Data import and manipulation in poppr version 1.1.5.99.583

Zhian N. Kamvar[1] and Niklaus J. Grünwald[1,2]

1) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR

2) Horticultural Crops Research Laboratory, USDA-ARS, Corvallis, OR

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Abstract

Poppr provides open-source, cross-platform tools for quick analysis of population genetic data enabling focus on data analysis and interpretation. While there are a plethora of packages for population genetic analysis, few are able to offer quick and easy analysis of populations with mixed reproductive modes. *Poppr*'s main advantage is the ease of use and integration with other packages such as *adegenet* and *vegan*, including support for novel methods such as clone correction, multilocus genotype analysis, calculation of Bruvo's distance, and the index of association. New features in version 2.0 include generation of minimum spanning networks with reticulation, calculation of the index of association for genomic data, and filtering multilocus genotypes based on genetic distance.

Contents

1 Introduction

1.1 Purpose

Poppr is an R package with convenient functions for analysis of genetic data with mixed modes of reproduction including sexual and clonal reproduction. While there are many R packages in CRAN and other repositories with tools for population genetic analyses, few are appropriate for populations with mixed modes of reproduction. There are several stand-alone programs that can handle these types of data sets, but they are often platform specific and often only accept specific data types. Furthermore, a typical analysis often involves switching between many programs, and converting data to each specific format.

Poppr is designed to make analysis of populations with mixed reproductive modes more streamlined and user friendly so that the researcher using it can focus on data analysis and interpretation. *Poppr* allows analysis of haploid and diploid dominant/co-dominant marker data including microsatellites, Single Nucleotide Polymorphisms (SNP), and Amplified Fragment Length Polymorphisms (AFLP). To avoid creating yet another file format that is specific to a program, *poppr* was created on the backbone of the popular R package *adegenet* and can take all the file formats that *adegenet* can take (Genpop, Genetix, Fstat, and Structure) and newly introduces compatibility with GenAlEx formatted files (exported to CSV). This means that anything you can analyze in *adegenet* can be further analyzed with *poppr*.

The real power of *poppr* is in the data manipulation and analytic tools. *Poppr* has the ability to bootstrap, clone-censor, and subset data sets. With *poppr* you can also quickly calculate Bruvo's distance, the index of association, and easily determine which multilocus genotypes are shared across populations.

In version 2.0, tools for genomic data were introduced (Kamvar *et al.* , 2015a). These tools allow researchers to define what it means to be a clone lost in a sea of genomic data, generate bootstrapped dendrograms with any genetic distance, and calculate minimum spanning networks with reticulations to reveal the underlying population structure of your clonal data.

1.2 A note about poppr version 1.1.5.99.583

In *poppr* version 1.1, the `genclone` object was introduced. This was an extension of *adegenet*'s `genind` object by adding two new slots:

1. **mlg** numeric classification of multilocus genotypes in the data set
2. **hierarchy** storage of multiple population factors.

In particular, the **hierarchy** slot played a large role in providing a more tractable way of performing analyses such as clone-correction and AMOVA. In March of 2015, The **hierarchy** slot was ported to the *adegenet* packaged and the name was changed to the **strata** slot. This means that code written before *poppr* 2.0.0 might need some small changes. Please see the Migrating from poppr version 1 vignette for more details:

```
vignette("how_to_migrate", package = "poppr")
```

1.3 Resources

This vignette will cover all of the material you need to know to efficiently analyze data in *poppr*. For information on methods of analysis (eg. index of association, distance measures, AMOVA, ...), please read the manual pages provided for each function.

As *poppr* expanded from version 1.0, the vignette also expanded to be 80+ pages. As a result, it became clear that over 22,000 was less of a manual and more of a novella with a terrible plot. To remedy this,

this vignette will focus only on data manipulation and a separate vignette, "algo", has been written to give algorithmic details of analyses introduced with *poppr*.

As of spring 2014, Drs. Niklaus J. Grünwald, Sydney E. Everhart, and I have co-authored a primer on using R for population genetic analysis. It is located at http://grunwaldlab.github.io//Population_Genetics_in_R and the source code can be found on our github site.

1.4 Getting Help

If you have any questions or feedback, feel free to send a message to the *poppr* forum at http://groups.google.com/group/poppr. You can submit bug reports there or on our github site: https://github.com/grunwaldlab/poppr

1.5 Acknowledgments

Much thanks goes to Sydney E. Everhart for alpha testing, beta testing, feature requests, proofreading, data contribution, and moral support throught the writing of this package and manual. Thanks also to Brian Knaus, Ignazio Carbone, David Cooke, Corine Schoebel, Jane Stewart, and Zaid Abdo for beta testing and feedback.

The following data sets are included in *poppr*:

- `Pinf` (SSR, *Phytophthora infestans*)(Goss *et al.* , 2014)
- `monpop` (SSR, *Monolinia fructicola*)(Everhart & Scherm, 2015)
- `Aeut` (AFLP, *Aphanomyces eutieches*)(Grünwald & Hoheisel, 2006)
- `Pram` (SSR, *Phytophthora ramorum*)(Kamvar *et al.* , 2015b)

1.6 Citation

The formal publication for the first version of *poppr* was published in the journal **PeerJ**: http://peerj.com/articles/281/. The new features in version 2.x were published in **Frontiers** http://dx.doi.org/10.3389/fgene.2015.00208. To cite *poppr*, please type in your R console:

```
citation(package = "poppr")
```

1.7 Installation

This manual assumes you have installed R. If you have not, please refer to The CRAN home page at http://cran.r-project.org/. We also recommend the Rstudio IDE (http://www.rstudio.com/), which allows the user to view the R console, environment, scripts, and plots in a single window.

1.7.1 From CRAN

To install *poppr* from CRAN, select "Package Installer" from the menu "Packages & Data" in the GUI or type:

```
install.packages("poppr", dependencies=TRUE)
```

All dependencies (*adegenet, pegas, vegan, ggplot2, phangorn, ape, reshape2* and *igraph*) will also be installed. In the unfortunate case this does not work, consult http://cran.r-project.org/doc/manuals/R-admin.html#Installing-packages.

1.7.2 From GitHub

GitHub is a repository where you can find all stable and development versions of *poppr*.

Since *poppr* contains C code, it needs to be compiled, which means that you need a working C compiler. If you are on Linux, you should have that, but if you are on Windows or OSX, you might need to download some special tools:

Windows Download Rtools: http://cran.r-project.org/bin/windows/Rtools/

OSX Download Xcode: https://developer.apple.com/xcode

To install from GitHub, you do not need to download the tarball since there is a package called *devtools* that will download and install the package for you directly from GitHub. After you have installed all dependencies (see above section), you should download *devtools*:

```
install.packages("devtools")
```

Now you can execute the command `install_github` with the user and repository name:

```
devtools::install_github("grunwaldlab/poppr")
```

If you are the adventurous type and are willing to test out unreleased versions of the package, you can also install the development version:

```
devtools::install_github("grunwaldlab/poppr@devel")
```

Users who install this version do so at their own risk. Since it is a development version, documentation may be incomplete or non-existent for new functions.

1.7.3 From Source

The tarball for *poppr* can be downloaded from CRAN: http://cran.r-project.org/package=poppr or via github at https://github.com/grunwaldlab/poppr.

You will need a C compiler to install this way. See the section above for details.

If you choose to install *poppr* from a source file, you should first make sure to install all of the dependencies with the following command:

```
pkgs <- c("adegenet", "pegas", "vegan", "ggplot2", "phangorn", "ape",
          "igraph", "reshape2", "dplyr", "shiny")
install.packages(pkgs)
```

After installing dependencies, download the package to your computer and then install it with:

```
install.packages("/path/to/poppr.tar.gz", type="source", repos=NULL)
```

1.8 Quick start

The author assumes that if you have reached this point in the manual, then you have successfully installed R and *poppr*. Before proceeding, you should be aware that R is case sensitive. This means that the words "Case" and "case" are different. You should also know where your R package library is located. In this section, you will learn how to locate a file, import it to R, and make a first analysis using the `poppr()` function.

What or where is my R package library?

R is as powerful as it is through a community of people who submit extra code called "packages" to help it do specific things. These packages live in a certain place on your computer called an R library. You can find out where this library is by typing `.libPaths()`

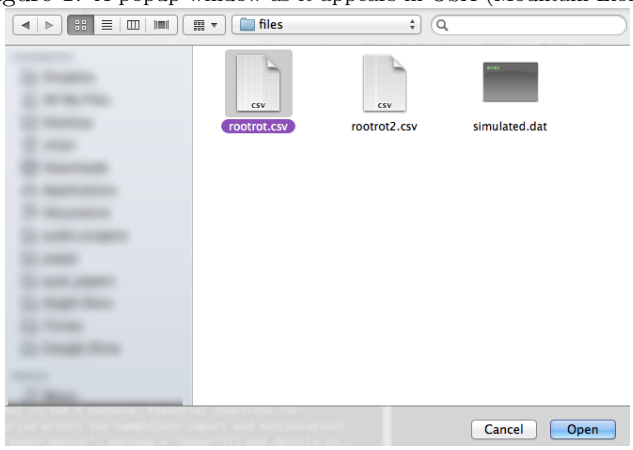
Importing a file into R involves you knowing the path to your file and then typing that into R's console. `getfile()` will help provide a point and click interface for selecting a file. This is simply a tool to help you get started. As you get better with R, you might feel that you don't need it at all.

