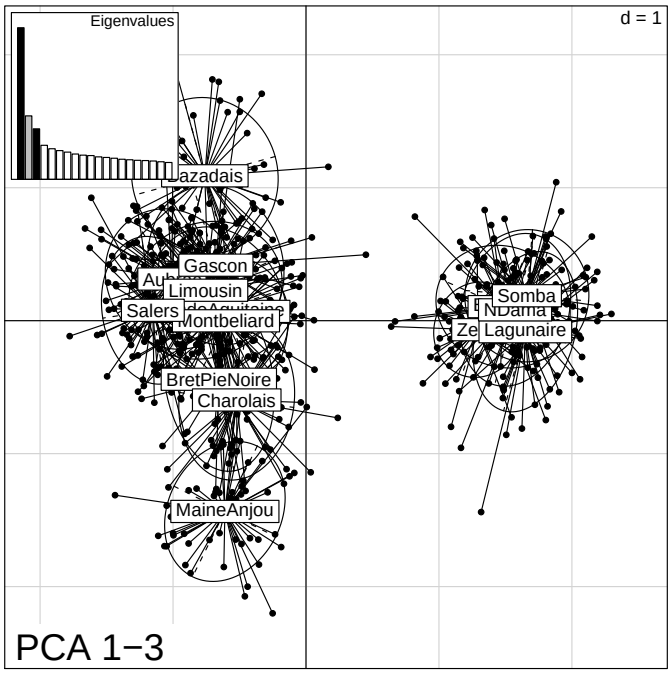
Here we represent the genotypes and 95% inertia ellipses for populations.

```
> 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 an 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.

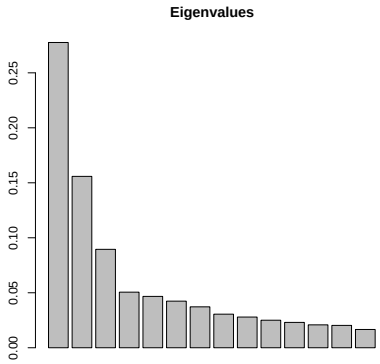
```
> s.class(pca1$li, obj$pop, xax = 1, yax = 3, lab = obj$pop.names,
+     sub = "PCA 1-3", csub = 2)
> add.scatter.eig(pca1$eig[1:20], nf = 3, xax = 1, yax = 3, posi = "top")
```



3.8 Performing a Correspondance Analysis on genpop objects

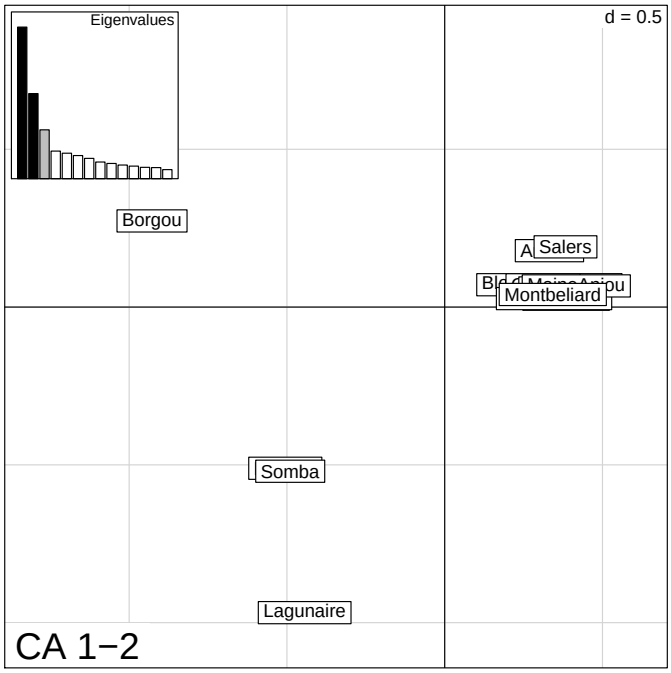
Being contingency tables, the `@tab` in `genpop` objects can be submitted to a Correspondance Analysis (CA) to seek a typology of populations. The approach is very similar to the previous one for PCA. Missing data are first replaced during convertion from `genind`, but one could create a `genpop` with 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")
```

