

# Analysing genome-wide SNP data using *adegenet*

## 1.3-0

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### **Abstract**

Genome-wide SNP data can quickly be challenging to analyse using standard computer. The package *adegenet* [1] for the R software [2] implements representation of these data with unprecedented efficiency using the classes `SNPbin` and `genlight`, which can require up to 60 times less RAM than usual representation using allele frequencies. This vignette introduces these classes and illustrates how these objects can be handled and analyzed in R. It also introduces more advanced features of an API in C language which may be useful to develop new method based on these objects.

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# 1 Introduction

Modern sequencing technologies now make complete genomes more widely accessible. The subsequent amounts of genetic data pose challenges in terms of storing and handling the data, making former tools developed for classical genetic markers such as microsatellite impracticable using standard computers. Adegenet has developed new object classes dedicated to handling genome-wide polymorphism (SNPs) with minimum rapid access memory (RAM) requirements.

Two new formal classes have been implemented: `SNPbin`, used to store genome-wide SNPs for one individual, and `genlight`, which stored the same information for multiple individuals. Information represented this way is binary: only biallelic SNPs can be stored and analyzed using these classes. However, these objects are otherwise very flexible, and can incorporate different levels of ploidy across individuals within a single dataset. In this vignette, we present these object classes and show how their content can be further handled and content analyzed.

## 2 Classes of objects

### 2.1 SNPbin: storage of single genomes

The class `SNPbin` is the core representation of biallelic SNPs which allows to represent data with unprecedented efficiency. The essential idea is to code binary SNPs not as integers, but as bits. This operation is tricky in R as there is no handling of bits, only bytes – series of 8 bits. However, the class `SNPbin` handles this transparently using sub-routines in C language. Considerable efforts have been made so that the user does not have to dig into the complex internal structure of the objects, and can handle `SNPbin` objects as easily as possible.

Like `genind` and `genpop` objects, `SNPbin` is a formal "S4" class. The structure of these objects is detailed in the dedicated manpage (`?SNPbin`). As all S4 objects, instances of the class `SNPbin` are composed of slots accessible using the `@` operator. This content is generic (it is the same for all instances of the class), and returned by:

```
> library(adegenet)
> getClassDef("SNPbin")
```

```
Class "SNPbin" [package "adegenet"]
```

```
Slots:
```

Name:	snp	n.loc	NA.posi	label	ploidy
Class:	list	integer	integer	charOrNULL	integer

The slots respectively contain:

- `snp`: SNP data with specific internal coding.
- `n.loc`: the number of SNPs stored in the object.
- `NA.posi`: position of the missing data (NAs).
- `label`: an optional label for the individual.
- `ploidy`: the ploidy level of the genome.

New objects are created using `new`, with these slots as arguments. If no argument is provided, an empty object is created:

```
> new("SNPbin")

=== S4 class SNPbin ===
0 SNPs coded as bits
Ploidy: 1
0 (NaN %) missing data
```

In practice, only the `snp` information and possibly the ploidy has to be provided; various formats are accepted for the `snp` component, but the simplest is a vector of integers (or numeric) indicating the number of second allele at each locus. The argument `snp`, if provided alone, does not have to be named:

```
> x <- new("SNPbin", c(0, 1, 1, 2, 0, 0, 1))
> x
```

```
=== S4 class SNPbin ===
7 SNPs coded as bits
Ploidy: 2
0 (0 %) missing data
```

If not provided, the ploidy is detected from the data and determined as the largest number in the input vector. Obviously, in many cases this will not be adequate, but ploidy can always be rectified afterwards; for instance:

```
> x

=== S4 class SNPbin ===
7 SNPs coded as bits
Ploidy: 2
0 (0 %) missing data

> ploidy(x) <- 3
> x

=== S4 class SNPbin ===
7 SNPs coded as bits
Ploidy: 3
0 (0 %) missing data
```

The internal coding of the objects is cryptic, and not meant to be accessed directly:

```
> x@snp
```

```
[[1]]
[1] 08

[[2]]
[1] 4e
```

Fortunately, data are easily converted back into integers:

```
> as.integer(x)

[1] 0 1 1 2 0 0 1
```

The main interest of this representation is its efficiency in terms of storage. For instance:

```
> dat <- sample(0:1, 1e+06, replace = TRUE)
> print(object.size(dat), unit = "auto")

3.8 Mb
```

```
> x <- new("SNPbin", dat)
> print(object.size(x), unit = "auto")

123.4 Kb
```

here, we converted a million SNPs into a `SNPbin` object, which turns out to be 32 smaller than the original data. However, the information in `dat` and `x` is strictly identical:

```
> identical(as.integer(x), dat)

[1] TRUE
```

The advantage of this storage is therefore being extremely compact, and allowing to analyse big datasets using standard computers.

While `SNPbin` objects are the very mean by which we store data efficiently, in practice we need to analyze several genomes at a time. This is made possible by the class `genlight`, which relies on `SNPbin` but allows for storing data from several genomes at a time.

