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Linking genomics and population genetics with R

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Abstract

Population genetics and genomics have developed and been treated as independent fields
3 of study despite having common roots. The continuous progress of sequencing
technologies is contributing to (re-)connect these two disciplines. We review the
challenges faced by data analysts and software developers when handling very big genetic
6 data sets collected on many individuals. We then expose how R, as a computing
language and development environment, proposes some solutions to meet these
challenges. We focus on some specific issues that are often encountered in practice:
9 handling and analysing SNP data, handling and reading VCF files, analysing haplotypes
and linkage disequilibrium, and performing multivariate analyses. We illustrate these
implementations with some analyses of three recently published data sets that contain
12 between 60,000 and 1,000,000 loci. We conclude with some perspectives on future
developments of R software for population genomics.

Keywords: multivariate analysis, NGS, R, SNP, VCF

15 **Introduction**

An exploration into the history of genetics reveals a complex pattern of interconnected concepts, hypotheses, and research programs. A remarkable historical fact is that
18 population geneticists laid the foundations of their discipline several decades before the physical support of heredity was known. Avery *et al.* (1944) demonstrated that DNA was the molecule coding for genes, a fact that was not immediately accepted by their
21 contemporary geneticists for several reasons, perhaps the main one being that, at this time, it was widely accepted that genes most likely consisted of proteins (Lederberg 1994; Deichmann 2004). Population genetics was a mature field at the time of Avery *et al.*'s finding as witnessed by the long-dated publication of seminal books on the subject
24 (Fisher 1930, e.g.). Thus, population and molecular genetics have had separate histories at some time and this certainly had an impact on how empirical studies were conducted or motivated. During almost half a century, population genetic studies were based on genotyping individuals on a limited number of loci, especially in conservation-oriented research (Ekblom & Wolf 2014). A crucial preliminary of these studies was to find the
30 'right genetic marker for the study' (Sunnucks 2000).

At the end of the century, genomic data from human populations started to accumulate making possible to address new questions in the emerging field of population
33 genomics, even though at this time we were "a long way from knowing all the SNPs, even in any given population" mainly because "exhaustive typing is currently prohibitive" (Goldstein & Weale 2001). Nevertheless, it appeared clearly that this wealth
36 of human genetic data could not be interpreted without traditional population genetic concepts such as linkage disequilibrium, genetic drift, or coalescent (Jorde *et al.* 2001). Another result that came out at this time was the peculiarity, from an evolutionary
39 point of view, of human populations compared to other species: low overall genetic diversity, considerable continental-level homogeneity, and small effective population size (Jorde *et al.* 2001). These features associated with the relatively simple biology of
42 humans (lack of polyploidy, long lifespan) and the considerable interest in medically oriented applications of genomic research resulted in a lot of developments for handling and analysing population genomic data. However, these tools did not appear flexible

45 enough to be applied to organisms with more complex biologies.

In the early twenty-first century, next generation sequencing technologies (NGS) have made possible to access the genotype of an individual at a very large number of loci and
48 even its complete genome sequence (Luikart *et al.* 2003). Even though there are still few species with their genome sequenced, several approaches have been proposed to apply NGS to any living species (Ellegren 2014). Furthermore, the decreasing costs of NGS
51 make increasingly easy to generate data on a large number of loci and to apply these technologies in studies on natural populations (Narum *et al.* 2013; Andrews & Luikart 2014). This is a radical change in point of view for population geneticists compared to
54 almost a century of practice of their discipline. In his review, Sunnucks (2000) listed three components in population genetics studies: genetic markers, statistical methods, and computer programs. We believe that the NGS revolution has solved, at least in
57 principle, the problem of genetic markers. The availability of vast amount of data clearly points to new developments in software. Furthermore, the perspective of merging population and molecular genetics calls for a unified approach to software development.

60 In this paper, we review the current state of progress in the analysis of population genomic data using R. We illustrate some recent developments with three recently published data sets: the human Y chromosome with 62,042 loci for 1233 individuals
63 (The 1000 Genomes Project Consortium 2015), a set of 1,055,818 phased genotypes for 121 fruit flies (*Drosophila melanogaster*, Kao *et al.* 2015ab), and a set of 61,951 SNPs for 113 dolphins (*Lagenorhynchus* spp., Fernández *et al.* 2016ab). In the next section, we
66 summarize the challenges ahead and explain how R can help meeting them. The following two sections explore some specific issues related to handling and analyses of large data sets in the context of population genomics, and how the R packages presented
69 in this paper integrate with other genomic bioinformatics software. We argue that R solves the issue of software in population genetics in the current context of NGS and in the perspective of a constant flux of genomic data. In the last section, we discuss the
72 future developments of statistical methods which will, hopefully, complete the merging of population and molecular genetics.

Challenges

75 NGS contrast sharply with their predecessor (the Sanger method) in two important
aspects: they generate far larger quantities of data, which is clearly a source of practical
difficulties for data storage and analysis (Stephens *et al.* 2015), and they evolve much
78 more quickly. The tremendous potential applications of NGS have stimulated a lot of
research and development (Erlich 2015). A new technology or platform can be created
and made available rapidly, but tests and validation for reproducibility take time.
81 Meanwhile, competing laboratories or companies may initiate another cycle of testing,
assessment, and validation for a new method. Furthermore, the commercialisation of a
technology and the training of laboratory staff to use this technology on an appropriate
84 scale also take time. All these time-lags result in a form of inertia in the deployment of
NGS in research laboratories. A now famous example is provided by the 454 platform
which was discontinued in 2013, just six years after being acquired by a large
87 company—some emerging platforms will probably have a shorter lifespan. It appears
that we are now in a situation where we can start sampling in the field without knowing
exactly what genotyping or sequencing method will be used in the laboratory.

90 Nowadays, it is possible to genotype a single individual at thousands or even millions
of loci, and population genomic studies typically do this for a few individuals (n), at least
far less than the number of loci that are analysed (p). This ‘small n , large p ’ problem
93 raises some difficulties, especially in statistical analyses (see section below on multivariate
methods). This situation contrasts with traditional population genetic studies which
generally used sampling protocols with $n > p$. With complete genome sequencing the
96 value of p is theoretically bounded by the size of the genome; however, the number of
variants (the sites in the genome which are variable among individuals in the sample)
depends on the sampling protocol. For instance, the 1000 Genomes Project found almost
99 38,000,000 variants from 1092 individuals sampled from 14 populations (The 1000
Genomes Project Consortium 2012); this number increased to more than 88,000,000
when 2504 individuals were sampled from 26 populations (The 1000 Genomes Project
102 Consortium 2015). With non-model organisms, we certainly have to expect even more
variation since most species are distributed among several subspecies over their range.

