Practical course using the \mathbf{Q} software

Multivariate analysis of genetic markers as a tool to explore the genetic diversity: some examples

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Abstract

This practical course aims at illustrating some possible applications of multivariate analyses to genetic markers data, using the R software [15]. Although a basic knowledge of the R language is assumed, most necessary commands are provided, so that coding should not be an obstacle. Two exercises are proposed, which go through different topics in genetic data analysis, respectively the study of spatial genetic structures, and the coherence of information coming from different markers. After going through the first section ('Let's start'), you should feel free to get to the exercise you want, as these are meant to be independent. This practical course uses mostly the *adegenet* [11] and *ade4* packages [4, 8, 7], but others like *adehabitat* [2, 1], genetics [16] and hierfstat [9] are also used.

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1 Let's start

1.1 Loading the packages

Before going further, we shall make sure that all we need is installed on the computer. Launch R, and make sure that the version being used is greater than 2.8.1 by typing:

```
> R.version.string
```

```
[1] "R version 2.11.1 (2010-05-31)"
```

The next thing to do is check that relevant packages are installed. To load an installed package, use the **library** instruction; for instance:

```
> library(adegenet)
```

loads *adegenet* if it is installed (and issues an error otherwise). To get the version of a package, use:

```
> packageDescription("adegenet", fields = "Version")
```

[1] "1.2-7"

adegenet version should read 1.2-6 or greater.

In case a package would not be installed, you can install it using install.packages. To install all the required dependencies, specify dep=TRUE. For instance, the following instruction should install *adegenet* with all its dependencies (it can take up to a few minutes, so don't run it unless *adegenet* is not installed):

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

Using the previous instructions, load (and install if required) the packages *adegenet*, *ade4*, *spdep*, *genetics*, and *hierfstat*.

1.2 How to get information?

There are several ways of getting information about R in general, or about *adegenet* in particular. The function **help.search** is used to look for help on a given topic. For instance:

```
> help.search("Hardy-Weinberg")
```

replies that there is a function HWE.test.genind in the *adegenet* package, other similar functions in *genetics* and *pegas*. To get help for a given function, use ?foo where 'foo' is the function of interest. For instance (quotes can be removed):

> `?`(spca)

will open the manpage of the spatial principal component analysis [12]. At the end of a manpage, an 'example' section often shows how to use a function. This can be copied and pasted to the console, or directly executed from the console using example. For further questions concerning R, the function RSiteSearch is a powerful tool to make an online research using keywords in R's archives (mailing lists and manpages).

adegenet has a few extra documentation sources. Information can be found from the website (http://adegenet.r-forge.r-project.org/), in the 'documents' section, including two tutorials, a manual which includes all manpages of the package, and a dedicated mailing list with searchable archives. To open the website from R, use:

```
> adegenetWeb()
```

The same can be done for tutorials, using **adegenetTutorial** (see manpage to choose the tutorial to open).

You will also find a listing of the main functions of the package typing:

```
> `?`(adegenet)
```

Note that you can also browse help pages as html pages, using:

> help.start()

To go to the *adegenet* page, click 'packages', 'adegenet', and 'adegenet-package'.

Lastly, several mailing lists are available to find different kinds of information on R; to name a few:

- R-help (https://stat.ethz.ch/mailman/listinfo/r-help): general questions about R
- R-sig-genetics (https://stat.ethz.ch/mailman/listinfo/r-sig-genetics): genetics in R
- adegenet forum (https://lists.r-forge.r-project.org/cgi-bin/mailman/ listinfo/adegenet-forum): adegenet and multivariate analysis of genetic markers

2 Spatial genetic structure of the chamois in the Bauges mountains

The chamois (*Rupicapra rupicapra*) is a conserved species in France. The Bauges mountains is a protected area in which the species has been recently studied. One of the most important questions for conservation purposes relates to whether individuals from this area form a single reproductive unit, or whether they are structured into sub-groups, and if so, what causes are likely to induce this structuring.

While field observations are very scarce and do not allow to answer this question, genetic data can be used to tackle the issue, as departure from panmixia should result in genetic structuring. The dataset *rupica* contains 335 georeferenced genotypes of Chamois from the Bauges mountains for 9 microsatellite markers, which we propose to analyse in this exercise.

2.1 An overview of the data

```
We first load the data:
> data(rupica)
> rupica
   ### Genind object ###
   genotypes of individuals -
@call: NULL
@tab: 335 x 55 matrix of genotypes
@ind.names: vector of
                       335 individual names
                       9 locus names
@loc.names: vector of
Cloc.nall: number of alleles per locus
Cloc.fac: locus factor for the 55 columns of Ctab
Call.names: list of 9 components yielding allele names for each locus
Cploidy: 2
@ploidy:
       codom
@type:
Optionnal contents:
Opop: - empty
@pop.names:
              empty -
Oother: a list containing: xy mnt showBauges
```

rupica is a typical genind object, which is the class of objects storing genotypes (as opposed to population data) in *adegenet*. rupica also contains topographic information about the sampled area, which can be displayed by calling rupica\$other\$showBauges. For instance, the spatial distribution of the sampling can be displayed as follows:

```
> rupica$other$showBauges()
> points(rupica$other$xy, col = "red", pch = 20)
```



This spatial distribution is clearly not random, but arranged into loose clusters; this can be confirmed by superimposing a kernel density curve (in blue) on the previous figure:

```
> rupica$other$showBauges()
> s.kde2d(rupica$other$xy, add.plot = TRUE)
> points(rupica$other$xy, col = "red", pch = 20)
```



Is geographical clustering strong enough to assign safely each individual to a group? Accordingly, shall we analyse these data at individual or group level?

