# An introduction to Discriminant Analysis of Principal Components (DAPC)

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**Abstract**

This vignette provides a tutorial for applying the Discriminant Analysis of Principal Components (DAPC [**?**]) using the *adegenet* package [**?**] for the R software [**?**]. This methods aims to identify and describe genetic clusters, although it can in fact be applied to any quantitative data. We illustrate how to use `find.clusters` to identify clusters, and `dapc` to describe the relationships between these clusters. More advanced topics are then introduced, such as the stability of DAPC results and supplementary individuals.

# Contents

# 1 Introduction

## 1.1 Rationale

Investigating genetic diversity using multivariate approaches relies on finding synthetic variables built as linear combinations of alleles (i.e. $a_1\text{allele}_1 + a_2\text{allele}_2 + ...$) and which reflect as well as possible the genetic variation between the studied individuals. However, most of the time we are not only interested in the diversity amongst individuals, but also and possibly more in the diversity between groups of individuals. Typically, one will be analysing individual data to identify populations, or more largely genetic clusters, and then describe these clusters.

A problem occuring in traditional methods is focussing on the entire variation. Genetic data can be described using a standard multivariate ANOVA model:

$$\text{total variance} = (\text{variance between groups}) + (\text{variance within groups})$$

or more simply, denoting $\mathbf{X}$ the data matrix:

$$VAR(\mathbf{X}) = B(\mathbf{X}) + W(\mathbf{X})$$

That is, usual approaches such as Principal Component Analysis (PCA) or Principal Coordinate Analysis (PCoA / MDS) focus on $VAR(\mathbf{X})$. That is, they only describe the global diversity, possibly overlooking differences between groups. On the contrary, DAPC optimizes $B(\mathbf{X})$ while minimizing $W(\mathbf{X})$: it seeks synthetic variables, the *discriminant functions*, which show differences between groups as best as possible while minimizing variation within clusters.

# 2 Identifying clusters using `find.clusters`

## 2.1 Rationale

DAPC in itself requires prior groups to be defined. However, groups are often unknown or uncertain, and there is a need for identifying genetic clusters before describing them. This can be achieved by using $k$-means, a clustering algorithm which finds $k$ groups maximizing the variation between groups, $B(\mathbf{X})$. To identify the optimal number of clusters, $k$-means is run sequentially with increasing values of $k$, and different clustering solutions are compared using Bayesian Information Criterion (BIC). Ideally, the optimal clustering solution should correspond to the lowest BIC. In practice, the 'best' BIC is often indicated by an elbow in the curve of BIC values as a function of $k$.

While $k$-means could be performed on the raw data, we prefer running the algorithm after transforming the data using PCA. This transformation has the major advantage of reducing the number of variables so as to speed up the clustering algorithm. Note this does not imply a loss of information and different results from the raw data, since one can retain all the principal components

(PCs) and therefore all the variation in the original data. However, in practice, a reduced number of PCs is often sufficient to identify the existing clusters, while allowing the clusters to be obtained essentially instantaneously.

## 2.2 In practice

Identification of the clusters is achieved by `find.clusters`. This function first transforms the data using PCA, asking the users to specify the number of retained PCs interactively unless the argument `n.pca` is provided. Then, it runs $k$-means algorithm (function `kmeans` from the *stats* package) with increasing values of $k$, unless the argument `n.clust` is provided. See `?find.clusters` for other arguments.

`find.clusters` is a generic function with methods for `data.frame`, and objects with the class `genind` (usual genetic markers) and `genlight` (genome wide SNP data). Here, we illustrate its use using a toy dataset simulated in [**?**], `dapcIllus`:

```
> library(adegenet)
> data(dapcIllus)
> class(dapcIllus)
```

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

```
> names(dapcIllus)
```

```
[1] "a" "b" "c" "d"
```

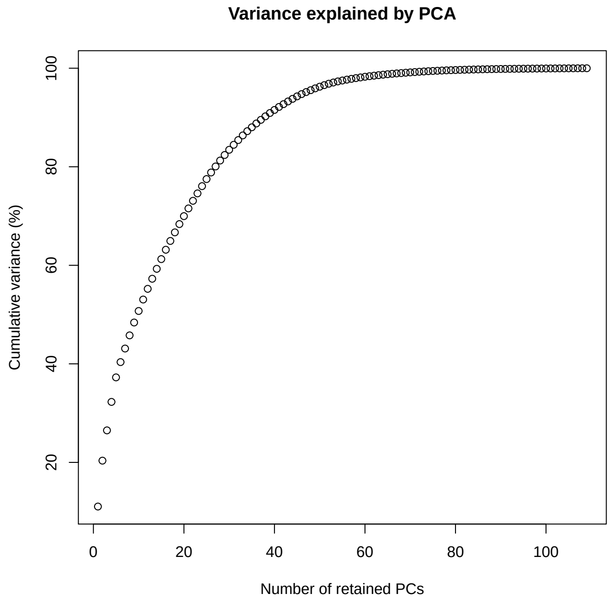
`dapcIllus` is a list containing four datasets; we shall only use the first one:

```
> x <- dapcIllus$a
> x
```

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

S4 class:  genind
@call: read.fstat(file = file, missing = missing, quiet = quiet)

@tab:  600 x 140 matrix of genotypes

@ind.names: vector of  600 individual names
@loc.names: vector of  30 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the  140 columns of @tab
@all.names: list of  30 components yielding allele names for each locus
@ploidy:  2
@type:  codom

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

@other: - empty -
```

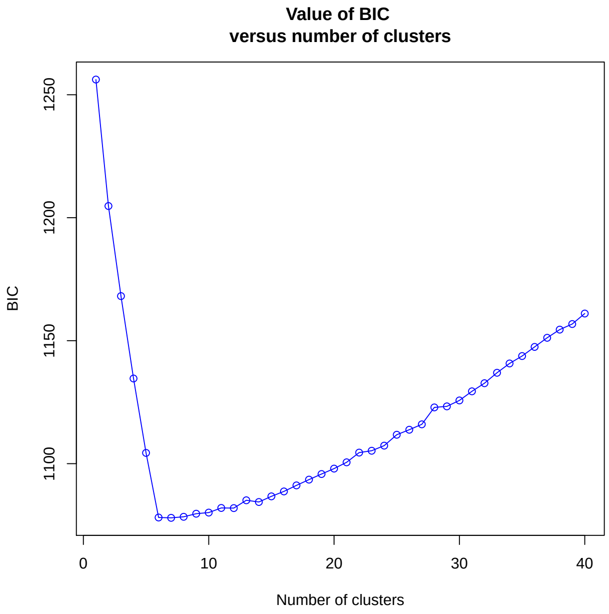
x is a dataset of 600 individuals simulated under an 6 island model for 30 microsatellite markers. We use find.clusters to identify clusters, although true clusters are, in this case, known. We specify that we want to evaluate up to $k = 40$ groups (max.n.clust=40):

```
> grp <- find.clusters(x, max.n.clust = 40)
```



The function displays a graph of cumulated variance explained by the eigenvalues of the PCA. Apart from computational time, there is no reason for keeping a small number of components; here, we keep all the information, specifying to retain 200 PCs (there are actually less PCs —around 110—, so all of them are kept).

Then, the function displays a graph of BIC values for increasing values of $k$:



This graph shows a clear decrease of BIC until $k = 6$ clusters, after which BIC increases. In this case, the elbow in the curve also matches the smallest BIC, and clearly indicates 6 clusters should be retained. In practice, the choice is often trickier to make.

The output of `find.clusters` is a list:

```
> names(grp)
```

```
[1] "Kstat" "stat"  "grp"   "size"
```

```
> head(grp$Kstat, 8)
```

```
     K=1      K=2      K=3      K=4      K=5      K=6      K=7      K=8
1256.185 1204.763 1168.137 1134.633 1104.379 1078.113 1077.659 1078.476
```

```
> grp$stat
```

```
     K=6
1078.113
```

```
> head(grp$grp, 10)
```

```
001 002 003 004 005 006 007 008 009 010
  3   3   3   1   3   3   3   3   3   3
Levels: 1 2 3 4 5 6
```

```
> grp$size
```

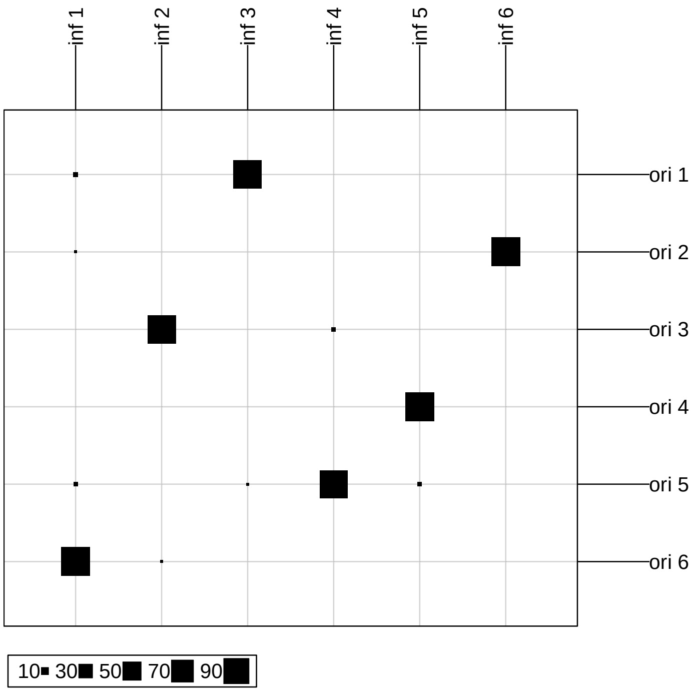
```
[1] 105  99  98  97 102  99
```

The components are respectively the chosen summary statistics (here, BIC) for different values of $k$ (slot `Kstat`), the selected number of clusters and the associated BIC (slot `stat`), the group memberships (slot `grp`) and the group sizes (slot `size`). Here, since we knew the actual groups, we can check how well they have been retrieved by the procedure. Actual groups are accessed using `pop`:

