

Introduction to dartR

Bernd Gruber, Peter Unmack, Oliver Berry & Arthur Georges

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1 What is dartR?

Package **dartR** is an R package for a) loading DArTTM SNP and SilicoDArT data generated from the commercial service provided by Diversity Arrays Technology Pty Ltd; (b) applying filters to those data based on locus metadata such as call rate, information content or reproducibility; (c) assigning individuals to populations and selecting subsets of individuals or populations; (d) visualization using Principal Coordinates Analysis (PCoA), (e) initial calculation of indices such as heterozygosity and F_{st} ; and (f) providing a conduit to a range of standard data formats and R packages for analysis. Though the majority of functions are.

Provided the data are in the **genlight** format (package **adegenet**), the package can be used to analyse SNP data from sources other than DArTTM. Please note we will give a detailed example below and outline several ways how to import data from other formats into **genlight**.

In most cases, the scripts in `{dartR}` are wrappers for scripts included in other already available packages, to provide easy access to these packages for analyzing DArT data, and to provide some enhanced output diagnostics. Relatively few scripts provide novel analyses. We make no apologies for this, as the objective of **dartR** is to provide fundamental tools for accessing and manipulating SNP data in preparation for analysis by the vast suite of packages available in R through the CRAN repository, and make them easier accessible for users not deeply accustomed with the R language, without “re-inventing the wheel”.

A summary of the capabilities of **dartR** is as follows:

- Intelligent interpretation and input of DArT comma-delimited files to a compact **genlight** form of the R `{adegenet}` package.
- Filtering loci and individuals on criteria drawn from the DArT locus metadata (such as `repAvg`, `AvgPIC`) or on computed statistics (such as call rate or Hamming distance).
- Relabelling individuals and recoding populations into new aggregations, and deleting selected individuals or populations.
- Visualization using Principal Coordinates Analysis (PCoA) and Neighbour-joining trees.
- Translation to other R packages (e.g. `NewHybrids`), to other `{adegenet}` objects (e.g. `genind`), and to standard data formats (e.g. `fastA`).
- A few specific analyses not available elsewhere (e.g. fixed difference analysis, assignment testing, fast calculation or linkage disequilibria per population).

2 Using this Vignette

To use this vignette interactively, you need to install and load the **dartR** package, and set the default directory using `setwd()`. To install the latest developmental version of the **dartR** package visit the github page for instructions (<https://github.com/green-striped-gecko/dartR>). Then we make the package available into the current project via `library(dartR)`. A brief description on the installation procedure is given below. There are two ways to install **dartR**, either via CRAN (preferred for the standard user) or via Github (latest, but untested version).

2.1 A) Installation via CRAN

As **dartR** is now a fully fledged package, simply type:

```
install.packages("dartR")
```

2.2 B) Manual installation via Github (with all dependencies)

```
# Install and attach library dartR
install.packages("devtools")
library(devtools)
source("http://bioconductor.org/biocLite.R")
biocLite("qvalue", suppressUpdates=T)
biocLite("SNPRelate", suppressUpdates=T)
install_github("green-striped-gecko/dartR")
```

To test if your installation was successful include the library by typing:

```
library(dartR)

## Loading required package: adegenet
## Loading required package: ade4

##
##    /// adegenet 2.0.1 is loaded //////////////////////////////////
##
##    > overview: '?adegenet'
##    > tutorials/doc/questions: 'adegenetWeb()'
##    > bug reports/feature requests: adegenetIssues()
```

Please note that library `adegenet` will output some lines as it is not easy to suppress those lines.

If some packages have not been loaded, an error message will be given and you will need to install those packages manually.

Once installed and invoked into your current R session, we need to prepare our session, so it points to a certain working directory. The working directory is the location on your hard drive, that holds your data and also is where intermediate and final output files will be exported if not directed otherwise.

To set the default directory, use the following with the appropriate directory specified:

```
# Set the default working directory (change this to suit)
setwd("c:/your.working.directory/")
```

Note that the file specification uses forward slashes, which works for the three main Operating Systems (Linux, Windows and MacOS).

The `dartR` library contains test datasets that form the basis of the commands and exercises below. We recommend that you rename the test data set to `gl`, in case you want to make alterations to it. If you wish to run the vignette code with your data, simply rename your data set to `gl`, after you have loaded it into R.

```
# Rename the test genlight object to gl, something simple
gl <- testset.gl
```

When working through the vignette, you should try the commands to see if you can replicate the output.

3 Genlight Format

The R package `dartR` relies on the SNP data being stored in a compact form using a bit-level coding scheme. SNP data coded in this way are held in a `genlight` object that is defined in R package `adegenet` (Jombart, 2008; Jombart and Ahmed, 2011). Refer to the tutorial prepared by Jombart and Collinson (2015) called *Analysing genome-wide SNP data using adegenet 2.0.0*, if you require further information. The complex storage arrangement of `genlight` objects is hidden from the user because it is accompanied by a number of

“accessors”. These allow the data to be accessed in a way similar to the manipulation of standard objects in R, such as lists, vectors and matrices. A `genlight` object can be considered to be a matrix containing the SNP data encoded in a particular way. The matrix entities (rows) are the individuals, and the attributes (columns) are the SNP loci. In the body of this individual x locus matrix are the SNP data, coded as 0 for homozygous reference state, 1 for heterozygous, and 2 for homozygous alternate (or SNP) state. You can access these data by converting to a standard matrix using

```
m <- as.matrix(gl)
```

This function allows normal R approaches for examination and manipulation. For example,

```
as.matrix(gl)[1:5,1:3]
```

```
##          100049687|12-A/G 100049698|16-C/T 100049728|23-T/G
## AA010915                2                NA                0
## UC_00126                2                NA                0
## AA032760               NA                NA                0
## AA013214                2                NA                0
## AA011723                2                NA                0
```

displays the SNP states for 5 rows [individuals] and 3 columns [loci]. Associated with the SNP genotypes in the `genlight` object is a `data.frame` of locus metadata (e.g. `CloneID`, `CallRate`, `TrimmedSequence`, etc). Each item in this list (`loc.metrics`) is a vector of length equal to the number of loci. Also associated with the SNP genotypes is a `data.frame` of individual metadata (individual id, population, sex, lat(itude), lon(gitude), etc). Each item in this `data.frame` (`ind.metrics`) is a vector of length equal to the number of individuals.

The information in the `genlight` object can be accessed using the following **adegenet** accessors:

- `nInd(gl)`: | returns the number of individuals in the `genlight` object.
- `nLoc(gl)`: returns the number of loci.
- `nPop(gl)` returns the number of populations to which the individuals are assigned.
- `indNames(gl)`: returns or sets labels for individuals.
- `locNames(gl)`: returns or sets labels for loci.
- `alleles(gl)`: returns or sets allelic states of each locus for each individual (e.g. “A/T”).
- `ploidy(gl)`: returns or sets the ploidy of the individuals (normally diploid or 2).
- `pop(gl)`: returns or sets the population to which each individual belongs. Try also `levels(pop(gl))` for a list of unique population names.
- `NA.posi`: returns the loci with missing values, that is, loci for which a sequence tag failed to amplify for each individual.
- `chr`: returns or sets the chromosome for each locus.
- `position`: returns or sets the position of each SNP in the sequence tag of each locus.
- `other(gl)`: returns of sets miscellaneous information stored as a list.



Task

Try some of these using commands on the R console

An alternative way to write these accessors is to use the form of `gl@pop` or `gl@other`, for example.

Some simple operations such as computing allele frequencies or diagnosing missing values can be problematic without representing the full dataset in memory. The package `{adegenet}` implements a few procedures that perform such basic tasks on `genlight` objects, processing one individual at a time, thereby minimizing memory requirements.

- `glSum`: counts the frequency of the alternate allele for each locus.

- glNA: counts the number of missing values for each locus.
- glMean: computes the relative frequency (a proportion in the range 0-1) of the second allele for each locus.
- glVar: computes the variance of the allele frequency distribution for each locus.
- glDotProd: computes the dot products between all pairs of individuals, with centering and scaling.

in each case taking advantage of the 0, 1, 2 coding of the SNP states for each locus – the coding essentially corresponds to the frequency of the alternate allele.



Task

Try some of these using commands on the R console



Hint

For further information on using genlight objects for SNP datasets, refer to the tutorial prepared by Thibaut Jombart and Caitlin Collinson (2015) entitled Analysing genome-wide SNP data using adegenet 2.0.0.

4 DArT Input Data Formats

Diversity Arrays Technology Pty Ltd (DArT™) supply your data as excel spreadsheets in comma delimited format (.csv). Several files are provided.

- SNP_1row.csv contains the SNP genotypes in one row format
- SNP_2row.csv contains the SNP genotypes in two row format
- SilicoDArT.csv contains the presence(1)/absence(0) of the sequence tag at a locus for each individual (analogous to AFLPs)
- metadata.csv contains a report of the success of the sequencing and an explanation of the locus metadata provided in the above spreadsheets.



Hint

Refer to the DArT documentation provided with your report for further information.

5 Reading DArT Files into a Genlight Object

SNP data can be read into a genlight object using read.dart(). This function intelligently interrogates the input csv file to determine * if the file is a 1-row or 2-row format, as supplied by Diversity Arrays Technology Pty Ltd. * the number of locus metadata columns to be input (the first typically being cloneID and the last repAvg). * the number of lines to skip at the top of the csv file before reading the specimen IDs and then the

SNP data themselves. * if there are any errors in the data. An example of the function used to input data is as follows:

```
gl <- gl.read.dart(filename = "testset.csv", covfilename = " ind_metrics.csv")
```

The filename specifies the csv file provided by Diversity Arrays Technology, and the covfilename specifies the csv file which contains metrics associated with each individual (id, pop, sex, etc).

Using the example data set provided in the package (accessed via an internal path to the files)

```
dartfile <- system.file("extdata","testset_SNPs_2Row.csv", package="dartR")
covfilename <- system.file("extdata","testset_metadata.csv", package="dartR")
gl <- gl.read.dart(filename=dartfile, covfilename = covfilename, probar=FALSE)
```

```
## Topskip not provided. Try to guess topskip...
## Set topskip to 3 . Trying to proceed...
## Trying to determine if one row or two row format...
## Found 2 row(s) format. Proceed...
## Added the following covmetrics:
## AlleleID SNP SnpPosition CallRate OneRatioSnp FreqHomRef FreqHomSnp FreqHets AvgCountRef AvgCountSnp
## Number of rows per Clone. Should be only 2 s: 2
## Recognised: 250 individuals and 255 SNPs in a 2 row format using C:/Users/s425824/AppData/Local/Temp/RtmpuUJcyg/Rinst15b49183020/dartR/extdata
## Start conversion....
## Format is 2 rows.
## Please note conversion of bigger data sets will take some time!
## Once finished, we recommend to save the object using save(object, file="object.rdata")
## Try to add covariate file: C:/Users/s425824/AppData/Local/Temp/RtmpuUJcyg/Rinst15b49183020/dartR/extdata
## Ids of covariate file (at least a subset of) are matching!
## Found 250 matching ids out of 250 ids provided in the covariate file. Subsetting snps now!.
## Added pop factor.
## Added latlon data.
## Added id to the other$ind.metrics slot.
## Added pop to the other$ind.metrics slot.
## Added lat to the other$ind.metrics slot.
## Added lon to the other$ind.metrics slot.
## Added sex to the other$ind.metrics slot.
## Added maturity to the other$ind.metrics slot.
```

The resultant genlight object, gl, can be interrogated to determine if the data have been input correctly.

To display (parts of) the genlight object we have the following options:

Display the structure of the genlight object

```
gl

## /// GENLIGHT OBJECT ///////////
##
## // 250 genotypes, 255 binary SNPs, size: 588.1 Kb
## 7868 (12.34 %) missing data
##
## // Basic content
## @gen: list of 250 SNPbin
## @ploidy: ploidy of each individual (range: 2-2)
##
## // Optional content
## @ind.names: 250 individual labels
## @loc.names: 255 locus labels
```

```
## @loc.all: 255 alleles
## @position: integer storing positions of the SNPs
## @pop: population of each individual (group size range: 1-11)
## @other: a list containing: loc.metrics latlong ind.metrics
```

Display the SNP genotypes for the first 3 individuals and 5 loci

```
as.matrix(gl)[1:3,1:3]
```

```
##          100049687-12-A/G 100049698-16-C/T 100049728-23-T/G
## AA010915                2                NA                0
## UC_00126                2                NA                0
## AA032760                NA                NA                0
```

Report the number of loci, individuals and populations

```
nLoc(gl)
```

```
## [1] 255
```

```
nInd(gl)
```

```
## [1] 250
```

```
nPop(gl)
```

```
## [1] 30
```

List the population labels (only the first 5)

```
levels(pop(gl))[1:5]
```

```
## [1] "EmmacBrisWive" "EmmacBurdMist" "EmmacBurnBara" "EmmacClarJack"
## [5] "EmmacClarYate"
```

To load your own data in, you need the csv file provided by DArT (e.g. mydata.csv) and (optional) an individual metadata file (e.g. my.metadata.csv).

```
gl <- gl.read.dart(filename="mydata.csv", covfilename = "my.metadata.csv")
```

6 Working with DArT Genlight Objects

Genlight objects for working with DArT genotypes have all of the general attributes described above, but also have some specific characteristics that derive from the DArT preparatory filtering and associated quality statistics. This is shown diagrammatically below.