2.2 Standard analyses

As a prior clustering of genotypes is not known, we cannot employ usual F_{ST} based approaches to detect genetic structuring. However, genetic structure could still result in a deficit of heterozygosity. The summary of genind objects provides expected and observed heterozygosity for each locus, which allows for a comparison:

```
> rupica.smry <- summary(rupica)
# Total number of genotypes: 335
# Population sample sizes:
335
# Number of alleles per locus:
L1 L2 L3 L4 L5 L6 L7 L8 L9
7 10 7 6 5 5 6 4 5
# Number of alleles per population:
1</pre>
```

```
55

# Percentage of missing data:

[1] 0

# Observed heterozygosity:

L1 L2 L3 L4 L5 L6 L7 L8

0.5880597 0.6208955 0.5253731 0.7582090 0.6597015 0.5283582 0.6298507 0.5552239

0.4149254

# Expected heterozygosity:

L1 L2 L3 L4 L5 L6 L7 L8

0.6076769 0.6532517 0.5314591 0.7259657 0.6601604 0.5706082 0.6412742 0.5473112

U 9

0.4070709
```

```
> plot(rupica.smry$Hexp, rupica.smry$Hobs, main = "Observed vs expected heterozygosity")
> abline(0, 1, col = "red")
```

Observed vs expected heterozygosity



The red line indicate identity between both quantities. What can we say about heterozygosity in this population? The following test provides further insights to answer this question:

```
8
```

95 percent confidence interval:

```
-0.01025068 0.02451318
sample estimates:
mean of the differences
0.00713125
```

We can seek a global picture of the genetic diversity among genotypes using a Principal Component Analysis (PCA, [14, 10], dudi.pca in ade4 package). The analysis is performed on a table of standardised alleles frequencies, obtained by scaleGen:

```
> rupica.X <- scaleGen(rupica, method = "binom")
> rupica.pca1 <- dudi.pca(rupica.X, cent = FALSE, scale = FALSE)</pre>
```



The function dudi.pca displays a barplot of eigenvalues and asks for a number of retained principal components. In general, eigenvalues represent the amount of genetic diversity — as measured by the multivariate method being used — represented by each principal component (PC). Here, each eigenvalue is the variance of the corresponding PC. This is also, up to a constant, the mean squared Euclidean distance between individuals. This is because (for $x \in \mathbb{R}^n$):

$$var(x) = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} (x_i - x_j)^2}{2n(n-1)}$$

This can be easily verified:

```
> rupica.pca1$eig[1]
[1] 1.560585
> pc1 <- rupica.pca1$li[, 1]
> var(pc1)
[1] 1.565257
> var(pc1) * (334/335)
[1] 1.560585
> 0.5 * mean(dist(pc1)^2) * (334/335)
```

```
[1] 1.560585
```

An abrupt decrease in eigenvalues is likely to indicate the boundary between true patterns and non-interpretable structures. In this case, we shall examin the first two principal components (though nothing really clear emerges from the eigenvalues).

```
> rupica.pca1
```

```
Duality diagramm
class: pca dudi
$call: dudi.pca(df = rupica.X, center = FALSE, scale = FALSE, scannf = FALSE,
    nf = 2)
$nf: 2 axis-components saved
$rank: 45
eigen values: 1.561 1.34 1.168 1.097 1.071 ...
  vector length mode
                        content
1 $cw
         55
                numeric column weights
2 $lw
         335
                numeric row weights
3 $eig
         45
                numeric eigen values
  data.frame nrow ncol content
1
  $tab
             335
                  55
                        modified array
             335
2 $1i
                  2
                        row coordinates
3 $11
                  2
             335
                        row normed scores
4
 $co
             55
                   2
                        column coordinates
 $c1
                   2
5
             55
                        column normed scores
other elements: cent norm
```

A dudi object contains various information; in the case of PCA, principal axes (loadings), principal components (synthetic variable), and eigenvalues are respectively stored in \$c1, \$li, and \$eig slots. The function s.label can be used to display to two first components; a kernel density (s.kde2d) is used for a better assessment of the distribution of the genotypes onto the principal axes:

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```
> s.label(rupica.pca1$li)
> s.kde2d(rupica.pca1$li, add.p = TRUE, cpoint = 0)
> add.scatter.eig(rupica.pca1$eig, 2, 1, 2)
```



What can we say about the genetic diversity among these genotypes as inferred by PCA? The function loadingplot allows to visualize the contribution of each allele, expressed as squared loadings, for a given principal component. This figure then gives further clues about the revealed structure: > loadingplot(rupica.pca1\$c1^2)



Loading plot

Variables

We can get back to the genotypes for the concerned markers (e.g., Bm203) to check whether the highlighted genotypes are indeed uncommon. **true-names** extracts the table of allele frequencies from a **genind** object (restoring original labels for markers, alleles, and individuals):

Only 4 genotypes possess one copy of this allele (the second result corresponds to a replaced missing data). Which individuals are they?