```
> table(pop(x), grp$grp)
```

```
      1   2   3   4   5   6
  1   3   0  97   0   0   0
  2   1   0   0   0   0  99
  3   0  98   0   2   0   0
  4   0   0   0   0 100   0
  5   2   0   1  95   2   0
  6  99   1   0   0   0   0
```

```
> table.value(table(pop(x), grp$grp), col.lab = paste("inf", 1:6),
+     row.lab = paste("ori", 1:6))
```

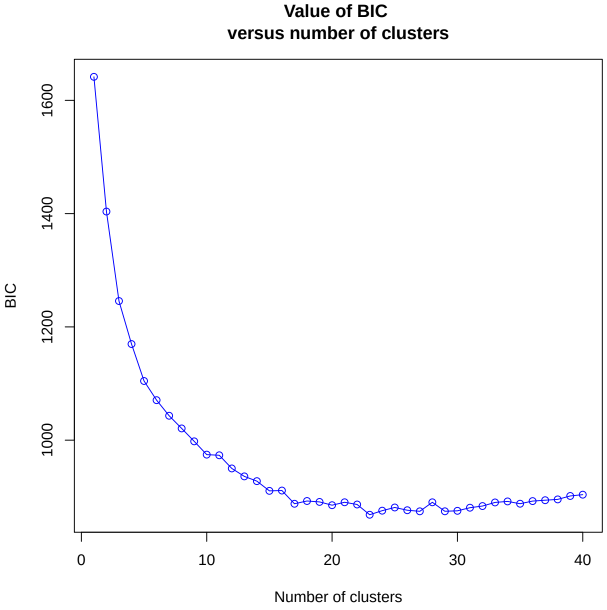


Rows correspond to actual groups (”ori”), while columns correspond to inferred groups (”inf”). Here, we can see that original groups have nearly been perfectly identified by the method.

## 2.3 How many clusters are there really in the data?

Although the most frequently asked when trying to find clusters in genetic data, this question is equally often meaningless. Clustering algorithms help making a caricature of a complex reality, which is most of the time far from following known population genetics models. Therefore, we are rarely looking for actual panmictic populations from which the individuals have been drawn. Genetic clusters can be biologically meaningful structures and reflect interesting biological processes, but they are still models.

A slightly different but probably more relevant question would be: ”How many clusters are useful to describe the data?”. A fundamental point in this question is that clusters are merely tools used to summarise and understand the data. There is no longer a ”true $k$”, but some values of $k$ are better, more efficient summaries of the data than others. For instance, in the following case:



, the concept of ”true $k$” is fairly hypothetical. This does not mean that clutering algorithms should necessarily be discarded, but surely the reality is more complex than a few clear-cut, isolated populations. What the BIC decrease says is that 10-20 clusters would provide useful summaries of the data. The actual number retained is merely a question of personnal taste.

# 3 Describing clusters using dapc

## 3.1 Rationale

DAPC aims to provide an efficient description of genetic clusters using a few synthetic variables. These are constructed as linear combinations of the original variables (alleles) which have the largest between-group variance and the smallest within-group variance. Coefficients of the alleles used in the linear combination are called *loadings*, while the synthetic variables are themselves referred to as *discriminant functions*.

Moreover, being based on the Discriminant Analysis, DAPC also provides membership probabilities of each individual to the different groups based on the retained discriminant functions. While these are different from the admixture coefficients of software like STRUCTURE, they can be still be interpreted as proximities of individuals to the different clusters. Membership probabilities also provide indications of how clear-cut genetic clusters are. Loose clusters will result in fairly flat distributions of membership probabilities of individuals across clusters, possibly sign of admixture in the case of island models.

Lastly, using the allele loadings, it is possible to represent new individuals (which have not participated to the analysis) onto the factorial planes, and derive membership probabilities as welll. Such individuals are referred to as *supplementary individuals*.

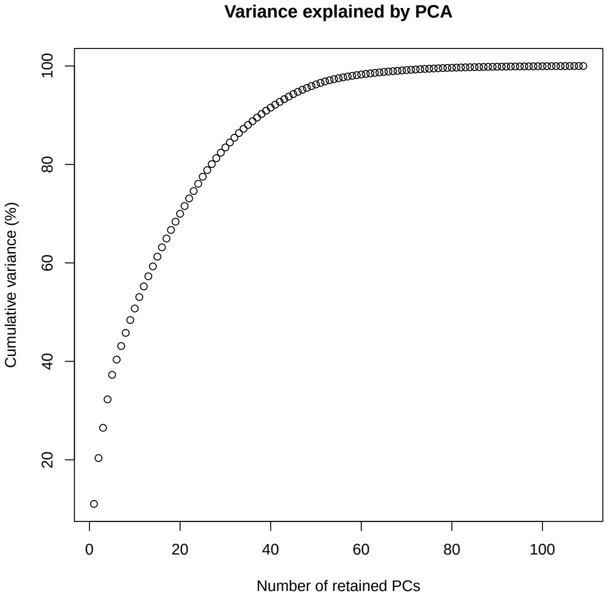
## 3.2 In practice

DAPC is implemented by the function `dapc`, which first transforms the data using PCA, and then performs a Discriminant Analysis on the retained principal components. Like `find.clusters`, `dapc` is a generic function with methods for texttttdata.frame, and objects with the class `genind` (usual genetic markers) and `genlight` (genome wide SNP data).

We run the analysis on the previous toy dataset, using the inferred groups stored in `grp$grp`:

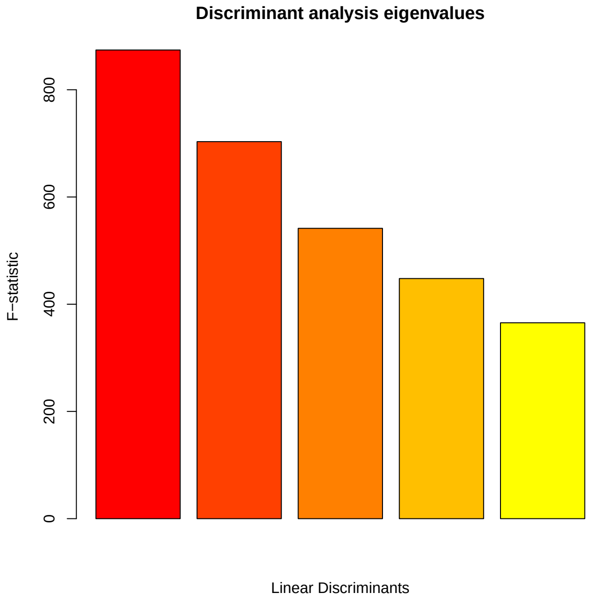
```
> dapc1 <- dapc(x, grp$grp)
```

The method displays the same graph of cumulated variance for the PCA step. However, unlike $k$-means, DAPC can benefit from not using too many PCs. Indeed, retaining too many components with respect to the number of individuals can lead to over-fitting and unstability in the membership probabilities returned by the method (see section below about the stability of DAPC results).



The bottomline is therefore retaining a few PCs without sacrificing too much information. Here, we can see that little information is gained by adding PCs after the first 40. We therefore retain 40 PCs.

Then, the method displays a barplot of eigenvalues for the discriminant analysis, asking for a number of discriminant functions to retain (unless argument `n.da` is provided).



For small number of clusters, all eigenvalues can be retained since all discriminant functions can be examined without difficulty. Whenever more (say, tens of) clusters are analysed, it is likely that the first few dimensions will carry more information than the others, and only those can then be retained and interpreted.

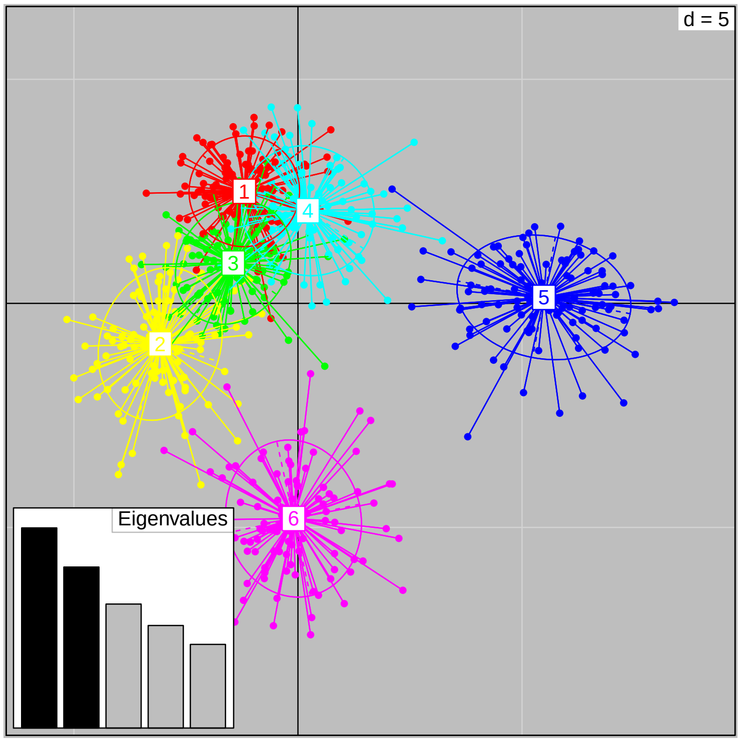
The object `dapc1` contains a lot of information:

```
> dapc1


        #########################################
        # Discriminant Analysis of Principal Components #
        #########################################
class: dapc
$call: dapc.genind(x = x, pop = grp$grp, n.pca = 40)

$n.pca: 40 first PCs of PCA used
$n.da: 5 discriminant functions saved
$var (proportion of conserved variance): 0.915

$eig (eigenvalues): 874.1 703.2 541.5 447.9 365.3  vector    length content
1 $eig      5      eigenvalues
2 $grp      600    prior group assignment
3 $prior    6      prior group probabilities
4 $assign   600    posterior group assignment
5 $pca.cent 140    centring vector of PCA
6 $pca.norm 140    scaling vector of PCA

  data.frame    nrow ncol content
1 $tab          600  40   retained PCs of PCA
2 $means        6    40   group means
3 $loadings     40   5    loadings of variables
4 $ind.coord    600  5    coordinates of individuals (principal components)
5 $grp.coord    6    5    coordinates of groups
```

```
6 $posterior    600  6    posterior membership probabilities
7 $pca.loadings 140  40   PCA loadings of original variables
8 $var.contr    140  5    contribution of original variables
```

For details about this content, please read the documentation (?dapc). Essentially, the slots ind.coord and grp.coord contain the coordinates of the individuals and of the groups used in scatterplots. Contributions of the alleles to each discriminant function are stored in the slot var.contr. Eigenvalues, corresponding to the ratio of the variance between groups over the variance within group for each discriminant function, are stored in eig. Basic scatterplots can be obtained using the function scatterplot:

```
> scatter(dapc1)
```



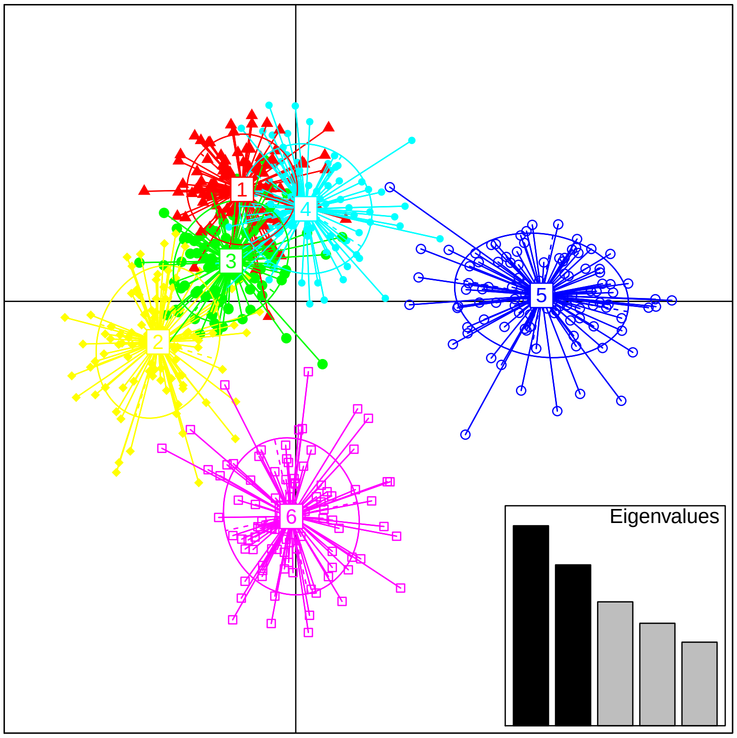
The obtained graph represents the individuals as dots and the groups as inertia ellipses. Eigenvalues of the analysis are displayed in inset. These graphs are fairly easy to customize, as shown below.

## 3.3 Customizing DAPC scatterplots

DAPC scatterplots are the main result of DAPC. It is therefore essential to ensure that information is displayed efficiently, and if possible to produce pretty figures. Possibility are almost unlimited, and here we just illustrate a few possibilities offered by scatter. Note that scatter is a generic function, with a dedicated method for objects produced by dapc. Documentation of this function can be accessed by typing ?scatter.dapc.

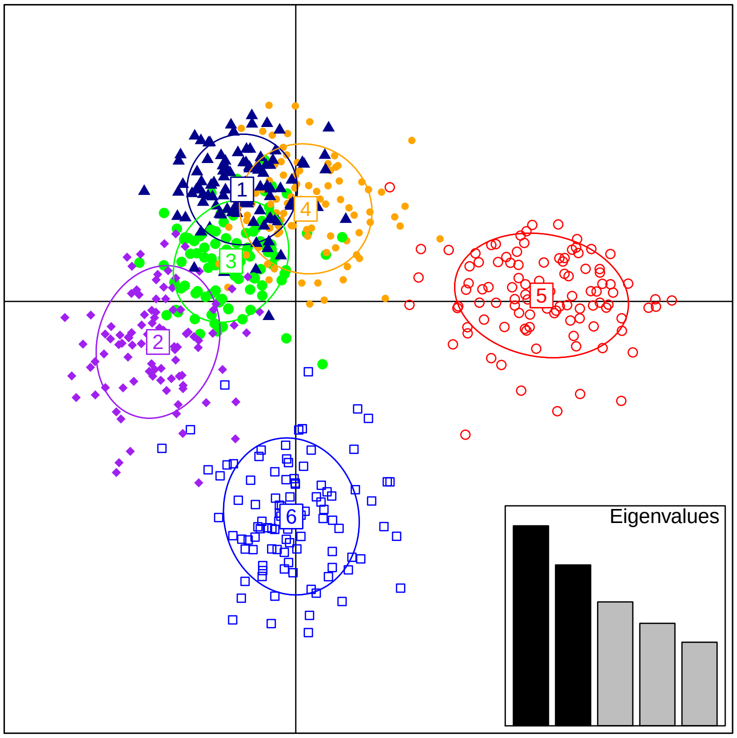
We illustrate some graphical possibilities trying to improve the display of the analysis presented in the previous section. While the default background (grey) allows to visualize rainbow colors (the default palette for the groups) more easily, it is not so pretty and is probably better removed for publication purpose. We also move the inset to a more appropriate place where it does not cover individuals, and use different symbols for the groups.

```
> scatter(dapc1, posi = "bottomright", grid = FALSE, bg = "white",
+     pch = 17:22)
```



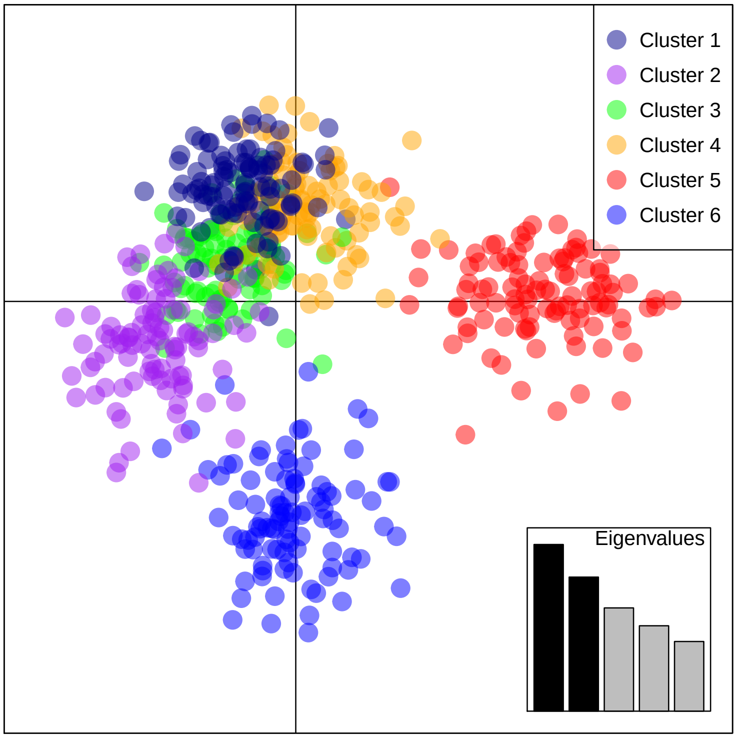
This is still not satisfying: we need to define other colors more visible over a white background, and we can remove the segments linking the points to their ellipses:

```
> myCol <- c("darkblue", "purple", "green", "orange", "red", "blue")
> scatter(dapc1, posi = "bottomright", grid = FALSE, bg = "white",
+     pch = 17:22, cstar = 0, axesel = FALSE, lwd = 2, col = myCol)
```



Another possibility is remove the labels within the ellipses and add a legend to the plot. We also use the same symbol for all individuals, but use bigger dots and transparent colours to have a better feel for the density of individuals on the factorial plane. We also add a customized barplot of eigenvalues in inset:

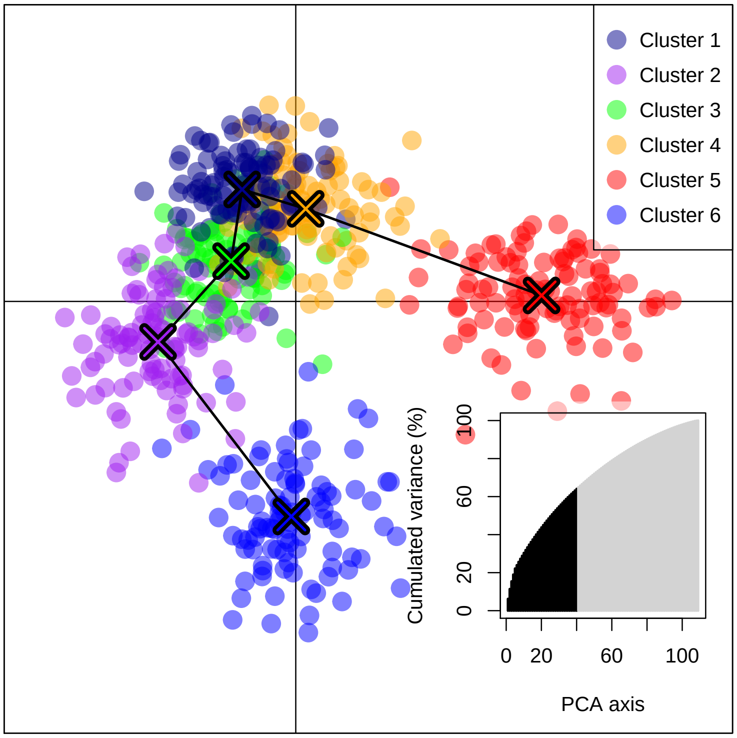
```
> scatter(dapc1, ratio = 0, grid = FALSE, bg = "white", pch = 20,
+     cell = 0, cstar = 0, lwd = 2, col = transp(myCol), cex = 3,
+     clab = 0)
> par(xpd = TRUE)
> legend("topright", pch = 20, cex = 1, col = transp(myCol), legend = paste("Cluster",
+     1:6), pt.cex = 3, bg = transp("white"))
> add.scatter.eig(dapc1$eig, 5, 1, 2, posi = "bottomright", inset = 0.03,
+     csub = 1)
```



We can also add a minimum spanning tree based on the (squared) distances between populations onto the DAPC scatterplot. This allows one to bear in mind the proximities between populations, which can be misinterpreted when looking at discriminant functions of lesser rank. We also add the centre of each group with crosses. Lastly, we remove the DAPC eigenvalues, not very useful in this case, and replace them by a graph of PCA eigenvalues retained in dimension-reduction step (retained eigenvalues in black).

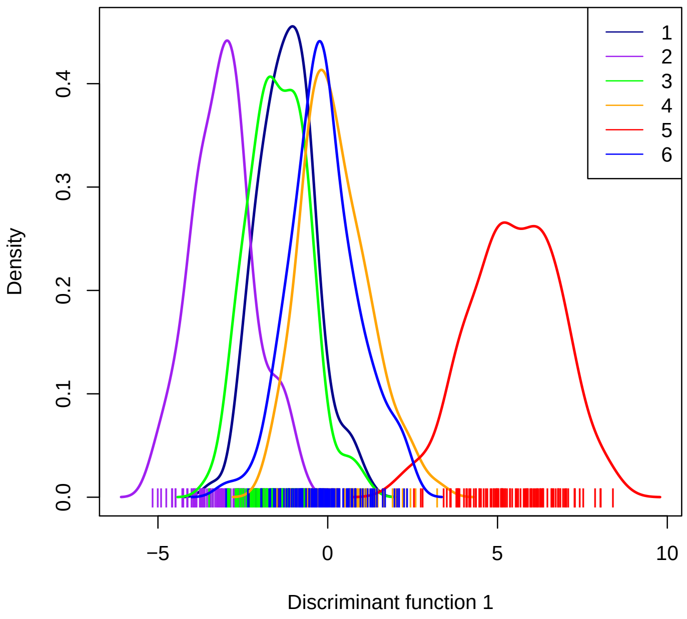
```
> pca.eig <- dudi.pca(scaleGen(x, scale = FALSE), scannf = FALSE,
+     nf = 1)$eig
> temp <- 100 * cumsum(pca.eig)/sum(pca.eig)
```

```
> scatter(dapc1, ratio = 0, grid = FALSE, bg = "white", pch = 20,
+     cell = 0, cstar = 0, lwd = 2, col = transp(myCol), cex = 3,
+     clab = 0, mstree = TRUE)
> par(xpd = TRUE)
> legend("topright", pch = 20, cex = 1, col = transp(myCol), legend = paste("Cluster",
+     1:6), pt.cex = 3, bg = transp("white"))
> points(dapc1$grp.coord[, 1], dapc1$grp.coord[, 2], pch = 4, cex = 3,
+     lwd = 8, col = "black")
> points(dapc1$grp.coord[, 1], dapc1$grp.coord[, 2], pch = 4, cex = 3,
+     lwd = 2, col = myCol)
> myInset <- function() {
+     plot(temp, col = rep(c("black", "lightgrey"), c(dapc1$n.pca,
+         1000)), ylim = c(0, 100), xlab = "PCA axis", ylab = "Cumulated variance (%)",
+         cex = 1, pch = 20, type = "h", lwd = 2)
+ }
> add.scatter(myInset(), posi = "bottomright", inset = c(-0.03,
+     -0.01), ratio = 0.28, bg = transp("white"))
```



Lastly, note that `scatter` can also represent a single discriminant function, which is especially useful when only one of these has been retained (e.g. in the case $k = 2$). This is achieved by plotting the densities of individuals on a given discriminant function with different colors for different groups:

```
> scatter(dapc1, 1, 1, col = myCol, bg = "white")
```



## 3.4 Interpreting variable contributions

In DAPC, the variable actually analyzed are principal components of a PCA. Loadings of these variables are generally uninformative. However, we also compute contributions of the alleles, which can be interpreted and sometimes very informative. In general, there are many alleles and their contribution is best plotted for a single discriminant function at a time.

Variable contributions are stored in the `var.contr` slot of a `dapc` object. They can be plotted using `loadingplot`. We illustrate this using the seasonal influenza dataset `H3N2`:

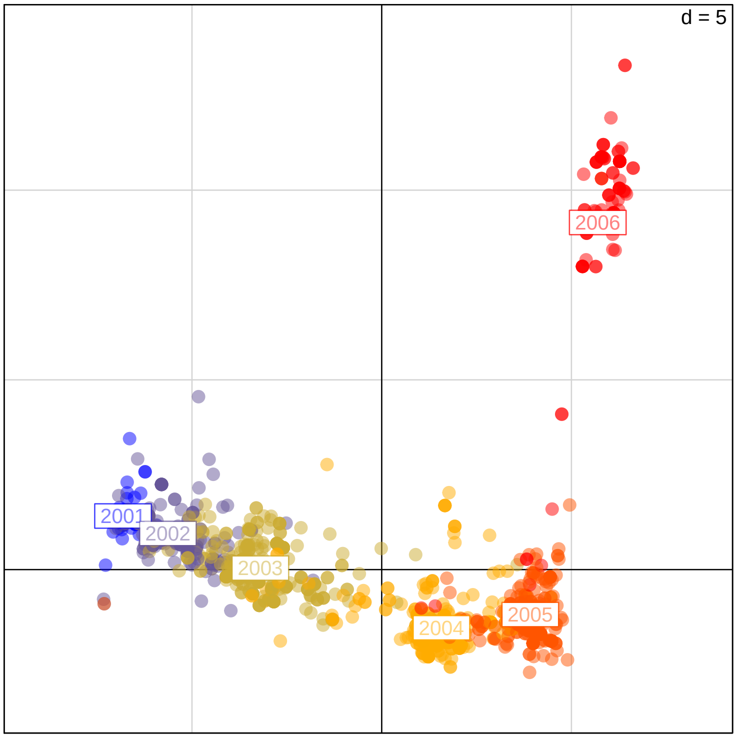
```
> data(H3N2)
> H3N2


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

S4 class:  genind
@call: .local(x = x, i = i, j = j, drop = drop)

@tab:  1903 x 334 matrix of genotypes

@ind.names: vector of  1903 individual names
@loc.names: vector of  125 locus names
@loc.nall: number of alleles per locus
@loc.fac: locus factor for the  334 columns of @tab
```

```
@all.names: list of  125 components yielding allele names for each locus
@ploidy:  1
@type:  codom

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

@other: a list containing: x  xy  epid
```

```
> pop(H3N2) <- H3N2$other$epid
> dapc.flu <- dapc(H3N2, n.pca = 30, n.da = 10)
```

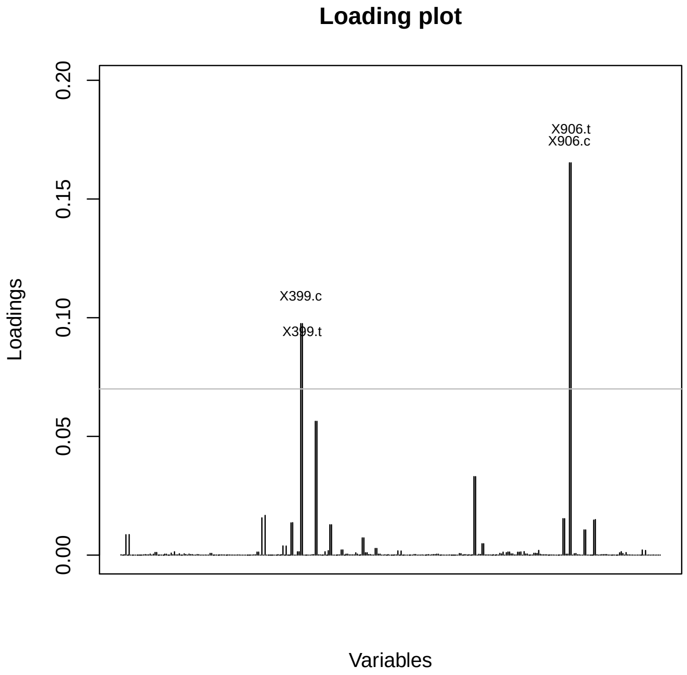
The first discriminant function shows the temporal evolution of the influenza virus, while the second one shows the originality of 2006 strains.