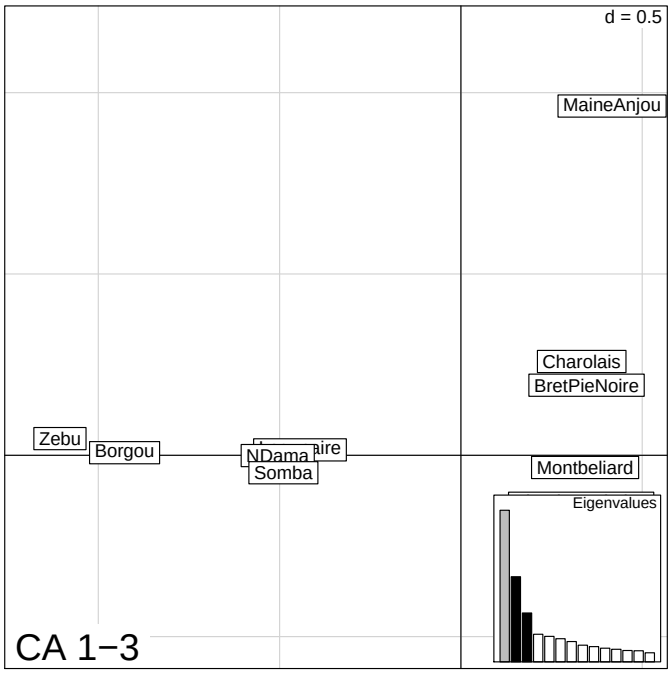


Now we display the resulting typologies:

```
> 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.

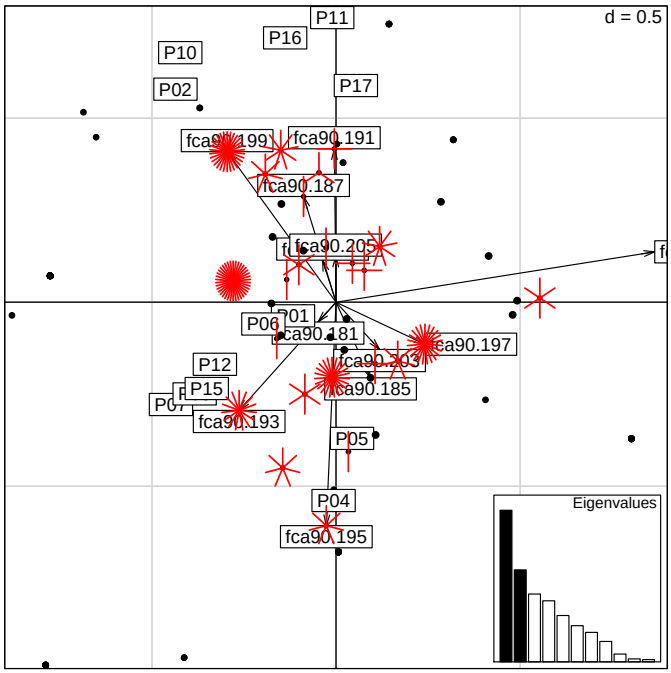
3.9 Analyzing a single locus

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

```
> data(nancycats)
> toto <- seploc(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 analyzis 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$ls, nancycats$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 enventually compute the corresponding F statistics. Note that we use the constructor `genind` to generate an object of this class from `X`:

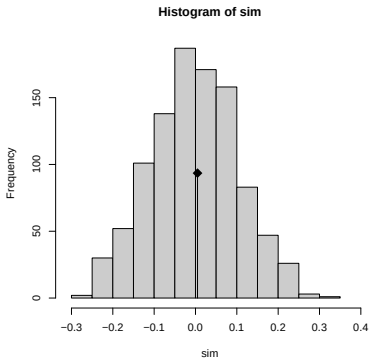
```
> fca90.ind <- genind(X, pop = nancycats$pop)
> gstat.randtest(fca90.ind, nsim = 999)


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
   14.31602   189.17050   300.00900


> 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 999 replicates
Simulated p-value: 0.476
Alternative hypothesis: greater

    Std.Obs Expectation    Variance
0.028829819 0.001876356 0.011150595

> plot(ibd)
```



Isolation by distance is clearly not significant.