```
> rownames(X)[bm203.221 == 0.5]
001 019 029 276
"8" "86" "600" "7385"
```

Conclusion?

Just to make sure that this analysis shows no spatial pattern, we can map geographically the principal components. The function **s.value** is wellsuited to do so, using black and white squares of variable size for positive and negative values. For instance:

```
> s.value(cbind(1:11, rep(1, 11)), -5:5, cleg = 0)
> text(1:11, rep(1, 11), -5:5, col = "red", cex = 1.5)
```



We can then apply this graphical representation to the first two principal components of the PCA:

```
> showBauges <- rupica$other$showBauges
> showBauges()
> s.value(rupica$other$xy, rupica.pca1$li[, 1], add.p = TRUE, cleg = 0.5)
> title("PCA - first PC", col.main = "yellow", line = -2, cex.main = 2)
```



- > showBauges()
 > s.value(rupica\$other\$xy, rupica.pca1\$li[, 2], add.p = TRUE, csize = 0.7)
 > title("PCA second PC", col.main = "yellow", line = -2, cex.main = 2)



What can we say about spatial genetic structure as inferred by PCA?

2.3 spatial Principal Component Analysis

PCA did not reveal any kind of spatial genetic structure, but is not anyway meant to do so; most likely, it will fail to detect spatial genetic structures that are not associated with the strongest genetic differentiation. The spatial Principal Component Analysis (sPCA, [12]) has been developed to include spatial information in the analysis of genetic data. Although implemented in *adegenet*, sPCA needs spatial methods from the *spdep* package, which should thus be loaded:

> library(spdep)

sPCA first requires the spatial proximities between genotypes to be modeled. The most convenient way to do so is to define geographic neighbours according to a given, preferably objective criterion. This amounts to constructing a spatial graph on which neighbours are linked by an edge. The function chooseCN proposes several spatial graphs (try example(chooseCN) for an example) that can be chosen interactively. In the case of the Chamois, we can use the intersection of home ranges as a criterion for neighbourhood; this amounts to considering as neighbours pairs of individuals separated by less than 2300 m. Knowing that spatial coordinates of individuals are stored in rupica\$other\$xy, use chooseCN to build the corresponding spatial graph. Save the resulting object as rupica.graph; this object should look like this (displaying it may take a few seconds):

> rupica.graph

Neighbour list object: Number of regions: 335 Number of nonzero links: 18018 Percentage nonzero weights: 16.05525 Average number of links: 53.78507

```
> plot(rupica.graph, rupica$other$xy)
> title("rupica.graph")
```



From there, we can use the **spca** function. Note that it would also be possible to specify the parameters of the spatial graph as arguments of **spca**. > rupica.spca1 <- spca(rupica, cn = rupica.graph)



This figure illustrates the fundamental difference between PCA and sPCA. Like dudi.pca, spca displays a barplot of eigenvalues, but unlike in PCA, eigenvalues of sPCA can also be negative. This is because the criterion optimized by the analysis can have positive and negative values, corresponding respectively to positive and negative autocorrelation. Positive spatial autocorrelation correspond to greater genetic similarity between geographically closer individuals. Conversely, negative spatial autocorrelation corresponds to greater dissimilarity between neighbours. The spatial autocorrelation of a variable is measured by Moran's I, and interpreted as follows:

- * $I_0 = -1/(n-1)$: no spatial autocorrelation (x is randomly distributed across space)
- * $I > I_0$: positive spatial autocorrelation
- * $I < I_0$: negative spatial autocorrelation

Principal components of PCA ensure that (ϕ referring to one PC) $var(\phi)$ is maximum. By contrast, sPCA provides PC which decompose the quantity $var(\phi)I(\phi)$. In other words, PCA focuses on variability only, while sPCA is a compromise between variability ($var(\phi)$) and spatial structure ($I(\phi)$).

In this case, only the principal components associated with the two first positive eigenvalues (in red) shall be retained. The printing of **spca** objects is more explicit than **dudi** objects, but named with the same conventions: > rupica.spca1

```
class: spca
$call: spca(obj = rupica, cn = rupica.graph, scannf = FALSE, nfposi = 2,
   nfnega = 0)
$nfposi: 2 axis-components saved
$nfnega: 0 axis-components saved
Positive eigenvalues: 0.03018 0.01408 0.009211 0.006835 0.004529 ...
Negative eigenvalues: -0.008611 -0.006414 -0.004451 -0.003963 -0.003329 ...
  vector length mode
                     content
1 $eig
       45
             numeric eigenvalues
 data.frame nrow ncol content
1 $c1
                     principal axes: scaled vectors of alleles loadings
            55
                2
2 $1i
           335 2
                     principal components: coordinates of entities ('scores')
3 $1s
           335 2
                     lag vector of principal components
4 $as
            2
                2
                    pca axes onto spca axes
$xy: matrix of spatial coordinates
$lw: a list of spatial weights (class 'listw')
other elements: NULL
```

Unlike usual multivariate analyses, eigenvalues of sPCA are composite: they measure both the genetic diversity (variance) and the spatial structure (spatial autocorrelation measured by Moran's I). This decomposition can also be used to choose which principal component to interprete. The function screeplot allows to display this information graphically:

> screeplot(rupica.spca1)



Spatial and variance components of the eigenvalues

While λ_1 indicates with no doubt a structure, the second eigenvalue, λ_2 is less clearly distinct from the successive values. Thus, we shall keep in mind this uncertainty when interpreting the second principal component of the analysis.

Let us now visualise the identified spatial structures, as we did for the PCA results:

```
> showBauges()
> s.value(rupica$other$xy, rupica.spca1$li[, 1], add.p = TRUE,
+ csize = 0.7)
> title("sPCA - first PC", col.main = "yellow", line = -2, cex.main = 2)
```



While the pattern is clear enough, we can still clarify the results using lagged scores, which allow a better perception of positively autocorrelated structures (by denoisifying data):

```
> showBauges()
> s.value(rupica$other$xy, rupica.spca1$ls[, 1], add.p = TRUE,
+ csize = 0.7)
> title("sPCA - first lagged PC", col.main = "yellow", line = -2,
+ cex.main = 2)
```



How would you interpret this result? How does it compare to results obtained by PCA? What likely inference can we make about the way the landscape influences this population of Chamois?

The second structure remains to be interpreted; using the same graphical representation as for the first principal component, try and visualise the second principal component. Some field observation suggest that it is not artefactual. How would you interprete this second structure?

To finish, you can try representing both structures at the same time using the color coding introduced by [3] (?colorplot). The final figure should ressemble this (although colors may change from one computer to another):



3 Different pictures of biodiversity: African and French cattle breeds

The study of the genetic diversity for conservation purposes asks the question of which markers should be used for such studies. In the case of domestic cattle breeds, the FAO (http://www.fao.org/) recommended using a panel of 30 microstallites for conservation genetics studies. The dataset microbov provides the genotypes of 704 cattles structured in two species and 15 breeds for the 30 microsatellites recommended by the FAO.

One question of interest, which can be asked through this dataset, relates to whether all these markers provide the same information, and whether a smaller subset of markers could be used to achieve the same level of resolution.

3.1 An overview of the data - basic analyses

```
We first load the data:
> data(microbov)
> microbov
   ### Genind object ###
   genotypes of individuals -
S4 class: genind
@call: genind(tab = truenames(microbov)$tab, pop = truenames(microbov)$pop)
Otab: 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
Cloc.fac: locus factor for the 373 columns of Ctab
Call.names: list of 30 components yielding allele names for each locus
Cploidy: 2
@ploidy:
@type: codom
Optionnal contents:
Opop: factor giving the population of each individual
Opop.names: factor giving the population of each individual
Oother: a list containing: coun breed spe
```

microbov is a typical genind object, which is the class of objects storing genotypes in *adegenet*. It also contains extra information (in microbov\$other) relating to the origin (coun, Africa or France), the breed (breed), and the species (spe, *Bos taurus* or *Bos indicus*) of the individuals.

The function summary gives an overview of the data:

```
> microbov.smry <- summary(microbov)</pre>
```

Total number of genotypes: 704 # Population sample sizes: Zebu Lagunaire NDama Somba Borgou 50 50 51 30 50 Bazadais BlondeAquitaine Charolais BretPieNoire Aubrac 47 55 50 61 31 Gascon Limousin MaineAnjou Montbeliard Salers 50 50 30 49 50 # Number of alleles per locus: L01 L02 L03 L04 L05 L06 L07 L08 L09 L10 L11 L12 L13 L14 L15 L16 L17 L18 L19 L20 9 7 12 5 11 9 7 12 13 9 13 16 14 14 14 10 10 19 11 13 L21 L22 L23 L24 L25 L26 L27 L28 L29 L30 17 12 16 13 12 15 8 22 21 # Number of alleles per population: 01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 251 235 143 179 194 212 146 196 176 200 213 186 191 168 188 # Percentage of missing data: [1] 2.320076 # Observed heterozygosity: 1.02 L03 L04 L05 L06 L07 L08 0.5530086 0.5399129 0.6905444 0.4508076 0.6415094 0.5974212 0.2904624 0.5860534 L09 L10 L11 L12 L13 L14 L15 L16 0.6848306 0.5771429 0.6603221 0.7054598 0.5953079 0.7052023 0.7979943 0.6384505 L19 L22 L17 L18 L20 L21 L23 1.24 0.4534884 0.6396527 0.6474074 0.6285714 0.6603499 0.6569343 0.5941807 0.7381295 1.27 L25L26 1.29 I.30 L28 0.6762178 0.7722063 0.6174785 0.6891117 0.6810730 0.4392387 # Expected heterozygosity: 101 L02 L03 L04 L05 L06 L07 L08 $0.7075198 \ 0.6004379 \ 0.7807931 \ 0.5373943 \ 0.7899071 \ 0.7613320 \ 0.4945057 \ 0.6859640$ L09 L10 L11 L12 L13 L14 L15 L16 0.8336124 0.7678602 0.7747632 0.8217379 0.7471427 0.7597794 0.8924578 0.7546062 L17 L18 L19 L20 L21 L22 L23 L24 0.6336998 0.7746696 0.7489997 0.7805834 0.7682354 0.7719260 0.7693717 0.8365613 1.27 L25 L.26 1.28 1.29 L.30 0.7417581 0.8921047 0.6876811 0.7718615 0.8882143 0.5648676

This allows, for instance, to compare observed and expected heterozygosity at each locus:

> plot(microbov.smry\$Hexp, microbov.smry\$Hobs, main = "Observed vs expected heterozygosity"
> abline(0, 1, col = "red")



Observed vs expected heterozygosity

What can we tell about these populations? Is this result surprising?

To infer genetic differentiation using F_{ST} -based approaches, we have to check that populations are at Hardy-Weinberg equilibrium for each locus. Given that we have 15 breeds for 30 loci to analyse, we have to perform 15x30=450 tests (!). Fortunately, the function HWE.test.genind does this job, returning either a list of detailed tests, or a matrix of p-values. In our case, interpreting each test and correcting for multiple testing would quickly become cumbersome. Rather, we shall describe how p-values are distributed across populations and across markers. We perform Hardy-Weinberg tests, asking for a matrix of p-values:

```
> microbov.HWE <- HWE.test.genind(microbov, res = "matrix")
> hist(microbov.HWE, col = "pink", main = "Distribution of HWE test p-values",
+ nclass = 60)
> points(as.vector(microbov.HWE), rep(1, 450), col = "red", pch = "|")
```



Distribution of HWE test p-values

While a majority of tests do not indicate deviation from Hardy-Weinberg equilibrium, some exceptions seem to exist. Are these structured by populations?

```
> barplot(apply(microbov.HWE, 1, mean), col = "deepskyblue1", main = "Distribution of HWE to
+ ylab = "mean p-value", las = 3)
```



Distribution of HWE test mean p-values per population

Are these structured by markers?

```
> barplot(apply(microbov.HWE, 2, mean), col = "green2", main = "Distribution of HWE test mean
+ ylab = "mean p-value", las = 3)
```



What would you conclude? Toward the end of this exercise, we shall remember that INRA35 seems to be a particular marker.

Genetic differentiation can be tested for multiallelic data using Goudet's G test, implemented in *hierfstat*, and wrapped for genind objects by gstat.randtest. Basically, we can test the significance of the genetic differentiation between breeds, which is the default 'population' of the genotypes. For simplicity (and because it does not alter the results), all markers (including INRA35) are kept in this test:

```
> microbov.gtest1 <- gstat.randtest(microbov, nsim = 199)
> microbov.gtest1
Monte-Carlo test
Call: gstat.randtest(x = microbov, nsim = 199)
Observation: 23534.67
Based on 199 replicates
```

Simulated p-value: 0.005 Alternative hypothesis: greater Std.Obs Expectation Variance 121.0326 5450.5525 22324.9217

27

> plot(microbov.gtest1)



The histogram shows the distribution of the test statistic obtained by a Monte Carlo procedure (permutation of the group factor). The original value of the statistic (on the right) being hugely superior to these values, there is no doubt that the genetic structuring is very significant. However, we can wonder if this structuration among breeds persists after accounting for the species differences. This can be tested using the same function:



Is their a significant genetic differentiation between breeds once species differentiation has been partialled out?

3.2 A first glance: Principal Component Analysis

Now that we know that strong genetic structures exists among the considered breeds, we can try to get a picture of it. Principal Component Analysis (PCA [14, 10]) is well suited for a first glance at the data. PCA is implemented in the dudi.pca function of the *ade4* package. The analysis is performed on a table of standardised relative alleles frequencies, obtained by scaleGen (which also replaces missing values adequately):

```
> microbov.X <- scaleGen(microbov, method = "binom")
> microbov.pca1 <- dudi.pca(microbov.X, cent = FALSE, scale = FALSE)</pre>
```



The function dudi.pca displays a barplot of eigenvalues and asks for a number of retained principal components. Eigenvalues represent the amount of variability (as measured by the variance/pairwise Euclidean distances) contained in each principal component. An abrupt decrease in eigenvalues is likely to indicate the boundary between strong and less interpretable structures. In this case, the first three eigenvalues clearly indicate strong structures; the first three principal components are thus retained.

A dudi object contains various information; in the case of PCA, principal axes (loadings), principal components (synthetic variable), and eigenvalues are respectively stored in microbov.pca1\$c1, microbov.pca1\$li, and microbov.pca1\$eig. The function s.class can be used to display to two first principal components, while grouping genotypes by populations:

```
> par(bg = "lightgrey")
 palette <- rainbow(50)
>
 s.class(microbov.pca1$li, pop(microbov), col = 1:15, sub = "PCA - PC 1 and 2",
>
     csub = 2)
+
 add.scatter.eig(microbov.pca1$eig[1:60], 3, 1, 2, posi = "top")
>
```



- > par(bg = "lightgrey")
 > s.class(microbov.pca1\$li, xax = 1, yax = 3, pop(microbov), col = 1:15,
 + sub = "PCA PC 1 and 3", csub = 2)
 > add.scatter.eig(microbov.pca1\$eig[1:60], 3, 1, 3, posi = "top")



These figures display the 'best' picture of genetic variability among the genotypes achievable in three dimensions. How would you interpret the resulting structures?

This ordination focuses on the whole variability among individuals; population information is just added *a posteriori*. Between-class analyses [6] are a familly of multivariate method which find optimal PC in terms of between-group inertia (i.e., the centroids of the ellipses in the previous scatterplot). Here, between-class PCA can be used to assess the between-breed structure (as illustrated in [11]):

```
> microbov.bpca1 = between(microbov.pca1, pop(microbov), scannf = FALSE,
+ nf = 3)
```