## 2.2 genlight: storage of multiple genomes

Like `SNPbin`, `genlight` is a formal S4 class. The slots of instances of this class are described by:

```
> getClassDef("genlight")
```

```

Class "genlight" [package "adegenet"]
Slots:
Name:      gen          n.loc    ind.names  loc.names  loc.all
Class:     list         integer  charOrNULL charOrNULL charOrNULL
Name:      chromosome   position  ploidy     pop        other
Class:     factorOrNULL intOrNULL intOrNULL  factorOrNULL list

```

As it can be seen, these objects allow for storing more information in addition to vectors of SNP frequencies. More precisely, their content is (see `?genlight` for more details):

- **gen**: SNP data for different individuals, each stored as a **SNPbin**; loci have to be identical across all individuals.
- **n.loc**: the number of SNPs stored in the object.
- **ind.names**: (optional) labels for the individuals.
- **loc.names**: (optional) labels for the loci.
- **loc.all**: (optional) alleles of the loci separated by `'/'` (e.g. `'a/t'`, `'g/c'`, etc.).
- **chromosome**: (optional) a factor indicating the chromosome to which the SNPs belong.
- **position**: (optional) the position of each SNPs in their chromosome.
- **ploidy**: (optional) the ploidy of each individual.
- **pop**: (optional) a factor grouping individuals into 'populations'.
- **other**: (optional) a list containing any supplementary information to be stored with the data.

Like **SNPbin** object, **genlight** object are created using the constructor **new**, providing content for the slots above as arguments. When none is provided, an empty object is created:

```

> new("genlight")

=== S4 class genlight ===
0 genotypes, 0 binary SNPs

```

The most important information to provide is obviously the genotypes (argument **gen**); these can be provided as:

- a **list** of integer vectors representing the number of second allele at each locus.
- a **matrix** / **data.frame** of integers, with individuals in rows and SNPs in columns.

- a list of `SNPbin` objects.

Ploidy has to be consistent across loci for a given individual, but individuals do not have to have the same ploidy, so that it is possible to have haploid, diploid, and tetraploid individuals in the same dataset; for instance:

```
> x <- new("genlight", list(indiv1 = c(1, 1, 0, 1, 1, 0), indiv2 = c(2,
+ 1, 1, 0, 0, 0), toto = c(2, 2, 0, 0, 4, 4)))
> x
```

```
=== S4 class genlight ===
3 genotypes, 6 binary SNPs
Ploidy statistics (min/median/max): 1 / 2 / 4
0 (0 %) missing data
```

```
> ploidy(x)
```

```
indiv1 indiv2  toto
      1      2      4
```

As for `SNPbin`, `genlight` objects can be converted back to integers vectors, stored as matrices or lists:

```
> as.list(x)
```

```
$indiv1
[1] 1 1 0 1 1 0

$indiv2
[1] 2 1 1 0 0 0

$toto
[1] 2 2 0 0 4 4
```

```
> as.matrix(x)
```

```
      [,1] [,2] [,3] [,4] [,5] [,6]
indiv1    1    1    0    1    1    0
indiv2    2    1    1    0    0    0
toto      2    2    0    0    4    4
```

In practice, `genlight` objects can be handled as if they were matrices of integers as the one above returned by `as.matrix`. However, they offer the advantage of efficient storage of the information; for instance, we can simulate 50 individuals typed for 1,00,000 SNPs each (including occasional NAs):

```
> dat <- lapply(1:50, function(i) sample(c(0, 1, NA), 1e+06, prob = c(0.5,
+ 0.499, 0.001), replace = TRUE))
> names(dat) <- paste("indiv", 1:length(dat))
> print(object.size(dat), unit = "auto")
```

```
381.5 Mb
```

```
> x <- new("genlight", dat)
> print(object.size(x), unit = "auto")
```

6.2 Mb

```
> object.size(dat)/object.size(x)
```

61.3365722669428 bytes

here again, the storage if the data is much more efficient in **genlight** than using integers: converted data occupy 61 times less memory than the original data.

The advantage of this storage is therefore being extremely compact, and allowing to analyse very large datasets using standard computers. Obviously, usual computations demand data to be at one moment coded as numeric values (as opposed to bits). However, most usual computations can be achieved by only converting one or two genomes back to numeric values at a time, therefore keeping RAM requirements low, albeit at a possible cost of increased computational time. This however is minimized by three ways:

1. conversion routines are optimized for speed using C code.
2. using parallel computation where multicore architectures are available.
3. handling smaller objects, thereby decreasing the possibly high computational time taken by memory allocation.

While this makes implementing methods more complicated. In practice, routines are implemented so as to minimize the amount of data converted back to integers, use C code where possible, and use multiple cores if the package *multicore* is installed and multiple cores are available. Fortunately, these underlying technical issues are oblivious to the user, and one merely needs to know how to manipulate **genlight** objects using a few key functions to be able to analyze data.

## 3 Data handling using **genlight** objects

### 3.1 Using accessors

In the following, we demonstrate how to manipulate and analyse **genlight** objects. The philosophy underlying formal (S4) classes in general, and **genlight** objects in particular, is that internal representation of the information can be complex as long as accessing this information is simple. This is made possible by decoupling storage and accession: the user is not meant to access the content of the object directly, but has to use **accessors** to retrieve or modify information.