First, tell your computer to search R's library, load the *poppr* package, and use `getfile()`:

```
library("poppr")
x <- getfile()
```

A pop up window will appear like this[1]:

Figure 1: A popup window as it appears in OSX (Mountain Lion).



[1]This window sometimes appears behind your current session of R, depending on the GUI and you will have to toggle to this window

Hey! My window doesn't look like that!

Now, this window will not match up to your window on your computer because you will probably not be in the right directory. Remember the first path in `.libPaths()`? Move to a folder called **poppr** in that path. In that folder, you will find another folder called **files**. Move there and your window will match the one displayed.

You should navigate to your R library and select the file called "rootrot.csv". If you don't know where your the poppr package lives, you can find it by typing `find.package('poppr')` into the R command line. Once we select a file, the file name and its path will be stored in the variable, `x`. We can confirm that by typing `x` into R's command line.

```
x

## $files
## [1] "/path/to/R/poppr/files/rootrot.csv"
##
## $path
## [1] "/path/to/R/poppr/files"
```

Here we can see that `x` is a list with two entries: `$files` shows the files you selected and `$path` shows the path to those files.

Not sure what I mean by path or working directory?

For anyone who has never used a command line, this is a new concept. You can think of the path as an address. So instead of `"/path/to/R"`, you could have `"/USA/Oregon/Corvallis"`. Or on your computer, it could be `"C:/users/poppr-user/R/win-library/3.1"` on Windows (where "poppr-user" is your username) or `"/Library/Frameworks/R.framework/Versions/3.1/Resources/library"` on OSX. Each slash represents a folder that you would click on when you are using the mouse.

A working directory is the folder that R is working in. It is where you can access and write files. When you tell R to read a file, it will only look for that file in your working directory. Note that you will not endanger your files by reading them into R. R works by making a copy of the file into memory. This means that you can manipulate the data in any way that you want without ever changing the original file.

To find what your current working directory is set to, type `getwd()` into the R console. Usually, you will start off a session in your "home" directory, which will look like this: `"~/"`. The command `setwd()` will change your working directory to any folder of your choice on your computer as indicated by the path that you provide. For more information, see Quick R at http://www.statmethods.net.

We will use `x$files` to access the file. The file is in the GenAlEx format, so we will import it using `read.genalex()` and then analyze it with the function `poppr()` to get a table of diversity indices per population.

```
myData  <- read.genalex(x$files)
myData
popdata <- poppr(x$files)
```

```
## | Athena_1
## | Athena_10
## | Athena_2
## | Athena_3
## | Athena_4
## | Athena_5
## | Athena_6
## | Athena_7
## | Athena_8
## | Athena_9
## | Mt. Vernon_1
## | Mt. Vernon_2
## | Mt. Vernon_3
## | Mt. Vernon_4
## | Mt. Vernon_5
## | Mt. Vernon_6
## | Mt. Vernon_7
## | Mt. Vernon_8
## | Total
```

The output of `poppr()` was assigned to the variable `popdata`, so let's look at the data.

```
popdata
```

```
##             Pop   N MLG  eMLG    SE     H     G lambda   E.5  Hexp    Ia rbarD
## 1      Athena_1   9   7  7.00 0.000 1.889  6.23  0.840 0.932 0.718  2.92 0.210
## 2     Athena_10   9   8  8.00 0.000 2.043  7.36  0.864 0.948 0.557  2.85 0.137
## 3      Athena_2  12  12 10.00   NaN 2.485 12.00  0.917 1.000 0.604  4.16 0.128
## 4      Athena_3  10   2  2.00 0.000 0.325  1.22  0.180 0.571 0.360  2.00 1.000
## 5      Athena_4  13   9  7.15 0.769 1.946  5.12  0.805 0.687 0.654  5.49 0.372
## 6      Athena_5  10   7  7.00 0.000 1.834  5.56  0.820 0.866 0.577  4.53 0.353
## 7      Athena_6   5   5  5.00 0.000 1.609  5.00  0.800 1.000 0.731  2.46 0.190
## 8      Athena_7  11  10  9.18 0.386 2.272  9.31  0.893 0.955 0.620  2.13 0.086
## 9      Athena_8   8   6  6.00 0.000 1.667  4.57  0.781 0.831 0.596  3.86 0.323
## 10     Athena_9  10  10 10.00 0.000 2.303 10.00  0.900 1.000 0.618  2.82 0.118
## 11 Mt. Vernon_1  10   9  9.00 0.000 2.164  8.33  0.880 0.952 0.659  7.13 0.276
## 12 Mt. Vernon_2   6   6  6.00 0.000 1.792  6.00  0.833 1.000 0.855 20.65 0.492
## 13 Mt. Vernon_3   8   6  6.00 0.000 1.667  4.57  0.781 0.831 0.482  2.12 0.106
## 14 Mt. Vernon_4  12   8  6.83 0.665 1.814  4.50  0.778 0.681 0.650  3.01 0.255
## 15 Mt. Vernon_5  17   7  5.54 0.828 1.758  5.07  0.803 0.848 0.637  2.68 0.340
## 16 Mt. Vernon_6  12  11  9.32 0.466 2.369 10.29  0.903 0.958 0.716 19.50 0.467
## 17 Mt. Vernon_7  12   9  7.82 0.649 2.095  7.20  0.861 0.870 0.685  1.21 0.153
## 18 Mt. Vernon_8  13   9  7.35 0.764 2.032  6.26  0.840 0.794 0.556  1.15 0.169
## 19        Total 187 119  9.61 0.612 4.558 68.97  0.986 0.720 0.726 14.37 0.271
##           File
## 1  rootrot.csv
## 2  rootrot.csv
## 3  rootrot.csv
## 4  rootrot.csv
## 5  rootrot.csv
## 6  rootrot.csv
## 7  rootrot.csv
## 8  rootrot.csv
## 9  rootrot.csv
## 10 rootrot.csv
```

```
## 11 rootrot.csv
## 12 rootrot.csv
## 13 rootrot.csv
## 14 rootrot.csv
## 15 rootrot.csv
## 16 rootrot.csv
## 17 rootrot.csv
## 18 rootrot.csv
## 19 rootrot.csv
```

The fields you see in the output include:

- `Pop` - Population name (Note that "Total" also means "Pooled").
- `N` - Number of individuals observed.
- `MLG` - Number of multilocus genotypes (MLG) observed.
- `eMLG` - The number of expected MLG at the smallest sample size ≥ 10 based on rarefaction. (Hurlbert, 1971)
- `SE` - Standard error based on `eMLG` (Heck *et al.* , 1975)
- `H` - Shannon-Wiener Index of MLG diversity. (Shannon, 1948)
- `G` - Stoddart and Taylor's Index of MLG diversity. (Stoddart & Taylor, 1988)
- `lambda` - Simpson's index, λ.
- `E.5` - Evenness, E_5. (Pielou, 1975)(Ludwig & Reynolds, 1988)(Grünwald *et al.* , 2003)
- `Hexp` - Nei's 1978 Expected Heterozygosity. (Nei, 1978)
- `Ia` - The index of association, I_A. (Brown *et al.* , 1980) (Smith *et al.* , 1993) (Agapow & Burt, 2001)
- `rbarD` - The standardized index of association, $\bar{r}_d$. (Agapow & Burt, 2001)

These fields are further described in the function `poppr`. You can access the help page for `poppr` by typing `?poppr` in your R console.

One thing to note about this output is the `NaN` in the column labeled `SE`. In R, `NaN` means "Not a number". This is produced from calculation of a standard error based on rarefaction analysis. Occasionally, this calculation will encounter a situation in which it must attempt to take a square root of a negative number. Since the root of any negative number is not defined in the set of real numbers, it must therefore have an imaginary component, i. Unfortunately, R will not represent the imaginary components of numbers unless you specifically tell it to do so. By default, R represents these as `NaN`.

1.9 Importing data into poppr {Get out of my dreams and into my R}

1.9.1 How does R represent data? {Objective: data}

Working with data in R means that these data have to be stored inside an "object", which is stored in the computer's memory. Because of this, it's important to know the difference between a **file** and an **object**. When anyone talking about importing a **file** into R, they are referring to a spreadsheet or text file that lives in a folder on your hard drive. Spreadsheet files (saved as *.csv files) are normally imported through the R function `read.table()`. The output of `read.table()` is a `data.frame`. A `data.frame` is an **object** represented in your computer's memory. This means that it only exists for as long as R is running.

The good thing about having objects stored in memory is that you can manipulate them in any way and not affect the source of those data. Since R is a scripted language (instead of point-and-click), any of your manipulations can be saved in a separate R file that can be easily adapted to new data. Of course, most data are not going to be entered into R manually. Usually they will be formatted in a manner that can be read by popular population genetics programs.

As previously mentioned, since *poppr* is based on *adegenet*, it's possible to read in the following file formats into a `genind` object with the function `import2genind()`:

- FSTAT
- GENEPOP
- GENETIX
- STRUCTURE

Here, we introduce a new way of importing data into a `genind` or `genclone` object from a GENALEX formatted file.