AlleleID	CloneID	AlleleSequence	SNP	SNPPosition	CallRate	OneRatioRef	OneRatioSnp	FreqHomRef	FreqHomSnp	FreqHets	PICRef	PICSnp	AvgPIC	AvgCountRef	AvgCountSnp	RepAvg	AS	B5	C5	D5	E5
100049687	[20131003	TGCAGAAACA		12	0.98358	0.000982	0.999018	0.000982	0.999018	0	0.00196	0.00196	0.00196	22	11.32	1	0.01E+11	9.01E+11	9.01E+11	9.01E+11	9.01E+11
100049687	[20131003	TGCAGAAACA	12:A-G	12	0.98358	0.000982	0.999018	0.000982	0.999018	0	0.00196	0.00196	0.00196	22	11.32	1	1	1	1	1	1
100049698	[20131004	TGCAGAAACC		16	0.44928	0.993548	0.030108	0.969892	0.006452	0.02366	0.01282	0.0584	0.03561	9.3304	7.8125	1	-	-	-	-	-
100049698	[20131004	TGCAGAAACC	16:C-T	16	0.44928	0.993548	0.030108	0.969892	0.006452	0.02366	0.01282	0.0584	0.03561	9.3304	7.8125	1	-	-	-	-	-
100049728	[20131006	TGCAGAAAGC		23	1	0.999034	0.016425	0.983575	0.000966	0.01546	0.00193	0.03231	0.01712	30.7213	17.6	1	1	1	1	1	1
100049728	[20131006	TGCAGAAAGC	23:T-G	23	1	0.999034	0.016425	0.983575	0.000966	0.01546	0.00193	0.03231	0.01712	30.7213	17.6	1	0	0	0	0	0
100049805	[20131010	TGCAGAAATT		56	0.99324	1	0.000973	0.999027	0	0.00097	0	0.00194	0.00097	7.0721	5	1	1	1	1	1	1
100049805	[20131010	TGCAGAAATT	56:A-T	56	0.99324	1	0.000973	0.999027	0	0.00097	0	0.00194	0.00097	7.0721	5	1	0	0	0	0	0
100049816	[11357138	TGCAGAAATT		51	0.98647	0.610186	0.400588	0.599412	0.389814	0.01077	0.47572	0.48023	0.47798	12.0158	9.17901	0.98995	1	1	1	1	1
100049816	[11357138	TGCAGAAATT	51:C-T	51	0.98647	0.610186	0.400588	0.599412	0.389814	0.01077	0.47572	0.48023	0.47798	12.0158	9.17901	0.98995	0	0	0	0	0
100049839	[20131014	TGCAGAACAA		39	0.90725	0.001065	0.998935	0.001065	0.998935	0	0.00213	0.00213	0.00213	7	4.32989	1	-	-	-	-	-
100049839	[20131014	TGCAGAACAA	39:A-T	39	0.90725	0.001065	0.998935	0.001065	0.998935	0	0.00213	0.00213	0.00213	7	4.32989	1	-	-	-	-	-
100049926	[20131016	TGCAGAACTG		33	0.89952	0.9087	0.105263	0.894737	0.0913	0.01396	0.16593	0.18837	0.17715	6.90047	4.47899	0.99327	1	1	1	1	1
100049926	[20131016	TGCAGAACTG	33:C-T	33	0.89952	0.9087	0.105263	0.894737	0.0913	0.01396	0.16593	0.18837	0.17715	6.90047	4.47899	0.99327	0	0	0	0	0
100049990	[20131018	TGCAGAGAG		20	0.99614	0.974782	0.059166	0.940834	0.025218	0.03395	0.04917	0.11133	0.08025	7.90997	6	1	1	1	1	1	1
100049990	[20131018	TGCAGAGAG	20:G-T	20	0.99614	0.974782	0.059166	0.940834	0.025218	0.03395	0.04917	0.11133	0.08025	7.90997	6	1	0	0	0	0	0
100050079	[11357397	TGCAGAGGCG		57	0.97778	1	0.000988	0.999012	0	0.00099	0	0.00197	0.00099	8.67041	8	1	1	1	1	1	1

gl.read.dart()

Dataframe	
LOCUS METADATA	
AlleleID, CloneID, AlleleSequence, SNP, SnpPosition, CallRate, OneRatioRef, OneRatioSnp, FreqHomRef, FreqHomSnp, FreqHets, PICRef, PICSnp, AvgPIC, AvgCountRef, AvgCountSnp, RepAvg	
LOCI	
	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
AA010915	2 0 0 0 2 1 2 2 2 0 0 0 2 0 0 0 2 1 1 2 2 2 2 0 0 0 0 2 2 0 0 0 0 1
UC_00126	2 - 2 0 0 2 1 2 2 2 2 0 0 2 0 0 0 2 1 1 2 2 2 2 0 0 0 0 2 2 0 0 0
AA032760	0 0 - 0 - 2 0 0 2 1 2 2 2 0 0 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 0 2
AA013214	0 2 0 0 0 2 2 0 0 0 1 1 2 2 2 0 0 2 0 0 2 0 0 0 2 1 1 2 2 2 2 0 0
AA011723	0 2 2 2 0 0 2 1 2 2 2 0 0 2 0 0 0 2 1 1 2 2 2 2 0 0 0 0 2 2 0 0
AA012411	2 0 2 2 0 2 - 2 0 0 2 1 2 2 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 2 0 0
AA019237	2 0 2 0 0 2 1 2 2 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 0 2 2 0 0 2 0 0
AA019238	0 0 0 2 2 2 0 2 0 0 2 0 0 2 1 2 2 2 0 0 2 0 0 0 2 1 1 2 2 2 2 0
AA019239	0 2 0 0 0 - 0 - 2 0 0 2 1 2 2 2 0 0 2 0 0 0 2 1 1 2 2 2 2 0 0
AA019235	0 2 0 0 0 0 2 0 0 2 1 2 2 2 0 0 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0
AA019240	1 0 - 0 0 2 2 0 0 2 1 2 2 2 0 0 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 0
AA019241	2 0 2 2 0 0 2 0 0 2 1 2 2 2 0 0 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0
AA019242	0 0 0 2 2 0 0 2 1 2 2 2 0 0 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 2 2
AA019243	0 1 2 0 0 2 1 2 2 2 0 0 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 2 2 0 0
AA019251	0 0 2 0 2 0 0 2 1 2 2 2 0 0 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 2 2
AA019252	2 0 0 0 0 2 1 2 2 2 0 0 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 2 2 0 0
AA012405	2 - 0 1 0 0 2 0 2 0 0 2 1 2 2 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 2 0 0
AA012406	0 0 0 2 2 2 0 0 2 1 2 2 2 0 0 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 2
AA012409	0 0 2 0 2 2 0 0 2 1 2 2 2 0 0 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 2
AA012499	0 2 2 2 2 0 0 0 2 2 0 0 0 1 - - 2 0 0 0 2 2 0 0 0 1 - - 2 - 2
AA012422	1 2 0 0 2 1 2 2 2 0 0 2 0 0 0 2 1 1 2 2 2 2 0 0 0 0 2 2 0 0 0 0
AA012434	2 2 0 2 0 0 2 1 2 2 2 0 0 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 2 2 0
AA012469	0 0 0 2 2 2 0 0 2 0 0 2 1 2 2 2 0 0 2 0 0 2 1 1 2 2 2 2 0 0 0 0
AA012500	2 0 1 2 1 2 2 2 0 0 2 0 0 0 2 1 1 2 2 2 2 0 0 0 2 2 0 0 0 2 0 0 1
AA032799	2 0 0 2 2 2 1 2 0 0 2 0 0 0 2 0 2 2 2 2 1 1 0 0 0 2 2 0 0 0 0 1

Dataframe	
INDIVIDUAL METADATA	
Latitude Longitude Maturity Sex	

INDIVIDUALS

0	Homozygous reference allele
1	Heterozygous
2	Homozygous alternate allele
-	Missing

6.1 Locus metadata

The locus metadata included in the genlight object are those provided as part of your DArT report. These metadata are obtained from the DArT csv file when it is read in to the genlight object. The locus metadata are held in an R `data.frame` that is associated with the SNP data as part of the genlight object.

The locus metadata would typically include:

identifier	explanation
CloneID:	Unique identifier for the sequence in which the SNP marker occurs

identifier	explanation
SNP:	In 2-row format, this column is blank in the Reference row, and contains the base position and base variant details in the SNP row. In 1-row format, this column contains the base position and base variant details
SnpPosition:	The position (zero is position 1) in the sequence tag at which the defined SNP variant base occurs
TrimmedSequence	(optional) The sequence containing the SNP or SNPs, trimmed of adaptors.
CallRate:	The proportion of samples for which the genotype call is non-missing (that is, not “_”)
OneRatioRef:	The proportion of samples for which the genotype score is “1”, in the Reference allele row of the 2-row format.
OneRatioSnp:	The proportion of samples for which the genotype score is “1”, in the SNP allele row of the 2-row format
FreqHomRef:	The proportion of samples homozygous for the Reference allele
FreqHomSnp:	The proportion of samples homozygous for the Alternate (SNP) allele
FreqHets:	The proportion of samples which score as heterozygous.
PICRef:	The polymorphism information content (PIC) for the Reference allele row
PICSnp:	The polymorphism information content (PIC) for the SNP allele row
AvgPIC:	The average of the polymorphism information content (PIC) of the Reference and SNP allele rows
AvgCountRef:	The sum of the tag read counts for all samples, divided by the number of samples with non-zero tag read counts, for the Reference allele row
AvgCountSnp:	The sum of the tag read counts for all samples, divided by the number of samples with non-zero tag read counts, for the Alternate (SNP) allele row
RepAvg:	The proportion of technical replicate assay pairs for which the marker score is consistent.

These metadata variables are held in the `genlight` object as part of a `data.frame` called `loc.metrics`, which can be accessed in the following form:

```
#Only the entries for the first ten individuals are shown
gl@other$loc.metrics$RepAvg[1:10]
```

```
## [1] 1.000000 1.000000 1.000000 1.000000 0.989950 1.000000 0.993274
## [8] 1.000000 1.000000 0.980000
```

You can check the names of all available `loc.metrics` via:

```
names(gl@other$loc.metrics)

## [1] "AlleleID"      "SNP"           "SnpPosition"   "CallRate"      "OneRatioSnp"
## [6] "FreqHomRef"    "FreqHomSnp"    "FreqHets"      "AvgCountRef"   "AvgCountSnp"
## [11] "RepAvg"        "clone"         "uid"
```

Depending on the report from DarT you may have additional (fewer) `loc.metrics` (e.g. Trimmed Sequence is available on request).

These metadata are used by the `{dartR}` package for various purposes, so if any are missing from your dataset, then there will be some analyses that will not be possible. For example, TrimmedSequence is used to generate output for subsequent maximum likelihood phylogeny analyses that require estimates of base frequencies and transition and transversion ratios.

CloneID is essential (with its very special format), and `dartR` scripts for loading your data sets will terminate with an error message if this is not present.

6.2 Individual Metadata

Individual (=specimen) metadata are user specified, and do not come from DArT. Individual metadata are held in a second dataframe associated with the SNP data in the genlight object. See the figure above.

Two special individual metrics are:

individual metric	explanation
id	Unique identifier for the individual or specimen that links back to the physical sample
pop	A label for the biological population from which the individual was drawn

These metrics are supplied by the user by way of a metafile, provided at the time of inputting the SNP data to the genlight object. A metafile is a comma-delimited file, usually named ind_metrics.csv or similar, that contains labelled columns. The file must have a column headed id, which contains the individual (=specimen or sample labels) and a column headed pop, which contains the populations to which individuals are assigned.

These special metrics can be accessed a:

`gl@pop` or `pop(gl)`

and

`gl@ind.names` or `indNames(gl)`

A number of other user-defined metrics can be included in the metadata file. Examples of user-defined metadata for individuals include:

metric	explanation
sex	Sex of the individual (Male, Female)
maturity	Maturity of the individual (Adult, Subadult, juvenile)
lat	Latitude of the location of collection
long	Longitude of the location of collection

These optional data are provided by the user in the same metafile used to assign id labels and assign individuals to populations. It is the excel csv file referred to above.

The individual metadata are held in the genlight object as a dataframe named ind.metrics and can be accessed using the following form:

```
#only first 10 entries shows  
gl@other$ind.metrics$sex[1:10]
```

```
## [1] Male   Male   Male   Male   Unknown Male   Female  Female  
## [9] Male   Female  
## Levels: Female Male Unknown
```

6.3 Importing data from other formats/sources

In the table below we list possible avenues to load data from other formats and most importantly also from text simple files, which opens the package in principle to any other source.

Import path	Package	Pathway*	Description
gl.read.dart	dartR	—	based on DaRT data [with optional meta data for individuals]
read.loci	pegas	loci2genind, gi2gl	data set are provided as a csv text file (?read.loci)
read.vcfR	pegas	vcfR2genlight	vcf text file (vcfR package)
read.fstat	adegenet	gi2gl	Fstat format (version 2.9.3) by Jerome Goudet
read.genetix	adegenet	gi2gl	Format Belkhir K., Borsa P., Chikhi L., Raufaste N. & Bonhomme F. (1996-2004) GENETIX
read.structure	adegenet	gi2gl	Structure format of Pritchard, J.; Stephens, M. & Donnelly, P. (2000)
read.PLINK	adegenet	—	Data provided in PLINK format
fasta2genlight	adegenet	—	Extracts SNPs data from fasta format (?adegenet)
read.genetable	PopGenReport	gi2gl	csv text file based on df2genind Adamack & Gruber (2014) (?read.genetable)

*Pathway provides the order of functions needed to convert data to genlight, — indicates that the function directly converts to a **genlight** object

Below we exemplify an import from a text file. Provided with the package is a simple data set in a text file format (editable via Excel or Calc) or any text editor. To convert your data into a genlight object you can arrange your data set in the same format and also attach meta data for loci such as call rates, sequences and individuals (will be stored in the @other slot under), such as coordinates, lat/lons and population structure.

To have a look at the format in the provided file, type:

```
read.csv( paste(.libPaths()[1], "/dartR/extdata/platy.csv", sep="" ))
```

```
##      ind  pop      lat      long  group    age loci1 loci2 loci3 loci4
## 1  T158 Black -40.86642 145.2836 Female   juv   A/A   G/C   A/T   A/A
## 2  T306 Black -40.85589 145.2764   Male    Ad   A/A   G/G   A/A   A/A
## 3  T305 Black -40.87889 145.2885 Female    Ad   A/A   G/G   A/T   A/A
## 4  T148 Black -40.99193 145.3757   Male    Ad   A/A   G/G   A/A   A/A
## 5  T149 Black -40.99193 145.3757 Female    Ad   A/A   G/C   A/T   A/A
## 6  T106 Brid -41.23205 147.4597   Male    Ad   A/A   G/G   A/A   A/A
## 7  T107 Brid -41.23205 147.4597   Male    Ad   A/A   G/G   A/T   A/A
## 8  T110 Brid -41.23205 147.4597 Female    Ad   A/A   G/G   A/A   A/A
## 9  T111 Brid -41.23205 147.4597 Female    Ad   A/T   G/C   A/T   A/A
## 10 T308  Cam -41.09567 145.7958   Male  Sub-Ad A/T   C/C   A/A   A/A
## 11 T307  Cam -41.06975 145.8152   Male    Ad   A/T   C/C   A/T   A/A
## 12 T302  Cam -41.05121 145.8280   Male    Ad   T/T   G/C   A/A   A/A
## 13 T303  Cam -41.04764 145.8230 Female    Juv   T/T   C/C   A/T   A/A
##      loci5 loci6
## 1     G/C    T/A
## 2     G/C    T/A
## 3     G/C    T/A
## 4     G/C    T/A
```

```
## 5      G/C    T/A
## 6      G/G    T/A
## 7      G/G    T/A
## 8      G/G    T/A
## 9      G/G    T/A
## 10     G/G    T/A
## 11     C/C    T/A
## 12     C/C    T/T
## 13     C/C    A/A
```

We now have to specify each of the columns to be able to read it into R as a genind format using `read.genetable` from the `PopGenReport` package. For a detailed explanation see `?read.genetable`.

```
#you might need to install PopGenReport via
#install.packages("PopGenReport")
library(PopGenReport)
```

```
## Loading required package: knitr
```

```
## Loading required package: raster
```

```
## Loading required package: sp
```

```
platy <- read.genetable( paste(.libPaths()[1],"/dartR/extdata/platy.csv",
sep="" ), ind=1, pop=2, lat=3, long=4, other.min=5, other.max=6, oneColPerAll=FALSE,sep="/")

platy
```

```
## /// GENIND OBJECT ///////////
```

```
##
```

```
## // 13 individuals; 6 loci; 11 alleles; size: 12.8 Kb
```

```
##
```

```
## // Basic content
```

```
## @tab: 13 x 11 matrix of allele counts
```

```
## @loc.n.all: number of alleles per locus (range: 1-2)
```

```
## @loc.fac: locus factor for the 11 columns of @tab
```

```
## @all.names: list of allele names for each locus
```

```
## @ploidy: ploidy of each individual (range: 2-2)
```

```
## @type: codom
```

```
## @call: df2genind(X = genes, sep = sep, ncode = ncode, ind.names = as.character(inds),
```

```
## loc.names = colnames(genes), pop = as.character(pops), NA.char = NA.char,
```

```
## ploidy = ploidy)
```

```
##
```

```
## // Optional content
```

```
## @pop: population of each individual (group size range: 4-5)
```

```
## @other: a list containing: latlong data
```

To convert the genind to a genlight object simply type:

```
platy.gl <- (gi2gl(platy))
```

```
## Loading required package: parallel
```

The resulting genlight object has already some of the meta data as provided in the table but not in the right place (pop, indNames ploidy, loc.n.all and latlong in the “@other“ slot are already there).