```
> par(bg = "lightgrey")
> s.class(microbov.bpca1$ls, xax = 1, yax = 2, pop(microbov), col = 1:15,
+     sub = "Between PCA - PC 1 and 2", csub = 2)
> add.scatter.eig(microbov.bpca1$eig, 3, 1, 2, posi = "top")
```



Is there any improvement in terms of between-breed differentiation? The slot microbov.bpca1\$as contains the projection of PCA axes onto betweenclass principal axes (use s.corcircle to display it). How more informative is the between-class analysis in the present case? What does it mean in terms of apportionment of the genetic diversity among these cattle breeds?

Now that clear patterns have been identified, we can ask how each marker contributes to showing these structures. The contribution of each marker (measured as squared loadings) can be displayed using loadingplot:

> loadingplot(microbov.pca1\$c1^2, main = "Allele contributions to the PC1")



Allele contributions to the PC1

Variables

From this picture, could you tell if some markers play a more important role in the analysis than others? This was the contribution of alleles to the first principal component. Using the same function and the argument **axis**, try to obtain the same figure for the second and third principal components. Are the conclusions any different (if yes, how)?

3.3 A deeper look: Multiple Co-Inertia Analysis

PCA is not the most appropriate tool to compare the information provided by different markers about the populations (*i.e.*, breeds). Indeed, it only seeks principal axes of maximum genetic variability from all alleles, while a more appropriate approach would seek different principal components for each marker separately, and then compare them. The Multiple Co-Inertia Analysis (MCOA, [5, 13]) is especially devoted to this task. It performs separate analyses for each marker, and coordinates these analyses so as to highlight the common information they provide about populations. From these coordinated analyses, it builds a compromise, that is, a typology of populations reflecting the consensus information provided by the markers. Denoting $\mathbf{u}_k \in \mathbb{R}^n$ the principal component of the coordinated analysis of the kth $(k = 1, \ldots, K)$ microsatellite marker, and $\mathbf{v} \in \mathbb{R}^n$ the score of the compromise, MCOA ensures:

$$\sum_{k=1}^{K} w_k cov(\mathbf{u}_k, \mathbf{v})^2 = \sum_{k=1}^{K} w_k var(\mathbf{u}_k) var(\mathbf{v}) cor(\mathbf{u}_k, \mathbf{v})^2$$

is maximum (w_k is a weight for the kth marker). Therefore, MCOA achieves a consenus typology reflecting the genetic variability ($var(\mathbf{v})$) which is common ($cor(\mathbf{u}_k, \mathbf{v})^2$) to a majority of markers ($var(\mathbf{u}_k)$). It also provides a direct measure of the contribution of each marker to this consensus information, as measured by:

$$\frac{w_k cov(\mathbf{u}_k,\mathbf{v})^2}{\lambda}$$

where λ is the eigenvalue corresponding to **v**. This quantity is genuinely the proportion of a given structure exhibited by a single marker.

First of all, given that within-breed variability seems negligible compared to between-breed variability, we reduce data to counts of alleles per populations (losing the distinction between individuals). Objects storing population data in *adegenet* are genpop objects. This transformation is achieved by genind2genpop:

```
> bov <- genind2genpop(microbov)</pre>
 Converting data from a genind to a genpop object...
...done.
> bov
       - Alleles counts for populations -
S4 class: genpop
@call: genind2genpop(x = microbov)
Otab: 15 x 373 matrix of alleles counts
@pop.names: vector of 15 population names
@loc.names: vector of 30 locus names
Cloc.nall: number of alleles per locus
Cloc.fac: locus factor for the 373 columns of Ctab
Call.names: list of 30 components yielding allele names for each locus
Call.or 2
@ploidy:
@type: codom
Oother: a list containing: coun breed spe
```

Data are then separated by marker using **seploc**, and only tables of allele counts are retained for further analysis:

```
> lbov <- seploc(bov)</pre>
> 1X <- lapply(lbov, truenames)
> class(1X)
[1] "list"
> names(1X)
                             "ETH225"
                                                    "HEL5"
                                                                            "INRA35"
 [1] "INRA63"
                 "INRA5"
                                         "ILSTS5"
                                                                "HEL1"
                                                                            "ETH3"
     "ETH152"
                 "INRA23"
                             "ETH10"
                                         "HEL9"
                                                    "CSSM66"
                                                                "INRA32"
 [8]
                                         "INRA37"
                                                                            "MM12"
[15]
     "BM2113"
                 "BM1824"
                             "HEL13"
                                                    "BM1818"
                                                                "ILSTS6"
     "CSRM60"
                 "ETH185"
                                         "HAUT27"
                                                    "TGLA227" "TGLA126" "TGLA122"
[22]
                             "HAUT24"
     "TGLA53"
                 "SPS115"
[29]
> 1X$INRA63
                   INRA63.167 INRA63.171 INRA63.173 INRA63.175 INRA63.177
                                          0
                                                      0
                                                                               27
Borgou
                             0
                                                                   4
                             0
                                                      Ō
                                                                    7
                                                                               16
Zebu
                                          1
                                                      0
                                          0
                                                                               44
Lagunaire
                             1
                                                                  16
                                                                  2
12
NDama
                             0
                                          0
                                                      0
                                                                               39
                             0
                                          0
                                                      0
                                                                               42
Somba
                             0
                                          0
                                                      0
                                                                  80
                                                                                0
Aubrac
                                                                               28
52
                             Ō
                                          0
                                                      Ō
                                                                  54
Bazadais
                             0
                                                      0
                                          0
                                                                  54
BlondeAquitaine
BretPieNoire
                             0
                                          0
                                                       1
                                                                  39
                                                                               18
                                                      5
0
                             0
                                          0
                                                                  46
                                                                               37
Charolais
                             0
                                          0
                                                                  77
                                                                                1
Gascon
                             0
                                          0
                                                      1
                                                                  45
                                                                               52
Limousin
                                                      0
                             0
                                                                  46
                                          1
                                                                               48
MaineAnjou
Montbeliard
                             0
                                          0
                                                      0
                                                                  25
                                                                               25
Salers
                             0
                                          0
                                                       0
                                                                  70
                                                                                0
                               INRA63.181
                                            INRA63.183
                   INRA63.179
                                                         INRA63.185
Borgou
                                                     60
                             1
                                          7
                                                                   1
                                                     47
                             4
                                         19
                                                                   6
Zebu
                                                                  23
Lagunaire
                             0
                                          2
                                                     16
NDama
                             5
                                         11
                                                      3
                                                                   0
Somba
                             3
                                          8
                                                     34
                                                                   1
                            20
                                          0
                                                      0
                                                                   0
Aubrac
                                          0
                                                     10
                                                                   0
Bazadais
                             0
7
2
                                                      7
2
BlondeAquitaine
                                          0
                                                                   0
BretPieNoire
                                          0
                                                                   0
                                                                   0
3
Charolais
                            15
                                          0
                                                      1
                                          0
                                                      8
Gascon
                            11
                                                                   Ō
                             2
1
                                          0
                                                      0
Limousin
                                                      0
MaineAnjou
                                          0
                                                                   0
Montbeliard
                             4
                                          0
                                                       6
                                                                   0
                            25
                                          0
                                                       5
Salers
                                                                   0
```

kbov contains counts of alleles per population separately for each marker. After turning these into allele frequencies, each table is analysed by a PCA. The method is applied to all 30 tables in a single command using lapply:

```
> 1X <- lapply(1X, prop.table, 1)
> 1PCA <- lapply(1X, dudi.pca, center = TRUE, scale = FALSE, scannf = FALSE,
+ nf = 3)
> class(1PCA)
```

[1] "list"

> names(1PCA)

```
[1] "INRA63"
                "INRA5"
                          "ETH225"
                                     "ILSTS5"
                                               "HEL5"
                                                          "HEL1"
                                                                    "INRA35"
                                                                    "ETH3"
 [8] "ETH152"
               "INRA23"
                          "ETH10"
                                     "HEL9"
                                               "CSSM66"
                                                         "INRA32"
[15] "BM2113"
[22] "CSRM60"
                "BM1824"
                          "HEL13"
                                     "INRA37"
                                               "BM1818" "ILSTS6"
                                                                   "MM12"
               "ETH185"
                                    "HAUT27"
                                               "TGLA227" "TGLA126" "TGLA122"
                          "HAUT24"
[29] "TGLA53"
               "SPS115"
> 1PCA$INRA63
Duality diagramm
class: pca dudi
$call: FUN(df = X[[1L]], center = TRUE, scale = FALSE, scannf = FALSE,
    nf = 3)
$nf: 3 axis-components saved
$rank: 8
eigen values: 0.09829 0.03924 0.005741 0.003492 0.001314 ...
  vector length mode
                       content
        9
1 $cw
               numeric column weights
2 $1w
         15
                numeric row weights
3 $eig
        8
                numeric eigen values
  data.frame nrow ncol content
1 $tab
                        modified array
             15
                  9
2 $1i
             15
                   3
                        row coordinates
3 $11
             15
                   3
                        row normed scores
4 $co
                   3
             9
                        column coordinates
5 $c1
             9
                   3
                        column normed scores
other elements: cent norm
```

To visualise the results of a given analysis (here, INRA63), one can use:

```
> s.label(lPCA$INRA63$li)
```

> add.scatter.eig(lPCA\$INRA63\$eig, 3, 1, 2)



Now, using a **for** loop (or a **lapply**, or less elegantly several copy-paste operations), try and display results of other markers. Can you compare the information they provide? Note that the situation is complicated by the fact that the first principal component of one marker might ressemble best the third of another marker, or even a mixture of several components.

Let us try coordinating these analyses using MCOA. The method is implemented as the function mcoa in the *ade4* package. It requires data to be stored as a ktab object, which we obtain by:

> bov.ktab <- ktab.list.dudi(1PCA)</pre>

> bov.mcoa1 <- mcoa(bov.ktab)</pre>



Proceed like in previous analyses to select the number of retained principal components.