1.9.2 Function: read.genalex

A very popular program for population genetics is GenAlEx (http://biology.anu.edu.au/GenAlEx/Welcome.html) (Peakall & Smouse, 2012, 2006). GenAlEx runs within the Excel environment and can be very powerful in its analyses. *Poppr* has added the ability to read *.CSV files[2] produced in the GenAlEx format. It can handle data types containing regions and geographic coordinates, but currently cannot import allelic frequency data from GenAlEx. Using the *poppr* function `read.genalex` will import your data into *adegenet*'s `genind` object or *poppr*'s `genclone` object (more information on that below). For ways of formatting a GenAlEx file, see the manual here: http://biology.anu.edu.au/GenAlEx/Download_files/GenAlEx%206.5%20Guide.pdf

Below is an example of the GenAlEx format. We will use the data set called `microbov` from the *adegenet* package to generate it. The data contains three demographic factors: Country, Species and Breed contained within the `@other` slot (detailed in THE OTHER SLOT). We will first set these as the population strata, define the population as the combination of the strata, and then save a file to the desktop.

```
library("poppr")
data(microbov)
strata(microbov) <- data.frame(other(microbov)) # set the strata
setPop(microbov) <- ~coun/breed/spe             # combine the strata
microbov
genind2genalex(microbov, file = "~/Desktop/microbov.csv")
```

```
## Extracting the table ... Writing the table to ~/Desktop/microbov.csv ... Done.
```

The GenAlEx format contains individuals in rows and loci in columns. Individual data begins at row 4. Column A always contains individual names and column B defines the population of each individual. Notice here that the three demographic factors from the data have been concatenated with a "_". This allows us to import more than one population factor to use as hierarchical levels in a GENCLONE OBJECT.

[2] *.CSV files are comma separated files that are easily machine readable.

Figure 2: The first 15 individuals and 4 loci of the microbov data set. The first column contains the individual names, the second column contains the population names, and each subsequent column represents microsatellite genetic data. Highlighted in red is a list of populations and their relative sizes.

	A	B	C	D	E	F	G	H	I	J
1	30	704	15	50	50	51	30	50	50	47
2	Unmodified Data			AF_BI_Borgou	AF_BI_Zebu	AF_BT_Lagunaire	AF_BT_NDama	AF_BT_Somba	FR_BT_Aubrac	FR_BT_Bazadais
3	Ind	Pop	INRA63		INRA5		ETH225		ILSTS5	
4	AFBIBOR9503	AF_BI_Borgou	183	183	137	141	147	157	190	190
5	AFBIBOR9504	AF_BI_Borgou	181	183	141	141	139	157	186	186
6	AFBIBOR9505	AF_BI_Borgou	177	183	141	141	139	139	194	194
7	AFBIBOR9506	AF_BI_Borgou	183	183	141	141	141	147	184	190
8	AFBIBOR9507	AF_BI_Borgou	177	183	141	141	153	157	184	186
9	AFBIBOR9508	AF_BI_Borgou	177	183	137	143	149	157	184	186
10	AFBIBOR9509	AF_BI_Borgou	177	181	139	141	147	157	184	190
11	AFBIBOR9510	AF_BI_Borgou	183	183	139	141	155	157	184	186
12	AFBIBOR9511	AF_BI_Borgou	177	183	139	141	139	143	182	190
13	AFBIBOR9512	AF_BI_Borgou	183	183	141	141	157	159	186	186
14	AFBIBOR9513	AF_BI_Borgou	177	177	141	141	147	157	184	190
15	AFBIBOR9514	AF_BI_Borgou	183	183	143	143	139	157	186	186
16	AFBIBOR9515	AF_BI_Borgou	183	183	137	137	143	157	0	0
17	AFBIBOR9516	AF_BI_Borgou	177	183	137	143	139	157	182	184
18	AFBIBOR9517	AF_BI_Borgou	177	183	141	141	157	157	186	194

The First three rows contain information pertaining to the global data set. The only important information for *poppr* is the information contained in row 3 and the first three columns of row 1.

	A	**B**	**C**	**D**
1	# of Loci	# of Individuals	# of Populations	Pop1 Size ...
2	-	-	-	Pop1 Name ...
3	-	-	Locus 1	...

Highlighted in red in figure 2 are definitions of the number of populations and their respective sizes. As this is redundant information, we can remove it. Below is an example of a valid data set that can be imported into *poppr*.

Figure 3: The first 15 individuals and 4 loci of the microbov data set. This is the same figure as above, however the populations and counts have been removed from the header row and the third number in the header has been replaced by 1.

	A	B	C	D	E	F	G	H	I	J
1	30	704	1	704						
2	Example Modified	Data		ALL						
3	Ind	Pop	INRA63		INRA5		ETH225		ILSTS5	
4	AFBIBOR9503	AF_BI_Borgou	183	183	137	141	147	157	190	190
5	AFBIBOR9504	AF_BI_Borgou	181	183	141	141	139	157	186	186
6	AFBIBOR9505	AF_BI_Borgou	177	183	141	141	139	139	194	194
7	AFBIBOR9506	AF_BI_Borgou	183	183	141	141	141	147	184	190
8	AFBIBOR9507	AF_BI_Borgou	177	183	141	141	153	157	184	186
9	AFBIBOR9508	AF_BI_Borgou	177	183	137	143	149	157	184	186
10	AFBIBOR9509	AF_BI_Borgou	177	181	139	141	147	157	184	190
11	AFBIBOR9510	AF_BI_Borgou	183	183	139	141	155	157	184	186
12	AFBIBOR9511	AF_BI_Borgou	177	183	139	141	139	143	182	190
13	AFBIBOR9512	AF_BI_Borgou	183	183	141	141	157	159	186	186
14	AFBIBOR9513	AF_BI_Borgou	177	177	141	141	147	157	184	190
15	AFBIBOR9514	AF_BI_Borgou	183	183	143	143	139	157	186	186
16	AFBIBOR9515	AF_BI_Borgou	183	183	137	137	143	157	0	0
17	AFBIBOR9516	AF_BI_Borgou	177	183	137	143	139	157	182	184
18	AFBIBOR9517	AF_BI_Borgou	177	183	141	141	157	157	186	194

All GenAlEx formatted data can be imported with the command `read.genalex`, detailed below:

Default Command:

```
read.genalex(genalex, ploidy = 2, geo = FALSE, region = FALSE,
    genclone = TRUE, sep = ",", recode = FALSE)
```

- `genalex` - a *.CSV file exported from GenAlEx on your disk (For example: `"my_genalex_file.csv"`).
- `ploidy` - a number indicating the ploidy for the data set (eg 2 for diploids, 1 for haploids).
- `geo` - GenAlEx allows you to have geographic data within your file. To do this for *poppr*, you will need to follow the first format outlined in the GenAlEx manual and place the geographic data AFTER all genetic and demographic data with one blank column separating it (See the GenAlEx Manual for details). If you have geographic information in your file, set this flag to `TRUE` and it will be included within the resulting genind object in the `@other` slot. (If you don't know what that is, don't worry. It will be explained later in The other slot.)
- `region` - To format your GenAlEx file to include regions, you can choose to include a separate column for regional data, or, since regional data must be in contiguous blocks, you can simply format it in the same way you would any other data (see the GenAlEx manual for details). If you have your file organized in this manner, select this option and the regional information will be stored in the `@other` slot of the resulting genind object or be incorporated into the hierarchy of the genclone object.
- `genclone` - This flag will convert your data into a `genclone` object (see Send in the clones for more info).
- `sep` - The separator argument for columns in your data. It defaults to ",".
- `recode` - If your data is polyploid data, this gives you the option to recode it. (See About Polyploid Data for details)

If you are unfamiliar with exporting data from Excel

1. Click the Microsoft Office Button in the top left corner of Excel. (Or go to the File menu if you have an older version)
2. Click Save As...
3. In the "Save as type" drop down box, select CSV (comma delimited).

Note that regional data and geographic data are not mutually exclusive. You can have both in one file, just make sure that they are on the same sheet and that the geographic data is always placed after all genetic and demographic data.

We have a short example of GenAlEx formatted data with no geographic or regional formatting. We will first see where the data is using the command `system.file()`

```
system.file("files/rootrot.csv", package="poppr")
```

```
## [1] "/path/to/R/library/poppr/files/rootrot.csv"
```

Now import the data into *poppr* like so:

```
rootrot <- read.genalex(system.file("files/rootrot.csv", package="poppr"))
```

Executing rootrot shows that this file is now in genclone format and can be used with any function in *poppr* and *adegenet*

```
rootrot

##
## This is a genclone object
## -------------------------
## Genotype information:
##
##    119 multilocus genotypes
##    187 diploid individuals
##     56 dominant loci
##
## Population information:
##
##      1 stratification - Pop
##     18 populations defined - Athena_1 Athena_10 Athena_2 ... Mt. Vernon_6 Mt. Vernon_7
## Mt. Vernon_8
```

1.9.3 Other ways of importing data

Adegenet already supports the import of FSTAT, STRUCTURE, GENPOP, and GENETIX formatted files, so if you have data in those formats, you can import them using the function `import2genind`. For sequence data, check if you can use `read.dna` from the *ape* package to import your data. If you can, then you can use the *adegenet* function `DNAbin2genind`. If you don't have any of these formats handy, you can still import your data using R's `read.table` along with `df2genind` from *adegenet*. For more information, see *adegenet*'s "Getting Started" vignette.