```
> myPal <- colorRampPalette(c("blue", "gold", "red"))
> scatter(dapc.flu, col = transp(myPal(6)), ratio = 0, cell = 0,
+     cex = 2, bg = "white", cstar = 0)
```



We can assess which alleles most highlight the originality of 2006 using `loadingplot`:

```
> set.seed(4)
> contrib <- loadingplot(dapc.flu$var.contr, axis = 2, thres = 0.07,
+     lab.jitter = 1)
```



`temp` is a list invisibly returned by `loadingplot` which contains the most contributing alleles. In this case, SNPs `906` and `399` reflect most the temporal evolution of the virus. We can look into their frequencies over 2002-2006:

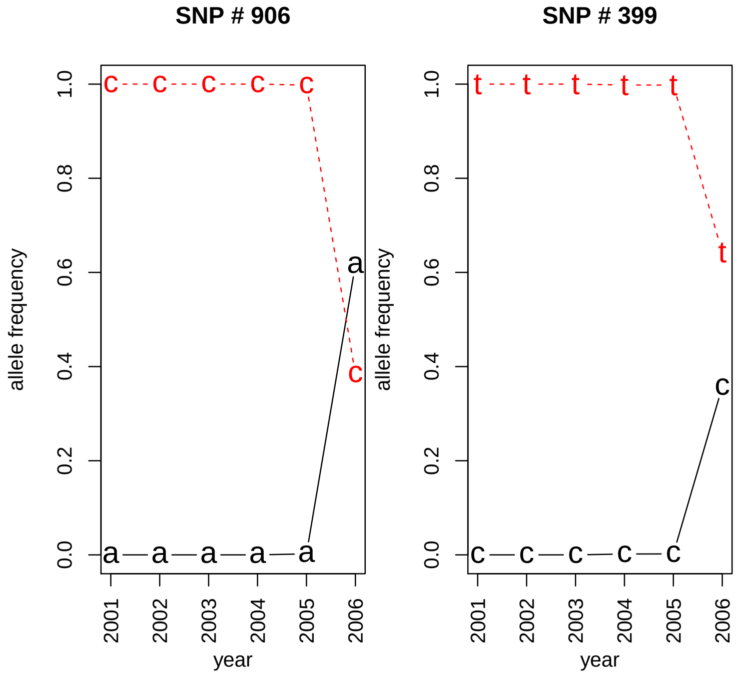
```
> temp <- seploc(H3N2)
> snp906 <- truenames(temp[["906"]])$tab
> snp399 <- truenames(temp[["399"]])$tab
> freq906 <- apply(snp906, 2, function(e) tapply(e, pop(H3N2),
+     mean, na.rm = TRUE))
> freq399 <- apply(snp399, 2, function(e) tapply(e, pop(H3N2),
+     mean, na.rm = TRUE))
> freq906
```

```
           906.c     906.t
2001 0.000000000 1.0000000
2002 0.000000000 1.0000000
2003 0.000000000 1.0000000
2004 0.000000000 1.0000000
2005 0.002155172 0.9978448
2006 0.616071429 0.3839286
```

```
> freq399
```

```
           399.c     399.t
2001 0.000000000 1.0000000
2002 0.000000000 1.0000000
2003 0.000000000 1.0000000
2004 0.001848429 0.9981516
2005 0.002079002 0.9979210
2006 0.357142857 0.6428571
```

```
> par(mfrow = c(1, 2), mar = c(5.1, 4.1, 4.1, 0.1), las = 3)
> matplot(freq906, pch = c("a", "c"), type = "b", xlab = "year",
+     ylab = "allele frequency", xaxt = "n", cex = 1.5, main = "SNP # 906")
> axis(side = 1, at = 1:6, lab = 2001:2006)
> matplot(freq399, pch = c("c", "t"), type = "b", xlab = "year",
+     ylab = "allele frequency", xaxt = "n", cex = 1.5, main = "SNP # 399")
> axis(side = 1, at = 1:6, lab = 2001:2006)
```



In both cases, a new allele appeared in 2005 at a very low frequency, and reached high or even dominant frequencies a year later. Irrespective of the mecanism underlying these changes (drift or selection), this illustrate that in seasonal influenza, specific nucleotides can undergo drastic changes within only a couple of years.

## 3.5 Interpreting group memberships

Besides scatterplots of discriminant functions, group memberships of DAPC can be exploited. Note that caution should be taken when interpreting group memberships of a DAPC based on many PCs, which can be unstable (see section below). Despite possible bias due to overfitting, group memberships can be used as indicators of how clear-cut genetic clusters are. Note that this is most useful for groups defined by an external criteria, i.e. defined biologically, as opposed to identified by $k$-means. It is less useful for groups identified using `find.clusters`, since we expect $k$-means to provide optimal groups for DAPC, and therefore both classifications to be mostly consistent.

Membership probabilities are based on the retained discriminant functions. They are stored in `dapc` objects as the slot `posterior`:

```
> class(dapc1$posterior)
```

```
[1] "matrix"
```

```
> dim(dapc1$posterior)
```

```
[1] 600   6
```

```
> round(head(dapc1$posterior), 3)
```

```
        1 2     3 4 5 6
001 0.000 0 1.000 0 0 0
002 0.000 0 1.000 0 0 0
003 0.000 0 1.000 0 0 0
004 0.984 0 0.016 0 0 0
005 0.000 0 1.000 0 0 0
006 0.000 0 1.000 0 0 0
```

Each row corresponds to an individual, each column to a group. This information can be summarized using `summary` on the `dapc` object:

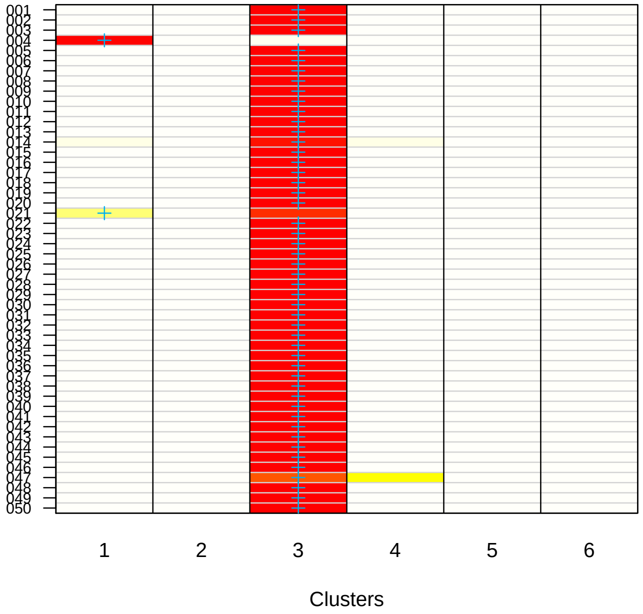
```
> summary(dapc1)
```

```
$n.dim
[1] 5

$n.pop
[1] 6

$assign.prop
[1] 0.9966667

$assign.per.pop
        1         2         3         4         5         6
0.9904762 1.0000000 1.0000000 1.0000000 0.9901961 1.0000000

$prior.grp.size

  1   2   3   4   5   6
105  99  98  97 102  99

$post.grp.size

  1   2   3   4   5   6
105  99  99  97 101  99
```

The slot `assign.per.pop` indicates the proportions of successful reassignment (based on discriminant functions) of individuals to their original clusters. Large values indicate clear-cut clusters, while low values suggest admixed groups.

This information can also be visualized using `assignplot` (see `?assignplot` for display options); here, we chose to represent only the first 50 individuals to make the figure readable:

```
> assignplot(dapc1, subset = 1:50)
```

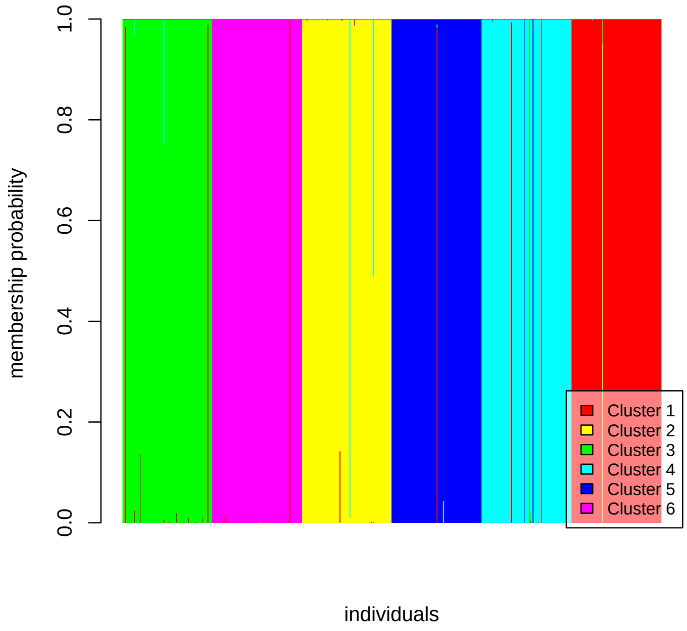


This figure is the simple graphical translation of the table above. Heat colors represent probabilities (red=1, white=0); blue crosses represent the prior cluster provided to DAPC. Here, we observe for most individuals that DAPC classification is consistent with the original clusters (blue crosses are on red rectangles), except one discrepancie for individual 21, classified in group 1 while DAPC would assign it to group 3.

Such figure is particularly useful when prior biological groups are used, as one may infer admixed or misclassified individuals.

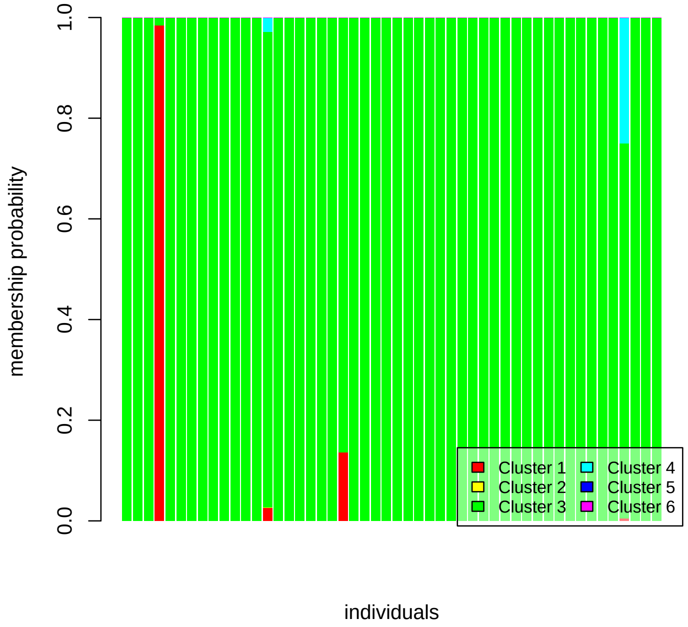
Note that this information can also be plotted in a STRUCTURE-like (!) way using `compoplot` (see `?compoplot` for customizing the plot). We can plot information of all individuals to have a global picture of the clusters composition.

```
> compoplot(dapc1, posi = "bottomright", leg.txt = paste("Cluster",
+     1:6), lab = "", ncol = 1, xlab = "individuals")
```



But we can have a closer look to a subset of individuals as easily; for instance, for the first 50 individuals:

```
> compoplot(dapc1, subset = 1:50, posi = "bottomright", leg.txt = paste("Cluster",
+     1:6), lab = "", ncol = 2, xlab = "individuals")
```

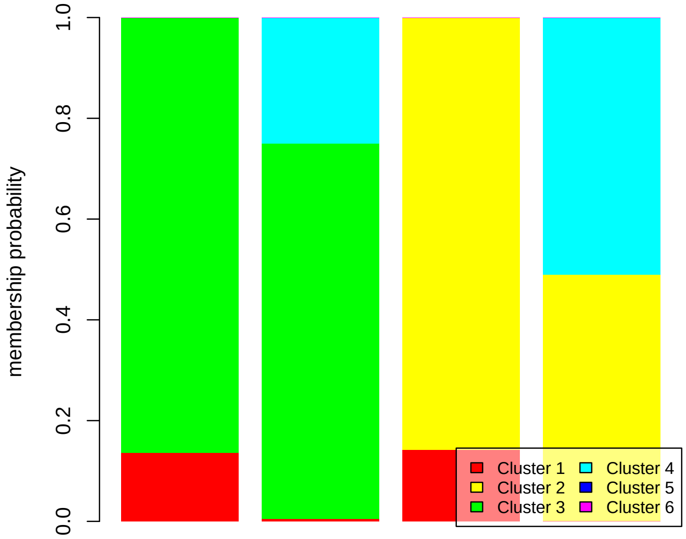


Obviously, we can use the power of R to lead our investigation further. For instance, which are the most 'admixed' individuals? Say admixed individuals are those having no more than 90% of probability of membership in a single cluster:

```
> temp <- which(apply(dapc1$posterior, 1, function(e) all(e < 0.9)))
> temp
```

```
021 047 243 280
 21  47 243 280
```

```
> compoplot(dapc1, subset = temp, posi = "bottomright", leg.txt = paste("Cluster",
+     1:6), lab = "", ncol = 2)
```



# 4 Ensuring stability of DAPC results

## 4.1 When and why group memberships can be unreliable

In DAPC, discriminant functions are linear combinations of variables (principal components of PCA) which optimize the separation of individuals into pre-defined groups. Based on the retained discriminant functions, it is possible to derive group membership probabilities, which can be interpreted in order to assess how clear-cut or admixed the clusters are. Unfortunately, retaining too many PCs with respect to the number of individuals can lead to over-fitting the discriminant functions. In such case, discriminant function become so ”flexible” that they could discriminate almost perfectly any cluster. While the main scatterplots are usually unaltered by this process, membership probabilities can become drastically inflated.

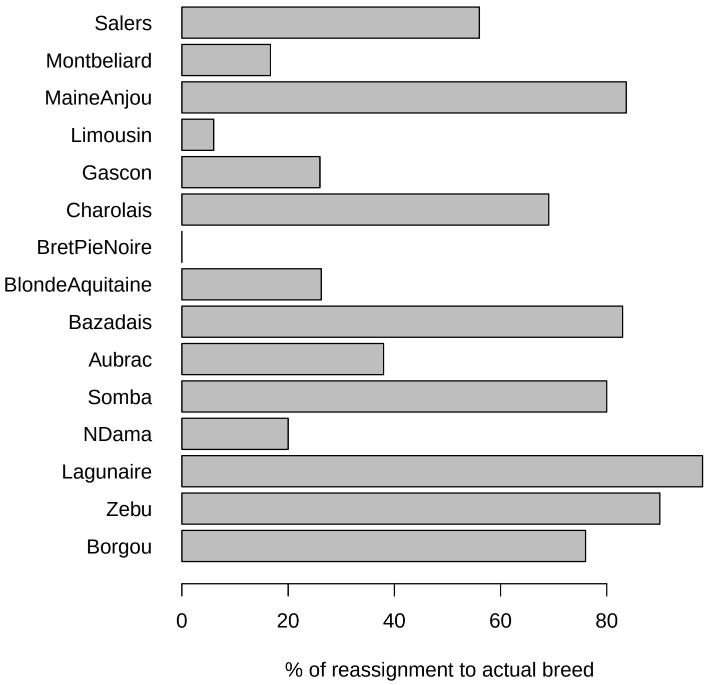
This point can be illustrated using the `microbov` dataset (704 cattles of 15 breeds typed for 30 microsatellite markers). We first examine the % of successful reassignment (i.e., quality of discrimination) for different numbers of retained PCs. First, retaining 3 PCs during the dimension-reduction step, and all discriminant functions:

```
> data(microbov)
> microbov
```

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

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

@tab:  704 x 373 matrix of genotypes

@ind.names: vector of  704 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
@type:  codom

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
```

```
> temp <- summary(dapc(microbov, n.da = 100, n.pca = 3))$assign.per.pop *
+     100
```

```
> par(mar = c(4.5, 7.5, 1, 1))
> barplot(temp, xlab = "% of reassignment to actual breed", horiz = TRUE,
+     las = 1)
```

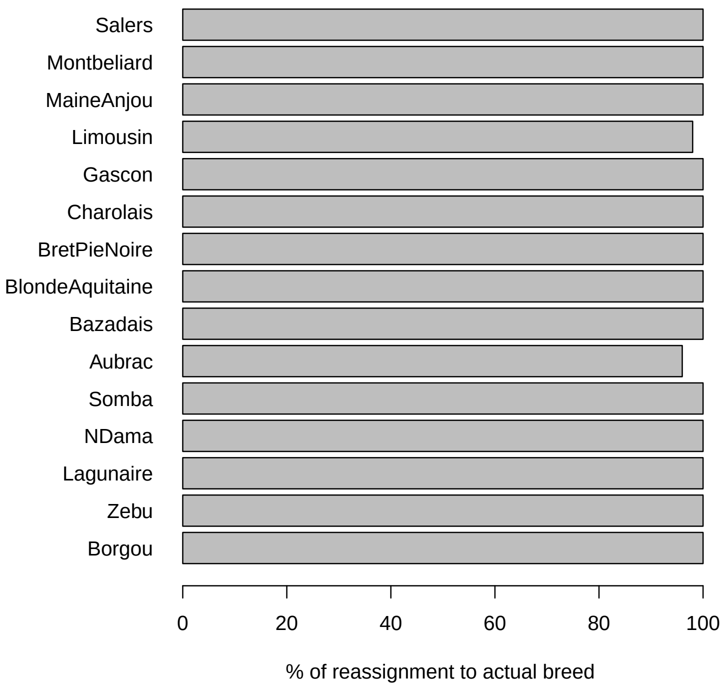


We can see that some breeds are well discriminated (e.g. Zebu, Lagunaire, > 90%) while others are entirely overlooked by the analysis (e.g. Bretone Pie

Noire, Limousin, <10%). This is because too much genetic information is lost when retaining only 3 PCs. We repeat the analysis, this time keeping 300 PCs:

```
> temp <- summary(dapc(microbov, n.da = 100, n.pca = 300))$assign.per.pop *
+     100
```