For example to put the individual meta data (group, age) into the right slot in a genlight object we need to move it into the `@other$ind.metrans` slot.

```
platy.gl@other$ind.metrics <- platy.gl@other$data
```

If there are additional data available for the loci (e.g. via a csv file) we can also attach that via the `@other$loc.metrics` slot using `read.csv`. For demonstration we will create some random loci meta data with the code below and store it into a `data.frame` called `df.loc`. We create two columns: `TrimmedSequence` (containing the sequence data for each loci, trimmed to a consistent length, and removing the adaptors), and a quality index (ranging from zero to one indicating the reproducibility of repeated runs for that loci).

```
ts <- sapply(1:nLoc(platy.gl), function(x) paste(sample(c("A","T","G","C"), 50, replace = T),
                                                    collapse = ""))
df.loc <- data.frame(RepAvg = runif(nLoc(platy.gl)), TrimmedSequence=ts)

platy.gl@other$loc.metrics <- df.loc
```

The last line of code stores the loci metadata in the right location and we can use our filter and report function now on the genlight object as before.

```
gl.report.callrate(platy.gl)
```

```
## Reporting for a genlight object
## Note: Missing values most commonly arise from restriction site mutation.
##
##   Loci with no missing values = 6 [100%]
## [1] "Completed"
```

```
gl2 <- gl.filter.repavg(platy.gl, t=0.5)
```

```
## Reporting for a genlight object
## Note: RepAvg is a DArT statistic reporting reproducibility averaged across alleles for each locus.
##
## Initial no. of loci = 6
## No. of loci deleted = 2
## Summary of filtered dataset
##   Reproducibility >= 0.5
##   No. of loci: 4
##   No. of individuals: 13
##   No. of populations: 3
```

Some of the functions require additional meta data, e.g. the `gl2fasta` need sequence data (`TrimmedSequence`), the position of the SNP in the sequence for each loci `@position` and the type of alleles for each loci `@loc.all`. If such data is available the information needs to be stored in the correct slots to work with the function. The code below exports our genlight object to a fasta format (again we create random meta data via the code below).

```
platy.gl@position <- as.integer(runif(nLoc(platy.gl),2,49))
platy.gl@loc.all <- testset.gl@loc.all[1:6]
```

And finally we can use the `gl2fasta` function (using method 1, see explanation in `?gl2fasta`):

```
gl2fasta(platy.gl)
```

7 Subsetting and Recoding Data

7.1 Filtering

A range of filters are available for selecting individuals or loci on the basis of quality metrics.

function	explanation
<code>gl.report.callrate()</code>	Calculate and report the number of loci or individuals for which the call rate exceeds a range of thresholds.
<code>gl.filter.callrate()</code>	Calculate call rate (proportion with non-missing scores) for each locus or individual and remove those loci or individuals below a specified threshold.
<code>gl.report.repavg()</code>	Report the number of loci or individuals for which the reproducibility (averaged over the two allelic states) exceeds a range of thresholds.
<code>gl.filter.repavg()</code>	Remove those loci or individuals for which the reproducibility (averaged over the two allelic states) falls below a specified threshold.
<code>gl.report.dups()</code>	Report the number of sequence tags with multiple SNP loci, and the number of SNP loci that are part of or individuals for which the reproducibility (averaged over the two allelic states) exceeds a range of thresholds.
<code>gl.filter.dups()</code>	Remove all but one locus where there is more than one locus per sequence tag.
<code>gl.report.monomorphs()</code>	Report the number of monomorphic loci and the number of loci for which the scores are all missing (NA).
<code>gl.filter.monomorphs()</code>	Remove all monomorphic loci, including loci for which the scores are all missing (NA).
<code>gl.report.hamming()</code>	Report the distribution of pairwise Hamming distances between trimmed sequence tags.
<code>gl.filter.hamming()</code>	Filter loci by taking out one of a pair of loci with Hamming distances less than a threshold.
<code>gl.filter.hwe</code>	Filters departure of Hardy-Weinberg-Equilibrium for every loci per population or overall
<code>gl.report.hwe</code>	Reports departure of Hardy-Weinberg-Equilibrium for every loci per population or overall

Refer to the help on each function for details of the parameters taken by each of these functions using `?nameoffunction`.

7.2 Examples of dartR code to filter a `gl` dataset

1. Filter on call rate, threshold = at least 95% loci called

```
gl2 <- gl.filter.callrate(gl, method = "loc", threshold = 0.95)
```

```
## Reporting for a genlight object
## Note: Missing values most commonly arise from restriction site mutation.
##
## Initial no. of loci = 255
##   No. of loci deleted = 82
## Summary of filtered dataset
##   Call Rate > 0.95
##   No. of loci: 173
##   No. of individuals: 250
##   No. of populations: 30
```


2. Filter individuals on call rate (threshold =90%)

```
gl2 <- gl.filter.callrate(gl, method="ind", threshold = 0.90)
```

```
## Reporting for a genlight object
## Note: Missing values most commonly arise from restriction site mutation.
##
## Initial no. of individuals = 250
## Filtering a genlight object
##   no. of individuals deleted = 206
## Individuals retained = 44
## List of individuals deleted because of low call rate
##   AA010915 AA032760 AA011723 AA012411 AA019237 AA019238 AA019239 AA019235 AA019240 AA019241 AA019243
##   from populations
##   EmmacMDBForb EmmacMDBMaci EmmacBurnBara EmmacCoopEulb EmmacBurdMist EmmacBurdMist EmmacBurdMist EmmacBurdMist
## Summary of filtered dataset
##   Call Rate > 0.9
##   No. of loci: 255
##   No. of individuals: 44
##   No. of populations: 17
```

3. Filter on reproducibility, threshold (here called t, do not ask why) 100% reproducible

```
gl2 <- gl.filter.repavg(gl, t=1)
```

```
## Reporting for a genlight object
## Note: RepAvg is a DArT statistic reporting reproducibility averaged across alleles for each locus.
##
## Initial no. of loci = 255
## No. of loci deleted = 41
## Summary of filtered dataset
##   Reproducibility >= 1
##   No. of loci: 214
##   No. of individuals: 250
##   No. of populations: 30
```

4. Filter out multiple snps in single sequence tags (!!!!!produces an error currently!!!!)

5. Filter out monomorphic loci

```
gl2 <- gl.filter.monomorphs(gl, v=0)
```

6. Filter out loci with trimmed sequence tags that are too similar (possible paralogues). Only works if TrimmedSequence is available in the loci metadata, therefore we use another test data set here.

```
gl2 <- gl.filter.hamming(testset.gl, t=0.25, probar = F)
```

```
## Analysing a genlight object
## Hamming distance ranges from zero (sequence identity) to 1 (no bases shared at any position)
## Calculating pairwise Hamming distances between trimmed reference sequence tags
##
##
## Summary of filtered dataset
##   Initial No. of loci: 255
##   Hamming d > 0.25
##   Loci deleted 17
##   Final No. of loci: 238
##   No. of individuals: 250
```



```
## No. of populations: 30
```

Note: This filter and its accompanying report function is slow when there are many loci. Recommended that it be applied after all other filtering, and only if less than 20,000 loci remain. May require an overnight run.

Please note in the examples we always stored the resulting filter into a new `genlight` object `g12`, `g13` etc. Though it is a bit of a waste in terms of memory, it avoids confusion which filter you have already applied. A series of filter could then look like:

```
g12 <- gl.filter.callrate(g1, method = "loc", threshold = 0.95)
g13 <- gl.filter.callrate(g12, method="ind", threshold = 0.90)
g14 <- gl.filter.repavg(g13, t=1)
```

7.3 Population (=higher level grouping) reassignment

Recall that the metafile contains information assigned to each individual including, often at a minimum, population assignment. Population recode tables are csv files (comma delimited text files) that can be used to reassign individuals to different populations (groups), thus amalgamating populations or deleting populations from the `genlight` object.

The initial population assignments via the metafile can be viewed via:

```
#population names (#30 populations)
levels(pop(g1))
```

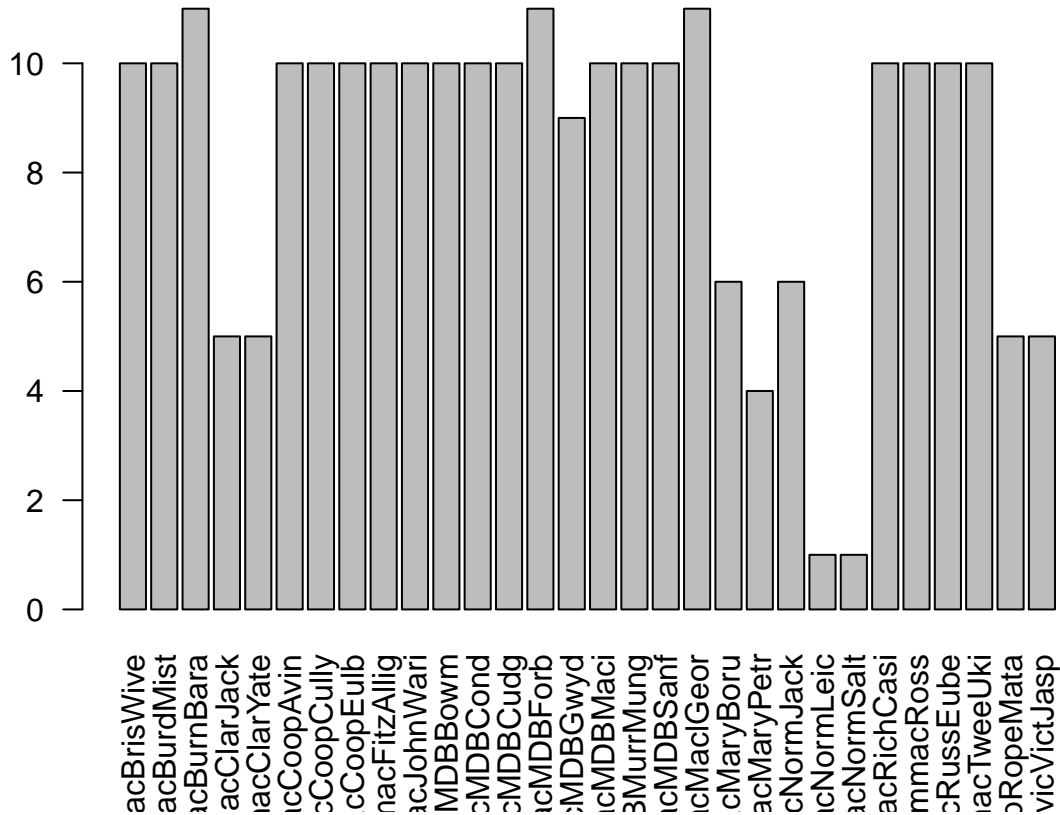
```
## [1] "EmmacBrisWive" "EmmacBurdMist" "EmmacBurnBara"
## [4] "EmmacClarJack" "EmmacClarYate" "EmmacCoopAvin"
## [7] "EmmacCoopCully" "EmmacCoopEulb" "EmmacFitzAllig"
## [10] "EmmacJohnWari" "EmmacMDBBowm" "EmmacMDBCond"
## [13] "EmmacMDBCudg" "EmmacMDBForb" "EmmacMDBGwyd"
## [16] "EmmacMDBMaci" "EmmacMDBMurrMung" "EmmacMDBSanf"
## [19] "EmmacMacIGeor" "EmmacMaryBoru" "EmmacMaryPetr"
## [22] "EmmacNormJack" "EmmacNormLeic" "EmmacNormSalt"
## [25] "EmmacRichCasi" "EmmacRoss" "EmmacRusseEube"
## [28] "EmmacTweeUki" "EmsubRopeMata" "EmvicVictJasp"
```

```
#table on individuals per population
table(pop(g1))
```

```
##
##   EmmacBrisWive  EmmacBurdMist  EmmacBurnBara  EmmacClarJack
##           10           10           11           5
##   EmmacClarYate  EmmacCoopAvin  EmmacCoopCully  EmmacCoopEulb
##           5           10           10           10
##   EmmacFitzAllig  EmmacJohnWari  EmmacMDBBowm  EmmacMDBCond
##           10           10           10           10
##   EmmacMDBCudg    EmmacMDBForb    EmmacMDBGwyd  EmmacMDBMaci
##           10           11           9           10
##   EmmacMDBMurrMung  EmmacMDBSanf  EmmacMacIGeor  EmmacMaryBoru
##           10           10           11           6
##   EmmacMaryPetr    EmmacNormJack  EmmacNormLeic  EmmacNormSalt
##           4           6           1           1
##   EmmacRichCasi      EmmacRoss    EmmacRusseEube  EmmacTweeUki
##           10           10           10           10
##   EmsubRopeMata    EmvicVictJasp
##           5           5
```

As a demonstration of the great ability of R it is easy to create a barplot on the number of individuals per population:

```
barplot(table(pop(gl)), las=2)
```



You can reassign individuals to new populations using a recode table. The quickest way to construct a recode table for an active genlight object is using

```
gl.make.recode.pop(gl, outfile = file.path(tempdir(), "new_pop_assignments.csv"))
```

Proforma recode table written to: C:\Users\s425824\AppData\Local\Temp\RtmpM1Pi2F/new_pop_assignments

```
## [1] "EmmacBrisWive"      "EmmacBurdMist"      "EmmacBurnBara"
## [4] "EmmacClarJack"      "EmmacClarYate"      "EmmacCoopAvin"
## [7] "EmmacCoopCully"     "EmmacCoopEulb"      "EmmacFitzAllig"
## [10] "EmmacJohnWari"      "EmmacMDBBowm"       "EmmacMDBCCond"
## [13] "EmmacMDBCudg"       "EmmacMDBForb"       "EmmacMDBGwyd"
## [16] "EmmacMDBMaci"       "EmmacMDBMurrMung"   "EmmacMDBSanf"
## [19] "EmmacMacIGeor"      "EmmacMaryBoru"      "EmmacMaryPetr"
## [22] "EmmacNormJack"      "EmmacNormLeic"      "EmmacNormSalt"
## [25] "EmmacRichCasi"      "EmmacRoss"           "EmmacRussEube"
## [28] "EmmacTweeUki"       "EmmacRopeMata"      "EmmacVictJasp"
```



Hint

Please note we are using the `tempdir()` to read/write files to a location in all examples. Feel free to change that to your needs by just providing a path to the folder of your liking.