Available accessors are documented in `?genlight`. Most of them are identical to accessors for **genind** and **genpop** objects, such as:



- `nInd`: returns the number of individuals in the object.
- `nLoc`: returns the number of loci (SNPs).
- `indNames†`: returns/sets labels for individuals.
- `locNames†`: returns/sets labels for loci (SNPs).
- `alleles†`: returns/sets alleles.
- `ploidy†`: returns/sets ploidy of the individuals.
- `pop†`: returns/sets a factor grouping individuals.
- `other†`: returns/sets misc information stored as a list.

where <sup>†</sup> indicates that a replacement method is available using `<-`; for instance:

```
> dat <- lapply(1:3, function(i) sample(0:2, 10, replace = TRUE))
> dat

[[1]]
[1] 1 0 1 2 1 0 0 0 1 2

[[2]]
[1] 0 1 0 0 0 2 1 1 1 0

[[3]]
[1] 2 0 1 2 0 0 2 2 1 1

> x <- new("genlight", dat)
> x

=== S4 class genlight ===
3 genotypes, 10 binary SNPs
Ploidy: 2
0 (0 %) missing data

> indNames(x)

NULL

> indNames(x) <- paste("individual", 1:3)
> indNames(x)

[1] "individual 1" "individual 2" "individual 3"

> locNames(x)
> locNames(x) <- paste("SNP", 1:nLoc(x), sep = ".")
> as.matrix(x)

      SNP.1 SNP.2 SNP.3 SNP.4 SNP.5 SNP.6 SNP.7 SNP.8 SNP.9 SNP.10
individual 1      1      0      1      2      1      0      0      0      1      2
individual 2      0      1      0      0      0      2      1      1      1      0
individual 3      2      0      1      2      0      0      2      2      1      1
```

In addition, some specific accessors are available for `genlight` objects:

- `NA.posi`: returns the position of missing values in each individual.
- `chromosome†`: returns/sets the chromosome of each SNP.
- `chr†`: same as `chromosome` — used as a shortcut.
- `position†`: returns/sets the position of each SNP.

Accessors are meant to be clever about replacement, meaning that they try hard to prevent replacement with inconsistent values. For instance, in object `x`:

```
> x
```

```
=== S4 class genlight ===
3 genotypes, 10 binary SNPs
Ploidy: 2
0 (0 %) missing data
@loc.names: labels of the SNPs
```

if we try to set information about the chromosomes of the SNPs, the instruction:

```
> chr(x) <- rep("chr-1", 7)
```

will generate an error because the provided factor does not match the number of loci (10), while:

```
> chr(x) <- rep("chr-1", 10)
> x
```

```
=== S4 class genlight ===
3 genotypes, 10 binary SNPs
Ploidy: 2
0 (0 %) missing data
@chromosome: chromosome of the SNPs
@loc.names: labels of the SNPs
```

```
> chr(x)
```

```
[1] chr-1 chr-1 chr-1 chr-1 chr-1 chr-1 chr-1 chr-1 chr-1 chr-1
Levels: chr-1
```

is a valid replacement.

## 3.2 Subsetting the data

`genlight` objects are meant to be handled as if they were matrices of allele numbers, as returned by `as.matrix`. Therefore, subsetting can be achieved using `[ idx.row , idx.col ]` where `idx.row` and `idx.col` are indices for rows (individuals) and columns (SNPs). For instance, using the previous toy dataset, we try a few classical subsetting of rows and columns:

```
> x
```

```

=== S4 class genlight ===
3 genotypes, 10 binary SNPs
Ploidy: 2
0 (0 %) missing data
@chromosome: chromosome of the SNPs
@loc.names: labels of the SNPs

> as.matrix(x)

      SNP.1 SNP.2 SNP.3 SNP.4 SNP.5 SNP.6 SNP.7 SNP.8 SNP.9 SNP.10
individual 1      1      0      1      2      1      0      0      0      1      2
individual 2      0      1      0      0      0      2      1      1      1      0
individual 3      2      0      1      2      0      0      2      2      1      1

> as.matrix(x[c(1, 3), ])

      SNP.1 SNP.2 SNP.3 SNP.4 SNP.5 SNP.6 SNP.7 SNP.8 SNP.9 SNP.10
individual 1      1      0      1      2      1      0      0      0      1      2
individual 3      2      0      1      2      0      0      2      2      1      1

> as.matrix(x[, c(TRUE, FALSE)])

      SNP.1 SNP.3 SNP.5 SNP.7 SNP.9
individual 1      1      1      1      0      1
individual 2      0      0      0      1      1
individual 3      2      1      0      2      1

> as.matrix(x[1:2, c(1, 1, 1, 2, 2, 2, 3, 3, 3)])

      SNP.1 SNP.1 SNP.1 SNP.2 SNP.2 SNP.2 SNP.3 SNP.3 SNP.3
individual 1      1      1      1      0      0      0      1      1      1
individual 2      0      0      0      1      1      1      0      0      0