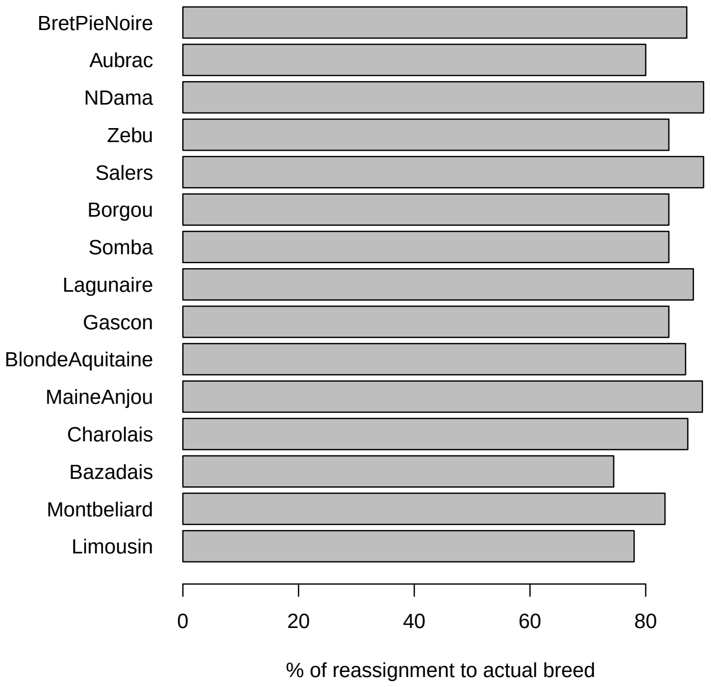
```
> par(mar = c(4.5, 7.5, 1, 1))
> barplot(temp, xlab = "% of reassignment to actual breed", horiz = TRUE,
+     las = 1)
```



We now obtain almost 100% of discrimination for all groups. Is this result satisfying? Actually not. The number retained PCs is so large that discriminant functions could model any structure and virtually any set of clusters would be well discriminated. This can be illustrated by running the analysis using randomized groups:

```
> x <- microbov
> pop(x) <- sample(pop(x))
> temp <- summary(dapc(x, n.da = 100, n.pca = 300))$assign.per.pop *
+     100
```

```
> par(mar = c(4.5, 7.5, 1, 1))
> barplot(temp, xlab = "% of reassignment to actual breed", horiz = TRUE,
+     las = 1)
```



Groups have been randomised, and yet we still get very good discrimination. There is therefore a trade-off between finding a space with a good power of discrimination using DAPC, and retaining too many dimensions and cause over-fitting.

## 4.2 Using the *a*-score

The trade-off between power of discrimination and over-fitting can be measured by the *a*-score, which is simply the difference between the % of successful reassignment of the analysis (observed discrimination) and values obtained using random groups (random discrimination). It can be seen as the % of successful reassignment corrected for the number of retained PCs. It is implemented by `a.score`, which relies on repeating the DAPC analysis using randomized groups, and computing *a*-scores for each group, and well as the average *a*-score:

```
> dapc2 <- dapc(microbov, n.da = 100, n.pca = 10)
> temp <- a.score(dapc2)
> names(temp)
```

```
[1] "tab"       "pop.score" "mean"
```

```
> temp$tab[1:5, 1:5]
```

```
      Borgou Zebu Lagunaire     NDama Somba
sim.1   0.74 0.84 0.8235294 0.5666667  0.58
sim.2   0.76 0.72 0.8627451 0.5333333  0.78
sim.3   0.60 0.78 0.8627451 0.5000000  0.70
sim.4   0.64 0.74 0.8627451 0.5333333  0.70
sim.5   0.72 0.76 0.9607843 0.5000000  0.80
```

```
> temp$pop.score
```

```
         Borgou            Zebu       Lagunaire           NDama           Somba
      0.6380000       0.7420000       0.8509804       0.5166667       0.7180000
         Aubrac        Bazadais BlondeAquitaine    BretPieNoire       Charolais
      0.4940000       0.8382979       0.3016393       0.4741935       0.5563636
         Gascon        Limousin      MaineAnjou     Montbeliard          Salers
      0.6640000       0.4280000       0.8653061       0.6733333       0.7720000
```

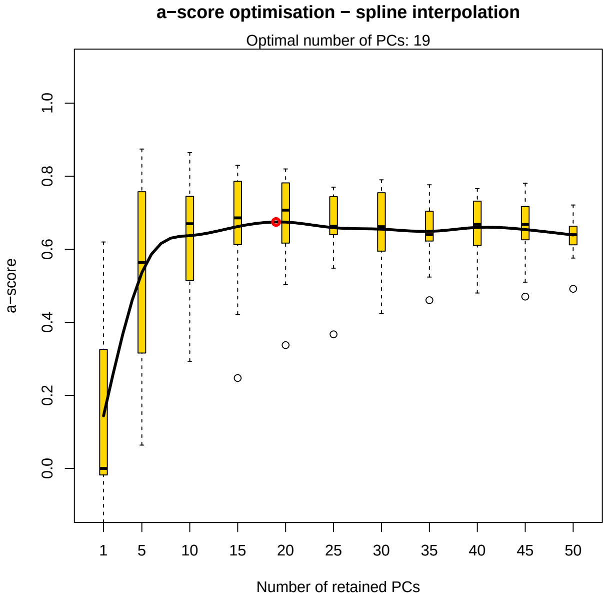
```
> temp$mean
```

```
[1] 0.6355187
```

The number of retained PCs can be chosen so as to optimize the *a*-score; this is achived by `optim.a.score`:

```
> dapc2 <- dapc(microbov, n.da = 100, n.pca = 50)
```

```
> temp <- optim.a.score(dapc2)
```

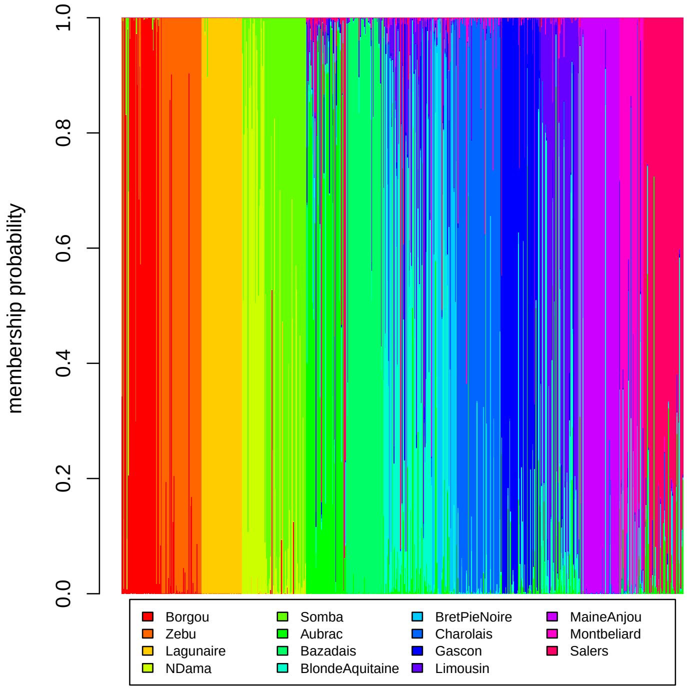


Since evaluating solutions for 1, 2, ... 100 retained PCs, as a first approximation the method evaluates a few numbers of retained PCs in this range, and uses spline interpolation to approximate the optimal number of PCs to retain. Then, one can evaluate all solutions within a restrained range using the argument `n.pca`. For the `microbov` dataset, we should probably retained between 10 and 30 PCs during the dimension-reduction step.

We perform the analysis with 20 PCs retained, and then map the membership probabilities as before:

```
> dapc3 <- dapc(microbov, n.da = 100, n.pca = 20)
> myCol <- rainbow(15)
```

```
> par(mar = c(5.1, 4.1, 1.1, 1.1), xpd = TRUE)
> compoplot(dapc3, lab = "", posi = list(x = 12, y = -0.01), cleg = 0.7)
```



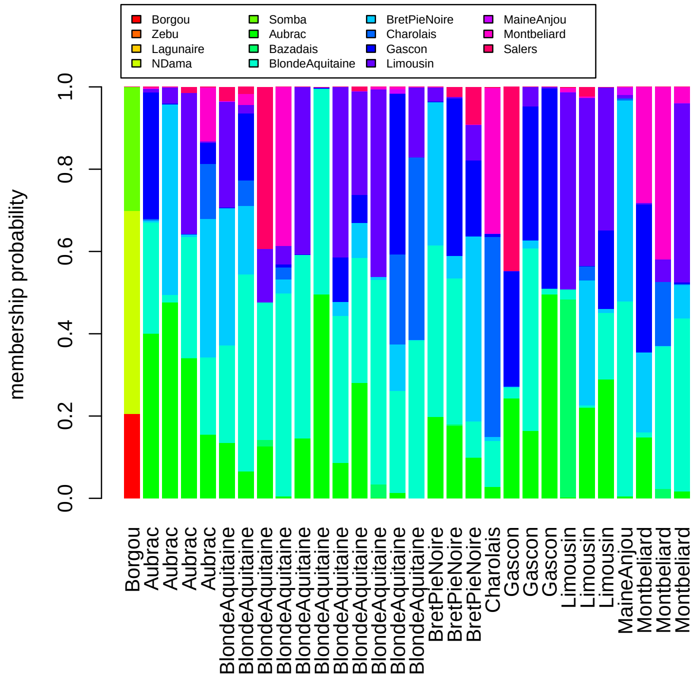
And as before, we can investigate further admixed individuals, which we arbitrarily define as those having no more than 0.5 probability of membership to any group:

```
> temp <- which(apply(dapc3$posterior, 1, function(e) all(e < 0.5)))
> temp
```

```
 AFBIBOR9511  FRBTAUB9062  FRBTAUB9070  FRBTAUB9078  FRBTAUB9225 FRBTBDA29851
           9          233          241          249          265          329
FRBTBDA29856 FRBTBDA29879 FRBTBDA35248 FRBTBDA35256 FRBTBDA35259 FRBTBDA35267
```

```
          334          354          361          363          365          368
FRBTBDA35278 FRBTBDA35281 FRBTBDA35877 FRBTBDA35941  FRBTBPN1906  FRBTBPN1913
          372          374          382          386          405          409
 FRBTBPN1915 FRBTCHA15957 FRBTGAS14183  FRBTGAS9173  FRBTGAS9200 FRBTLIM30832
          411          422          477          498          520          543
FRBTLIM30839 FRBTLIM30855  FRBTMA25298  FRBTMBE1496  FRBTMBE1514  FRBTMBE1544
          550          566          579          625          636          651
```

```
> lab <- pop(microbov)
> par(mar = c(8, 4, 5, 1), xpd = TRUE)
> compoplot(dapc3, subset = temp, cleg = 0.6, posi = list(x = 0,
+     y = 1.2), lab = lab)
```