1.9.4 Function: genind2genalex (exporting data)

Of course, being able to export data is just as useful as being able to import it, so we have this handy little function that will write a GenAlEx formatted file to wherever you desire.
WARNING: This will overwrite any file that exists with the same name.

Default Command:

```
genind2genalex(pop, filename = "genalex.csv", quiet = FALSE,
    geo = FALSE, geodf = "xy", sep = ",")
```

- `pop` - a `genind` object.
- `filename` - This is where you specify the path to the new file you wish to create. If you specify only a filename with no path, it will place the file in your current working directory.
- `quiet` - If this is set to `FALSE`, a status message will be printed to the console as the extraction progresses.

- `geo` - Set to `TRUE`, if you have a data frame or matrix in the `@other` slot of your genind object that contains geographic coordinates for all individuals or all populations. Setting this to `TRUE` means the resulting file will have two extra columns at the end of your file with geographic coordinates.
- `geodf` - The name of the data frame or matrix containing the geographic coordinates.
- `sep` - A separator to separate columns in the resulting file.

First, a simple example for the rootrot data we demonstrated in section 1.4.2:

```
genind2genalex(rootrot, "~/Desktop/rootrot.csv")
```

```
## Extracting the table ... Writing the table to ~/Desktop/rootrot.csv ... Done.
```

Here's an example of exporting the nancycats data set into GenAlEx format with geographic information. If we look at the nancycats geographic information, we can see it's coordinates for each population, but not each individual:

```
data(nancycats)
nancycats@other$xy
```

```
##            x         y
## P01 263.3498 171.10939
## P02 183.5028 122.40790
## P03 391.1050 254.70148
## P04 458.6121  41.72336
## P05 182.7769 219.08398
## P06 335.2121 344.83557
## P07 359.1662 375.36486
## P08 271.3345  67.89132
## P09 256.8169 150.02964
## P10 270.6086  17.00917
## P11 493.4544 237.25618
## P12 305.4510  85.33663
## P13 462.9674  86.79040
## P14 429.5768 291.04587
## P15 531.2003 115.13903
## P16 407.8003  99.87438
## P17 345.3745 251.79393
```

To export it:

```
genind2genalex(nancycats, "~/Desktop/nancycats_pop_xy.csv", geo = TRUE)
```

```
## Extracting the table ... Writing the table to ~/Desktop/nancycats_pop_xy.csv ... Done.
```

If we wanted to assign a geographic coordinate to each individual, we can use this trick knowing that there are 17 populations in the data set:

```
nan2 <- nancycats
nan2@other$xy <- nan2@other$xy[rep(1:17, table(pop(nan2))), ]
head(nan2@other$xy)
```

```
##            x        y
## P01 263.3498 171.1094
## P01 263.3498 171.1094
## P01 263.3498 171.1094
## P01 263.3498 171.1094
## P01 263.3498 171.1094
## P01 263.3498 171.1094
```

Now we can export it to a different file.

```
genind2genalex(nan2, "~/Desktop/nancycats_inds_xy.csv", geo = TRUE)
```

```
## Extracting the table ... Writing the table to ~/Desktop/nancycats_inds_xy.csv ... Done.
```

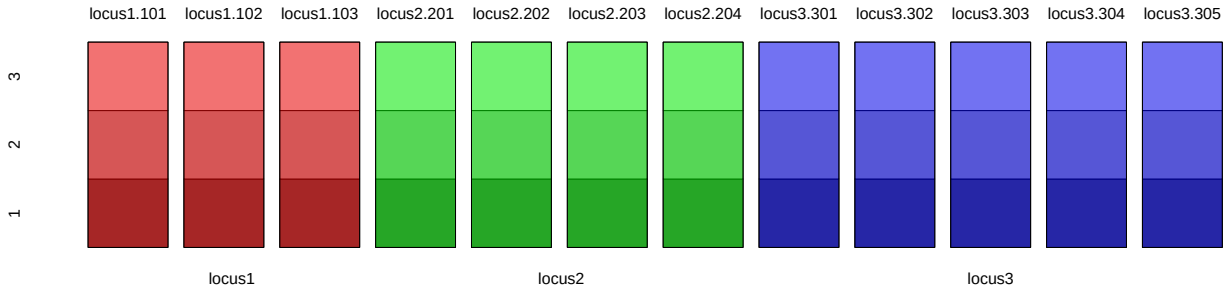
1.10 Getting to know *adegenet*'s genind object

Since *poppr* was built around *adegenet*'s framework, it is important to know how *adegenet* stores data in the genind object, as that is the object used by *poppr*. To create a genind object, *adegenet* takes a data frame of genotypes (rows) across multiple loci (columns) and converts them into a matrix of individual allelic frequencies at each locus (Jombart, 2008).

For example, Let's say we had data with 3 diploid individuals each with 3 loci that had 3, 4, and 5 allelic states respectively:

	locus1	locus2	locus3
1	101/101	201/201	301/302
2	102/103	202/203	301/303
3	102/102	203/204	304/305

The resulting `genind` object would contain a matrix that has 3 rows and 12 columns. Below is a schematic of what that would look like. Each column represents a separate allele, each row represents an individual and each color represents a different locus.



When we look at the data derived from table 1.10, we see that we have a matrix of individual allele frequencies at each locus.

```
##   locus1.101 locus1.102 locus1.103 locus2.201 locus2.202 locus2.203 locus2.204 locus3.301
## 1          2          0          0          2          0          0          0          1
## 2          0          1          1          0          1          1          0          1
## 3          0          2          0          0          0          1          1          0
##   locus3.302 locus3.303 locus3.304 locus3.305
```

```
## 1          1          0          0          0
## 2          0          1          0          0
## 3          0          0          1          1
```

At each locus, the allele frequencies for each individual sum to one. Homozygotes are denoted as having an allele frequency of 1 at a particular allele while heterozygotes have their allele frequencies represented as $1/p$ wher p = ploidy. Along with this matrix, are elements that define the names of the individuals, loci, alleles, and populations. If you wish to know more, see the *adegenet* "Getting Started" manual.

1.10.1 The other slot

The other slot is a place in the genind object that can be used to store useful information about the data. We saw earlier that it could store demographic information, now let's explore a different example. Bruvo's distance is based off of a stepwise mutation model for microsatellites. This requires us to know the length of the repeat of each locus. We could store the repeat lengths in a separate variable in our R environment, but we are at risk of losing that. One wa to prevent it from being lost would be to place it in the "other" slot. For the purpose of this example, we will use the "nancycats" data set from the *adegenet* package and assume that it has dinucleotide repeats at all of its loci.

```
data(nancycats) # Load the data
other(nancycats) # geographical coordinates

## $xy
##            x         y
## P01 263.3498 171.10939
## P02 183.5028 122.40790
## P03 391.1050 254.70148
## P04 458.6121  41.72336
## P05 182.7769 219.08398
## P06 335.2121 344.83557
## P07 359.1662 375.36486
## P08 271.3345  67.89132
## P09 256.8169 150.02964
## P10 270.6086  17.00917
## P11 493.4544 237.25618
## P12 305.4510  85.33663
## P13 462.9674  86.79040
## P14 429.5768 291.04587
## P15 531.2003 115.13903
## P16 407.8003  99.87438
## P17 345.3745 251.79393

repeats <- rep(2, nLoc(nancycats)) #nLoc = number of loci
repeats

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

other(nancycats)$repeat_lengths <- repeats
other(nancycats) # two items named xy and repeat_lengths

## $xy
##            x         y
## P01 263.3498 171.10939
## P02 183.5028 122.40790
## P03 391.1050 254.70148
```

```
## P04 458.6121  41.72336
## P05 182.7769 219.08398
## P06 335.2121 344.83557
## P07 359.1662 375.36486
## P08 271.3345  67.89132
## P09 256.8169 150.02964
## P10 270.6086  17.00917
## P11 493.4544 237.25618
## P12 305.4510  85.33663
## P13 462.9674  86.79040
## P14 429.5768 291.04587
## P15 531.2003 115.13903
## P16 407.8003  99.87438
## P17 345.3745 251.79393
##
## $repeat_lengths
## [1] 2 2 2 2 2 2 2 2 2
```

1.11 The genclone object {send in the clones}

The `genclone` class was defined in order to make working with hierarchies more intuitive. It is built off of the `genind` object and has dedicated slots for defined multilocus genotypes. The name genclone refers to the fact that it has the ability to handle genotypes of clonal organisms (but it is also used for sexual populations).

In previous versions of *poppr*, the genclone object contained a hierarchy slot as well. This slot was moved to *adegenet* and its name was changed to "strata". This slot allows you to carry around several definitions for populations in the same data set.

The function `as.genclone` allows the user to convert a `genind` object to a `genclone` object. The following example will demonstrate that the `genclone` object is an extension of the `genind` object as well as the advantages of having populations pre-defined in your data set.

1.11.1 Function: as.genclone

Default Command:

```
as.genclone(x, ..., mlg, mlgclass = TRUE)
```

- **x -** a `genind` object to be converted.
- **... -** any arguments to be passed to the genind constructor.
- **mlg -** a vector representing the multilocus genotype definitions of your data.
- **mlgclass -** if `TRUE`, the MLGs represented in your object will be converted to an MLG class object, which allows for custom MLG definitions.