```

Moreover, one can split data into blocks of SNPs using `seplloc`. This can be achieved by specifying either a number of blocks (argument `n.block`) or the size of the blocks (argument `block.size`). The function also allows for randomizing the distribution of the SNPs in the blocks (argument `random=TRUE`), which is especially useful to replace computations that cannot be achieved on the whole dataset with parallelized computations performed on random blocks. For instance:

```

> x

=== S4 class genlight ===
3 genotypes, 10 binary SNPs
Ploidy: 2
0 (0 %) missing data
@chromosome: chromosome of the SNPs
@loc.names: labels of the SNPs

> as.matrix(x)

```

	SNP.1	SNP.2	SNP.3	SNP.4	SNP.5	SNP.6	SNP.7	SNP.8	SNP.9	SNP.10
individual 1	1	0	1	2	1	0	0	0	1	2
individual 2	0	1	0	0	0	2	1	1	1	0
individual 3	2	0	1	2	0	0	2	2	1	1

```
> seploc(x, n.block = 2)
```

```
$block.1
=== S4 class genlight ===
3 genotypes, 5 binary SNPs
Ploidy: 2
0 (0 %) missing data
@chromosome: chromosome of the SNPs
@loc.names: labels of the SNPs

$block.2
=== S4 class genlight ===
3 genotypes, 5 binary SNPs
Ploidy: 2
0 (0 %) missing data
@chromosome: chromosome of the SNPs
@loc.names: labels of the SNPs
```

```
> lapply(seploc(x, n.block = 2), as.matrix)
```

```
$block.1
      SNP.1 SNP.2 SNP.3 SNP.4 SNP.5
individual 1      1      0      1      2      1
individual 2      0      1      0      0      0
individual 3      2      0      1      2      0

$block.2
      SNP.6 SNP.7 SNP.8 SNP.9 SNP.10
individual 1      0      0      0      1      2
individual 2      2      1      1      1      0
individual 3      0      2      2      1      1
```

splits the data into two blocks of contiguous SNPs, while:

```
> lapply(seploc(x, n.block = 2, random = TRUE), as.matrix)
```

```
$block.1
      SNP.8 SNP.5 SNP.2 SNP.6 SNP.1
individual 1      0      1      0      0      1
individual 2      1      0      1      2      0
individual 3      2      0      0      0      2

$block.2
      SNP.4 SNP.9 SNP.10 SNP.7 SNP.3
individual 1      2      1      2      0      1
individual 2      0      1      0      1      0
individual 3      2      1      1      2      1
```

generates blocks of randomly selected SNPs.

### 3.3 Data conversions

#### 3.3.1 The .snp format

*adegenet* has defined its own format for storing biallelic SNP data in text files with extension `.snp`. This format has several advantages: it is fairly compact

(more so than usual non-compressed formats), allows for any information about individuals or loci to be stored, allows for comments, and is easily parsed — in particular, not all information has to be read at a time, again minimizing RAM requirements for import procedures.

An example file of this format is distributed with *adegenet*. Once the package has been installed, the file can be accessed by typing:

```
> file.show(system.file("files/exampleSnpDat.snp", package = "adegenet"))
```

Otherwise, this file is also accessible from the *adegenet* website (section 'Documents'). A complete description of the *.snp* format is provided in the comment section of the file.

The structure of a *.snp* file can be summarized as follows:

- a (possibly empty) **comment section**
- **meta-information**, i.e. information about loci or individuals, stored as named vectors
- **genotypes**, stored as named vectors

The *comment section* can start with the line:

```
>>>> begin comments - do not remove this line <<<<
```

and ends with the line:

```
>>>> end comments - do not remove this line <<<<}
```

While this section can be left empty, these two lines have to be present for the format to be valid. Each *meta-information* is stored using two lines, the first starting as:

```
>> name-of-the-information
```

and the second containing the information itself, each item separated by a single space. Any label can be used, but some specific names will be recognized and interpreted by the parser:

- **position**: the following line contains integers giving the position of the SNPs on the sequence
- **allele**: character strings representing the two alleles of each loci separated by "/"
- **population**: character strings indicating a group memberships of the individuals

- **ploidy**: integers indicating the ploidy of each individual; alternatively, one single integer if all individuals have the same ploidy
- **chromosome**: character strings indicating the chromosome on which the SNP are located

Each *genotype* is stored using two lines, the first being `> label-of-the-individual`, and the second being integers corresponding to the number of second allele for each loci, without separators; missing data are coded as `'-'`.