This will generate a csv file with two columns, the first containing the existing population assignments, and the second also containing those assignments ready for editing. This editing is best done in Excel.

The population reassignments are then applied using:

```
glnew <- gl.recode.pop(gl, pop.recode=file.path(tempdir(),"new_pop_assignments.csv"))
```

You can check that the new assignments have been applied with:

```
levels(pop(gl))
```

```
## [1] "EmmacBrisWive" "EmmacBurdMist" "EmmacBurnBara"
## [4] "EmmacClarJack" "EmmacClarYate" "EmmacCoopAvin"
## [7] "EmmacCoopCully" "EmmacCoopEulb" "EmmacFitzAllig"
## [10] "EmmacJohnWari" "EmmacMDBBowm" "EmmacMDBCond"
## [13] "EmmacMDBCudg" "EmmacMDBForb" "EmmacMDBGwyd"
## [16] "EmmacMDBMaci" "EmmacMDBMurrMung" "EmmacMDBSanf"
## [19] "EmmacMacIGeor" "EmmacMaryBoru" "EmmacMaryPetr"
## [22] "EmmacNormJack" "EmmacNormLeic" "EmmacNormSalt"
## [25] "EmmacRichCasi" "EmmacRoss" "EmmacRussEube"
## [28] "EmmacTweeUki" "EmmacRopeMata" "EmmacVictJasp"
```



Task

Try this using commands in the R editor to create the comma-delimited recode file, edit in Excel to remove the Emmac prefix from populations, then apply it using the above command from the R editor. Check your results.

Another way of population reassignment, and probably the easiest, is to use:

```
glnew2 <- gl.edit.recode.pop(gl, pop.recode = file.path(tempdir(),"new_pop_assingments.csv"))
```

This command will bring up a window with a table showing the existing population assignments, with a second column available for editing. When the window is closed, the assignments will be applied. If you have optionally nominated a pop.recode file, a recode table will be written to file for future use. Again, you can check that the new assignments have been applied with `levels(pop(gl))`.

7.4 Deleting populations

You can delete selected populations from a genlight object using the “Delete” keyword in the population recode file. By reassigning populations to Delete, you are flagging them for deletion, and when the recode table is applied, individuals belonging to those populations will be deleted from the genlight object, and any resultant monomorphic loci will be removed.

Again, you can check that the new assignments have been applied and requested populations deleted with `levels(pop(gl))`.



Task

Try deleting some populations, say the outgroup populations (EmsubRopeMata and EmvicVictJasp) using `gl.edit.recode.pop()` from the R editor. Check your results.

7.5 Relabeling individuals

Recall that the `genlight` object contains labels for each individual. It obtains these names from the csv datafile provided by DArT at the time of reading these data in. There may be reasons for changing these individual labels – there may have been a mistake, or new names need to be provided in preparation for analyses to be included in publications.

Individual recode tables are csv files (comma delimited text files) that can be used to rename individuals in the `genlight` object or for deleting individuals from the `genlight` object. These population assignments can be viewed using

```
#only first 10 entries are shown  
indNames(gl)[1:10]
```

```
## [1] "AA010915" "UC_00126" "AA032760" "AA013214" "AA011723" "AA012411"  
## [7] "AA019237" "AA019238" "AA019239" "AA019235"
```

The quickest way to rename individuals in to construct a recode table for an active `genlight` object is using

```
gl.make.recode.ind(gl, outfile=file.path(tempdir(), "new_ind_assignments.csv"))
```

```
## Proforma recode table written to: C:\Users\s425824\AppData\Local\Temp\RtmpM1Pi2F/new_ind_assignments  
## [1] "AA010915" "UC_00126" "AA032760" "AA013214" "AA011723"  
## [6] "AA012411" "AA019237" "AA019238" "AA019239" "AA019235"  
## [11] "AA019240" "AA019241" "AA019242" "AA019243" "AA019251"  
## [16] "AA019252" "AA012405" "AA012406" "AA012409" "AA012499"  
## [21] "AA012422" "AA012434" "AA012469" "AA012500" "AA032799"  
## [26] "AA032826" "AA010795" "AA010796" "AA032800" "AA032801"  
## [31] "AA032808" "AA032809" "AA032811" "AA032812" "AA032822"  
## [36] "AA032825" "AA010797" "AA010752" "AA010754" "AA010756"  
## [41] "AA010798" "AA010799" "AA010800" "AA010802" "AA010803"  
## [46] "AA010804" "AA010809" "AA010749" "AA010758" "AA010763"  
## [51] "AA010765" "AA010771" "AA010772" "AA010781" "AA032762"  
## [56] "AA032763" "AA032756" "AA032757" "AA032758" "AA032761"  
## [61] "AA032765" "AA010931" "AA010937" "AA010940" "AA032764"  
## [66] "AA032768" "AA010936" "AA010909" "AA010916" "AA010917"  
## [71] "AA010920" "AA010921" "AA020651" "AA020652" "AA020667"  
## [76] "AA020669" "AA020655" "AA020656" "AA020644" "AA020645"  
## [81] "AA020646" "AA020649" "AA013203" "AA013217" "AA013220"  
## [86] "AA013202" "AA013225" "AA018496" "AA018497" "AA018513"  
## [91] "AA013231" "AA013261" "AA013265" "AA013270" "AA018492"  
## [96] "AA018493" "AA018494" "AA018495" "AA018514" "AA018515"  
## [101] "AA018516" "UC_00125" "UC_00126a" "UC_00146" "UC_00149"  
## [106] "AA018640" "AA018658" "AA011729" "UC_00132" "UC_00137"
```

```
## [111] "UC_00143" "UC_00157" "UC_00161" "AA018637" "AA018638"
## [116] "AA018639" "AA011731" "AA033576" "AA033577" "AA011732"
## [121] "AA011737" "AA011741" "AA011744" "AA011745" "AA011746"
## [126] "AA011749" "AA033575" "AA033578" "AA012411a" "AA033579"
## [131] "AA033582" "AA033593" "AA033602" "AA033609" "AA033617"
## [136] "AA010915a" "AA011723a" "AA019158" "AA020379" "UC_01044"
## [141] "AA018380" "AA018371" "AA004553" "AA000328" "AA000311"
## [146] "AA019159" "AA020378" "UC_01060" "AA018379" "AA018365"
## [151] "AA004554" "AA000303" "AA000320" "AA019160" "AA020377"
## [156] "UC_01053" "AA018375" "AA004555" "AA000304" "AA019165"
## [161] "AA019161" "AA020376" "UC_01062" "AA018374" "AA04523"
## [166] "AA000305" "AA019164" "AA020375" "AA018373" "AA032875"
## [171] "AA000309" "AA019163" "AA020374" "AA018368" "AA032878"
## [176] "AA000302" "AA019162" "AA020365" "UC_00150" "AA018369"
## [181] "AA004551" "AA032880" "AA000307" "AA019156" "AA020371"
## [186] "UC_01051" "AA018370" "AA004552" "AA032882" "AA000310"
## [191] "AA019157" "AA019075" "AA004864" "AA019071" "AA004868"
## [196] "AA019083" "AA019072" "AA004858" "AA004869" "AA019082"
## [201] "AA019073" "AA004859" "AA004866" "AA019077" "AA004860"
## [206] "AA019080" "AA004861" "AA019079" "AA004862" "AA019078"
## [211] "AA004863" "UC_00267" "UC_00205" "UC_00206" "UC_00208"
## [216] "UC_00243" "UC_00209" "UC_00254" "UC_00210" "UC_00259"
## [221] "UC_00126c" "AA063718" "AA063720" "AA063722" "AA063726"
## [226] "AA063732" "AA063708" "AA063710" "AA063712" "AA063714"
## [231] "AA063716" "AA020735" "AA032442" "AA032441" "AA020749"
## [236] "AA020746" "AA020744" "AA020743" "AA020739" "AA020738"
## [241] "AA001451" "AA01452" "AA001454" "AA001455" "AA001446"
## [246] "AA001456" "AA001447" "AA001448" "AA001449" "AA001450"
```

This will generate a csv file with two columns, the first containing the existing individual names, and the second also containing those names ready for editing. This editing is best done in Excel.

The population reassignments are then applied using

```
glnew3 <- gl.recode.ind(gl, ind.recode=file.path(tempdir(),"new_ind_assignments.csv"))
```

You can check that the new assignments have been applied with `indNames(gl)`

Another way of individual reassignment is to use

```
gl <- gl.edit.recode.ind(gl, ind.recode=file.path(tempdir(),"new_ind_assignments.csv"))
```

This command will bring up a window with a table showing the existing individual labels, with a second column available for editing. When the window is closed, the renaming will be applied. If you have optionally nominated a `ind.recode` file, a recode table will be written to file for future use. Again, you can check that the new assignments have been applied with `indNames(gl)`.

7.6 Deleting individuals

You can delete selected individuals from a genlight object using the “Delete” keyword in the individual recode file. By renaming individuals to Delete, you are flagging them for deletion, and when the recode table is applied, those individuals will be deleted from the genlight object, and any resultant monomorphic loci will be removed. Again, you can check that the new assignments have been applied and requested populations deleted with `indNames(gl)`.

7.7 Using R commands to manipulate the genlight object

With your data in a genlight object, you have the full capabilities of the adegenet package at your fingertips for subsetting your data, deleting SNP loci and individuals, selecting and deleting populations, and for recoding to amalgamate or split populations. Refer to the manual Analysing genome-wide SNP data using adegenet. For example:

```
gl_new <- gl[gl$pop!="EmmacBrisWive", ]
```

removes all individuals of the population EmmacBrisWive from the data set.

The basic idea is here that we can use the indexing function [] on the genlight object gl to subset our data set by individuals(=rows) and loci(=columns) in the same manner as we can subset a matrix in R.

For example:

```
glsub <- gl[1:7, 1:3]
glsub
```

```
## /// GENLIGHT OBJECT //////////
##
## // 7 genotypes, 3 binary SNPs, size: 107.8 Kb
## 8 (38.1 %) missing data
##
## // Basic content
##   @gen: list of 7 SNPbin
##   @ploidy: ploidy of each individual (range: 2-2)
##
## // Optional content
##   @ind.names: 7 individual labels
##   @loc.names: 3 locus labels
##   @loc.all: 3 alleles
##   @position: integer storing positions of the SNPs
##   @pop: population of each individual (group size range: 1-1)
##   @other: a list containing: loc.metrics latlong ind.metrics
```

Subsets the data to the first seven individuals and the first three loci.

!!!Be aware that the accompanying meta data for individuals are subsetted, but the metadata for loci are not!!!!. So if you check the dimensions of the meta data of the subsetted data set via:

```
dim(glsub@other$ind.metrics)
```

```
## [1] 7 6
```

```
dim(glsub@other$loc.metrics)
```

```
## [1] 255 13
```

you see that the subsetting of the meta data for individuals worked fine (we have seven individuals (=rows)). But we have still all the metadata for all loci (in the rows for the (=107 instead of 3). This “bug/feature” is how the adegenet package implemented the genlight object.

To take care for the correct filtering for loci and individuals we suggest therefore to use the following approach:

1. create an index for individuals (if you want to subset by individuals)
2. create an index for loci (if you want to subset by loci))

For example you want to have only individuals of two populations (“EmmacRussEube” or “EmvicVictJasp”) and 30 randomly selected loci you could type:

```
index.ind <- pop(gl)=="EmmacRussEube" | pop(gl)=="EmvicVictJasp"
#check if the index worked
table( pop(gl), index.ind)
```

```
##           index.ind
##           FALSE TRUE
##   EmmacBrisWive      10    0
##   EmmacBurdMist      10    0
##   EmmacBurnBara      11    0
##   EmmacClarJack       5    0
##   EmmacClarYate       5    0
##   EmmacCoopAvin      10    0
##   EmmacCoopCully      10    0
##   EmmacCoopEulb      10    0
##   EmmacFitzAllig      10    0
##   EmmacJohnWari      10    0
##   EmmacMDBBowm       10    0
##   EmmacMDBCond       10    0
##   EmmacMDBCudg       10    0
##   EmmacMDBForb       11    0
##   EmmacMDBGwyd        9    0
##   EmmacMDBMaci       10    0
##   EmmacMDBMurrMung    10    0
##   EmmacMDBSanf       10    0
##   EmmacMacIGeor      11    0
##   EmmacMaryBoru       6    0
##   EmmacMaryPetr       4    0
##   EmmacNormJack       6    0
##   EmmacNormLeic       1    0
##   EmmacNormSalt       1    0
##   EmmacRichCasi      10    0
##   EmmacRoss          10    0
##   EmmacRussEube       0    10
##   EmmacTweeUki       10    0
##   EmsubRopeMata       5    0
##   EmvicVictJasp       0    5
```

```
index.loc <- sample(nLoc(gl), 30, replace = F)
index.loc
```

```
## [1] 253 192 107 37 1 170 67 144 3 104 167 128 111 52 145 10 40
## [18] 15 98 38 22 80 50 17 171 184 57 247 149 41
```

and then

3. apply the indices to the genlight object and the meta data at the same time:

```
glsub2 <- gl[index.ind, index.loc]
glsub2@other$ind.metrics <- gl@other$ind.metrics[index.ind,] #not necessary
glsub2@other$loc.metrics <- gl@other$loc.metrics[index.loc,] #necessary
```

We can check the result via:

```
glsub2
```

```
## /// GENLIGHT OBJECT //////////
##
```

```
## // 15 genotypes, 30 binary SNPs, size: 105.3 Kb
## 57 (12.67 %) missing data
##
## // Basic content
##   @gen: list of 15 SNPbin
##   @ploidy: ploidy of each individual (range: 2-2)
##
## // Optional content
##   @ind.names: 15 individual labels
##   @loc.names: 30 locus labels
##   @loc.all: 30 alleles
##   @position: integer storing positions of the SNPs
##   @pop: population of each individual (group size range: 5-10)
##   @other: a list containing: loc.metrics latlong ind.metrics
dim(glsb2@other$ind.metrics)

## [1] 15 6
dim(glsb2@other$loc.metrics)

## [1] 30 13
```

For those not fully versed in R, there are the above {dartR} filters to achieve the same end and the advantage is that the filters do handle subsets of data correctly without any additional need to subset the meta data. The advantage of the R approach is that it is much more useful in case you want to script your analysis without intervention of a user when recoding your data set.

8 Genetic Distance

SNP data are multivariable data, in the sense that each individual (entity) has an allele profile (state) for each of several loci (attributes). It is a simple data matrix because SNPs are bi-allelic, that is, each locus can have one of two allelic states – aa, ab or bb, coded in a genlight object as 0, 1 or 2 respectively. One allele (the most common allele) is assigned to the reference allele, and the other to the alternate (or in DArT documentation, the SNP allele).

An obvious first choice in exploring the data is to construct a distance matrix between individuals based on the genetic profiles, or to construct a distance matrix between populations based on their allele frequency profiles.

There are a very many measures of genetic distance, but they collapse in number when applied to bi-allelic SNP data. For example, Rogers D and Euclidean D differ only by a constant multiplier when the data are biallelic. Distance matrices can be generated by a number of R packages, the most popular of which are `dist()` from package `stats` and `vegdist` from package `vegan`. The function `gl.dist` is a wrapper for those two functions, applying them to allele frequencies calculated for each locus at each population defined in the genlight object.