Let's show an example of a `genclone` object. First, we will take an existing `genind` object and convert it using the function `as.genclone` (We can also use the function `read.genalex` to import as `genclone` or `genind` objects). We will use the `Aeut` data set because it is a clonal data set that has a simple population

strata (Grünwald & Hoheisel, 2006). The data set is here: http://dx.doi.org/10.6084/m9.figshare.877104 and it is AFLP data of the root rot pathogen *Aphanomyces euteiches* collected from two different fields in NW Oregon and W Washington, USA. These fields were divided up into subplots from which samples were collected. The fields represent the population and the subplots represent the subpopulation. Let's take a look at what the `genind` object looks like:

```
library("poppr")
data(Aeut)
Aeut

## /// GENIND OBJECT /////////
##
##  // 187 individuals; 56 loci; 56 alleles; size: 66.5 Kb
##
##  // Basic content
##    @tab:  187 x 56 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 56-56)
##    @ploidy: ploidy of each individual  (range: 2-2)
##    @type:  PA
##    @call: old2new_genind(object = x, donor = new(class(x)))
##
##  // Optional content
##    @pop: population of each individual (group size range: 90-97)
##    @other: a list containing: population_hierarchy
```

We can see that there is a data frame in the `@other` slot called "population_hierarchy". This is left over from *poppr* version 1.x behavior. Since the `genind` object now has a `@strata` slot, we can use it to set the stratification (previously called "hierarchy").

```
strata(Aeut) <- other(Aeut)$population_hierarchy[-1]
Aeut

## /// GENIND OBJECT /////////
##
##  // 187 individuals; 56 loci; 56 alleles; size: 70.1 Kb
##
##  // Basic content
##    @tab:  187 x 56 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 56-56)
##    @ploidy: ploidy of each individual  (range: 2-2)
##    @type:  PA
##    @call: old2new_genind(object = x, donor = new(class(x)))
##
##  // Optional content
##    @pop: population of each individual (group size range: 90-97)
##    @strata: a data frame with 2 columns ( Pop, Subpop )
##    @other: a list containing: population_hierarchy
```

Now we can convert this to a `genclone` object, which will store information about our multilocus genotypes for us.

```
agc <- as.genclone(Aeut)
agc

##
```

```
## This is a genclone object
## -------------------------
## Genotype information:
##
##    119 multilocus genotypes
##    187 diploid individuals
##     56 dominant loci
##
## Population information:
##
##      2 strata - Pop Subpop
##      2 populations defined - Athena Mt. Vernon
```

We can see here that it shows less information, but it gives us a very simple overview of our data. Don't be fooled, however, because it contains the same information as a `genind` object.

```
c(is.genind(Aeut), is.genclone(Aeut), is.genind(agc), is.genclone(agc))

## [1]  TRUE FALSE  TRUE  TRUE

# Adegenet functions work the same, too
c(nInd(Aeut), nInd(agc))

## [1] 187 187
```

If we wanted to, we could also convert it back to a genind object.

```
genclone2genind(agc)

## /// GENIND OBJECT /////////
##
##  // 187 individuals; 56 loci; 56 alleles; size: 79.6 Kb
##
##  // Basic content
##    @tab:  187 x 56 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 56-56)
##    @ploidy: ploidy of each individual  (range: 2-2)
##    @type:  PA
##    @call: genclone2genind(x = agc)
##
##  // Optional content
##    @pop: population of each individual (group size range: 90-97)
##    @strata: a data frame with 2 columns ( Pop, Subpop )
##    @other: a list containing: population_hierarchy
```

1.12 About polyploid data

WARNING

Treat polyploid data with care. Please read this section carefully and consult the help pages for all functions mentioned here.

With diploid or haploid data, genotypes are unambiguous. It is often clear when it is homozygous or heterozygous. With polyploid data, genotypes can be ambiguous. For example, a tetraploid individual with

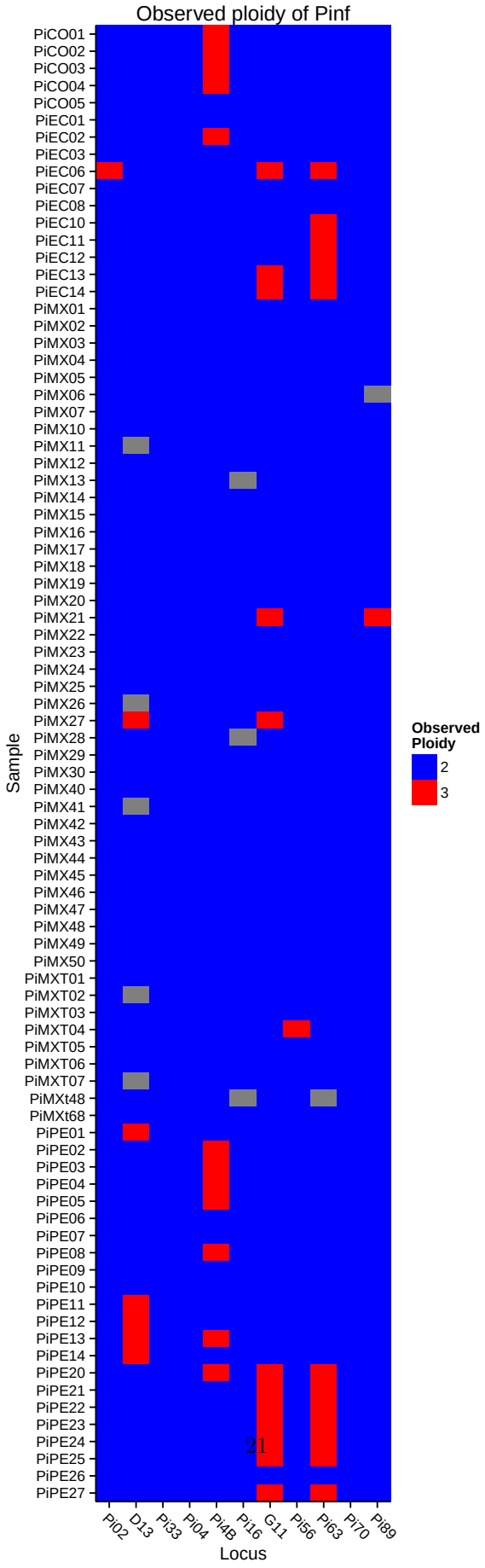
the apparent genotype of **A/B** could actually have one of three genotypes: **A/A/A/B**, **A/A/B/B**, or **A/B/B/B**. This ambiguity prevents a researcher from accurately calling all alleles present. In *adegenet*, it was previously difficult to import polyploid data because of this ambiguity as data was required to be unambiguous or missing.

A solution to this problem is to code missing alleles as "0". An example of this is found within the `Pinf` data set in *poppr* (Goss *et al.* , 2014). First, we will look at where we have polyploid allele calls.

```
data(Pinf)
Pinf

##
## This is a genclone object
## -------------------------
## Genotype information:
##
##    72 multilocus genotypes
##    86 tetraploid individuals
##    11 codominant loci
##
## Population information:
##
##     2 strata - Continent Country
##     2 populations defined - South America North America

ptab <- info_table(Pinf, type = "ploidy", plot = TRUE)
```


Observed ploidy of Pinf
PiCO01
PiCO02
PiCO03
PiCO04
PiCO05
PiEC01
PiEC02
PiEC03
PiEC06
PiEC07
PiEC08
PiEC10
PiEC11
PiEC12
PiEC13
PiEC14
PiMX01
PiMX02
PiMX03
PiMX04
PiMX05
PiMX06
PiMX07
PiMX10
PiMX11
PiMX12
PiMX13
PiMX14
PiMX15
PiMX16
PiMX17
PiMX18
PiMX19
PiMX20
PiMX21
PiMX22
PiMX23
PiMX24
PiMX25
PiMX26
PiMX27
PiMX28
PiMX29
PiMX30
PiMX40
PiMX41
PiMX42
PiMX43
PiMX44
PiMX45
PiMX46
PiMX47
PiMX48
PiMX49
PiMX50
PiMXT01
PiMXT02
PiMXT03
PiMXT04
PiMXT05
PiMXT06
PiMXT07
PiMXt48
PiMXt68
PiPE01
PiPE02
PiPE03
PiPE04
PiPE05
PiPE06
PiPE07
PiPE08
PiPE09
PiPE10
PiPE11
PiPE12
PiPE13
PiPE14
PiPE20
PiPE21
PiPE22
PiPE23
PiPE24
PiPE25
PiPE26
PiPE27
Sample
Pi02
D13
Pi33
Pi04
Pi4B
Pi16
G11
Pi56
Pi63
Pi70
Pi89
Locus
Observed Ploidy
2
3
21

We look at the last six samples over two loci, Pi63 (3 alleles, triploid) and Pi70 (3 alleles, diploid) to examine how the data is represented.

```
tail(tab(Pinf[loc = locNames(Pinf)[9:10]]))
```

```
##        Pi63.000 Pi63.148 Pi63.151 Pi63.157 Pi70.000 Pi70.189 Pi70.192 Pi70.195
## PiPE22        1        1        1        1        2        1        1        0
## PiPE23        1        1        1        1        2        1        1        0
## PiPE24        1        1        1        1        2        1        1        0
## PiPE25        1        1        1        1        2        1        1        0
## PiPE26        2        0        0        2        2        0        1        1
## PiPE27        1        1        1        1        2        1        1        0
```

Each column in this data represents a different allele at a particular locus. `Pi63.148` is the allele `148` at locus `Pi63`. Each row is an individual. The numbers represent the fraction of a given allele that makes up the individual genotype at a particular locus. What we can see here is that the number of columns is 8 when we expect only 6 (2 loci $\times$ 3 alleles). The first allele at each locus is `000`. Let's take a look at the data in a human-readable format.

```
Pinfdf <- genind2df(Pinf, sep = "/")
tail(Pinfdf[10:11])
```

```
##                    Pi63            Pi70
## PiPE22 000/148/151/157 000/000/189/192
## PiPE23 000/148/151/157 000/000/189/192
## PiPE24 000/148/151/157 000/000/189/192
## PiPE25 000/148/151/157 000/000/189/192
## PiPE26 000/000/157/157 000/000/192/195
## PiPE27 000/148/151/157 000/000/189/192
```

It's more clear now that we have a data set of tetraploid individuals where some genotypes appear diploid (`000/000/157/157`) and some appear triploid (`000/148/151/157`). The tetraploid genotype is padded with zeroes to make up the difference in ploidy.