`.snp` files can be read in R using `read.snp`, which converts data into `genlight` objects. The function reads data by chunks of a several individuals (minimum 1, no maximum besides RAM constraints) at a time, which allows one to read massive datasets with negligible RAM requirements (albeit at a cost of computational time). The argument `chunkSize` indicates the number of genomes read at a time; larger values mean reading data faster but require more RAM. We can illustrate `read.snp` using the example file mentioned above. The non-comment part of the file reads:

```
[...]
>> position
1 8 11 43
>> allele
a/t g/c a/c t/a
>> population
Brit Brit Fren monster NA
>> ploidy
2
> foo
1020
> bar
0012
> toto
10-0
> Nyarlathotep
0120
> an even longer label but OK since on a single line
1100
```

We read the file in using:

```
> obj <- read.snp(system.file("files/exampleSnpDat.snp", package = "adegenet"),
+               chunk = 2)
```

```
Reading biallelic SNP data file into a genlight object...
```

```
Reading comments...
```

```

Reading general information...
Reading 5 genotypes...
...
Checking consistency...
Building final object...
...done.

> obj

=== S4 class genlight ===
5 genotypes, 4 binary SNPs
Ploidy: 2
1 (0.05 %) missing data
@pop: individual membership for 4 populations
@position: position of the SNPs
@alleles: alleles of the SNPs

> as.matrix(obj)

              1.a/t.a/t 8.g/c.g/c
foo              1         0
bar              0         0
toto             1         0
Nyarlahotep      0         1
an even longer label but OK since on a single line 1         1
              11.a/c.a/c 43.t/a.t/a
foo              2         0
bar              1         2
toto            NA         0
Nyarlahotep      2         0
an even longer label but OK since on a single line 0         0

> alleles(obj)

[1] "a/t" "g/c" "a/c" "t/a"

> pop(obj)

[1] Brit   Brit   Fren   monster NA
Levels: Brit Fren monster NA

> indNames(obj)

[1] "foo"
[2] "bar"
[3] "toto"
[4] "Nyarlahotep"
[5] "an even longer label but OK since on a single line"

```

Note that `system.file` is generally useless: it is only used in this example to access a file installed alongside the package. Usual calls to `read.snp` will resemble:

```
> obj <- read.snp("path-to-my-file.snp")
```

### 3.3.2 Importing data from PLINK

Genome-wide SNP data of diploid organisms are frequently analyzed using PLINK, whose format is therefore becoming a standard. Data with PLINK format (`.raw`) can be imported into `genlight` objects using `read.PLINK`. This function requires the data to be saved in PLINK using the `'-recodeA'` option (see details section in `?read.PLINK`). More information on exporting from PLINK can be found at <http://pngu.mgh.harvard.edu/~purcell/plink/dataman.shtml#recode>.

Like `read.snp`, `read.PLINK` has the advantage of reading data by chunks of a few individuals (down to a single one at a time, no upper limits), which minimizes the amount of memory needed to read information before its conversion to `genlight`; however, using more chunks also means more computational time, since the procedure has to re-read the same file several time. Note that meta information about the loci also known as `.map` can also be read alongside a `.raw` file using the argument `map.file`. Alternatively, such information can be added to a `genlight` object afterwards using `extract.PLINKmap`.

### 3.3.3 Extracting SNPs from alignments

In many cases, raw genomic data are available as aligned sequences, in which case extracting polymorphic sites can be non-trivial. The biggest issue is again memory: most software extracting SNPs from aligned sequences require all the sequences to be stored in memory at a time, a duty that most common computers cannot undertake. *adegenet* implements a more parsimonious alternative which allows for extracting SNPs from alignment while processing a reduced number of sequences (down to a single) at a time.

The function `fasta2genlight` extracts SNPs from alignments with *fasta* format (file extensions `'.fasta'`, `'.fas'`, or `'.fa'`). Like `read.snp` and `read.PLINK`, `fasta2genlight` processes data by chunks of individuals so as to save memory requirements. It first scans the whole file for polymorphic positions, and then extracts all biallelic SNPs from the alignment.

`fasta2genlight` is illustrated like `read.snp` using a toy dataset distributed alongside the package. The file is first located using `system.file`, and then processed using `fasta2genlight`:

```
> myPath <- system.file("files/usflu.fasta", package = "adegenet")
> obj <- fasta2genlight(myPath, chunk = 10)
```

```
Converting FASTA alignment into a genlight object...
```

```
Looking for polymorphic positions...
```

```
.....
Extracting SNPs from the alignment...
.....
```



```

Building final object...
...done.

> obj

=== S4 class genlight ===
80 genotypes, 274 binary SNPs
Ploidy: 1
26 (0 %) missing data
@position: position of the SNPs
@alleles: alleles of the SNPs