To calculate Euclidean distances (only for the first five individuals and the first 10 loci):

```
d <- gl.dist(gl[1:5,1:10])

## Using SNP data from a genlight object
## Tallying allele frequencies, this may take some time
## Calculation of allele frequencies complete
##
## Calculating distances: euclidean
## Refer to dist {stats} documentation for algorithm
```


d

```
##           EmmacBurnBara EmmacMDBForb EmmacMDBMaci EmmacMDBSanf
## EmmacBurnBara           0
## EmmacMDBForb           0           0
## EmmacMDBMaci           0           0           0
## EmmacMDBSanf           0           0           0           0
## EmmacMacIGeor          0           0           0           0
##           EmmacMacIGeor
## EmmacBurnBara
## EmmacMDBForb
## EmmacMDBMaci
## EmmacMDBSanf
## EmmacMacIGeor           0
```

Distances available for computation include:

method = “manhattan”, “euclidean”, “canberra”, “bray”, “kulczynski”, “jaccard”, “gower”, “altGower”, “morisita”, “horn”, “mountford”, “raup”, “binomial”, “chao”, “cao”, “mahalanobis”, “maximum”, “binary” or “minkowski”.

Refer to the documentation for functions `?dist` and `?vegdist` for computational formulae.

8.1 F Statistics

Several packages have already implemented the calculations of F statistics. Therefore we just provide different ways how to convert our data sets and use already existing packages:

For Fst and Neis Gst we recommend the use of functions of the StAMPP package as they allow for the use of parallel computing, which is much faster. It also works on genlight objects directly and therefore no conversion is necessary. For a more detailed explanation on options to achieve also confidence intervals via bootstrap refer to the StAMPP help pages) `stampFst` calculates pairwise Fst values between populations. (due to performance reasons we use only the first 30 individuals, which result in 8 populations :

```
library(StAMPP) #you may need to install the package
pwfst <- stampFst(gl[1:20,], nboots=1, percent=95, nclusters=1)
round(pwfst,3)
```

For Neis Gst use

```
pwGst <- stampNeisD(gl[1:20,]) #no parallel version :-(
round(pwGst,3)
```

For Jost’s D and G’st we suggest to use functions from the `mmmod` package. The disadvantage here is that it is much slower as we need to convert our data set from a `genlight` object and then use a non-parallel function from `mmmod` (depending on your computer the example will take some time).

```
library(mmmod) #you may need to install the package first
#for performance reason use only a subset (and recode the populations)
recpops <- factor(rep(LETTERS[1:5],50))
glsub <- gl
pop(glsub) <- recpops

gi <- gl2gi(glsub, probar = FALSE)
```

```
## Start conversion....
```

```
## Please note conversion of bigger data sets will take some time!
```

```
## Once finished, we recommend to save the object using >save(object, file="object.rdata")
##
## Matrix converted.. Prepare genind object...
## Finished! Took 1 seconds.
```

```
round(pairwise_D(gi),4)
```

```
##           A           B           C           D
## B -1e-04
## C  2e-04 -4e-04
## D  5e-04 -1e-04  2e-04
## E  0e+00 -4e-04 -1e-04  0e+00
```

```
round(pairwise_Gst_Hedrick(gi),4)
```

```
##           A           B           C           D
## B -0.0037
## C  0.0064 -0.0113
## D  0.0133 -0.0015  0.0064
## E -0.0011 -0.0118 -0.0028 -0.0014
```

```
round(pairwise_Gst_Nei(gi),4)
```

```
##           A           B           C           D
## B -0.0018
## C  0.0031 -0.0054
## D  0.0065 -0.0007  0.0031
## E -0.0005 -0.0057 -0.0014 -0.0007
```

9 Visualisation

Genetic similarity of individuals and populations can be visualized by way of Principal Coordinates Analysis (PCoA) ordination (Gower, 1966). Individuals (entities) are represented in a space defined by loci (attributes) with the position along each locus axis determined by genotype (0 for homozygous reference SNP, 2 for homozygous alternate SNP, and 1 for the heterozygous state). Alternatively, populations can be regarded as the entities to be plotted in a space defined by the loci, with the position along each locus axis determined by the relative frequency of the alternate allele.

Orthogonal linear combinations of the original axes are calculated and ordinated such that the first PCoA axis explains the most variation, PCoA-2 is orthogonal to PCoA-1 and explains the most residual variation, and so on. A scree plot of eigenvalues provides an indication of the number of informative axes to examine, viewed in the context of the average percentage variation explained by the original variables. The data are typically presented in two or three dimensions in which emergent structure in the data is evident.

9.1 PCoA in dartR

The script `gl.pcoa()` is essentially a wrapper for `glPca()` of package `ade4` with default settings apart from setting `parallel=FALSE`, converting the eigenvalues to percentages and some additional diagnostics.

```
pc <- gl.pcoa(gl, nfactors=5)
```

```
## Performing a PCoA, individuals as entities, SNP loci as attributes
## Ordination yielded 14 informative dimensions from 249 original dimensions
## PCoA Axis 1 explains 23.3 % of the total variance
## PCoA Axis 1 and 2 combined explain 42.8 % of the total variance
```

```
## PCoA Axis 1-3 combined explain 54.4 % of the total variance
```

The resultant object `pc` contains the eigenvalues, factor scores and factor loadings that can be accessed for subsequent analyses.

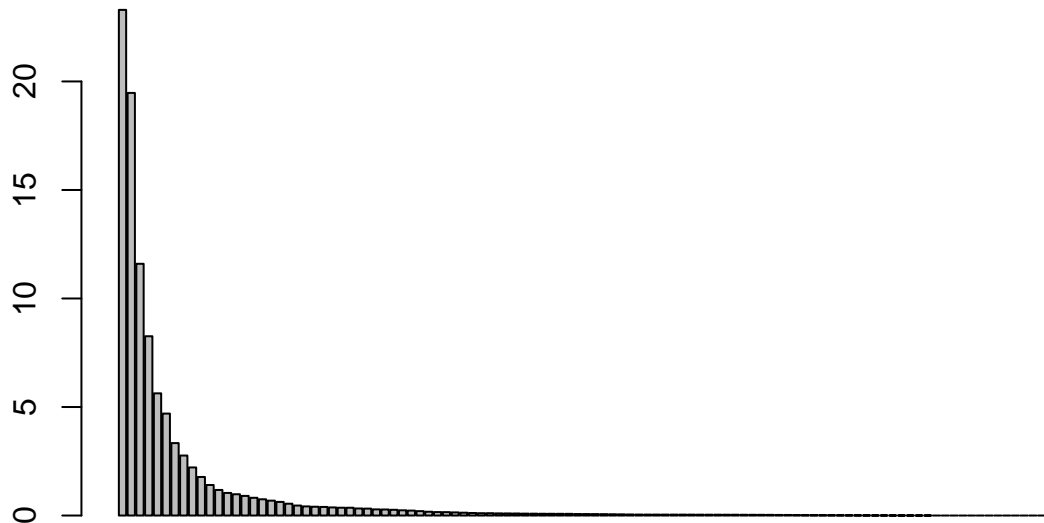
```
names(pc)
```

```
## [1] "eig"      "scores"   "loadings" "call"
```

The eigenvalues give the scaling factor for the eigenvectors (PCoA axis 1 ... n), the scores give the coordinates of the points (the entities, be they individuals or populations) in the new ordinated space, and the loadings give the correlations of the original variables (the loci) against the new axes. Loci that load high on axis 1 are influential in discrimination among the entities in the direction of axis 1.

For example the percentage of variation the is represented by the axes can be calculated and visualised via:

```
barplot(pc$eig/sum(pc$eig)*100, )
```



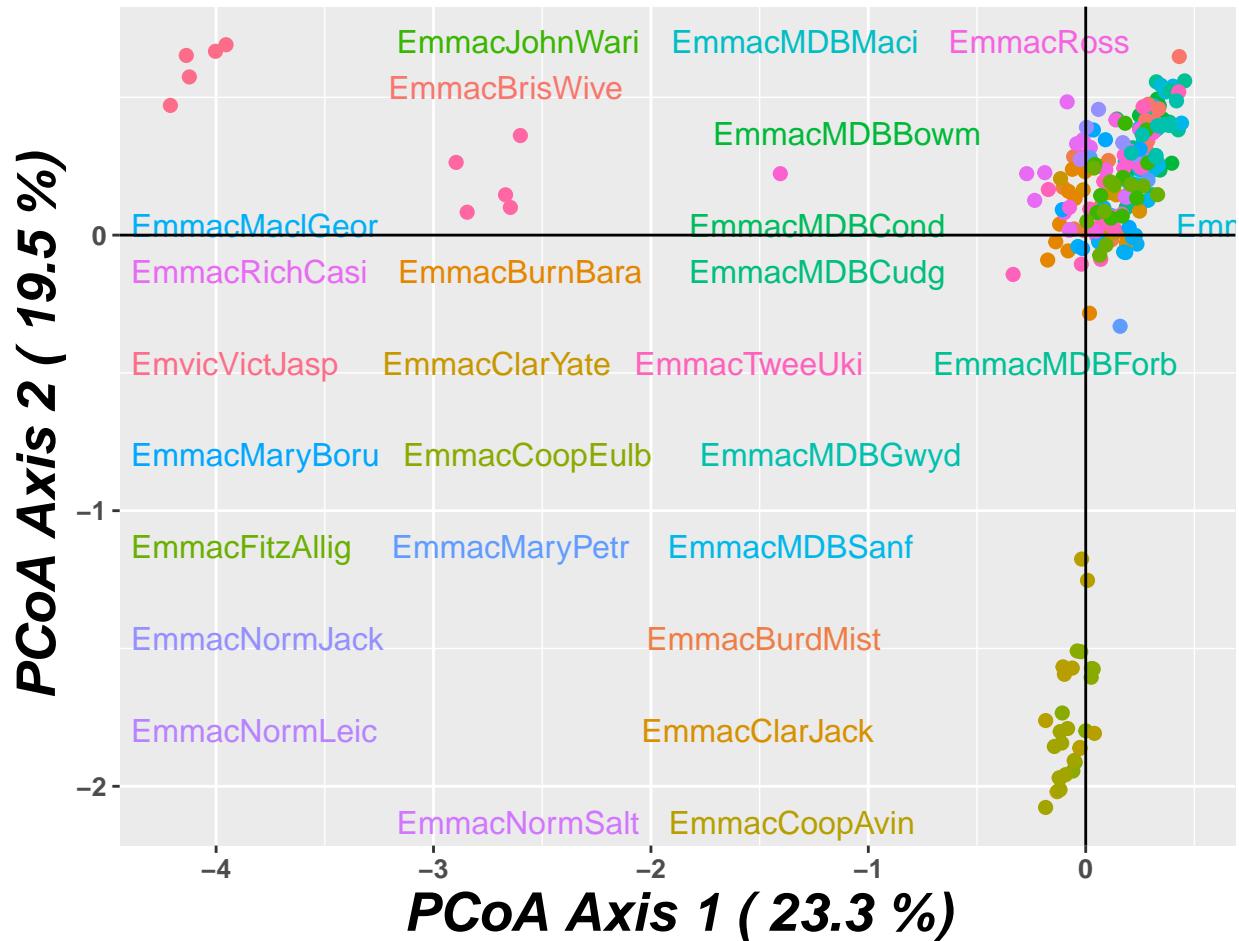
9.2 Plotting the results of PCoA

The results of the PCoA can be plotted using `gl.pcoa.plot()` with a limited range of options. The script is essentially a wrapper for `plot {ggplot2}` with the added functionality of `{directlabels}` and `{plotly}`.

The plotting script is not intended to produce publication quality plots, but should form a basis for importing the plots to illustrator for subsequent amendment. The command

```
gl.pcoa.plot(pc, gl, labels="pop", xaxis=1, yaxis=2)
```

```
## Plotting populations
```



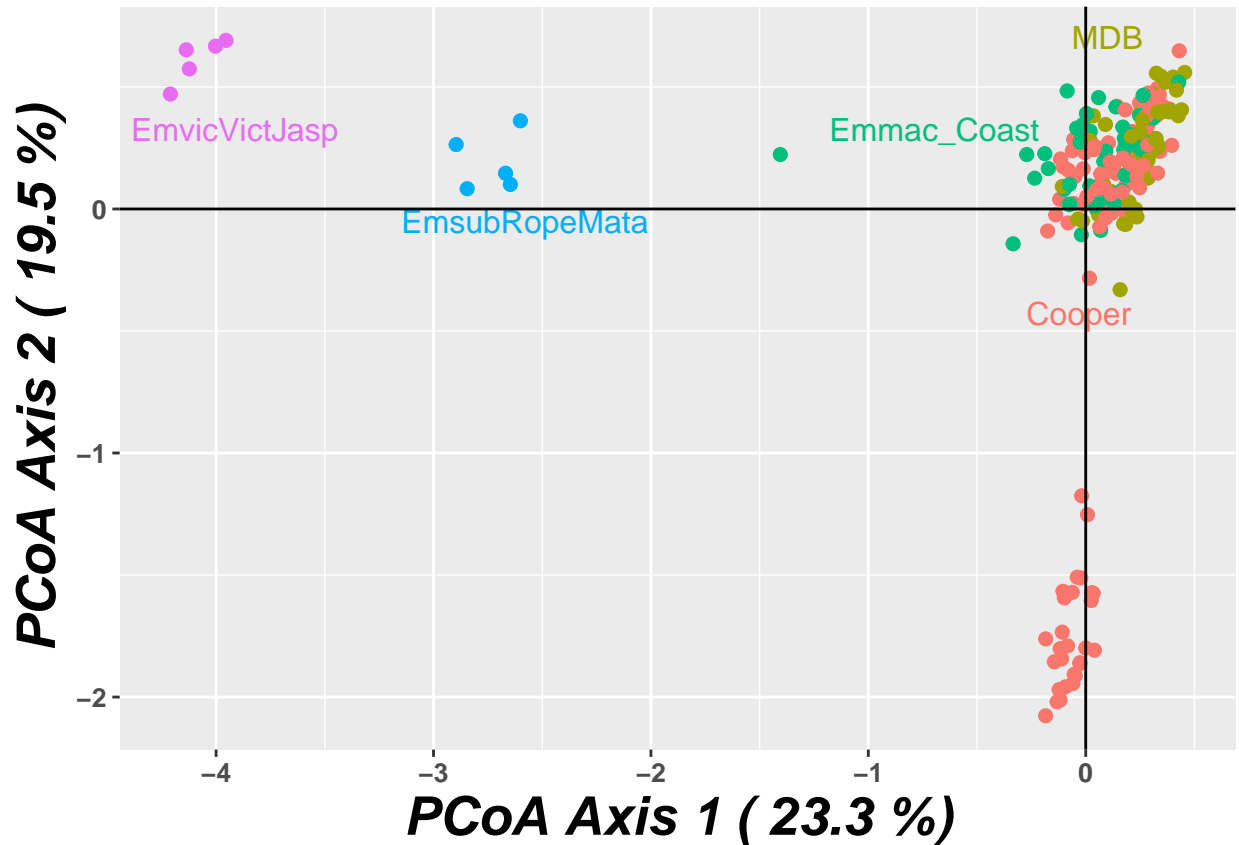
You can see that this plot is very busy, and that the many labels are displaced quite some distance from their associated points. This is because there is a tradeoff between avoiding overlap of the labels and proximity of the labels – you can use colour to identify which labels go with which points. More sensibly, recoding populations would be in order. We could use

```
glnew <- gl.edit.recode.pop(gl)
```

or using R

```
glnew <- gl
levels(pop(glnew)) <- c(rep("Cooper",13), rep("MDB", 8 ), rep("Emmac_Coast",7),"EmsubRopeMata" , "Emvi
gl.pcoa.plot(pc, glnew, labels="pop", xaxis=1, yaxis=2)
```

```
## Plotting populations
```



Note that we did not need to re run the PCoA analysis, only to recode the pop labels in the genlight object that we hand to the plotting routine. Much clearer plot now.