3.11 Using Monmonier's algorithm to define genetic boundaries

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

Instead of using Voronoi tesselation 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.

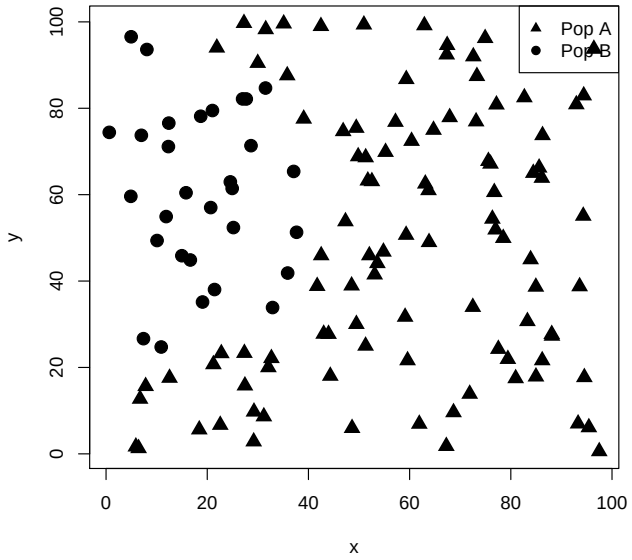
Let's take the example from the function's manpage and detail it. The dataset used is sim2pop.

```
> 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
@ploidy:  2

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)
```

```
P01 P02
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 inequal 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)
```

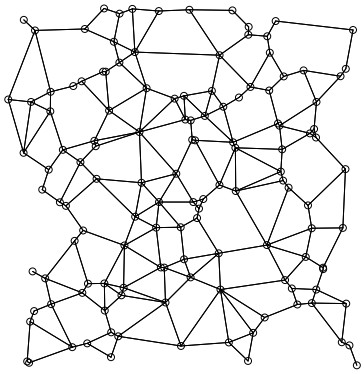
```
function (xy, dist, cn, threshold = NULL, bd.length = NULL, nrun = 1,
    skip.local.diff = rep(0, nrun), scanthres = is.null(threshold),
    allowLoop = TRUE)
NULL
```

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)
```

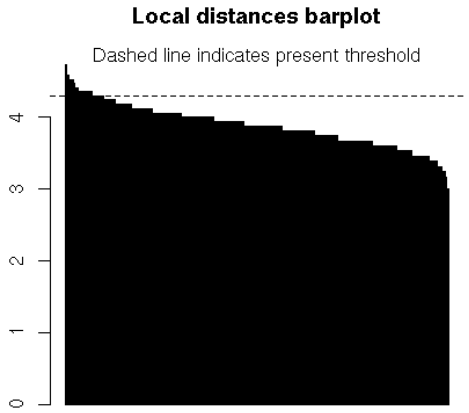
The next argument (cn) is a connection network. As existing routines to build such networks are spread over several packages, the function chooseCN will help you choose one. This is an interactive function, so difficult to demonstrate here (see ?chooseCN). Here we ask the function not to ask for a choice (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
```

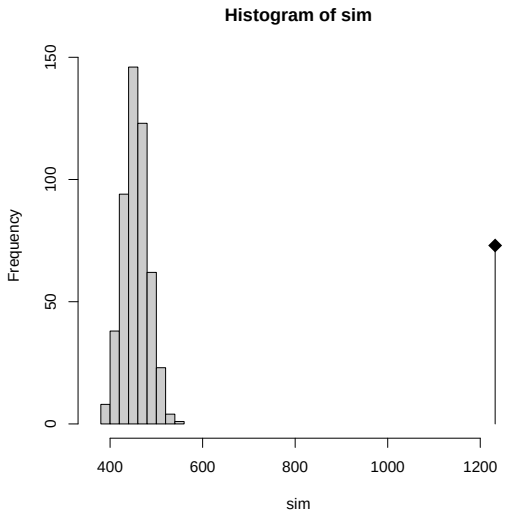
```
             Pop         Ind
Total 0.03824374 -0.07541793
Pop   0.00000000 -0.11818137
```