The fast pace of NGS development is usually accompanied with a rapid development
105 of bioinformatics tools often resulting in the appearance of a wide variety of data
formats. A problem is that very different technologies are used by the different NGS
platforms, so that raw data and their assembly differ a lot even though the final goal is
108 the same. For instance, though the FASTQ file format has been adopted as the *de facto*
standard for storing raw DNA reads, the emerging NGS platforms in the late 2000's used
subtle differences in their respective FASTQ files making the development of common
111 bioinformatics tools problematic (see: https://en.wikipedia.org/wiki/FASTQ_format
for a review of the variation in FASTQ formats). Another difficulty comes from the fact
that population genetics software have traditionally been developed independently of
114 each other and often created their own data file formats, resulting in burdensome
conversions necessary along bioinformatics workflows (see Lischer & Excoffier 2012, for a
tool to work around this problem). Besides, because of the conceptual separation
117 between population and molecular genetics, most software for population genetics has
been developed with classical genetic markers and cannot handle large genomic data sets
simply because of memory limitations.

120 *Why R*

The quest for standardization is a common and recurrent issue in software development,
and applications for data analysis are no exception to this. The 1990's were a key period
123 in this respect: efficient hardware (e.g., 32-bit processors) were widely available, Internet
became common, and a new generation of software started to spread. R was developed
in this exciting context (Ihaka & Gentleman 1996), and started to be progressively
126 adopted by a wide community of scientists for analysing their data, but also for
developing new applications (Vance 2009). Several reasons can explain the success of R:
we can cite a few that are relevant for population genetics. R is available for all common
129 platforms and operating systems. Its fast graphical capabilities make it ideal for
exploratory analyses. Some generic features of R are extremely useful when
manipulating data, such as factors or data structures indexed with names. R is modular
132 so that building new packages or pipelines, or conducting simple analyses is
straightforward. R can be easily interfaced with code written in C, C++, and some

other languages, so that computations which are not efficient in R can be recoded in
135 these languages without losing the advantages of the R environment. R has a standard
documentation system which makes very easy to find the relevant information when
using a new package or a new function (of course, providing the package developers have
138 written the documentation appropriately). R has been widely adopted in statistics
courses in many countries so that most students have a fair knowledge of this language.
Finally, and not the least, several user interfaces (Emacs+ESS, JGR, R-commander,
141 RKWard, RStudio, Tinn-R, among others) are available which considerably ease the
work flow while accomodating each researcher's personal taste.

Naturally, all these features (and others) apply to the analysis of population genetic
144 data which has led to the progressive development of a software environment that is
attractive to a wide community of population geneticists. Two remarkable features of
this software are worth mentioning here. First, these R packages consider all kinds of
147 genetic markers: DNA sequences, SNP, micro-satellites, allozymes. Second, all situations
can be handled: genotypes with single or multiple loci, locus with more than two alleles,
all levels of ploidy including cases where ploidy level varies within the same data set,
150 phased and unphased genotypes (Table 1). By contrast to some applications which have
been developed specifically for diploid organisms with simple life histories and relatively
low polymorphism, R packages are developed to meet the needs of a wide community of
153 molecular ecologists. This underlines the philosophy of R to be inclusive in its
developments: from the simplest to the most complex or challenging situations.

Data and files

156 R has a wide range of tools to handle and analyse DNA sequences (mostly in the
package *ape*; Paradis *et al.* 2004), and traditional allelic data (mostly in the package
adegenet; Jombart 2008). In this section, we detail how some other kinds of data that are
159 more specific to NGS can be handled with R.

SNP Data

Molecular genetic studies have revealed that variation in genetic materials can take many forms. In humans, among 88,332,015 genetic variants identified in a sample of 2504 individuals, 95.53% were biallelic single nucleotide polymorphism (SNP), 4.07% insertions–deletions (indels), 0.33% multiallelic SNPs, and 0.07% structural variants (The 1000 Genomes Project Consortium 2015). Thus, biallelic SNPs are by far the most common form of genetic variation in natural populations (see also the examples below). Among the technologies developed to acquire this kind of variants, two are most frequently used: sequencing and genotyping arrays, the second one being able to acquire only biallelic SNPs (which are often called ‘SNPs’ in short). The approach based on genotyping SNPs can be seen as an intermediate stage between the use of traditional population genetic markers and the more modern genomic sequencing-based approaches which consider the whole set of genetic variation in populations.

Typically, SNP data files store genotypes of individuals at many loci in a matrix form with additional information for each locus such as its position along the chromosome. A difficulty with these files is that there is no widely accepted standard format. For instance, a genotype can be coded in the usual form (e.g., A/A, A/T, or T/T), or as the number of minor alleles in the genotype (0, 1, or 2, if T is the minor allele). However, it appears that the VCF format has recently been adopted as a standard for all kinds of SNP data files (see the example with the dolphin data).

The package `adegenet` provides several tools to read and handle SNP data (Jombart & Ahmed 2011). The core of these tools is the class `"genlight"` designed to store SNP data in a compact way: for example, such an object with one million SNPs for 100 individuals uses about 16 Mb of RAM (or active memory) which is less than 1% of the available RAM on most modern computers. Besides, `adegenet` has several functions specially designed for the analysis of `"genlight"` data, such as `glPca` which performs a principal components analysis (PCA; see below), `glPlot` which plots a set of SNPs, or `glSim` which simulates efficiently SNP data. The function `read.PLINK` in the same package can read some SNP files. More generally, SNPs are stored in plain-text files in tabular form, so standard R functions (e.g., `read.table`) can be used to parse such files.

VCF files

Over the last few years, the Variant Call Format (VCF) has appeared as a standard for
192 storing population genetic data from NGS sequencing or genotyping (Danecek *et al.*
2011). By contrast to SNP data files, VCF files can contain data on all types of genetic
variation. VCF files are text files storing a lot of information, including on the process
195 used to create it from the raw sequencing reads (variant calling). The genetic data are
stored in a matrix-like layout where the individuals are the columns and the loci are the
rows.

198 Several R packages have tools to analyse VCF files: two of them, `stacksr` and `vcfR`, are
described in this issue. Bioconductor has the package `VariantAnnotation` (Obenchain *et al.*
2014) for annotating VCF files. We focus here on the functions in `pegas` because this
201 package is dedicated to population genetics (Paradis 2010). `vcfR` provides functions to
assess the quality of the VCF file (Knaus & Grünwald 2017) together with functions for
data conversion (Table 2).

204 VCF files can be very big, so a strategy is to first scan the files before reading more
detailed information such as the genotypes. `pegas` follows this strategy by providing
several functions: the main ones are `VCFloci` and `read.vcf`. The first function scans a
207 VCF file and extracts the information on the loci; the second one reads the individual
genotypes with the possibility to select a subset. `VCFloci` uses a specific mechanism to
read big files: the file is scanned sequentially by chunks of one billion bytes (1 Gb; the
210 chunk size can be modified by the user). The function then stores a small table with the
name of the file, the size of each chunk adjusted to meet the nearest end of a line, and
the number of loci in each chunk. With this mechanism, not more than 1 Gb of RAM is
213 used at the same time whatever the size of the file. The running time increases roughly
linearly with the file size: it takes about 20 sec to scan an 8 Gb file (compressed or not)
with about 500,000 loci. For smaller files, the running time depends on whether the file
216 is compressed or not: for a 200 Mb file with 62,000 loci, this takes 1 sec or 0.2 sec,
respectively.