This method allows Bruvo's Distance (Bruvo *et al.* , 2004) and the Index of Association (Brown *et al.* , 1980; Smith *et al.* , 1993; Agapow & Burt, 2001) to work with polyploids as they specifically recognize the zeroes as being missing data. A side effect, unfortunately is that the extra zeroes appear as extra alleles. As this affects all frequency-based statistics (except for the ones noted above), the user should reformat their data set with the function `recode_polyploids`, which will remove the zeroes.

```
Pinf_rc <- recode_polyploids(Pinf, newploidy = TRUE)
tail(tab(Pinf_rc[loc = locNames(Pinf_rc)[9:10]]))
```

```
##        Pi63.148 Pi63.151 Pi63.157 Pi70.189 Pi70.192 Pi70.195
## PiPE22        1        1        1        1        1        0
## PiPE23        1        1        1        1        1        0
## PiPE24        1        1        1        1        1        0
## PiPE25        1        1        1        1        1        0
## PiPE26        0        0        2        0        1        1
## PiPE27        1        1        1        1        1        0
```

Below, we show the observed genotypes:

```
Pinfrcdf <- genind2df(Pinf_rc, sep = "/")
tail(Pinfrcdf[10:11])
```

```
##               Pi63    Pi70
## PiPE22 148/151/157 189/192
## PiPE23 148/151/157 189/192
## PiPE24 148/151/157 189/192
## PiPE25 148/151/157 189/192
## PiPE26     157/157 192/195
## PiPE27 148/151/157 189/192
```

If you have imported your data as recoded polyploid data, you can use the argument "addzero" to fill out the ploidy:

```
tail(tab(recode_polyploids(Pinf_rc[loc = locNames(Pinf_rc)[9:10]], addzero = TRUE)))

##        Pi63.0 Pi63.157 Pi63.148 Pi63.151 Pi70.0 Pi70.192 Pi70.189 Pi70.195
## PiPE22      0        1        1        1      1        1        1        0
## PiPE23      0        1        1        1      1        1        1        0
## PiPE24      0        1        1        1      1        1        1        0
## PiPE25      0        1        1        1      1        1        1        0
## PiPE26      1        2        0        0      1        1        0        1
## PiPE27      0        1        1        1      1        1        1        0
```

2 Data Manipulation

One tedious aspect of population genetic analysis is the need for repeated data manipulation. *Poppr* includes novel functions for clone- censoring your data sets, removing genotypes or loci with missing data, removing uninformative loci, and shuffling populations.

2.1 Replace or remove missing data {Inside the golden days of missing data}

A data set without missing data is always ideal, but often not achievable. The *poppr* function `missingno` exists to handle missing data. Missing data can mean different things based on your data type. For microsatellites, missing data might represent any source of error that could cause a PCR product to not amplify in gel electrophoresis, which may or may not be biologically relevant. For a DNA alignment, missing data could mean something as simple as an insertion or deletion, which is biologically relevant. The choice to exclude or estimate data has very different implications for the type of data you have.

Treatment of Missing data is a non-trivial task. You should understand the nature of missing data in your data set before treatment.

2.1.1 Function: missingno

`missingno` is a function that serves as a way to exclude specific areas that contain systematic missing data. There are four methods available,

Default Command:

```
missingno(pop, type = "loci", cutoff = 0.05, quiet = FALSE, freq = FALSE)
```

- `pop` - a `genind` object.
- `type` - This could be one of four options:

  "loci" This is to be used for a data set that has systematic problems with certain loci that contain null alleles or simply failed to amplify. This will remove loci with a defined threshold of missing data from the data set.

  "geno" This is to be used for genotypes (individuals) in your data set where many null alleles are present. Individuals with a defined threshold missing data will be removed.

  "ignore" This function is used internally for functions like `aboot`. This will retain the missing data when using these functions.

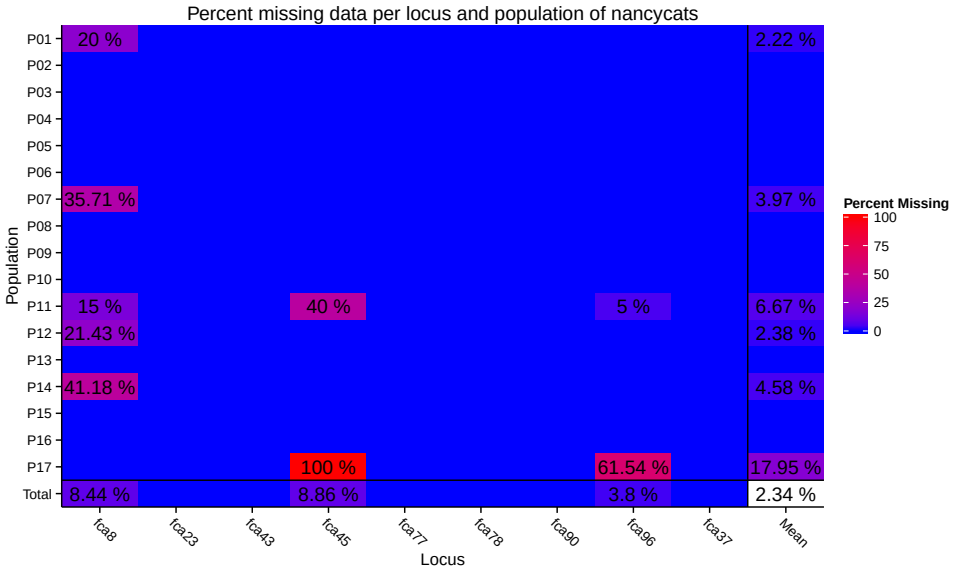
  "mean" This replaces missing data with the mean allele frequencies in the entire data set (This is for backwards compatibility, please don't use this).

  "zero" or "0" This replaces missing data with zero, signifying a new allele (This is for backwards compatibility, please don't use this).

- `cutoff` - This is a numeric value from 0 to 1 indicating the percent allowable missing data for either loci or genotypes. If you have, for example, two loci containing missing 5% and 10% missing data, respectively and you set `cutoff = 0.05`, `missingno` will remove the second locus. Percent missing data for genotypes is considered the percent missing loci over number of total loci.
- `quiet` - When this is set to `FALSE`, the number of missing values replaced will be printed to screen if the method is "zero" or "mean". It will print the number of loci or individuals removed if the method is "loci" or "geno".

Let's take a look at what this does by focusing in on areas with missing data. We'll use the data set `nancycats` as an example. Using the *poppr* function `info_table`, we can assess missing data within populations.

```
library("poppr")
data(nancycats)
info_table(nancycats, plot = TRUE)
```



```
##           Locus
## Population  fca8 fca23 fca43 fca45 fca77 fca78 fca90 fca96 fca37  Mean
##      P01   0.200     .     .     .     .     .     .     .     . 0.022
##      P02       .     .     .     .     .     .     .     .     .     .
##      P03       .     .     .     .     .     .     .     .     .     .
##      P04       .     .     .     .     .     .     .     .     .     .
##      P05       .     .     .     .     .     .     .     .     .     .
##      P06       .     .     .     .     .     .     .     .     .     .
##      P07   0.357     .     .     .     .     .     .     .     . 0.040
##      P08       .     .     .     .     .     .     .     .     .     .
##      P09       .     .     .     .     .     .     .     .     .     .
##      P10       .     .     .     .     .     .     .     .     .     .
##      P11   0.150     .     . 0.400     .     .     . 0.050     . 0.067
##      P12   0.214     .     .     .     .     .     .     .     . 0.024
##      P13       .     .     .     .     .     .     .     .     .     .
##      P14   0.412     .     .     .     .     .     .     .     . 0.046
##      P15       .     .     .     .     .     .     .     .     .     .
##      P16       .     .     .     .     .     .     .     .     .     .
##      P17       .     .     . 1.000     .     .     . 0.615     . 0.179
##      Total 0.084     .     . 0.089     .     .     . 0.038     . 0.023
```