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

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

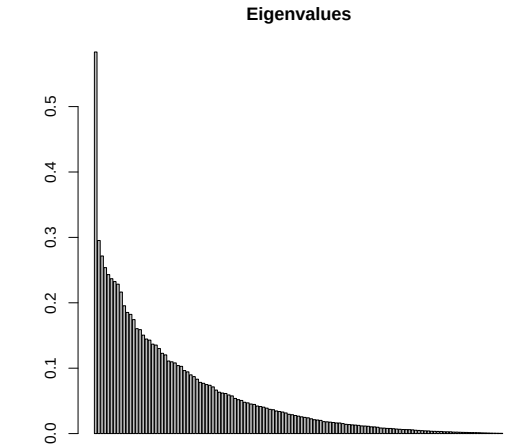
```
Monte-Carlo test
Call: gstat.randtest(x = sim2pop)

Observation: 1232.192

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

    Std.Obs Expectation    Variance
   25.80125   459.32510   897.27961
```

```
> plot(gtest)
```



Yes, it is very significant. The two samples are indeed genetically differenciated. 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.

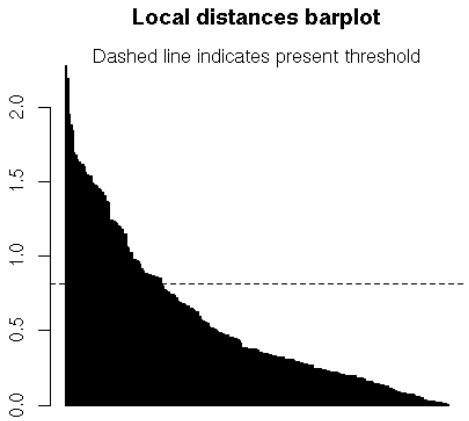
```
> library(ade4)
> pco1 <- dudi.pco(D, scannf = FALSE, nf = 1)
> barplot(pco1$eig, main = "Eigenvalues")
```



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

```
> 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
$nrun (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

$values:
 2.281778
# Second 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 ...
```

This may take some time... but never more than five minutes on an 'ordinary' personnal 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

$values
[1] 2.281778
```

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

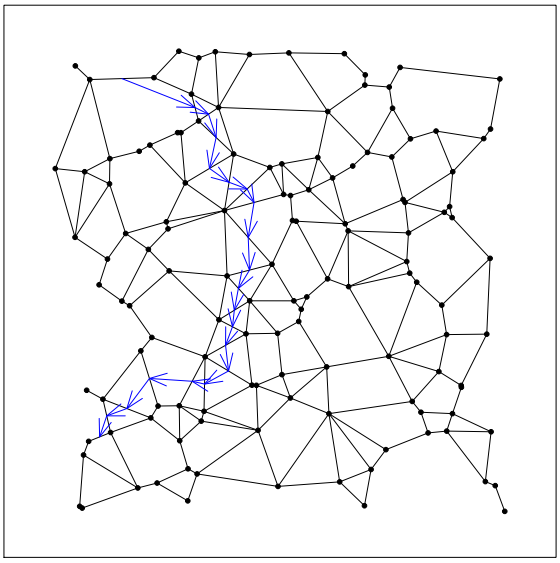
```
> coords.monmonier(mon1)


$run1
$run1$dir1
            x.hw     y.hw first second
Point_1 14.98299 93.81162    11    125