The output of `VCFloci` is a data frame (R's usual data table) with nine columns
219 where each row is a locus and the columns are the information for each locus from the

VCF file. The information correspond to the mandatory VCF header fields (see Danecek *et al.* 2011): chromosome (CHROM), position (POS), unique identifier (ID), reference
222 and alternative alleles (REF and ALT), phred-scaled quality score (QUAL), site-filtering
information (FILTER), a semicolon-separated list of additional annotation metadata
(INFO) and the FORMAT field containing genotype data information (often a
225 semicolon-separated list). The data frame can be manipulated to extract information
with standard R functions (Box 1). The function `VCFheader` prints the header of a VCF
file which, usually, contains details on these different fields.

228 The genotypes are then read by `read.vcf`. At this stage, it is good to think if it is
interesting or feasible to read a complete VCF file. `pegas` takes roughly four bytes of
RAM to store a single genotype; so (using the above notation), about $4np$ bytes are
231 needed for a given genetic data set. With the rule of thumb that data in R should not
use more than 25% of the available RAM to perform “comfortable” analyses, one million
loci for 1000 individuals appears as a reasonable upper limit on a computer with 16 Gb
234 of RAM. Furthermore, the user may be interested in only some loci depending on the
type of genetic variant, position on the chromosome, or else. A strategy is to locate these
loci by finding the rows of the output of `VCFloci` using standard R data manipulation
237 (Box 1). Similar operations can be done on the field "CHROM" (if loci from several
chromosomes are in the same file), "QUAL", or "FILTER". The field "INFO" requires a
special attention because it includes detailed information on each locus. To make this
240 information easier to digest, `pegas` provides the function `getINFO` to extract a specific
information from this character string; by default this is the sequencing depth (DP), but
the option `what` makes possible to extract another information, for instance, the variant
243 type (Box 2).

For small VCF files, `read.vcf` can be used directly without calling `VCFloci` first, but
the latter is still needed to get the information on the loci.

Haplotypes and Linkage Disequilibrium

The possibility to investigate the genetics of populations at many loci of individuals
249 brings the perspective to study the dynamics of genomes in populations. An exciting
opportunity is to identify parts of species genomes which evolve as a single unit
(Andrews & Luikart 2014). Several approaches with NGS allow one to infer genotype
252 phasing for diploid or polyploid organisms, either directly by sequencing long fragments
of DNA (e.g., McCoy *et al.* 2014), or with high coverage sequencing of shorter fragments
(Nielsen *et al.* 2011). With phased genotypes, it is straightforward to reconstruct the
255 haplotypes of each individual. `pegas` has the generic function `haplotype` which does this
operation (it also works with DNA sequences). Linkage disequilibrium (LD) is the most
widely used statistical method to identify alleles at different loci that are statistically
258 associated. It is also of central importance in association studies of genetic diseases
(Goldstein & Weale 2001; Jorde *et al.* 2001). The function `LDscan` permits to analyse LD
by calculating pairwise correlation coefficients (r^2) for a series of biallelic loci. The
261 output of this function can be plotted with `LDmap`; this function has an option `POS` to plot
the correlation coefficients together with the positions of the loci on the chromosome.
Figure 1 shows the LD at two scales for the chromosome 2L of the fruit fly data: for the
264 first 100 loci and for 100 loci equally spaced on the chromosome (Box 3). Two other
functions analyse LD for a pair of loci with any number of alleles: `LD` and `LD2` for phased
and unphased genotypes, respectively. These two functions return detailed statistics and
267 tests (Schaid 2004; Zaykin *et al.* 2008).

Multivariate Methods

Multivariate methods are common when analysing big data sets because of their abilities
270 to provide dimension reduction, that is to provide a summary of many variables into a
few, usually linear, combinations of them. Thus, population genetics has used these
methods for some time (Westfall & Conkle 1992; Guinand 1996; Moazami-Goudarzi &
273 Laloë 2002). The package `adegenet` implements a lot of tools for the multivariate analysis
of genetic data (Jombart *et al.* 2009). Some of the methods of particular interest here

include the spatial principal components analysis (sPCA; Jombart *et al.* 2008) and the
276 discriminant analysis of principal components (DAPC; Jombart *et al.* 2010) which is
illustrated below.

In spite of their efficiency with large tables, multivariate methods are penalized when
279 analysing very large data sets, for instance, when n and p are larger than 10,000. Some
approaches have been developed to decompose very large matrices without using
traditional methods such as eigen or singular value decomposition (Halko *et al.* 2011).
282 These approaches are based on random matrices and aim to approximate the first few
principal components. Abraham & Inouye (2014) implemented this approach to perform
a PCA on genomic microarray data collected on a large sample of individuals ($n >$
285 10,000). Their implementation is available as the R package `flashpca` distributed on
GitHub (<https://github.com/gabraham/flashpca>). This approach is quite
straightforward to code in R and is available in several forms with more or less accuracy
288 (Halko *et al.* 2011).

Discriminant analysis (DA) is a multivariate method which searches for linear
combinations of variables resulting in the strongest discrimination of groups identified
291 from the data (Fisher 1936). Under some assumptions, it is possible to quantify the
reliability of the inferred assignments. Thus, DA offers a powerful approach to assess the
presence of structure in genetic data with many loci. However, because it is based on
294 matrix decomposition algorithms, it suffers from the same limitations than PCA with
very big data sets, and these methods need to be adapted to be used on genomic data.
The DAPC gives a solution to this problem: it can be used to assess group structure in
297 genetic diversity with large datasets, and is also implemented separately for "`genlight`"
objects (Jombart & Ahmed 2011). The main idea behind the DAPC is to first perform a
data reduction using a PCA. Typically, less than 100 principal axes are sufficient. If
300 group priors are missing, a sequential k-means clustering with model selection based on
the Bayesian information criterion (BIC) is done to infer prior clusters. The next step is
to perform a discriminant analysis in the reduced data space using the prior clusters
303 returned by the k-means clustering (or the cluster definition provided by the user if
available). The results can be graphically visualized in two ways: by plotting the
projections of the individuals on discriminant axes (as usual in multivariate analyses), or

306 by examining the relative posterior probabilities to the different clusters inferred from
the discriminant analysis (as usual in stochastic assignment methods).

We consider here the dolphin data and try to show evidence for geographical structure
309 (Fernández *et al.* 2016a). The data are provided as a VCF file (simply named ‘vcf’)
available from Dryad (Fernández *et al.* 2016b). Because the multivariate methods are
implemented in *adegenet*, we have to convert the data read by *pegas* into the appropriate
312 class (Box 4). Remarkably, none of these analyses take significant running time (in fact,
the longest operation is to convert from the class "loci" to the class "genind").