We can see that locus fca8 has a lot of missing data. To demonstrate the function `missingno`, we will zoom into the first five individuals at the first locus.

```
tab(nancycats)[1:5, 8:13]

##      fca8.133 fca8.135 fca8.137 fca8.139 fca8.141 fca8.143
## N215       NA       NA       NA       NA       NA       NA
## N216       NA       NA       NA       NA       NA       NA
## N217        0        1        0        0        0        1
## N218        1        1        0        0        0        0
## N219        1        1        0        0        0        0
```

When looking at this data set, recall how a `genind` object is formatted. You have a matrix representing counts of alleles. For diploids, if you see 1, that means it is heterozygous at that allele, and a 2 means it's homozygous. Here we see three heterozygotes and two individuals with missing data (indicated by NA). Let's look at what happens when we exclude loci and genotypes with >5% missing data).

```
nanloci <-  missingno(nancycats, "loci")

##
##  2 loci contained missing values greater than 5%.
##  Removing 2 loci : fca8 fca45

nangeno <-  missingno(nancycats, "geno")

##
##  38 genotypes contained missing values greater than 5%.
##  Removing 38 genotypes : N215 N216 N188 N189 N190 N191 N192 N298 N299 N300
## N301 N302 N303 N304 N310 N195 N197 N198 N199 N200 N201 N206 N182 N184 N186 N282
## N283 N288 N291 N292 N293 N294 N295 N296 N297 N281 N289 N290

tab(nanloci)[1:5, 8:13]

##      fca23.144 fca23.146 fca23.148 fca23.150 fca43.133 fca43.135
## N215         0         1         0         0         0         0
```

```
## N216         0         2         0         0         0         0
## N217         0         1         0         0         0         0
## N218         0         0         0         0         0         0
## N219         0         1         0         0         0         0
```

Notice how we now see columns named "fca23.128" and "fca23.130". This is showing us another locus because we have removed the first. Recall from the summary table that the first locus had 16 alleles, and the second had 11. Now that we've removed loci containing missing data, all others have shifted over.
Let's look at the loci names and number of individuals.

```
nInd(nanloci)     # Individuals

## [1] 237

locNames(nanloci) # Names of the loci

## [1] "fca23" "fca43" "fca77" "fca78" "fca90" "fca96" "fca37"
```

You can see that the number of individuals stayed the same but the loci "fca8", "fca45", and "fca96" were removed.

Let's look at what happened when we removed individuals.

```
tab(nangeno)[1:5, 8:13]

##      fca8.133 fca8.135 fca8.137 fca8.139 fca8.141 fca8.143
## N217        0        1        0        0        0        1
## N218        1        1        0        0        0        0
## N219        1        1        0        0        0        0
## N220        0        1        0        0        0        1
## N221        0        2        0        0        0        0

nInd(nangeno)     # Individuals

## [1] 199

locNames(nangeno) # Names of the loci

## [1] "fca8"  "fca23" "fca43" "fca45" "fca77" "fca78" "fca90" "fca96" "fca37"
```

We can see here that the number of individuals decreased, yet we have the same number of loci. Notice how the frequency matrix changes in both scenarios? In the scenario with "loci", we removed several columns of the data set, and so with our sub-setting, we see alleles from the second locus. In the scenario with "geno", we removed several rows of the data set so we see other individuals in our sub-setting.

2.2 Extract populations {Divide (populations) and conquer (your analysis)}

This *poppr* function `popsub` makes subsetting genind or genlight objects by population easier:

2.2.1 Function: popsub

The command `popsub` is powerful in that it allows you to choose exactly what populations you choose to include or exclude from your analyses. As with many R functions, you can also use this within a function to avoid creating a new variable to keep track of.

Default Command:

```
popsub(gid, sublist = "ALL", blacklist = NULL, mat = NULL, drop = TRUE)
```

- `pop` - a `genind` object.
- `sublist` - vector of populations or integers representing the populations in your data set you wish to **retain**. For example: `sublist = c("pop_z", "pop_y")` or `sublist = 1:2`.
- `blacklist` - vector of populations or integers representing the populations in your data set you wish to **exclude**. This can take the same type of arguments as sublist, and can be used in conjunction with sublist for when you want a range of populations, but know that there is one in there that you do not want to analyze. For example: `sublist = 1:15, blacklist = "pop_x"`. One very useful thing about the blacklist is that it allows the user to be extremely paranoid about the data. You can set the blacklist to contain populations that are not even in your data set and it will still work!
- `mat` - (see section Multilocus Genotype Analysis for more information) A matrix produced from the `mlg.table` function. This overrides the *pop* argument and subsets this table instead.

To demonstrate this tool, we'll use the H3N2 virus data set provided in *adegenet*. It contains a data frame in the "other" slot called "x" that contains information about the year of epidemic, country, etc.

```
data("H3N2", package = "adegenet")
strata(H3N2) <- data.frame(other(H3N2)$x)
H3N2

## /// GENIND OBJECT /////////
##
##  // 1,903 individuals; 125 loci; 334 alleles; size: 3.9 Mb
##
##  // Basic content
##    @tab:  1903 x 334 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 2-4)
##    @loc.fac: locus factor for the 334 columns of @tab
##    @all.names: list of allele names for each locus
##    @ploidy: ploidy of each individual  (range: 1-1)
##    @type:  codom
##    @call: .local(x = x, i = i, j = j, drop = drop)
##
##  // Optional content
##    @strata: a data frame with 17 columns ( accession, length, host, segment, subtype, country, ... )
##    @other: a list containing: x  xy  epid
```

We will demonstrate the `popsub` functionality by setting the population factor to "country". Note, in this section, I am naming the variables staring with "v" indicating "virus".

```
setPop(H3N2) <- ~country
popNames(H3N2) # Only two countries from North America.

##  [1] "Japan"          "USA"            "Finland"        "China"          "South Korea"
##  [6] "Norway"         "Taiwan"         "France"         "Latvia"         "Netherlands"
## [11] "Bulgaria"       "Turkey"         "United Kingdom" "Denmark"        "Austria"
```

```
## [16] "Canada"         "Italy"          "Russia"         "Bangladesh"     "Egypt"
## [21] "Germany"        "Romania"        "Ukraine"        "Czech Republic" "Greece"
## [26] "Iceland"        "Ireland"        "Sweden"         "Nepal"          "Saudi Arabia"
## [31] "Switzerland"    "Iran"           "Mongolia"       "Spain"          "Slovenia"
## [36] "Croatia"        "Algeria"

v_na <- popsub(H3N2, sublist = c("USA", "Canada"))
popNames(v_na)

## [1] "USA"    "Canada"
```

If we want to see the population size, we can use the *adegenet* function `nInd()`:

```
c(NorthAmerica = nInd(v_na), Total = nInd(H3N2))

## NorthAmerica        Total
##          665         1903
```

You can see that the population factors are correct and that the size of the data set is considerably smaller. Let's see the data set without the North American countries.

```
v_na_minus <- popsub(H3N2, blacklist = c("USA", "Canada"))
popNames(v_na_minus)

##  [1] "Japan"          "Finland"        "China"          "South Korea"    "Norway"
##  [6] "Taiwan"         "France"         "Latvia"         "Netherlands"    "Bulgaria"
## [11] "Turkey"         "United Kingdom" "Denmark"        "Austria"        "Italy"
## [16] "Russia"         "Bangladesh"     "Egypt"          "Germany"        "Romania"
## [21] "Ukraine"        "Czech Republic" "Greece"         "Iceland"        "Ireland"
## [26] "Sweden"         "Nepal"          "Saudi Arabia"   "Switzerland"    "Iran"
## [31] "Mongolia"       "Spain"          "Slovenia"       "Croatia"        "Algeria"
```

Let's make sure that the number of individuals in both data sets is equal to the number of individuals in our original data set:

```
(nInd(v_na_minus) + nInd(v_na)) == nInd(H3N2)

## [1] TRUE
```

Now we have data sets with and without North America. Let's try something a bit more challenging. Let's say that we want the first 10 populations in alphabetical order, but we know that we still don't want any countries in North America. We can easily do this by using the *base* function `sort`.

```
vsort <- sort(popNames(H3N2))[1:10]
vsort

##  [1] "Algeria"        "Austria"        "Bangladesh"     "Bulgaria"       "Canada"
##  [6] "China"          "Croatia"        "Czech Republic" "Denmark"        "Egypt"

valph <- popsub(H3N2, sublist = vsort, blacklist = c("USA", "Canada"))
popNames(valph)

## [1] "China"          "Bulgaria"       "Denmark"        "Austria"        "Bangladesh"
## [6] "Egypt"          "Czech Republic" "Croatia"        "Algeria"
```

And that, is how you subset your data with poppr!

2.3 Clone-censor data sets {Attack of the clone correction}

Clone correction refers to the ability of keeping one observation of each MLG in a given population (or sub-population). Clone correcting can be hazardous if its done by hand (even on small data sets) and it requires a defined population hierarchy to get relevant results. *Poppr* has a clone correcting function that that will correct down to the lowest level of any defined population hierarchy. Note that clone correction in *poppr* is sensitive to missing data, as it treats all missing data as a single extra allele.

This function will create new data sets, but it is also utilized by the functions `poppr` and `poppr.amova` natively.

2.3.1 Function: clonecorrect

This function will return a clone corrected data set corrected for the lowest population level. Population levels are specified with the `hier` flag. You can choose to combine the population hierarchy to analyze at the lowest population level by choosing `combine = TRUE`.