```

obj is a **genlight** object containing SNPs of 80 isolates of seasonal influenza (H3N2) sampled within the US over the last two decades; sequences correspond to the hemagglutinin (HA) segment. Besides genotypes, obj contains the positions of the SNPs and the alleles at each retained loci. Names of the loci are constructed as the combination of both:

```

> position(obj)

[1] 7 12 31 32 36 37 44 45 52 60 62 72 73 78 96
[16] 99 105 108 121 128 140 146 147 150 165 177 181 187 190 191
[31] 193 196 197 210 216 218 219 232 234 246 249 255 273 286 289
[46] 295 310 323 327 328 365 402 405 412 417 418 419 424 438 439
[61] 441 444 445 447 450 451 460 462 466 467 472 478 479 483 499
[76] 508 510 511 512 513 514 516 518 520 522 524 531 537 538 547
[91] 549 563 564 571 577 585 591 603 604 606 609 614 616 622 623
[106] 624 625 626 628 630 635 636 638 642 648 650 652 669 671 672
[121] 687 689 690 693 694 696 702 705 706 712 714 715 716 717 721
[136] 722 723 727 729 732 733 734 735 744 754 759 778 780 786 811
[151] 822 831 833 837 854 855 859 872 875 876 881 882 884 885 894
[166] 903 913 920 927 930 945 948 954 963 973 981 983 988 990 993
[181] 1005 1009 1011 1017 1023 1030 1044 1047 1053 1056 1059 1080 1087 1089 1092
[196] 1107 1116 1125 1128 1134 1140 1146 1149 1158 1163 1169 1170 1171 1173 1176
[211] 1180 1182 1185 1188 1191 1197 1200 1201 1205 1206 1218 1221 1230 1233 1246
[226] 1263 1266 1275 1290 1296 1320 1323 1325 1338 1347 1353 1389 1397 1401 1403
[241] 1404 1413 1416 1431 1446 1453 1458 1474 1475 1485 1512 1518 1524 1527 1530
[256] 1536 1548 1557 1563 1572 1574 1613 1614 1620 1627 1637 1642 1644 1652 1653
[271] 1683 1687 1688 1700

> alleles(obj)

[1] "a/g" "c/t" "t/c" "t/c" "t/c" "c/a" "t/c" "c/t" "a/g" "c/t" "g/t" "c/a"
[13] "a/g" "a/g" "a/g" "c/t" "a/g" "g/a" "c/a" "a/g" "a/g" "a/g" "a/c" "t/c"
[25] "t/c" "t/a" "a/g" "t/c" "g/a" "c/t" "g/a" "a/g" "g/a" "t/c" "c/t" "g/a"
[37] "a/g" "a/g" "a/g" "g/a" "a/t" "t/c" "t/g" "c/a" "a/g" "g/a" "g/a" "a/c"
[49] "t/c" "t/c" "c/t" "g/a" "g/a" "a/g" "a/g" "g/a" "a/g" "a/g" "c/a" "g/a"
[61] "t/c" "g/a" "g/a" "t/c" "g/a" "a/g" "g/t" "t/a" "a/g" "a/t" "g/a" "g/a"
[73] "t/a" "c/a" "t/c" "t/c" "g/a" "c/a" "a/c" "c/t" "a/c" "a/c" "t/c" "g/a"
[85] "a/c" "a/t" "t/c" "g/a" "c/t" "a/t" "t/c" "g/a" "c/a" "g/a" "t/c" "t/c"
[97] "g/a" "g/a" "a/g" "c/t" "g/a" "g/a" "g/a" "a/g" "c/t" "c/t" "a/t" "g/t"
[109] "c/t" "a/g" "t/c" "t/c" "g/a" "a/t" "g/a" "g/a" "g/a" "a/g" "g/t" "a/g"
[121] "a/g" "t/c" "c/t" "g/a" "a/g" "t/c" "g/a" "t/c" "a/g" "t/a" "g/a" "g/a"
[133] "t/g" "a/g" "g/a" "g/a" "t/c" "t/c" "c/t" "t/c" "a/c" "g/t" "a/g" "c/a"
[145] "a/t" "a/g" "g/a" "t/c" "c/a" "c/t" "a/g" "a/g" "g/a" "g/a" "g/a" "g/a"
[157] "g/a" "g/a" "a/c" "c/a" "g/a" "t/c" "c/t" "t/c" "c/t" "t/c" "c/t" "a/g"
[169] "t/a" "t/c" "g/a" "c/t" "t/c" "c/t" "g/a" "a/g" "a/c" "c/t" "g/a" "a/g"
[181] "g/a" "c/a" "g/a" "a/g" "g/t" "a/g" "c/t" "c/t" "c/t" "a/g" "t/c" "g/a"
[193] "g/a" "a/g" "c/t" "c/t" "a/g" "g/a" "c/a" "a/g" "a/t" "t/c" "t/c" "t/a"
[205] "c/a" "c/t" "c/a" "g/a" "c/t" "a/g" "a/g" "c/t" "g/a" "a/g" "g/a" "g/a"
[217] "a/g" "a/g" "a/g" "g/a" "g/a" "a/g" "a/g" "c/t" "t/a" "a/g" "t/c" "c/t"
[229] "a/g" "t/c" "c/t" "g/a" "a/g" "c/t" "t/c" "t/c" "g/a" "g/a" "a/t" "g/a"
[241] "g/a" "g/a" "g/a" "c/t" "c/t" "a/g" "c/t" "g/a" "c/t" "g/a" "t/c" "a/g"