Population Genetics and Genomic Bioinformatics

315 The main strength of R, and maybe the most attractive one for data analysis, is the
possibility to integrate a great variety of methods and tools while using the same
programming language. In the past few years, the analysis of genetic data has changed
318 quite radically. Until the early twenty-first century, population geneticists used to get
their data from the laboratory on their physical support (e.g., electrophoresis gels), then
input the data in the computer, and analyse them with specialised software. With the
321 advent of NGS, genetic data are directly acquired on the computer, but an additional
step is required: the identification of alleles from the many (hundreds of millions)
sequencing reads. This step is called ‘variant calling’ and requires highly specialised
324 software. Because NGS technologies are evolving very fast (see above), this software is
also evolving very fast. Fortunately, the past few years have seen the development of
more integrated bioinformatics tools to accomplish such tasks. The R package *poRe* has
327 been recently released to analyse data from the the mobile nanopore sequencer MinION
(Watson *et al.* 2015). This package takes profit of R’s tools such as scanning a directory
with many files, searching for patterns in file names, and reading files in a specific format
330 (here HDF5), to extract and plot summary statistics from the sequencing runs, as well as
export data into FASTQ or FASTA format.

Bioconductor is a long-term effort to produce R packages for the analysis of
333 large-scale genomic data sets (Gentleman *et al.* 2005; Huber *et al.* 2015). Bioconductor’s
website (www.bioconductor.org) hosts a suite of integrated R packages mostly
dedicated to the analysis of expression data. Several packages have been released for the

336 analysis of DNA microarray data (e.g., Dunning *et al.* 2007; Ritchie *et al.* 2009; Morgan
2015). The package `ape` has functions to convert DNA data from Bioconductor classes
into the "DNAbin" class from which population and evolutionary analyses can be
339 performed.

A crucial aspect of R packages which permits the integration of different tasks is the
stability of the packages themselves and especially of the data classes defined therein
342 (Tables 1, 3). Several R packages for evolutionary genetic analyses are now more than
one decade-old and have been widely used and tested, so that their contents are now
reliable for a wide range of applications, and package developers have the possibility to
345 work with them to develop new tools and methods (e.g., Paradis 2012; Lawrence &
Morgan 2014).

Perspectives

348 Remarkable progress has been accomplished in the development of software for
population genetics in the context of analysing data sets from NGS. There is no doubt
that this will continue in the years to come where population genetic data will become
351 more and more important to assess biodiversity dynamics and adaptations of species in a
changing environment. We discuss below three specific areas where significant progress is
likely to be achieved in the coming years.

354 As apparent from the contributions to this Special Issue, a lot of effort has been
devoted to improve the overall quality of the software dedicated to population genetics.
On the other hand, hardware has continued to improve. For instance, multi-core
357 processors are now the rule rather than the exception, so that parallel computing can be
accomplished on almost all computers. The adaptation of genetic software to this has
been uneven. Lawrence & Morgan (2014) give some examples using Bioconductor,
360 illustrating the issue that implementing a parallel algorithm to accomplish a given task
may not always be beneficial. R has a built-in capacity to parallelize computations using
the package `parallel`. The functions in `parallel` can be used with most R packages described
363 in this article, for instance, by distributing a calculation to several cores of a processor.
However, there is certainly some improvements possible since a lot of the computations

used in population genetics implies repetitive and independent calculations (e.g., for
366 allele frequencies by population or for LD).

NGS raw data are more or less noisy because they are generated through some
biological and electrical processes which may be affected by random variation. Besides
369 these measurement errors, intrinsic biological processes induce another form of variation,
for instance because of cell-specific genetic changes. Lynch (2008) addressed the issue of
inferring population genetic parameters using NGS data (see Korneliussen *et al.* 2013,
372 for a more recent contribution). Blischak *et al.* (2016) developed a hierarchical Bayesian
model to assess genotype uncertainty in autopolyploids using raw read counts. They
implemented their method in the R package *polyfreqs* (available on CRAN). We take this
375 opportunity to emphasize the importance of open source software for the future of
population genomics. O’Rawe *et al.* (2015) warned about the risk that commercial NGS
applications tend to ignore uncertainty in genotyping inference. As a sign of this risk,
378 these authors mention that several applications for haplotype or variant call are
proprietary and closed-source. Considering the continuously increasing diversity of NGS
technologies and the increasing range of their applications, a special attention to
381 uncertainty in statistical inference using NGS data will be crucial.

Environmental monitoring already allows acquisition of data in real time, and recent
developments in NGS (e.g., nanopore sequencing) strongly suggest that similar things
384 will soon be accomplished with population genetic data. This is a crucial challenge for
developing efficient software and making sense of the results in real time. Facing this
forthcoming challenge will mean having to combine data analysis with theoretical
387 population genetics in order to help us meet issues related to global change, biodiversity
conservation, and pathogen dynamics. It will surely be useful to remember the words of
caution—if not wisdom—from Gower (2008): “Automatic instrumentation ensures that
390 there is no lack of large data sets. This is something new but I sometimes think that we
are getting perilously close to the search for the philosophers stone. If only we could find
the right recipe, great truths would be revealed by analysing vast masses of data.”

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Box 1 *Scanning and reading VCF files*

First we scan the human Y chromosome data with `VCFloci`, print the number of loci and the labels of the columns with `names`:

```
> info.Y <- VCFloci("chrY.vcf.gz")
Scanning file chrY.vcf.gz
 171.6615 Mb
Done.
> nrow(info.Y)
[1] 62042
> names(info.Y)
[1] "CHROM" "POS" "ID" "REF" "ALT" "QUAL"
[7] "FILTER" "INFO" "FORMAT"
```

The number of individuals in the VCF file are obtained with the function `VCFlabels` which extracts their labels (or identifiers):

```
> labs <- VCFlabels("chrY.vcf.gz")
> length(labs)
[1] 1233
> head(labs)
[1] "HG00096" "HG00101" "HG00103" "HG00105" "HG00107"
[6] "HG00108"
```

Then we read the genotypes with `read.vcf`. Here, we select the loci in a 1 Mb region of the Y chromosome between positions 5,000,000 and 6,000,000 which can be found with:

```
> sel <- which(info.Y$POS > 5e6 & info.Y$POS <= 6e6)
> length(sel)
[1] 5
```

`read.vcf` has two ways to specify which loci to read: either using the options `from` and `to` (which are set to 1 and 10,000 by default), or using the option `which.loci` which requires a vector of integers specifying the indices of the loci to read:

```
> x <- read.vcf("chrY.vcf.gz", which.loci = sel)
Reading 5 / 5 loci.
Done.
> x
Allelic data frame: 1233 individuals
                    5 loci
```

If the user wants to read all loci with a single command, the following can be done:

```
> x <- read.vcf("chrY.vcf.gz", to = nrow(info.Y))
```

Box 2 Going further with the INFO field in VCF files

Here we use the information returned by `VCFloci`, such as the field `CHROM` (if loci from several chromosomes are in the same file), to select some loci to be read. The field `INFO` requires a special attention because it includes detailed information on each locus:

```
> info.Y$info[1]
[1] "AA=G;AC=22;AF=0.0178427;AN=1233;DP=84761;NS=1233;\
AMR_AF=0.0000;AFR_AF=0.0000;EUR_AF=0.0000;SAS_AF=0.0000;\
EAS_AF=0.0451;VT=SNP;EX_TARGET"
```