$run1$dir2
            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
```

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.

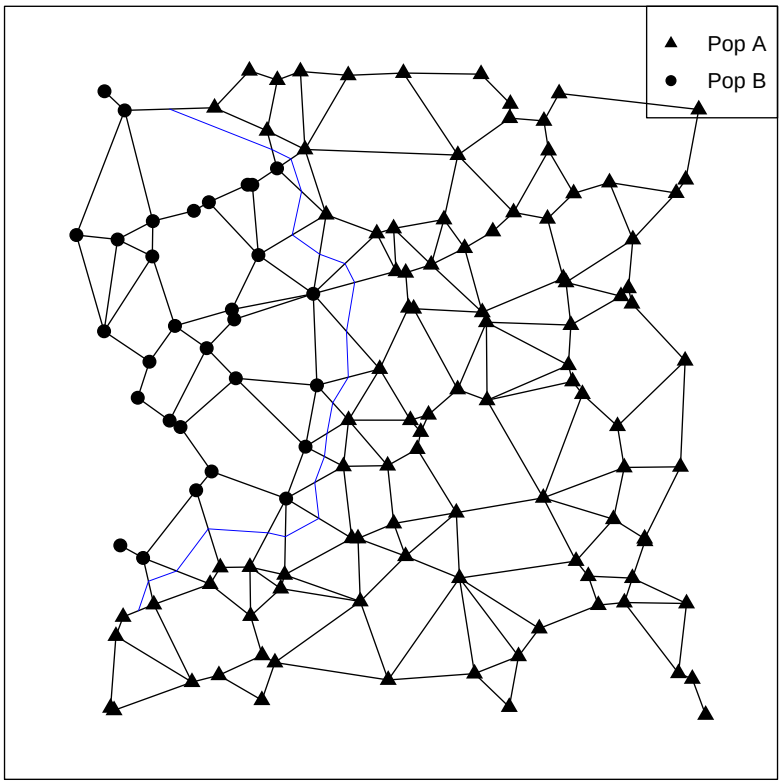
Finally, you can plot very simply the obtained boundary using the method `plot`:

```
> plot(mon1)
```



see arguments in `?plot.monmonier` to customize this representation. Last, we can compare the infered 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")
```

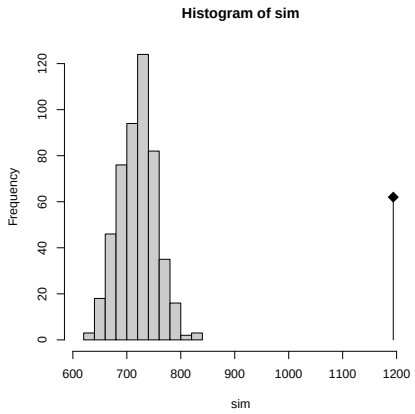
A first generation (F1) of hybrids 'zebler' is obtained. Is it possible to perform a backcross, say, with 'salers' population? Yes, here it is:

```
> 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 0.01656461 -0.03223344
Pop   0.00000000 -0.04961998
```



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

3.13 Reading AFLP data

Adegenet was primarly suited to handle codominant markers like microsatellites. However, dominant markers like AFLP can be used as well. This is a particular case of genind object where each locus possesses only one allele, and where values in the `@tab` slot are no longer frequencies, but presence/absence indications.

Here is an example using a toy dataset 'AFLP.txt' that can be downloaded from the adegenet website, section 'Documentation':

```
> dat <- read.table("AFLP.txt", header = TRUE)
> dat
```

```
     loc1 loc2 loc3 loc4
indA    1    0    1    1
indB    0    1    1    1
indC    1    1    0    1
indD    0   NA    1   NA
indE    1    1    0    0
indF    1    0    1    1
indG    0    1    1    0
```

The genind constructor is used to build a genind object:

```
> obj <- genind(dat)
> obj
```

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

S4 class:  genind
@call: genind(tab = dat)

@tab:  7 x 4 matrix of genotypes

@ind.names: vector of  7 individual names
@loc.names: vector of  4 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the  4 columns of @tab
@all.names: list of  4 components yielding allele names for each locus
@ploidy:  2