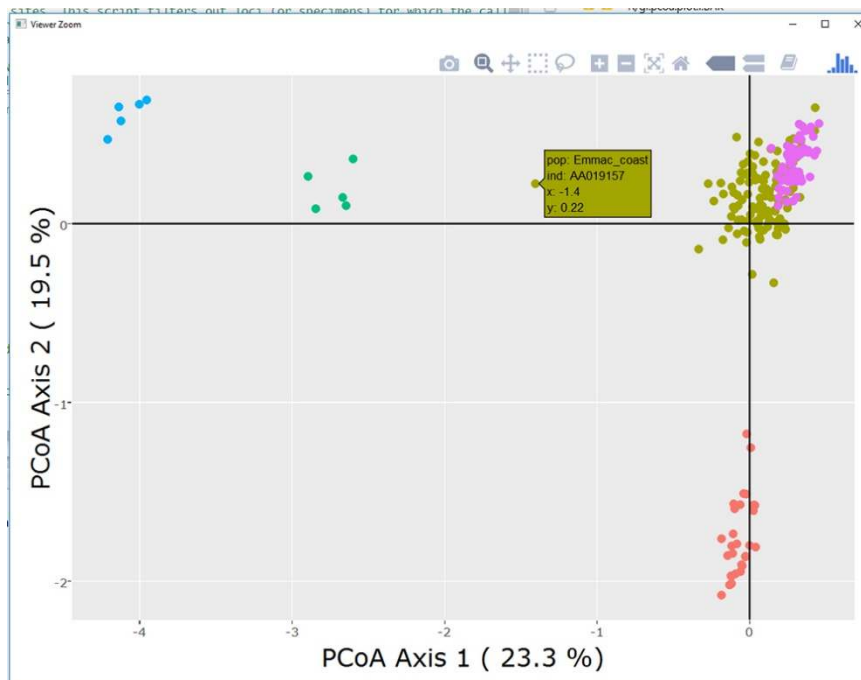
There are other options for `gl.pcoa.plot()` that allow the axes to be scaled on the basis of proportion of variation explained, to select other combinations of axes to plot, and for adding confidence ellipses. Use the R help facility to explore these additional options.

Note that there is one point that seems intermediate between *Emydura macquarii* from the coast, and *Emydura subglobosa* (from northern Australia west of the Great Dividing Range). How do we find out what individual that point represents? Replot the data using `labels="interactive"` to prime the plot for analysis using `ggplotly {plotly}`:

The commands

```
gl.pcoa.plot(pc, gl.new, labels="interactive", xaxis=1, yaxis=2)
ggplotly()
```

will plot the individuals in the top two dimensions of the ordinated space, colour the points in accordance to the population to which they belong, and allow points to be identified interactively using the mouse.



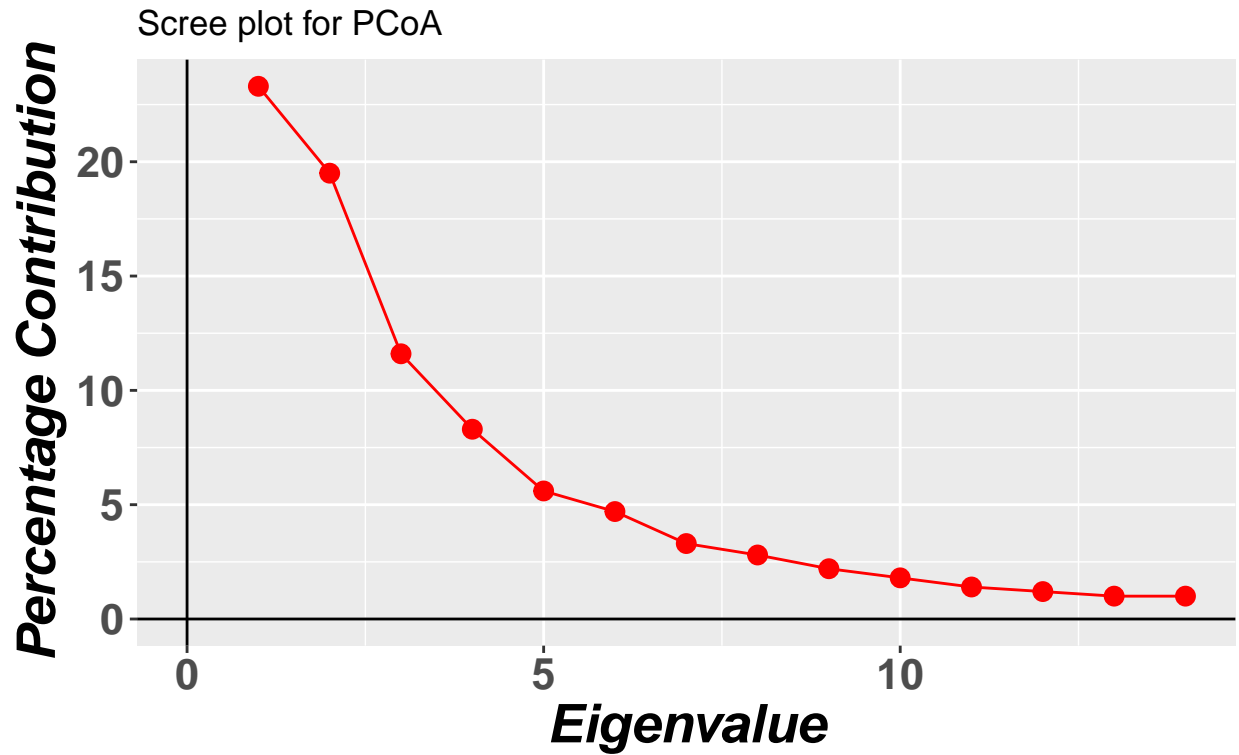
Now moving the mouse over the point reveals its identity. The animal is AA19157, from the coastal populations, and further scrutiny reveals it is from the Barron River in northern Queensland. Seems there has been some allelic exchange there.

9.3 The Scree Plot

The number of dimensions with substantive information content can be determined by examining a scree plot (Cattell, 1966).

```
gl.pcoa.scree(pc)
```

```
## Note: Only eigenvalues for dimensions that explain more than the average of the original variables are shown.
## No. of axes each explaining 10% or more of total variation: 3
```

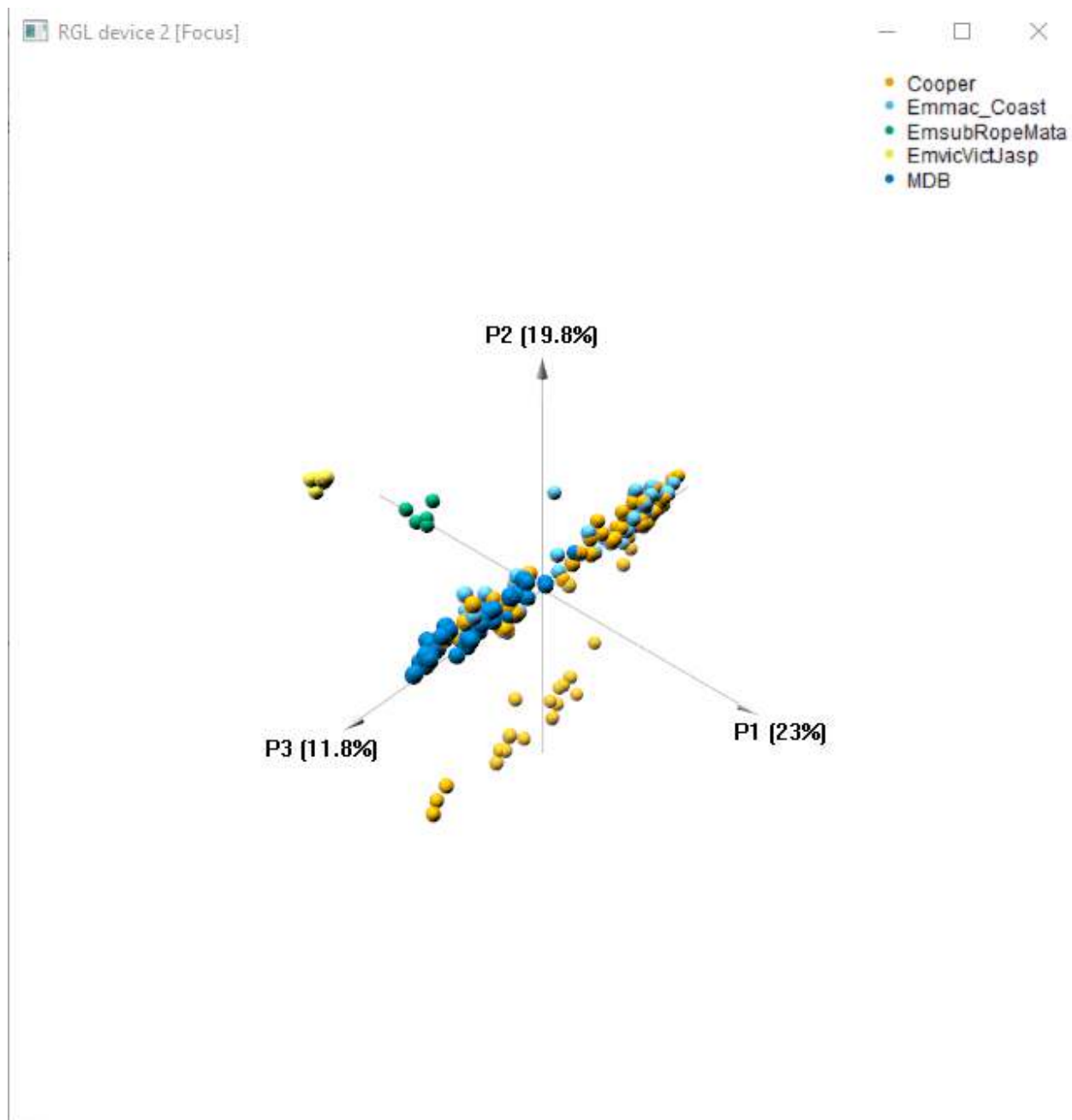


This plot, by default, will show the percentage variation in the data explained by each axis successively where the amount of variation is substantive. By substantive, I mean explaining more than the original variables did on average. As a rule of thumb, one should examine all dimensions that explain more than 10% of the variation in the data.

9.4 3D Plot

Should you find that 2 dimensions are insufficient to capture all substantive variation, you can examine a plot of PCoA axis 2 against axis 1 and axis 3 against axis 1 and so on, taking care to note the proportion of variation explained by each axis. Alternatively, when the data cluster tightly, additional dimensions can be examined by removing all individuals from the analysis except those belonging to a single cluster and re-running the PCoA (Georges and Adams, 1992). If three dimensions are indicated by the scree plot, as in our current case, an interactive 3D plot can be produced

```
gl.pcoa.plot.3d(pc, glnew)
```



Note that the plot appears in a new window, outside R Studio, and that it is interactive in the sense that you can rotate the plot using the mouse to obtain the most discriminatory view.

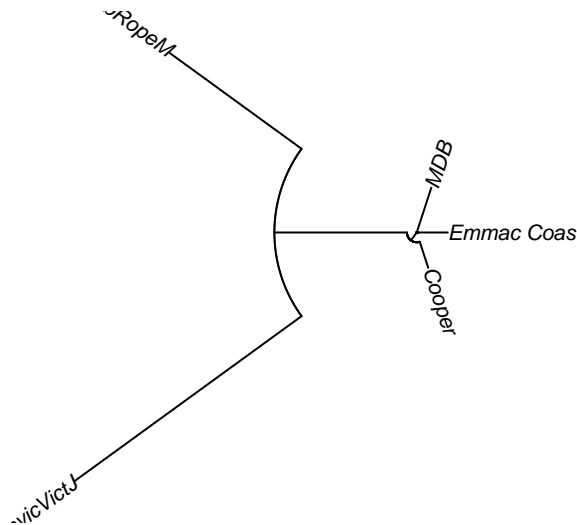
This function is essentially a wrapper for the corresponding function in `{pca3d}`, adding percentage variation explained to each axis and fixing some parameters.

9.5 Neighbour-joining trees

Another way of visualizing genetic similarity among entities is to construct a phenetic tree using a neighbor-joining approach or UPGMA. This script is essentially a wrapper for `nj {ape}` applied to Euclidean distances between OTUs.


```
gl.tree.nj(glnew, type="fan")
```

```
## Converting to a matrix of frequencies, locus by populations
## Computing Euclidean distances
```



```
##
## Phylogenetic tree with 5 tips and 3 internal nodes.
##
## Tip labels:
## [1] "Cooper"      "MDB"          "Emmac_Coas"  "EmsubRopeM"  "EmvicVictJ"
##
## Unrooted; includes branch lengths.
```

10 Fixed Difference Analysis

The PCoA approach outlined above considers allele frequency differences between individuals or populations as a basis for constructing a distance matrix which is then used by the PCoA algorithms to execute the ordination. There is some advantage in considering only fixed differences between populations, that is, allelic differences where the alleles have come to fixation to alternative states in populations taken pairwise.

A fixed difference between two populations at a specific locus occurs when the population share no alleles at that locus. Gene frequencies may ebb and wane, but once a locus becomes fixed for an allele or suite of alleles, there is no returning. The accumulation of fixed differences between two populations is considered a robust indication of lack of gene flow. In a nutshell, fixed differences are summed over populations taken pairwise, and when two populations have no fixed differences (or insubstantial fixed differences), the populations are

amalgamated and the process repeated until there is no further reduction (Georges and Adams, 1996). The final set of taxa are diagnosable by the presence or absence of a set of alleles at multiple loci. The script

```
gl.collapse.recursive(gl, t=0)
```

will ultimately yield a grouping of aggregate populations that are diagnosable from each other by one or more fixed allelic differences. Here is the output, abridged: Let's refresh the data again, to bring back in the outgroups, then run the analysis.

A full series of distance matrices and associated recode tables have been filed to disk for later interrogation and use.

The outcome is unequivocal. All of the coastal *Emydura macquarii* and those from the Murray-Darling basin amalgamate into one OTU on the basis of no fixed differences between them (Group 1.1). The Cooper Creek animals form a separate diagnosable unit (OTU) (Group 1.2). The populations that did not amalgamate – outgroup taxa *Emydura victoriae* and *Emydura subglobosa* – remain well supported.