To make this information easier to digest, the function `getINFO` helps to extract a specific information from this character string; by default this is the sequencing depth (`DP`), but the option `what` makes possible to extract any other information, for instance, the variant type:

```
> VT.Y <- getINFO(info.Y, what = "VT")
> table(VT.Y)
```

```
INDEL  MNP  SNP   SV
1314   113 60505  110
```

The function `VCFheader` reads the header of the VCF file where details on the abbreviations used can be found:

```
> cat(VCFheader("chrY.vcf.gz"))
....
##INFO=<ID=VT,Number=.,Type=String,Description="indicates what type\
of variant the line represents"
....
```

Thus, we can now locate which loci are SNPs:

```
> sel.snp <- which(VT.Y == "SNP")
> length(sel.snp)
[1] 60505
```

As usual with R, it is possible to combine these logical comparisons with the AND operator (`&`), for instance, to locate the SNPs within a 2 Mb region:

```
> sel <- which(VT.Y == "SNP" & info.Y$POS > 1e6 & info.Y$POS <= 3e6)
> length(sel)
[1] 1231
```

To illustrate how to use other information returned by `VCFloci`, we scan the fruitfly data (Kao *et al.* 2015a) which include genotypes from all chromosomes in a single file:

```
> fl <- "global.pop.GATK.SNP.hard.filters.V3.phased_all.pop.maf.05.recode.vcf.gz"
> info.fly <- VCFloci(fl)
> table(info.fly$CHROM)
```

```
      2L      2R      3L      3R      R      X
224253 193675 214235 270619      1 153035
```


Box 3 *Analysis of linkage disequilibrium*

We read the fruitfly data and call the two functions `LDscan` and `LDmap` on the first 100 loci (we could use the option `to` as above to read only 100 loci, but this gives us the opportunity to show how to subset a data frame of genotypes). The positions of the loci are stored in the column `POS` output by `VCFloci` (Box 2), so we extract the positions for these 100 loci and pass them as the (optional) second argument of `LDmap` (Fig. 1a):

```
> x <- read.vcf(fl)
> res <- LDscan(x[, 1:100])
> LDmap(res, info.fly$POS[1:100])
```

549

We now select 100 loci regularly spaced from 1 to 224,253 (this value comes from the last tabulation in Box 2) and using `ceiling` to ensure we have only integers:

```
> s <- ceiling(seq(1, 224253, length.out = 100))
```

We then repeat the LD analysis using the appropriate POS information (Fig. 1b):

```
> xs <- read.vcf(fl, which.loci = s)
> res2 <- LDscan(xs)
> LDmap(res2, info.fly$POS[s])
```

Box 4 DAPC with the dolphin data

We read 1000 loci and convert them to the class "genind":

```
> x2 <- read.vcf("vcf", to = 1000)
> g2 <- loci2genind(x2)
```

We can now call the method implemented in the function `find.clusters`:

```
> o2 <- find.clusters(g2)
```

When called with no other option, the function displays two graphs (Fig. 2) and asks the user to input the number of principal components (PCs) and the number of clusters to retain. The first graph shows that 40 PCs retain about 80% of the variance, so we select this number. The second graph shows a lowest value of BIC with seven clusters. However, the present data has eight populations (see below) so we select this number of clusters (K) for this analysis (we could use simply the original populations).

The output is a list with several elements, including `grp` giving the cluster assignment of the individuals. The labels of the individuals include the geographical origin of the samples (as often the case with this kind of data set). So it is simple to extract this information, and cross-tabulate it with the cluster assignment done by `find.clusters`:

```
> head(names(o2$grp))
[1] "FAE_2" "FAE_609" "FAE_660" "FAE_667" "FAE_668" "FAE_671"
> pop <- gsub("_.*", "", names(o2$grp))
> cluster <- o2$grp
> table(pop, cluster)
      cluster
pop    1  2  3  4  5  6  7  8
DEN    0  0  0  0  4  2  0  0
FAE    0  9  1  3  2  0  2  1
FRA    0  0  0  0  1  0  1  0
GER    3  0  0  0  5  3  1  0
ICE    0  0  0  0 15  2  0  0
IRE    0  9  0  0  2  2  3  0
NOR    0  0  0  0  5  2  0  0
SCO    0  5  0  0 24  1  2  0
```

There does not seem to be a very good match between the originally sampled populations and the cluster assignments. Thus, we repeat the analysis with a smaller number of clusters ($K = 4$) in a non-interactive way by specifying the required options:

```
> o2b <- find.clusters(g2, n.pca = 40, n.clust = 4)
```

As above, we extract the 'pop' and 'cluster' information (not shown):

```
> table(pop, cluster)
      cluster
pop    1  2  3  4
DEN    0  6  0  0
FAE    0  6  4  8
FRA    0  1  0  1
GER    6  5  0  1
ICE    2 15  0  0
IRE    2  4  0 10
NOR    2  5  0  0
SCO    0 26  0  6
```

We now perform the DAPC with 4 groups asking to output two discriminant axes:

```
> d2b <- dapc(g2, cluster, n.pca = 40, n.d = 2)
```

The output contains several elements, including the coordinates of the individuals and the group means. The plots can now be done with R's standard functions (see Supplementary Information) or with `adegenet`'s functions (Figs. 3, 4):

```
> col <- c("red", "blue", "gold", "darkgreen")
> scatter(d2b, posi.da = "topright", col = col)
> compoplot(d2b, legend = FALSE, col = col)
```

Table 1: Major R data classes for population genetics and genomics. The package in parentheses is where the class is defined and which contains the main functions for data manipulation and visualization.

Class	Data type	Main features	Missing data
DNAbin (ape)	DNA sequences	Single or multiples sequences, aligned or not	Ambiguity code + ?*
genind (adegenet)	Allelic (allele-centered)	Any level of ploidy; ploidy may vary among loci and among individuals	NA†; null allele(s) can be specified at input
genlight (adegenet)	biallelic SNP	Any level of ploidy	Not supported
loci (pegas)	Allelic (locus-centered)	Any level of ploidy; ploidy may vary among loci and among individuals; genotypes may be phased or not	NA†; null allele(s) can be specified from R

*The question mark (?) is not part of the IUPAC ambiguity code.

†Standard missing value in R.

Table 2: Data inter-operability.

Class	File		Class conversion	
	Input	Output	From	To
DNABin	FASTA Phylip Clustal	FASTA Phylip	Bioconductor*	genlight
genind	Genetix Structure Fstat Genepop GenAlex		loci	loci
genlight	PLINK		DNABin	
loci	Tabular files† Genetix	Tabular files†	genind	genind
vcfR	VCF	VCF		DNABin genind loci

*Several classes are supported.

†Tab-delimited, CSV, and similar formats.

Table 3: Main R packages for population genetics and genomics. The packages not cited in the text are hierfstat (Goudet 2005), poppr (Kamvar *et al.* 2014), and mmod (Winter 2012).