Optionnal contents:
@pop:  - empty -
@pop.names:  - empty -

@other: - empty -
```

```
> truenames(obj)
```

```
     loc1.loc1 loc2.loc2 loc3.loc3 loc4.loc4
indA         1         0         1         1
indB         0         1         1         1
indC         1         1         0         1
indD         0        NA         1        NA
indE         1         1         0         0
indF         1         0         1         1
indG         0         1         1         0
```

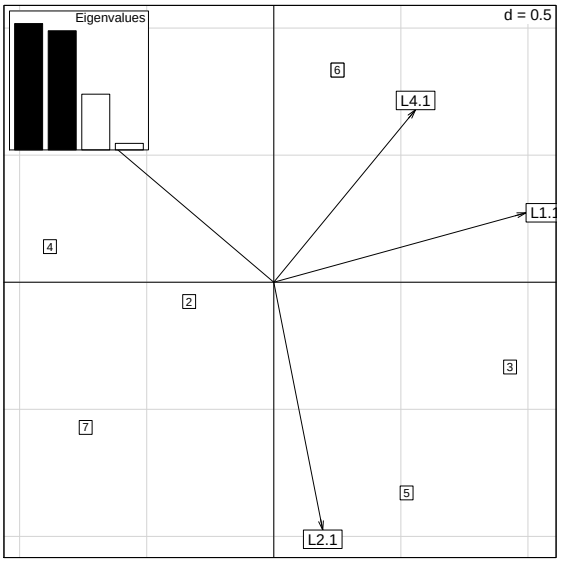
To continue with the toy example, we can proceed to a simple PCA. NAs are first replaced:

```
> objNoNa <- na.replace(obj, met = 0)
```

```
 Replaced 2 missing values

> objNoNa

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

S4 class:  genind
@call: genind(tab = dat)

@tab:  7 x 4 matrix of genotypes

@ind.names: vector of  7 individual names
@loc.names: vector of  4 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the  4 columns of @tab
@all.names: list of  4 components yielding allele names for each locus
@ploidy:  2

Optionnal contents:
@pop:  - empty -
@pop.names:  - empty -

@other: - empty -
```

Now the PCA is performed:

```
> library(ade4)
> pca1 <- dudi.pca(objNoNa, scannf = FALSE, scale = FALSE)
> scatter(pca1)
```



More generally, multivariate analyses from ade4, the sPCA (`spca`), the global and local tests (`global.rtest`, `local.rtest`), or the Monmonier's algorithm (`monmonier`) will work without problem with AFLP data. See more information about non-diploid data in adegenet in the next section.

3.14 Handling non-diploid data

As said above, adegenet was primarly suited to handle codominant markers like microsatellites. Version 1.2-0 handles different levels of ploidy. This is reflected by the fact that genind objects now have a @ploidy slot, which contains an integer indicating the number of copies of each gene. Note that to change this value, an integer must be assigned, and not a numeric. For instance:

```
> obj <- new("genind")
> obj


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

S4 class:  genind
@call: NULL

@tab:  0 x 0 matrix of genotypes

@ind.names: vector of  0 individual names
@loc.names: vector of  0 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the  0 columns of @tab
@all.names: list of  0 components yielding allele names for each locus
@ploidy:  2

Optionnal contents:
@pop:  - empty -
@pop.names:  - empty -

@other: - empty -


> is.integer(1)


[1] FALSE


> obj@ploidy <- as.integer(1)
> obj@ploidy


[1] 1
```

Basically, most features of adegenet are now available for different levels of ploidy. The list of functions that do not work with non-diploid data is provided on the adegenet website (section 'Documents'). In general, these functions do not work because they rely on other softwares that do not support different levels of ploidy. For instance, there will not be any read.genetix for non-diploid data because such data are not handled by the GENETIX software.

Reading non-diploid genotypes is done using df2genind: data are read from a genotype x loci data.frame, where each element is a character string indicating alleles, possibly separated by a particular character. This can be illustrated using the toy datasets `haplo.txt` and `triplo.txt`, available from the adegenet website (section 'Documents').

First we read haploid data.