[253] "a/g" "c/t" "a/g" "a/g" "c/t" "a/g" "t/c" "g/a" "c/t" "t/c" "a/g" "c/t"
[265] "c/t" "t/c" "c/t" "g/t" "t/c" "c/t" "g/a" "a/g" "a/g" "g/a"

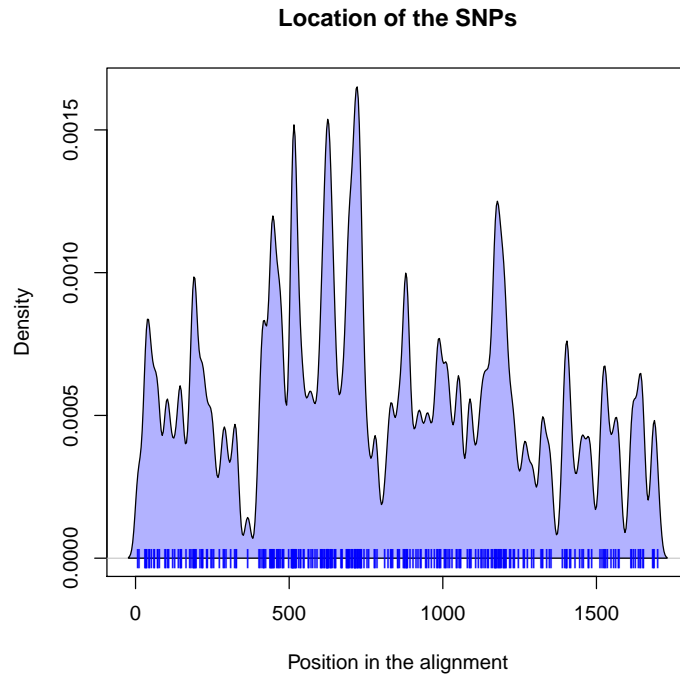
```

```
> locNames(obj)
```

```
[1] "7.a/g" "12.c/t" "31.t/c" "32.t/c" "36.t/c" "37.c/a"
[7] "44.t/c" "45.c/t" "52.a/g" "60.c/t" "62.g/t" "72.c/a"
[13] "73.a/g" "78.a/g" "96.a/g" "99.c/t" "105.a/g" "108.g/a"
[19] "121.c/a" "128.a/g" "140.a/g" "146.a/g" "147.a/c" "150.t/c"
[25] "165.t/c" "177.t/a" "181.a/g" "187.t/c" "190.g/a" "191.c/t"
[31] "193.g/a" "196.a/g" "197.g/a" "210.t/c" "216.c/t" "218.g/a"
[37] "219.a/g" "232.a/g" "234.a/g" "246.g/a" "249.a/t" "255.t/c"
[43] "273.t/g" "286.c/a" "289.a/g" "295.g/a" "310.g/a" "323.a/c"
[49] "327.t/c" "328.t/c" "365.c/t" "402.g/a" "405.g/a" "412.a/g"
[55] "417.a/g" "418.g/a" "419.a/g" "424.a/g" "438.c/a" "439.g/a"
[61] "441.t/c" "444.g/a" "445.g/a" "447.t/c" "450.g/a" "451.a/g"
[67] "460.g/t" "462.t/a" "466.a/g" "467.a/t" "472.g/a" "478.g/a"
[73] "479.t/a" "483.c/a" "499.t/c" "508.t/c" "510.g/a" "511.c/a"
[79] "512.a/c" "513.c/t" "514.a/c" "516.a/c" "518.t/c" "520.g/a"
[85] "522.a/c" "524.a/t" "531.t/c" "537.g/a" "538.c/t" "547.a/t"
[91] "549.t/c" "563.g/a" "564.c/a" "571.g/a" "577.t/c" "585.t/c"
[97] "591.g/a" "603.g/a" "604.a/g" "606.c/t" "609.g/a" "614.g/a"
[103] "616.g/a" "622.a/g" "623.c/t" "624.c/t" "625.a/t" "626.g/t"
[109] "628.c/t" "630.a/g" "635.t/c" "636.t/c" "638.g/a" "642.a/t"
[115] "648.g/a" "650.g/a" "652.g/a" "669.a/g" "671.g/t" "672.a/g"
[121] "687.a/g" "689.t/c" "690.c/t" "693.g/a" "694.a/g" "696.t/c"
[127] "702.g/a" "705.t/c" "706.a/g" "712.t/a" "714.g/a" "715.g/a"
[133] "716.t/g" "717.a/g" "721.g/a" "722.g/a" "723.t/c" "727.t/c"
[139] "729.c/t" "732.t/c" "733.a/c" "734.g/t" "735.a/g" "744.c/a"
[145] "754.a/t" "759.a/g" "778.t/c" "780.g/a" "786.t/c" "811.c/a"
[151] "822.c/t" "831.a/g" "833.a/g" "837.g/a" "854.g/a" "855.g/a"
[157] "859.g/a" "872.g/a" "875.a/c" "876.c/a" "881.g/a" "882.t/c"
[163] "884.c/t" "885.t/c" "894.c/t" "903.t/c" "913.c/t" "920.a/g"
[169] "927.t/a" "930.t/c" "945.g/a" "948.c/t" "954.t/c" "963.c/t"
[175] "973.g/a" "981.a/g" "983.a/c" "988.c/t" "990.g/a" "993.a/g"
[181] "1005.g/a" "1009.c/a" "1011.g/a" "1017.a/g" "1023.g/t" "1030.a/g"
[187] "1044.c/t" "1047.c/t" "1053.c/t" "1056.a/g" "1059.t/c" "1080.g/a"
[193] "1087.g/a" "1089.a/g" "1092.c/t" "1107.c/t" "1116.a/g" "1125.g/a"
[199] "1128.c/a" "1134.a/g" "1140.a/t" "1146.t/c" "1149.t/c" "1158.t/a"
[205] "1163.c/a" "1169.c/t" "1170.c/a" "1171.g/a" "1173.c/t" "1176.a/g"
[211] "1180.a/g" "1182.c/t" "1185.g/a" "1188.a/g" "1191.g/a" "1197.g/a"
[217] "1200.a/g" "1201.a/g" "1205.a/g" "1206.g/a" "1218.g/a" "1221.a/g"
[223] "1230.a/g" "1233.c/t" "1246.t/a" "1263.a/g" "1266.t/c" "1275.c/t"
[229] "1290.a/g" "1296.t/c" "1320.c/t" "1323.g/a" "1325.a/g" "1338.c/t"
[235] "1347.c/t" "1353.t/c" "1389.g/a" "1397.g/a" "1401.a/t" "1403.g/a"
[241] "1404.g/a" "1413.g/a" "1416.g/a" "1431.c/t" "1446.c/t" "1453.a/g"
[247] "1458.c/t" "1474.g/a" "1475.c/t" "1485.g/a" "1512.t/c" "1518.a/g"
[253] "1524.a/g" "1527.c/t" "1530.a/g" "1536.a/g" "1548.c/t" "1557.a/g"
[259] "1563.t/c" "1572.g/a" "1574.c/t" "1613.t/c" "1614.a/g" "1620.c/t"
[265] "1627.c/t" "1637.t/c" "1642.c/t" "1644.g/t" "1652.t/c" "1653.c/t"
[271] "1683.g/a" "1687.a/g" "1688.a/g" "1700.g/a"
```