Admixture appears to be the strongest between a few breeds (Blonde d'Aquitaine, Bretonne Pie-Noire, Limousine and Gascone). Some features are fairly surprising; for instance, the last individual is fairly distant from its cluster, but almost 50% chances to be assigned to two other breeds.

# 5 Using supplementary individuals

## 5.1 Rationale

Statistically speaking, supplementary individuals are observations which did not participate to the analysis, but which we would like to predict using our model of the data. In the context of DAPC, we may know groups for most individuals, but some individuals could be of unknown or uncertain group. In this case, we need to exclude individuals from the analysis, and then project them as supplementary individuals on the discriminant functions. The only requirement for this operation is that supplementary individuals have been typed as the same loci as the rest of the dataset.

Technically, using supplementary individuals consist in transforming the new data using the centring and scaling of the contributing individuals, and then using the same discriminant coefficients as for the contributing individuals to predict the position of the new individuals onto the discriminant functions.

## 5.2 In practice

We will illustrate the practice of supplementary individuals using the cattle breeds data previously analyzed (microbov dataset). We first split the dataset into two parts: one used for the analysis, and one used as supplementary individuals:

```
> data(microbov)
> set.seed(2)
> kept.id <- unlist(tapply(1:nInd(microbov), pop(microbov), function(e) sample(e,
+     30, replace = FALSE)))
> x <- microbov[kept.id]
> x.sup <- microbov[-kept.id]
> nInd(x)
```

```
[1] 450
```

```
> nInd(x.sup)
```

```
[1] 254
```

x is a genind containing the data to be analyzed; x.sup contains the supplementary individuals.

We perform the DAPC of x, and use predict to predict results for the supplementary individuals:

```
> dapc4 <- dapc(x, n.pca = 20, n.da = 15)
> pred.sup <- predict.dapc(dapc4, newdata = x.sup)
> names(pred.sup)
```

```
[1] "assign"     "posterior"  "ind.scores"
```

```
> head(pred.sup$assign)
```

```
[1] Zebu   Borgou Borgou Borgou Borgou Borgou
15 Levels: Borgou Zebu Lagunaire NDama Somba Aubrac ... Salers
```

```
> pred.sup$ind.scores[1:5, 1:3]
```

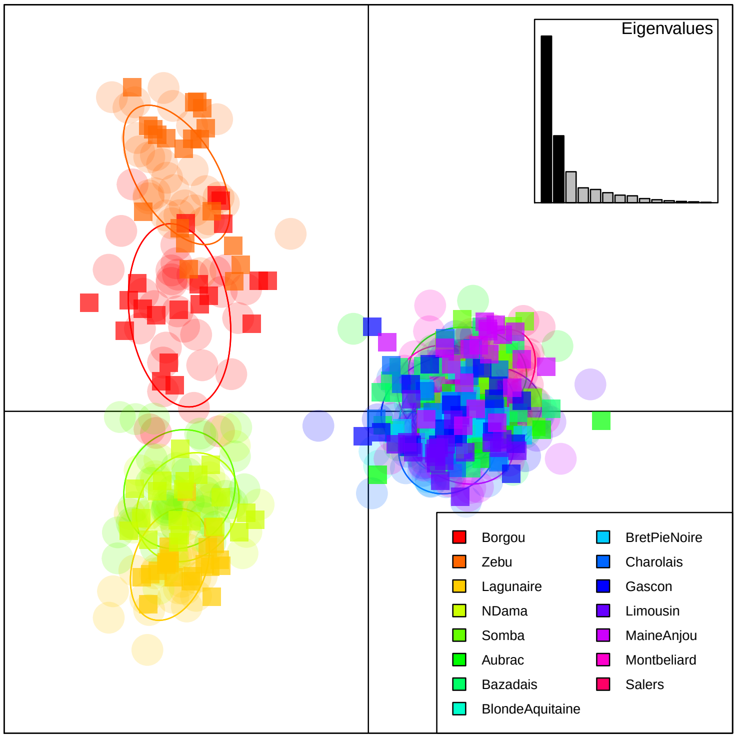
```
          LD1      LD2         LD3
001 -4.161913 5.935408  0.05654973
002 -2.755717 3.590551 -0.14428830
003 -5.212104 2.786854 -0.60542873
004 -4.375756 3.175634  0.92918315
005 -3.014747 3.592078  0.92285817
```

```
> round(pred.sup$posterior[1:5, 1:5], 3)
```

```
    Borgou  Zebu Lagunaire NDama Somba
001  0.129 0.871         0     0     0
002  0.995 0.005         0     0     0
003  0.999 0.001         0     0     0
004  0.999 0.001         0     0     0
005  0.995 0.005         0     0     0
```

The list pred.sup contains all the predictions about the new data based on the analysis stored in dapc4. The slot assign contains the assignment of new individuals to groups; ind.scores contains the coordinates of the new individuals on the discriminant functions; posterior contains the posterior membership probabilities. We can visualize the information by different ways. First, we can represent the new individuals using a scatterplot, and see :

```
> col <- rainbow(length(levels(pop(x))))
> col.points <- transp(col[as.integer(pop(x))], 0.2)
> scatter(dapc4, col = col, bg = "white", ratio = 0, grid = FALSE,
+     pch = "", cstar = 0, axesel = FALSE, clab = 0, lwd = 3, xlim = c(-10,
+         10))
> par(xpd = TRUE)
> points(dapc4$ind.coord[, 1], dapc4$ind.coord[, 2], pch = 20,
+     col = col.points, cex = 5)
> col.sup <- col[as.integer(pop(x.sup))]
> points(pred.sup$ind.scores[, 1], pred.sup$ind.scores[, 2], pch = 15,
+     col = transp(col.sup, 0.7), cex = 2)
> legend("bottomright", fill = col, leg = levels(pop(x)), ncol = 2,
+     cex = 0.7, bg = transp("white"))
> add.scatter.eig(dapc4$eig, 15, 1, 2, posi = "topright", inset = 0.02)
```



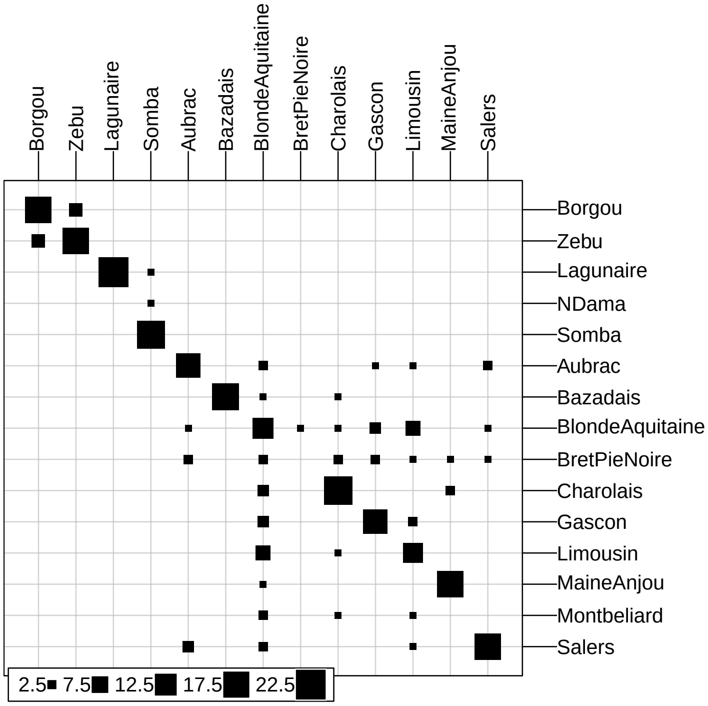
Light dots and ellipses correspond to the original analysis, while more solid squares indicate supplementary individuals. Results are fairly satisfying:

```
> mean(as.character(pred.sup$assign) == as.character(pop(x.sup)))
```

```
[1] 0.7322835
```

Around 73 of individuals have been assigned to their actual cluster. For more details about which breed was assigned to which cluster, we can display the contingency table of the actual cluster vs the inferred one:

```
> table.value(table(pred.sup$assign, pop(x.sup)), col.lab = levels(pop(x.sup)))
```



Columns correspond to actual clusters of the supplementary individuals, while rows correspond to inferred clusters. Overall, groups are fairly well retrieved, but we can notice that individuals of Blonde d'Aquitaine breed are poorly identified compared to other breeds.