You can see the power of this approach for defining diagnosable taxa, taxa that are free of contemporary or recent geneflow and so on independent evolutionary trajectories.

One challenge to this approach stems from low sample sizes. The probability of all alleles in one such population having one allelic state, say the homozygous reference allele 0/0 (2-row format), and all the individuals in the other population having the alternate allelic state, homozygous 1/1, by chance alone depends on the allelic frequency in the parent populations and on the sample size ($2n$ for diploids).



Hint

The consequence of low sample sizes is to generate spurious fixed differences, that is, fixed differences arising via sampling error. The probability of such errors compounds as more and more SNP loci are examined

When the number of loci examined gets up into the 10s of thousands and the sample sizes are small (say < 5 individuals per population), the probability of a spurious fixed difference between two populations rises to unacceptable levels. To manage this, it is wise to ensure that all populations are sampled with 10 or more individuals ($2n=20$), whereby the probability of generating spurious fixed differences is vanishingly small. The compounded probability of one spurious fixed difference in 100,000 loci is complicated to calculate without detailed knowledge of parental allele frequencies, but remains manageably small for $n=10$.

11 Isolation by distance plot

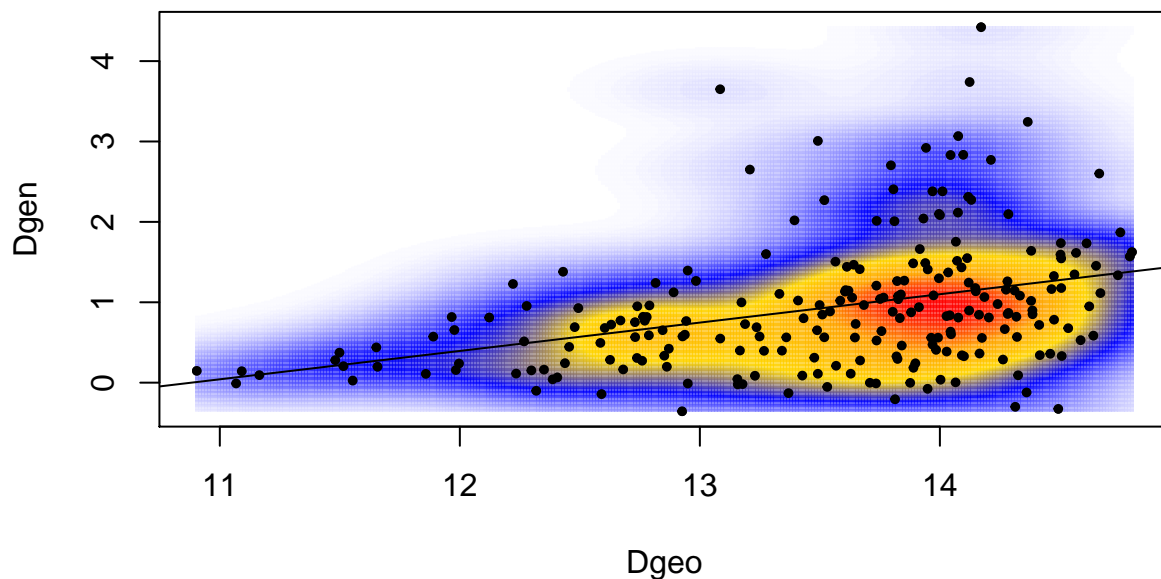
This functions performs an isolation by distance analysis based on a mantel test and also produces an isolation by distance plot. If a `genlight` object with coordinates (in the `@other$latlong` slot) is provided, then a Euclidean and genetic distance matrix are calculated (currently only pairwise F_{st} between population is implemented as default). Coordinates are expected as lat long and converted to Google Earth Mercator projections to avoid distortions. If coordinates are already projected in a suitable coordinate system, you can set `projected=TRUE` and no projection is applied. An isolation by distance analysis will be carried out and a plot will be returned, based on $\log(\text{Euclidean distance})$ against between population pairwise $F_{st}/1-F_{st}$ (see Rousseau's distance measure. *Genetics* April 1, 1997 vol. 145 no. 4 1219-1228) You can also provide your own genetic and Euclidean distance matrix, then simply the mantel and isolation by distance plot will be returned. The function is based on code provided by the adegenet tutorial (<http://adegenet.r-forge.r-project.org/files/tutorial-basics.pdf>), using the functions `mantel` (package `vegan`), `stamppFst` (package `StAMPP`) and `Mercator` (package `dismo`).

An example run (using only a subset of the test data):

```
gl <- gl.ibd(gl=testset.gl[1:180,])

## Standard analysis performed on the genlight object. Mantel test and plot will be Fst/1-Fst versus log
## Coordinates transformed to Mercator (google) projection to calculate distances in meters.
##
## Mantel statistic based on Pearson's product-moment correlation
##
## Call:
## mantel(xdis = Dgen, ydis = Dgeo, permutations = 999, na.rm = TRUE)
##
## Mantel statistic r: 0.3655
##      Significance: 0.001
##
## Upper quantiles of permutations (null model):
##   90%   95%  97.5%  99%
## 0.140 0.170 0.189 0.214
## Permutation: free
## Number of permutations: 999
```

Isolation by distance



12 Phylogenetic Analysis

The objective of phylogenetic analysis is to extract relationships between taxonomic entities, be they species, evolutionarily significant units (ESUs) or other diagnosable units (Felsenstein, 2004; Swofford and Berlocher, 1987). The goal is thus to extract the pattern of ancestry and descent among such taxonomic entities (call them operational taxonomic units or OTUs). The OTUs need to be diagnosable because one assumes that differences among them reflect divergence through time, unobscured by contemporary or recent tokogenic exchange. That is, they are considered to be on evolutionary trajectories that are independent by virtue of

reproductive or long-standing geographic isolation.

The true evolutionary history of the OTUs is in the form of a bifurcating tree, that is, the true divergences among OTUs satisfy both the conditions of a metric and the four-point condition (Buneman, 1973). Metric is used here in the sense that, given the positions of two OTUs to represent the distance between them, the distances to a third OTU uniquely defines its position. The four-point condition is used here in the sense that for any four OTUs, there exists a simple tree accurately depicting the distances between them (that is, there exists a non-negative internal branch). A set of OTUs and pairwise distances among them that satisfy the metric and four-point conditions will define a unique bifurcating tree.

In practice, homoplasy obscures the true phylogeny by distorting measures of genetic distance between taxa so that they no longer meet the metric and four-point criteria. The challenge becomes to estimate the most parsimonious (least steps), most likely (maximum likelihood) or the best-bet (Bayesian estimate) tree in terms of consistency with available data. Because information contained in the genetic code can be over-written by new mutations, the true evolutionary history may not be recoverable even with the most comprehensive of contemporary data. Instead, the exercise is to generate the phylogeny most consistent with available data, and use this solution as the best available hypotheses for future testing, in whole or in part, as new data or new methods of analyzing those data come to hand.

There are a number of approaches and a large range of software packages for recovering phylogenies which are not covered here (Felsenstein, 1989; Swofford, 2002). The objective of `{dartR}` is to generate files in a format that can be read into the packages of choice. We export to fastA format.

12.1 Distance Methods

Under ideal conditions, the true phylogeny can be uniquely recovered from the true measures of divergence between OTUs. So one approach to recovering phylogenies is to extract the phylogeny that is most consistent with the estimated distances between OTUs. To avoid introducing artificial departure of the distances from the underlying tree, a metric distance needs to be chosen, and for SNP datasets we choose Euclidean Distance. This differs from Rogers D (Rogers, 1972) by a constant multiplier, and so the two measures are essentially the same.

The script for distance phylogeny is `gl2phylip()` which calculates Euclidean distances using `dist {stats}` then outputs the data in a form suitable for input to the Phylip package written by Joseph Felsenstein (<http://evolution.genetics.washington.edu/phylip.html>) (Felsenstein, 1989). The input file can include replicated distance matrices for the purpose of bootstrapping.

Assuming the data have been appropriately filtered before saving, the commands to do this are

```
gl <- testset.gl
phy <- gl2phylip(gl, outfile="turtle.phy", bstrap=1000)
```

The output file, `turtle.phy` or whatever you decide to call it, is available for input to Phylip and the variety of commands for handling distance matrices (e.g. `fitch`). Refer to the Phylip documentation.

12.2 Character-based Methods

As with allozyme data sets of the past, SNP datasets present particular challenges for phylogenetic analysis. The challenges arise because of difficulty in handling heterozygotes. Individuals homozygous for the reference state (0) or homozygous for the alternate state (2) are unambiguous in their character state, but heterozygotes (1) present both character states.

12.3 Converting Diploid to Haploid

One way of overcoming this is to randomly allocate the SNP state for heterozygous loci to one or the other homozygous states, that is, to add background noise to the data from which the phylogenetic signal can still be extracted without introducing a systematic bias. We convert diplotypes to haplotypes amenable to phylogenetic analysis with `gl2fasta()` using method 1 to 4 (see `?gl2fasta` for an explanation of the various methods available)

The way to do this is use the command:

```
gl2fasta(gl, method=2, outfile="nohets.fasta")
```

concatenates the sequences of the DNA fragments, trimmed of adaptors, into a fastA file, having first randomly allocated the heterozygous states. This set of “composite haplotypes” is then suitable for analysis using the diverse range of phylogenetic packages available. The reason for including the full trimmed sequences, most of the bases of which are monomorphic across individuals, is to allow for application of various mutational models in likelihood analysis. These require estimates of base frequencies and the frequency of transitions and transversions and the software to generate these estimates and factor them in to the phylogenetic analysis (e.g. ModelTest (Posada and Crandall 1998) require the full sequence information.

The base frequencies and transition and transversion ratios can in any case be obtained using

```
gl.report.bases(testset.gl)
```

```
## Key variable TrimmedSequence found.
##
## Average trimmed sequence length: 51 ( 20 to 69 )
## Total number of trimmed sequences: 255
## Base frequencies (%)
##   A: 27.83
##   G: 24.71
##   T: 26.18
##   C: 21.29
##
## Transitions   : 52.55
## Transversions: 47.45
## tv/ts ratio: 1.1074
##
##      col1 col2
## [1,] "A"  "27.83"
## [2,] "G"  "24.71"
## [3,] "T"  "26.18"
## [4,] "C"  "21.29"
## [5,] "tv" "47.45"
## [6,] "ts" "52.55"
```

Monomorphic sites are parsimony uninformative, so a more compact output fastA file, for parsimony analyses, can be generated with

```
gl2fasta(gl, method=4, outfile="nohets.fasta")
```

12.4 Using Ambiguity Codes

Another common approach is to output the concatenated sequences using ambiguity codes for the heterozygous SNP bases. This can be done with

```
gl2fasta(gl, method=1, outfile="ambcodes.fasta")
```

Maximum likelihood packages like RAXML cater for the ambiguities by adjusting tip likelihoods as described by Felsenstein (2004:255). Again, it is possible to output only the variable sites to the fastA file.

```
gl2fasta(gl, method=3, outfile="ambcodes.fasta")
```

These data can be used in some Maximum Likelihood software by providing base frequencies and transition and transversion ratios separately. They can be calculated with

```
gl.report.bases(testset.gl)
```

```
## Key variable TrimmedSequence found.
##
## Average trimmed sequence length: 51 ( 20 to 69 )
## Total number of trimmed sequences: 255
## Base frequencies (%)
##   A: 27.83
##   G: 24.71
##   T: 26.18
##   C: 21.29
##
## Transitions   : 52.55
## Transversions: 47.45
## tv/ts ratio: 1.1074
##
##      col1 col2
## [1,] "A"  "27.83"
## [2,] "G"  "24.71"
## [3,] "T"  "26.18"
## [4,] "C"  "21.29"
## [5,] "tv" "47.45"
## [6,] "ts" "52.55"
```

13 Population Assignment

Assigning individuals of unknown provenance to populations of known provenance is a challenging exercise, and several approaches have been suggested. Perhaps the simplest is to calculate the probabilities of yielding the observed genotype of the unknown individual given the observed allele frequencies in each the target population. Using this approach, the individual is assigned notionally to those populations for which this probability is highest; populations for which the probability is lower than some level of significance are eliminated from further consideration. This approach was first applied in a study of microsatellite markers in bear populations (Paetkau et al. 2004) and subsequently applied using classical and Bayesian approaches to estimating probabilities (Götz and Thaller 1998; Blanchong et al. 2002).

Unfortunately, the sheer number of SNPs generated by next generation sequencing technologies, often in the 10s or 100s of thousands, makes the assumption of independence of the loci untenable. Linkage is the problem and for most organisms, there is insufficient genomic knowledge to overcome this directly. Similarly, the sample sizes used in studies of population assignment are typically small, and inappropriate for tests of sufficient power to identify loci that can be regarded as independent. Lack of independence is a problem computationally because it is a prerequisite for combining the probabilities (or likelihoods) of the observed genotype at each locus as a product (or by summation of log values) to yield an overall probability of assignment for the unknown genotype. We have taken an alternate approach.

The approach taken here is to first eliminate from consideration those target populations where a SNP allele

is present in the unknown individual but not in the target. When the unknown individual possesses such a private allele, the target population is unlikely to be the source population. This analysis can be done with script `gl.report.pa`. The analysis is also undertaken by `gl.assign`.

In many cases, examining private alleles will narrow down the possible source populations considerably, and depending on the spatial resolution required for the assignment (say, Australia or New Guinea), may provide a satisfactory answer.

A second approach is to examine the position of the unknown individual relative to the target populations in a reduced ordinated locus space using PCoA. This graphic representation is provided by `gl.assign()`. Addition of confidence ellipses then allows a decision to eliminate some populations from consideration as the source of the unknown individual.

The converse is not true. This approach does not allow assignment of the unknown to populations that contain the unknown within their confidence ellipse. The overall confidence envelope is multidimensional, and separation of the unknown from a target population may occur in deeper dimensions. Hence, as with the private alleles approach, this graphical approach serves to narrow down the candidates for the source of the unknown, and may in that sense, provide a satisfactory answer.

A third approach is to address the issue of non-independence (linkage) among the SNP loci by ordinating the space defined by those loci. The resultant axes, linear combinations of the information contained in each locus, are orthogonal and so can be regarded as independent. Subsequent standardization can achieve independent and identically distributed variates, which simplifies analysis of probabilities and likelihoods.

The script `gl.assign` first eliminates populations on the basis of private alleles. It then ordinated the space defined in locus space for the remaining populations.

A limited number of dimensions is retained in the final solution, the decision based on consideration of (a) the number of substantive eigenvalues (greater in explanatory power than the original variables before ordination), (b) the number of populations including the unknown, (c) an operational maximum number of dimensions specified in the code (`dim=7`) or (d) a user specified value. The script selects the minimum of these values to set the dimension of the reduced ordination space used subsequently.

A 95% confidence envelope (or some other level of confidence specified by the user) is defined in the reduced ordinated space, and the likelihood of the unknown genotype occurring is estimated for each dimension under Normal distribution assumptions. These likelihoods are logged for computational reasons, weighted by the eigenvalue for their respective dimension, and summed to yield an Assignment Index for the unknown against each population. Summing the weighted logged likelihoods is supported by the independence of each of the ordinated axes, but the result should be nevertheless regarded as an assignment index rather than an accurate likelihood.