```
> bov.mcoa1
Multiple Co-inertia Analysis
list of class mcoa
$pseudoeig: 15 pseudo eigen values
11.4 3.467 2.274 0.8631 0.4978 ...
$call: mcoa(X = bov.ktab, scannf = FALSE, nf = 3)
$nf: 3 axis saved
   data.frame nrow ncol content
   $SynVar
               15
                     3
                          synthetic scores
1
23
               373
   $axis
                     3
                           co-inertia axis
                     33332223
   $Tli
               450
                          co-inertia coordinates
4
   $T11
               450
                           co-inertia normed scores
5
   $Tax
               120
                           inertia axes onto co-inertia axis
6
   $Tco
               373
                           columns onto synthetic scores
7
   $TL
               450
                           factors for Tli Tl1
8
   $TC
               373
                           factors for Tco
9
   $T4
               120
                          factors for Tax
10 $lambda
               30
                           eigen values (separate analysis)
11 $cov2
               30
                     3
                          pseudo eigen values (synthetic analysis)
other elements: NULL
```

The content of a mcoa object is a bit more complicated than that of PCA (dudi object), but only bits are useful here. bov.mcoa1\$Tli contains principal components of coordinated analyses for the different markers, while

bov.mcoal\$SynVar contains the compromise, *i.e.* the typology of populations emerging as a consensus among the markers. bov.mcoal\$cov2 gives the contribution of each marker to each structure of the compromise, and can be used to assess discrepancies in the information yielded by the different loci.

Coordinated analyses can be displayed like separated analyses:

		•]	4 05
	u = 0.3		u = 0.5
NDama.1		MaineAnjou	.2
Ma	aineAnjou.1	Lagunaire.2	in.2
Lagunaire.1 Blonde	Aquitaine.1	NDama 2] BlondeAq	Charolais 2
			Aubras
Somba.1	Bazauais.		BretPieNoire
			Salers.2
Borgen 4	Salers.1	Zebu.2)u.2	Montbeliard.2
Zebu.1			
INRA63		IINRA5	
	d = 0.8		d = 0.5
	d = 0.5	Bazadais.4	d = 0.5
	d = 0.5	Bazadais.4	d = 0.5
[NDama.3]	d = 0.5	Bazadais.4	d = 0.5
[NDama.3]	Lagunaire.3	Bazadais.4	d = 0.5
[NDama.3]	Lagunaire.3	Bazadais.4	d = 0.5
NDama.3	d = 0.5	Bazadais.4 [Nt Montbeliard.4] [Somba.4] [Lagunaire_4] [Ga	d = 0.5
[NDama.3]	d = 0.5	Bazadais.4 [Bazadais.4] [Nt Montbeliard.4] [Somba.4] [Lagunaire_4] Ga	d = 0.5 scon.4
NDama.3	d = 0.5	Bazadais.4 [Bazadais.4] [N[Montbeliard.4] [Somba.4] [Lagunaire.4] Ga	d = 0.5 scon.4 Bid Charolais.4 }.4 Brownshairs 1
NDama.3	d = 0.5	Bazadais.4 [Nt Montbeliard.4] [Somba.4] [Lagunaire_4] Ga Borgou.4]	d = 0.5 scon.4 Bld Charolais.4 }.4 M Salers.4).4 Emoustrie
[Zebu 3]	d = 0.5	Bazadais.4 [Nt Montbeliard.4] [Somba.4] [Lagunaire 4] Ga Borgou.4]	d = 0.5 scon.4 Bid Charolais.4 }.4 Brat Dia Marian 1 M Salers.4 J.4 Linnoustrie
NDama.3] [Zebu.3]	d = 0.5	Bazadais.4 [Bazadais.4] [N[Montbeliard.4] [Somba.4] [Lagunaire.4] Ga Borgou.4]	d = 0.5 scon.4 Bid Charolais.4 2.4 BretPicAlaise 1 M Salers.4 1.4 Limnoustrie
NDama.3 Zebu.3 Borgou.3	d = 0.5	Bazadais.4 [Nt Montbeliard.4] [Somba.4] [Lagunaire_4] Ga Borgou.4]	d = 0.5 scon.4 Bld Charolais.4 }.4 Brot Dicable income M Salers.4 }.4 Emnoushine
[NDama.3] [Zebu.3] [Borgou.3]	d = 0.5	Bazadais.4 [Nt Montbeliard.4] [Lagunaire 4] [Lagunaire 4] [Ga Borgou.4]	d = 0.5 scon.4 Bid Charolais.4 }.4 Brathic Maine 4 M Salers.4].4 Lamoushire

Use the commands above to plot results of different markers, making sure to visualise the plan of the first and third principal components as well. How does it compare to the results obtained with previous (uncoordinated) analyses?

The compromise between all these analyses is very similar to the usual PCA of all data:

> s.label(bov.mcoa1\$SynVar)

> add.scatter.eig(bov.mcoal\$pseudoeig, 3, 1, 2)

					d = 1
	Lagunaire]			
	NDama Somba		Montbeliard	.e	
			Charolais MaineAniou	ר]	
			Biorig Gascon	ne	
			Aubra	ac Iers	
	Borgou				
Eigenva	alues				
I	Zeb	1			

> s.label(bov.mcoal\$SynVar, xax = 1, yax = 3)
> add.scatter.eig(bov.mcoal\$pseudoeig, 3, 1, 3)



However, we now gained further information about how markers contribute to this figure. Try and represent graphically the marker contributions stored in bov.mcoal\$cov2 for the three structures of the compromise. First, observe that:

```
> sum(bov.mcoa1$cov2[, 1])
```

[1] 11.40258

```
> bov.mcoal$pseudoeig[1]
```

[1] 11.40258

Then, plot the contribution of each marker to each of the three scores of the compromise; an example of result for the first structure would be:



Contribution to the Africa/France differentiation

What can we say about the general consistency of these markers? Are there redundant markers? Are there 'outlying' markers? Would it be possible to achieve the same structuring without using the full panel of 30 microsatellites recommended by the FAO?

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