Package	Main methods implemented
adegenet*	Multivariate methods (sPCA, DAPC)
ape	Evolutionary distances, distance-based phylogenetics, phylogenetic bootstrap
hierfstat*	Hierarchical F -statistics
pegas	Population differentiation (HWE,, AMOVA), haplotype networks, linkage maps
poppr*	Applications to clonal organisms, distances for microsatellites
mmod*	Population differentiation (F_{ST} , G_{ST} , Φ_{ST} , D)
stacksR	Interface to STACKS
vcfR	Tools for FASTA, GFF, and VCF files

*These packages use the class "genind".

Fig. 1 LD maps at two different genomic scales for the fruit fly data: (a) for the 100
552 first loci on the chromosome 2L, and (b) for 100 loci regularly spaced along this
chromosome. The horizontal axis indicates the position of the loci on the chromosome.
The linkage coefficients (r^2) between each pair of loci are indicated as coloured squares:
555 the squares at the bottom of the triangle are for nearby loci, whereas the square at the
top is for the two most distant loci.

Fig. 2 Results of finding clusters with the dolphin data. (a) Cumulated variance
558 explained by the principal components (PCs). (b) Bayesian information criterion (BIC)
for the different values of K (number of clusters).

Fig. 3 Results of the DAPC with the dolphin data.

561 **Fig. 4** Posterior probabilities of group membership from the DAPC with the dolphin
data. The color scheme is the same than in Fig 3.

Figure 1:

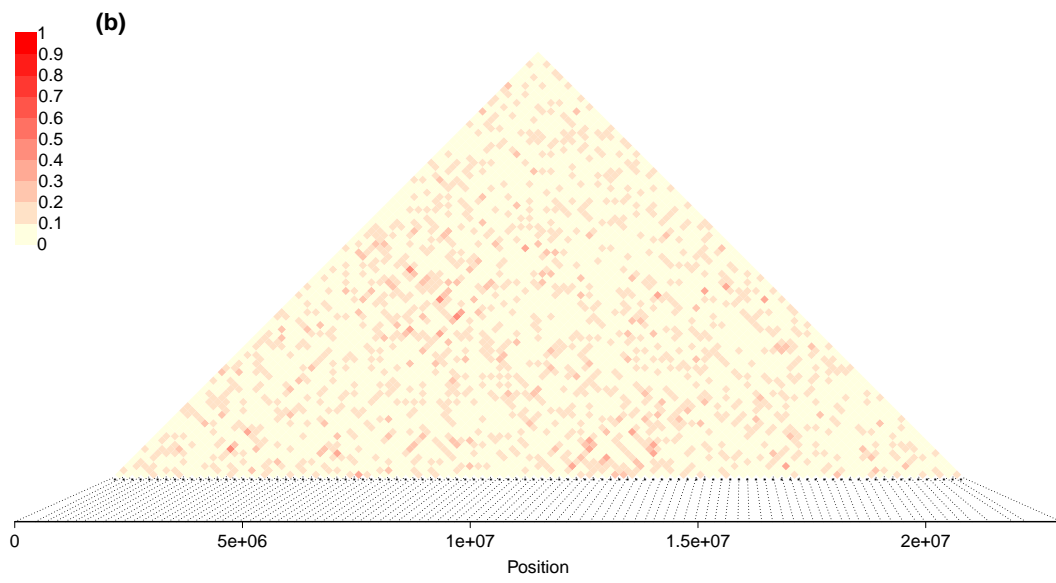
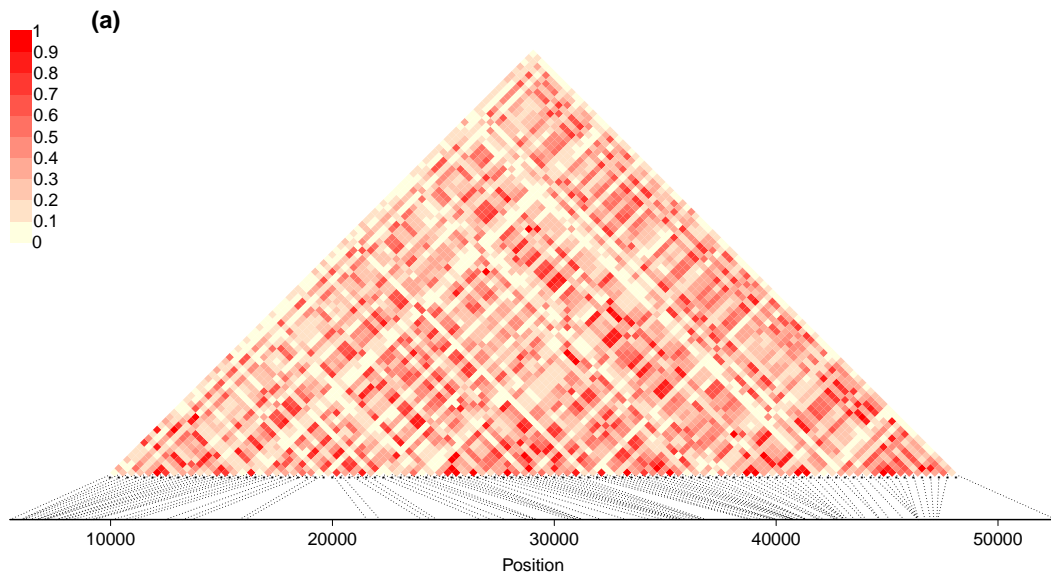


Figure 2:

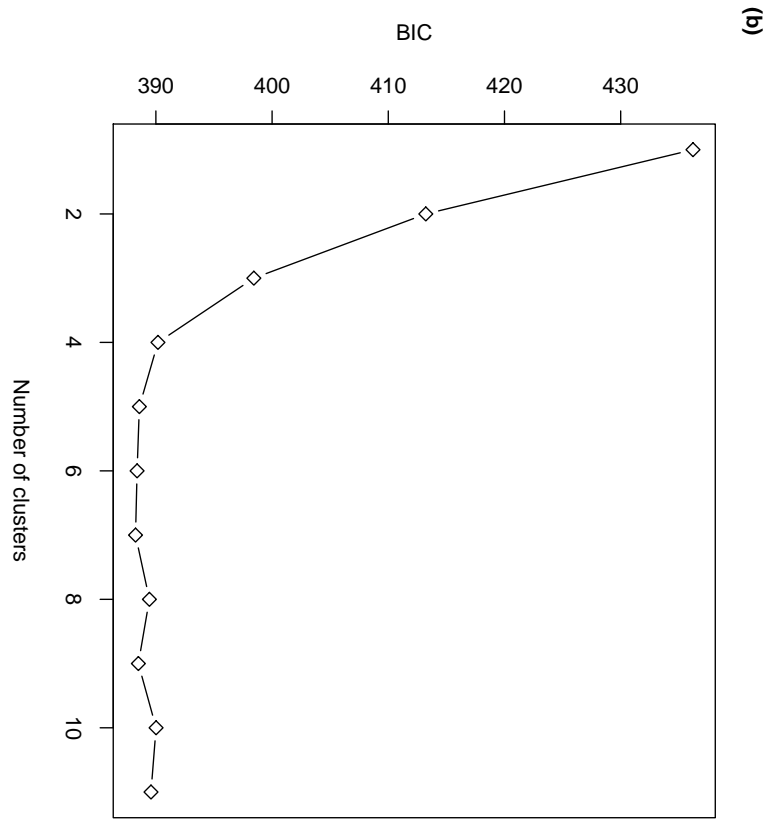
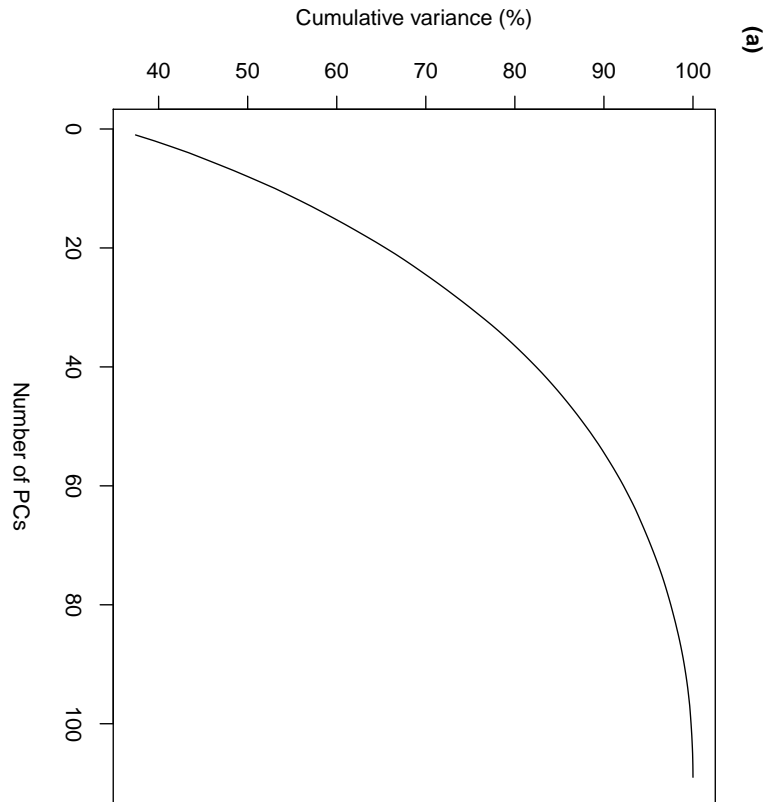


Figure 3:

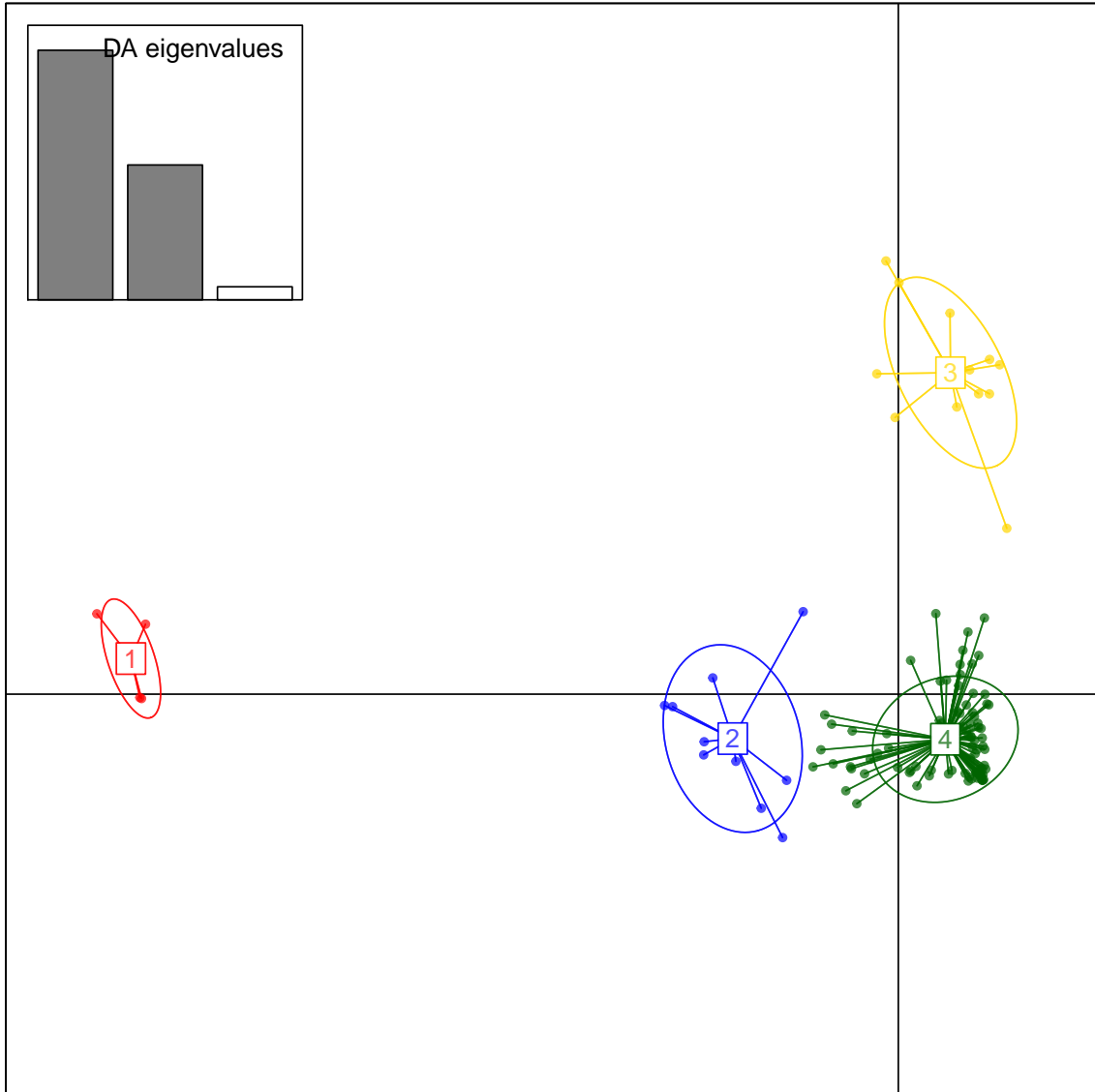
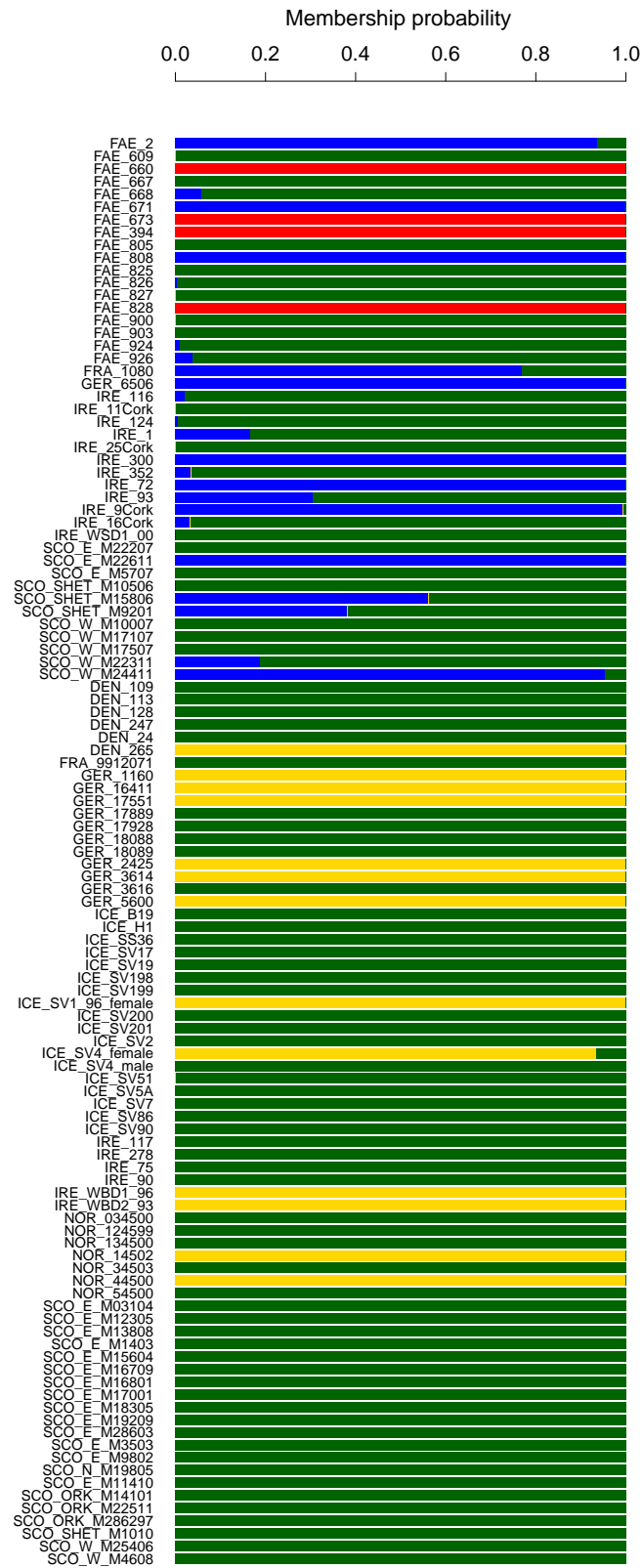