An Assignment Index is calculated in the same way for a notional individual residing on the boundary of the confidence envelope. Comparing the assignment index for each population with that of the notional boundary individual provides a basis for a decision on assignment. If the Assignment Index for the unknown is less than the critical value for the Assignment Index (that of the boundary individual), then the unknown is assigned to that population. Where more than one population is selected, the population with the greatest Assignment Index is the most likely. This will become evident in the examples that follow.

```
x <- gl.report.pa(testset.gl, id="UC_00146", nmin=10, t=0)
```

```
## IDENTIFYING LOCI WITH PRIVATE ALLELES
```

```
##
```

```
## Discarding 10 populations with sample size less than 10 : EmmacClarJack EmmacClarYate EmmacMDBGwyd E
```

```
##
```

```
## Retaining 20 populations with sample size greater than or equal to 10 : EmmacBrisWive EmmacBurdMist 1
```

```
##
```

```
## Assigning 1 unknown individual(s) to 20 target populations
```

```
## Unknown individual: UC_00146
```

```
## Total number of SNP loci: 255
```

```
##
## Table showing number of loci with private alleles
##      0 EmmacMacIGeor
##      1 EmmacBurnBara EmmacRichCasi EmmacTweeUki
##      2 EmmacBrisWive EmmacMDBBowm EmmacMDBCudg EmmacMDBForb EmmacMDBMaci EmmacMDBMurrMung
##      3 EmmacFitzAllig EmmacMDBCCond EmmacMDBSanf EmmacRussEube
##      4 EmmacBurdMist EmmacJohnWari EmmacRoss
##      6 EmmacCoopEulb
##      7 EmmacCoopCully
##     16 EmmacCoopAvin
##
## Data retained for the unknown individual and remaining candidate source populations ( 0 or less loci
```

In this example, the unknown individual, UC_00146, is assigned to only one population, EmmacIGeor, so the assignment problem is resolved. This individual does indeed come from the Georges tributary of the Macleay River.

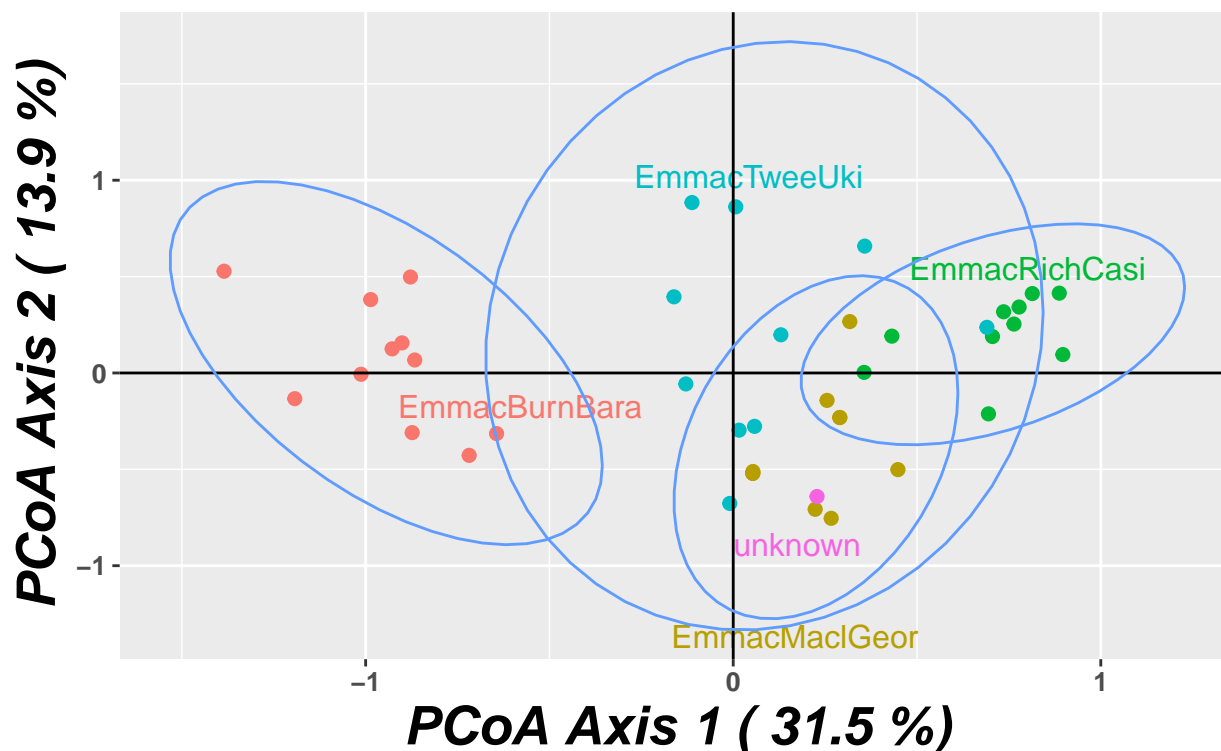
If we are worried that the 1 locus with a private allele out of 255 loci for populations EmmacBurnBara, EmmacRichCasi, and EmmacTweeUki is less than substantial evidence, we can proceed to analyse the data further using `gl.assign`. We would take this step also if there were more than one population scoring 0 private alleles. The code below yields the results of the private alleles analysis again, but in addition computes assignment based on confidence envelopes:

```
x <- gl.assign(testset.gl, id="UC_00146", nmin=10, alpha=0.95, t=1)
```

```
## IDENTIFYING LOCI WITH PRIVATE ALLELES
##
## Discarding 10 populations with sample size less than 10 : EmmacClarJack EmmacClarYate EmmacMDBGwyd E
##
## Retaining 20 populations with sample size greater than or equal to 10 : EmmacBrisWive EmmacBurdMist 1
##
## Assigning 1 unknown individual(s) to 20 target populations
## Unknown individual: UC_00146
## Total number of SNP loci: 255
##
## Table showing number of loci with private alleles
##      0 EmmacMacIGeor
##      1 EmmacBurnBara EmmacRichCasi EmmacTweeUki
##      2 EmmacBrisWive EmmacMDBBowm EmmacMDBCudg EmmacMDBForb EmmacMDBMaci EmmacMDBMurrMung
##      3 EmmacFitzAllig EmmacMDBCCond EmmacMDBSanf EmmacRussEube
##      4 EmmacBurdMist EmmacJohnWari EmmacRoss
##      6 EmmacCoopEulb
##      7 EmmacCoopCully
##     16 EmmacCoopAvin
##
## Data retained for the unknown individual and remaining candidate source populations ( 1 or less loci
##
##
## COMPUTING ASSIGNMENT BASED ON CONFIDENCE ENVELOPES
##
## Number of populations, including the unknown: 5
## Number of dimensions with substantial eigenvalues: 7
## Hard coded upper limit to dimensions: 8
## User specified dimensions to retain:
## Dimension of confidence envelope set at 5
## Performing a PCoA, individuals as entities, SNP loci as attributes
```



```
## Ordination yielded 7 informative dimensions from 41 original dimensions
## PCoA Axis 1 explains 31.5 % of the total variance
## PCoA Axis 1 and 2 combined explain 45.4 % of the total variance
## PCoA Axis 1-3 combined explain 56.6 % of the total variance
## Plotting populations
```



```
##
## Likelihood Index for assignment of unknown UC_00146 to listed populations
##
##      Population      Index      CE Assign
## 2 EmmacMacIGeor -1.1294 -2.6193    yes
## 4 EmmacTweeUki  -1.2056 -2.6193    yes
## 3 EmmacRichCasi -3.9405 -2.6193    no
## 1 EmmacBurnBara -7.8446 -2.6193    no
## Index is a weighted log-likelihood
## CE is the value of the Index on the boundary of the 95 % confidence envelope
## Best assignment is the population with the largest value of the Index, in this case EmmacMacIGeor
```

The plot allows us to eliminate populations EmmacRichCasi and EmmacBurnBara from consideration as the unknown individual does not fall within or near their confidence ellipses. EmmacTweeUki and EmmacMacIGeor remain in contention.

Output from further analysis using the ordination and assignment index yields the following. Both EmmacTweeUki and EmmacMacIGeor are potential sources for the unknown individual, but the most likely source is EmmacMacIGeor, as supported by the private allele analysis.

A critical issue is whether the number of individuals in the target populations are sufficient to characterize them in the critical considerations made here. Is the sample size sufficient to support the identification of a private allele in the unknown? Is it sufficient to confidently construct confidence ellipses in the PCoA plot? Is it sufficient to provide a robust estimate of the distribution of individuals along each axis of the ordination in order to adequately estimate the likelihood of the unknown on that axis? We have set a default of `nmin=10`,

but this is a matter of judgement that needs to be considered when planning a study.

14 Genlight Conversion

The genlight objects for managing SNP data are a relatively recent development, and the analysis options are limited. Conversion to {adegenet} genind object opens up a greater range of analysis options, and this conversion can be achieved with

```
gl <- testset.gl
gi <- gl2gi(gl, probar=FALSE)

## Start conversion....
## Please note conversion of bigger data sets will take some time!
## Once finished, we recommend to save the object using >save(object, file="object.rdata")
##
## Matrix converted.. Prepare genind object...
## Finished! Took 1 seconds.
```

Conversion in the opposite direction can be achieved with

```
gl2 <- gi2gl(gi)
```

14.1 Interfaces to Other Software

Package **dartR** does not pretend to provide a comprehensive range of analyses, but rather to provide avenues from SNP data stored as a genlight object to other available software packages. The following conversion functions are available:

function	explanation
gl2fasta()	Outputs the concatenated trimmed sequences to fastA format after first converting heterozygous SNPs to ambiguity codes or randomly assigning the heterozygous state to one or the other homozygous states (diplotypes to haplotypes).
gl2genind()	Converts a genlight object to a genind object as defined by the {adegenet} package.
genind2gl()	Converts a genind object as defined by the {adegenet} package to a genlight object.
gl2nhyb()	Outputs 200 loci selected according to user specified criteria for input to the package NewHybrids (Anderson and Thompson, 2002).

gl2faststructure() gl2 gdsfmt() | Outputs a gl object to a file in gds format that can subsequently be used with {SNPRelate}.

14.2 NewHybrids

NewHybrids (Anderson and Thompson, 2002) employs a statistical method for identifying species hybrids using data on multiple, unlinked markers which does not require that allele frequencies be known in the parental species. The probability model used is one in which parentals and various classes of hybrids (F1's, F2's, and various backcrosses) form a mixture from which the sample is drawn. Using the framework of Bayesian model-based clustering, NewHybrids computes, using Markov chain Monte Carlo, the relative likelihood (posterior probability) that each individual belongs to each of the distinct hybrid classes. NewHybrids is

limited to ca 200 loci because of memory constraints, so the best 200 available loci were selected on the basis of their being different and fixed in each of the two nominated parental populations, and then on avgPIC.

To generate an input file for NewHybrids, use the function

```
glnew <- gl2nhyb(gl, outfile = file.path(tempdir(),"nhyb.txt"))

## Extracting the SNP data
##   No parental population specified
##   Selecting ca 200 random loci
## Subsampling at random, approximately 200 loci from genlight object
##   No. of loci retained = 128
##   Note: SNP metadata discarded
## Converting data to NewHybrids format
##   Adding sequential number
## Writing the NewHybrids input file C:\Users\s425824\AppData\Local\Temp\RtmpM1Pi2F/nhyb.txt
##   NumIndivs 250
##   NumLoci 128
##   Digits 1
##   Format Lumped
```

with appropriate options set, for example:

```
gl.new <- gl2nhyb(gl, outfile = "nhyb.txt", p0 = NULL, p1 = NULL, t = 0, m = "random")
```

Refer to help (?gl2nhyb) for further information. This function compares two sets of parental populations to identify loci that exhibit a fixed difference, returns an genlight object with the reduced data, and creates an input file for the program NewHybrids using the top 200 loci. In the absence of two identified parental populations, the script will select a random set 200 loci only (method=random) or the first 200 loci ranked on information content (AvgPIC).

The resultant NewHybrids input file is then passed to the program NewHybrids outside the R environment. Refer to the NewHybrids documentation to continue.

14.3 Phylip

PHYLIP (Felsenstein, 1989) is a package of programs for inferring phylogenies (evolutionary trees). Methods that are available in the package include parsimony, distance matrix, and likelihood methods, including bootstrapping and consensus trees. The {dartR} scripts produce Euclidean distance matrices in a form that can be input to Phylip distance analyses (e.g. program Fitch).

To generate an input matrix for Phylip, use the function

```
glnew <- gl2phylip(outfile = "phyinput.txt")
```

To generate an input file containing resampled input matrices for the purposes of bootstrapping, use:

```
gl.new <- gl2phylip(outfile = "phyinput.txt", bstrap = 1000)
```

The resultant output file can be passed to Phylip programs for execution. Refer to the Phylip documentation.

14.4 SNPRelate

R package SNPRelate is available to undertake principal components analysis and relatedness analysis (Zheng et al., 2012). {SNPRelate} expects the data to be in gds format. As with the genlight format of package {adegenet}, the gds format supports efficient memory management and storage of SNP genotypes and associated metadata. In this format each byte encodes up to four SNP genotypes thereby reducing file

size and access time. The GDS format supports data blocking so that only the subset of data that is being processed needs to reside in memory. GDS formatted data is also designed for efficient random access to large data sets.

Data conversion from a `genlight` object to `gds` format is via

```
gl2gds(gl, outfile="test.gds")
```

Once this file is created, you can open it for analysis with `{SNPRelate}` using

```
gds <- snpgdsOpen("gl2gds.gds")
```

and undertake the range of analyses available in `{SNPRelate}`, including

option	explanation
LD-pruning	A pruned set of loci which are in approximate linkage equilibrium will avoid the strong influence of locus clusters on relatedness analysis
PCoA	with some nice diagnostic plots
Relatedness	Identity-by-descent estimation can be done by either the method of moments (MoM) or maximum likelihood estimation (MLE)
IBS	Identity by State analysis and Multidimensional Scaling (MDS) for displaying relationships

14.5 Faststructure

STRUCTURE is one of the most widely used population analysis tools that allows researchers to assess patterns of genetic structure in a set of samples (Porrás-Hurtado et al., 2013). STRUCTURE is freely available software for population analysis developed (Pritchard et al., 2000). STRUCTURE analyses differences in the distribution of genetic variants amongst populations and places individuals into groups where they share similar patterns of variation. STRUCTURE both identifies populations from the data and assigns individuals to those populations. fastSTRUCTURE is an improved implementation to analyse large quantities of data (Raj et al., 2014).

To generate an input file for fastSTRUCTURE, use the function (this format can also be used for input to STRUCTURE, though the meta data options are not supported yet):

```
gl2faststructure(gl, outfile=file.path(tempdir(), "myfile.fs"), probar = FALSE)
```

```
## Saved faststructure file: C:/Users/s425824/AppData/Local/Temp/RtmpuUJcyg/Rbuild15b422824050/dartR/vi
## Consists of 250 individuals and 255 loci.
## NULL
```

15 References

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