It is usually informative to assess the position of the polymorphic sites within the genome; this is very easily done in R, using `density` with an appropriate bandwidth:

```
> temp <- density(position(obj), bw = 10)
> plot(temp, type = "n", xlab = "Position in the alignment", main = "Location of the SNPs")
> polygon(c(temp$x, rev(temp$x)), c(temp$y, rep(0, length(temp$x))),
+       col = transp("blue", 0.3))
> points(position(obj), rep(0, nLoc(obj)), pch = "|", col = "blue")
```



Note that retaining only biallelic sites may cause minor loss of information, as sites with more than 2 alleles are discarded from the data. It is however possible to ask `fasta2genlight` to keep track of the number of alleles for each site of the original alignment, by specifying:

```
> obj <- fasta2genlight(myPath, chunk = 10, saveNbAlleles = TRUE,
+   quiet = TRUE)
> obj
```

```
=== S4 class genlight ===
80 genotypes, 274 binary SNPs
Ploidy: 1
26 (0 %) missing data
@position: position of the SNPs
@alleles: alleles of the SNPs
@other: a list containing: nb.all.per.loc
```

The output object `obj` now contains the number of alleles of each position, stored in the `other` slot:

```
> head(other(obj)$nb.all.per.loc, 20)

[1] 1 1 1 1 1 1 2 1 1 1 1 2 1 1 1 1 1 1 1 1

> 100 * mean(unlist(other(obj)) > 1)

[1] 17.81305
```

About 18% of the sites are polymorphic, which is fairly high. This is not entirely surprising, given that the HA segment of influenza is known for its high mutation rate. What is the nature of this polymorphism?

```
> temp <- table(unlist(other(obj)))
> barplot(temp, main = "Distribution of the number \nof alleles per loci",
+         xlab = "Number of alleles", ylab = "Number of sites")
```

Most polymorphic loci are biallelic, but a few loci with 3 or 4 alleles were lost. We can estimate the loss of information very simply:

```
> temp <- temp[-1]
> temp <- 100 * temp/sum(temp)
> round(temp, 1)
```

```
      2      3      4
90.4  8.3  1.3
```

In this case, 90.4% of the polymorphic sites were biallelic, the others being essentially triallelic. This is probably a fairly exceptional situation due to the high mutation rate of the HA segment.

## 4 Data analysis using genlight objects

In the following, we illustrate some methods for the analysis of **genlight** objects, ranging from simple tools for diagnosing allele frequencies or missing data to recently developed multivariate approaches. Throughout these examples, we use **glSim** to simulate **genlight** objects. This simple simulation tool allows for simulating large SNPs data with possibly contrasted structures between two groups of individuals. See `?glSim` for more details on this tool.

### 4.1 Simple operations

### 4.2 Principal Component Analysis (PCA)

### 4.3 Principal Component Analysis (PCA)

### 4.4 Discriminant Analysis of Principal Components (DAPC)

## References

- [1] Jombart, T. (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24: 1403-1405.
- [2] R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.