```
> haplo <- read.table("haplo.txt", header = TRUE, colClasses = "character")
> haplo
```

```
      locTruc locYo LocMiss loc11
gen1        3    04    <NA>    11
gen2       03     4    <NA>    10
toto     <NA>    05    <NA>    10
youpi      45    01    <NA>  <NA>
foo        02    04    <NA>    11
mrNA     <NA>  <NA>    <NA>  <NA>
last       04    04    <NA>    09
```

Now we build a genind object:

```
> obj <- df2genind(haplo, ploidy = 1)
> obj
```

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

S4 class:  genind
@call: df2genind(X = haplo, ploidy = 1)

@tab:  6 x 10 matrix of genotypes

@ind.names: vector of  6 individual names
@loc.names: vector of  3 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the  10 columns of @tab
@all.names: list of  3 components yielding allele names for each locus
@ploidy:  1

Optionnal contents:
@pop:  - empty -
@pop.names:  - empty -

@other: - empty -
```

```
> summary(obj)
```

```
 # Total number of genotypes:  6

 # Population sample sizes:
P1
 6
```

```
 # Number of alleles per locus:
L1 L2 L3
 4  3  3

 # Number of alleles per population:
P1
10

 # Percentage of missing data:
[1] 11.11111

 # Observed heterozygosity:
[1] 0

 # Expected heterozygosity:
[1] 0
```

The warning rightfully tells that entirely non-typed individuals and markers that were removed. To check that the reading was correct, we can do the converse operation:

```
> genind2df(obj)
```

```
      locTruc locYo loc11
gen1       03    04    11
gen2       03    04    10
toto     <NA>    05    10
youpi      45    01  <NA>
foo        02    04    11
last       04    04    09
```

Note that some alleles were wrongly coded (`haplo[1,1]` was '3' instead of '03') and were automatically recoded cleanly by `df2genind`.

The same example can be adapted to triploid data (file `triplo.txt`).

```
> triplo <- read.table("triplo.txt", header = TRUE, colClasses = "character")
> triplo
```

```
       locTruc    locYo  LocMiss    loc11
gen1   3/04/03 04/05/05     <NA> 11/11/11
gen2   03/3/03    4/4/5 NA/NA/NA 10/10/10
toto  NA/NA/NA  05/6/06     <NA> 10/11/11
youpi 45/40/42 01/02/03     <NA> NA/11/NA
foo   02/02/03  04/04/4     <NA> 11/12/11
mrNA  NA/NA/NA NA/NA/NA NA/NA/NA NA/NA/NA
last  04/04/04 04/01/02     <NA> 09/08/07
```

Note how nasty this file is: some alleles are miscoded ('3' instead of '03'), NAs are sometimes repeated ('NA/NA/NA' instead of simply 'NA'), etc. As far as the right ploidy level and the right separator are specified, `df2genind` should overcome these problems:

```
> obj <- df2genind(triplo, ploidy = 3, sep = "/")
> obj

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

S4 class:  genind
@call: df2genind(X = triplo, sep = "/", ploidy = 3)

@tab:  6 x 18 matrix of genotypes

@ind.names: vector of  6 individual names
@loc.names: vector of  3 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the  18 columns of @tab
@all.names: list of  3 components yielding allele names for each locus
@ploidy:  3

Optionnal contents:
@pop:  - empty -
@pop.names:  - empty -

@other: - empty -

> summary(obj)

 # Total number of genotypes:  6

 # Population sample sizes:
P1
 6

 # Number of alleles per locus:
L1 L2 L3
 6  6  6

 # Number of alleles per population:
P1
18

 # Percentage of missing data:
[1] 11.11111

 # Observed heterozygosity:
       L1        L2        L3
0.6000000 0.8333333 0.6000000

 # Expected heterozygosity:
       L1        L2        L3
0.7377778 0.7592593 0.6933333

> genind2df(obj, sep = "-")

       locTruc    locYo    loc11
gen1  03-03-04 04-05-05 11-11-11
gen2  03-03-03 04-04-05 10-10-10
toto      <NA> 05-06-06 10-11-11
youpi 40-42-45 01-02-03     <NA>
foo   02-02-03 04-04-04 11-11-12
last  04-04-04 01-02-04 07-08-09
```

It worked!

3.15 Searching scales of spatial genetic variation: Mantel correlogram

One way to investigate the scales of spatial genetic patterns is to compute a Mantel correlogram. This approach consist in computing Mantel correlation at different distance classes. The obtained values can be tested using a Monte Carlo procedure, like the classical Mantel test (see `mantel.randtest` and above section about isolation-by-distance).