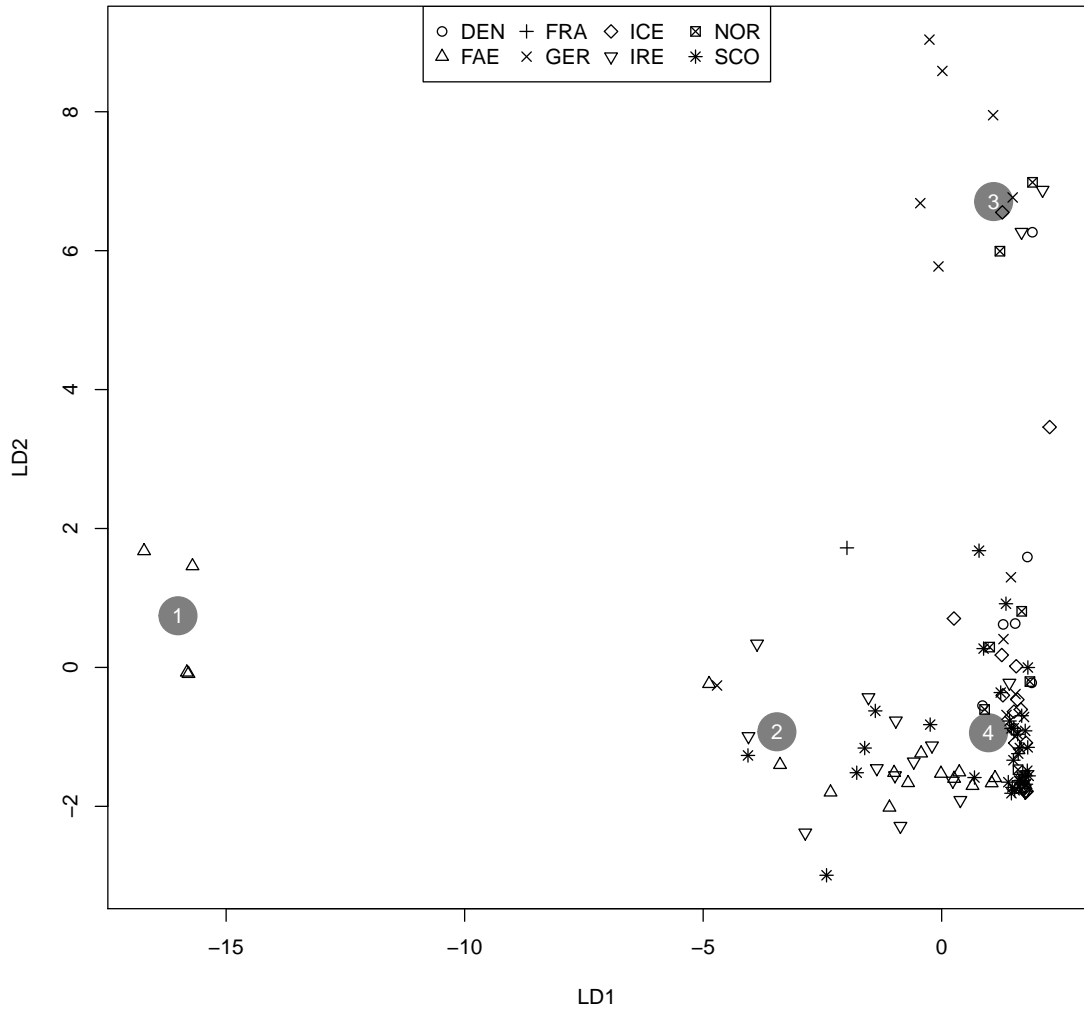
Figure 4:



Supplementary Information

564 R code for custom plot of the results from the DAPC on the dolphin data. This example shows how to extract the coordinates of the individuals and plot them with a symbol representing the original populations (Fig. S1):

```
567 plot(d2b$ind.coord, type = "n")
    points(d2b$grp.coord, pch = 19, col = "grey50", cex = 4)
    text(d2b$grp.coord, labels = 1:4, col = "white")
570 points(d2b$ind.coord, pch = (1:8)[pop])
    legend("top", legend = levels(pop), pch = 1:8, ncol = 4)
```



573 Fig. S1: Results of DAPC with the dolphin data. The group means are shown with white numbers on grey.