The Mantel correlogram is implemented by the function `mgram` of the package `ecodist`. We illustrate the procedure using the dataset `spcaIllus`.

```
> library(ecodist)
> data(spcaIllus)
```

First two distance matrices are constructed, from genetic data and geographic coordinates:

```
> Dgen <- dist(spcaIllus$dat2A$tab)
> Dgeo <- dist(spcaIllus$dat2A$other$xy)
```

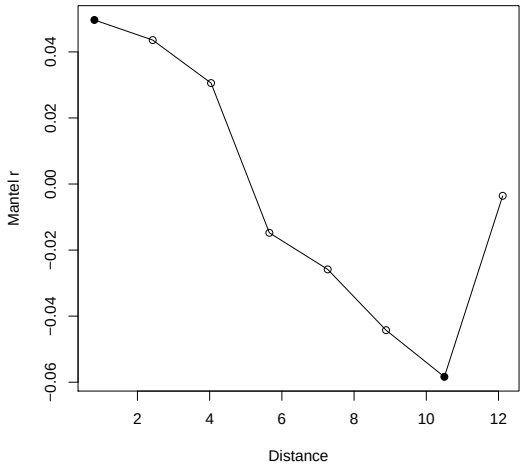
Now we compute the Mantel correlogram:

```
> mantCor <- mgram(Dgen, Dgeo, nperm = 499, nclass = 8)
> mantCor
```

```
$mgram
            lag ngroup      mantelr       pval        llim         ulim
[1,]  0.8077331    245  0.049673053 0.01603206  0.03304318  0.068778327
[2,]  2.4231993    482  0.043577934 0.08617234  0.02372699  0.065920089
[3,]  4.0386655    729  0.030560430 0.25651303  0.01018246  0.052298301
[4,]  5.6541317    649 -0.014773410 0.47695391 -0.03548419  0.003565284
[5,]  7.2695979    529 -0.025844957 0.26252505 -0.04768001 -0.003750427
[6,]  8.8850641    383 -0.044243413 0.12625251 -0.07194333 -0.017892634
[7,] 10.5005303    123 -0.058370091 0.03607214 -0.07769896 -0.033057362
[8,] 12.1159965     19 -0.003567667 0.88176353 -0.02973976  0.019060074

$resids
[1] NA

attr(,"class")
[1] "mgram"
```

The function `mgram` offers several options, that should be looked at into details for real application (`?mgram`). Here, the argument `nperm` specifies the number of permutations of data used in the Monte Carlo testing procedure, and `nclass` gives the number of distance classes to be used. Now we can look at the correlogram:

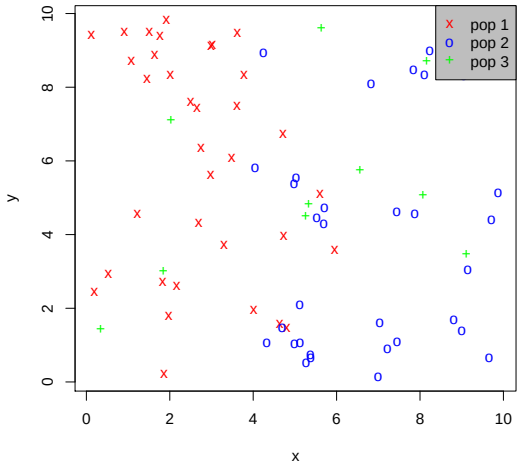
```
> plot(mantCor)
```



Plain dots indicate significant correlations. The Mantel correlogram shows that genotypes are relatively similar at short distance, while this similarity decreases with distance. The negative correlation around a distance of 10 indicates the genetic differenciation between some patches.

This is consistent with the actual structuring:

```
> xy <- spcaIllus$dat2A$other$xy
> pop <- spcaIllus$dat2A$pop
> levels(pop) <- c("x", "o", "+")
> col <- pop
> levels(col) <- c("red", "blue", "green")
> plot(xy, pch = as.character(pop), col = as.character(col))
> legend("topright", pch = c("x", "o", "+"), col = c("red", "blue",
+     "green"), legend = paste("pop", 1:3), bg = "grey")
```


pop 1
pop 2
pop 3
x
y
0
2
4
6
8
10