Default Command:

```
clonecorrect(pop, strata = 1, combine = FALSE, keep = 1)
```

- `pop` - a `genclone` object with a defined hierarchy or a `genind` object that has a population hierarchy data frame in the `@other` slot. Note, the `genind` object does not necessarily require a population factor to begin with.
- `strata` - A hierarchical formula (eg. `~Pop/Subpop`), representing the hierarchical levels in your data.
- `combine` - Do you want to combine the population hierarchy? If it's set to `FALSE` (default), you will be returned an object with the top most hierarchical level as a population factor unless the **keep** argument is defined. If set to `TRUE`, the hierarchy will be returned combined.
- `keep` - This flag is to be used if you set `combine = FALSE`. This will tell clone correct to return a specific combination of your hierarchy defined as integers. For example, imagine a hierarchy that needs to be clone corrected at three levels: *Population* by *Year* by *Month*. If you wanted to only run an analysis on the *Population* level, you would set `keep = 1` since *Population* is the first level of the hierarchy. On the other hand, if you wanted to run analysis on *Year* by *Month*, you would set `keep = 2:3` since those are the second and third levels of the hierarchy.

Let's look at ways to clone-correct our data. We'll look at our *A. euteichies* data that we used in the QUICKSTART since that data set is known to include clonal populations (Grünwald & Hoheisel, 2006). Try playing around with the data and see what different combinations of the `hier`, and `keep` flags produce. Below, I will give three examples of clone corrections at the sample level with respect to field, at the field level, and finally, at the level of the entire data set.

First, we will examine the original data set.

```
data(Aeut)
strata(Aeut) <- data.frame(other(Aeut)$population_hierarchy[-1])
Aeut

## /// GENIND OBJECT /////////
##
##  // 187 individuals; 56 loci; 56 alleles; size: 70.1 Kb
```

```
##
##  // Basic content
##    @tab:  187 x 56 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 56-56)
##    @ploidy: ploidy of each individual  (range: 2-2)
##    @type:  PA
##    @call: old2new_genind(object = x, donor = new(class(x)))
##
##  // Optional content
##    @pop: population of each individual (group size range: 90-97)
##    @strata: a data frame with 2 columns ( Pop, Subpop )
##    @other: a list containing: population_hierarchy
```

When you read in data with `read.genalex`, the default is to represent it in a genclone object. Since the `clonecorrect` function works on multilocus genotype definitions, It's more efficient to convert it to a genclone object first. We will also rename the strata to "field" and "sample" to make the biological relevance of the data clearer.

```
aphan <- as.genclone(Aeut)
nameStrata(Aeut) <- ~field/sample
```

Now we correct by sample with respect to field and keep the field as the population.

```
clonecorrect(aphan,  strata = ~Pop/Subpop)

##
## This is a genclone object
## -------------------------
## Genotype information:
##
##    119 multilocus genotypes
##    141 diploid individuals
##     56 dominant loci
##
## Population information:
##
##      2 strata - Pop Subpop
##      2 populations defined - Athena Mt. Vernon

# Your turn: Use the same stratification and use combine = TRUE and then
# keep = 1:2. Is there any difference?
```

Correcting by field. Notice how the number of MLG is much closer to our census.

```
clonecorrect(aphan, strata = ~Pop)

##
## This is a genclone object
## -------------------------
## Genotype information:
##
##    119 multilocus genotypes
##    120 diploid individuals
##     56 dominant loci
##
```

```
## Population information:
##
##      2 strata - Pop Subpop
##      2 populations defined - Athena Mt. Vernon
```

Correcting over whole data set. Our MLG is equal to our census.

```
clonecorrect(aphan, strata = NA)

##
## This is a genclone object
## -------------------------
## Genotype information:
##
##    119 multilocus genotypes
##    119 diploid individuals
##     56 dominant loci
##
## Population information:
##
##      2 strata - Pop Subpop
##      2 populations defined - Athena Mt. Vernon
```

2.4 Permutations and bootstrap resampling {every day I'm shuffling (data sets)}

A common null hypothesis for populations with mixed reproductive modes is panmixia, or to put it simply: lots of sex. *Poppr* randomly shuffles data sets in order to calculate P-values for the index of association (I_A and $\bar{r}_d$) (Agapow & Burt, 2001) using 4 different methods:

method	strategy	units sampled
1	permutation	alleles
2	simulation	alleles
3	simulation	alleles
4	permutation	genotypes

These methods are detailed below. We will create a dummy data set to be shuffled by each example below. Let's assume a single diploid locus with four alleles (1, 2, 3, 4) with the frequencies of 0.1, 0.2, 0.3, and 0.4, respectively:

	A1/A2
1	4/4
2	4/1
3	4/3
4	2/2
5	3/3

Table 1: Original

The 4 methods are detailed below.

2.4.1 Function: shufflepop

Default Command:

```
shufflepop(pop, method = 1)
```

- `pop` - a `genind` object.
- `method` - a number indicating the method of sampling you wish to use. The following methods are available for use:

1. **Permute Alleles (default)** This is a sampling scheme that will **permute alleles within the locus.**

   The example above might become tables 2 and 3.

	A1/A2
1	3/4
2	2/3
3	4/4
4	2/1
5	3/4

Table 2: Permute 1

	A1/A2
1	1/3
2	2/4
3	3/4
4	4/3
5	4/2

Table 3: Permute 2

   As you can see, The heterozygosity has changed, yet the allelic frequencies remain the same. Overall this would show you what would happen if the sample you had underwent panmixis within this sample itself.

2. **Parametric Bootstrap** The previous scheme reshuffled the observed sample, but the parametric bootstrap **draws samples from a multinomial distribution using the observed allele frequencies as weights.** Tables 4 and 5 are examples of what I mean.

	A1/A2
1	1/3
2	3/3
3	3/2
4	4/4
5	4/2

Table 4: Parametric 1

	A1/A2
1	3/4
2	2/3
3	4/2
4	4/4
5	4/2

Table 5: Parametric 2

   Notice how the heterozygosity has changed along with the allelic frequencies. The frequencies for alleles 3 and 4 have switched in the first data set, and we've lost allele 1 in the second data set purely by chance! This type of sampling scheme attempts to show you what the true population would look like if it were panmictic and your original sample gave you a basis for estimating expected allele frequencies. Since estimates are made from the observed allele frequencies, small samples will produce skewed results.

3. **Non-Parametric Bootstrap** The third method is sampling with replacement, again **drawing from a multinomial distribution, but with no assumption about the allele frequencies** (tables 6 and 7).

   Again, heterozygosity and allele frequencies are not maintained, but now all of the alleles have a 1 in 4 chance of being chosen.

	A1/A2
1	1/3
2	3/3
3	3/1
4	2/2
5	3/1

Table 6: Non-parametric 1

	A1/A2
1	1/3
2	3/1
3	2/3
4	2/1
5	4/3

Table 7: Non-parametric 2

4. **Multilocus permutation** This is called Multilocus permutation because it does the same thing as the permutation analysis in the program *multilocus* by Paul Agapow and Austin Burt (Agapow & Burt, 2001). This will shuffle the genotypes at each locus. Using our example above, tables 8 and 9 are shuffled with method 4.

	A1/A2
1	3/3
2	4/1
3	2/2
4	4/4
5	4/3

Table 8: ML 1

	A1/A2
1	4/4
2	2/2
3	3/3
4	4/3
5	4/1

Table 9: ML 2

Note that you have the same genotypes after shuffling, so at each locus, you will maintain the same allelic frequencies and heterozygosity. So, in this sample, you will only see a homozygote with allele 2. This also ensures that the P-values associated with I_A and $\bar{r}_d$ are exactly the same. This method **assumes that alleles are not independently assorting within individuals.** This strategy is **useful if you suspect the population is inbreeding** (Jerome Goudet, personal communication).

These shuffling schemes have been implemented for the index of association, but there may be other summary statistics you can use `shufflepop` for. All you have to do is use the function `replicate`. Let's use average Bruvo's distance with the first population of the data set `nancycats` as an example:

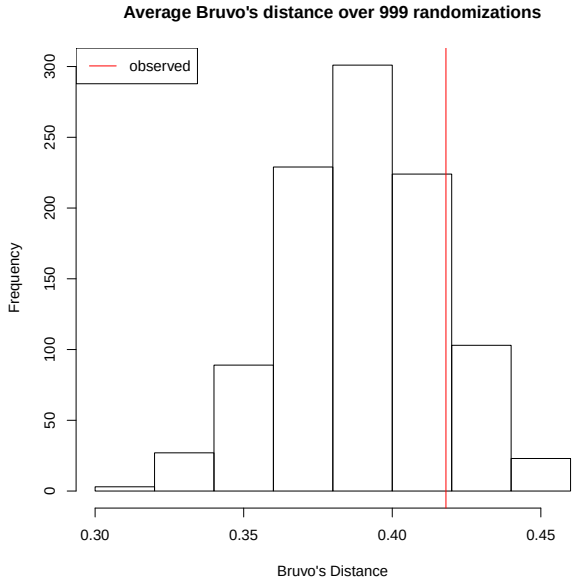
```
data(nancycats)
nan1 <- popsub(nancycats, 1)
reps <- rep(2, 9) # Assuming dinucleotide repeats.
observed <- mean(bruvo.dist(nan1, replen = reps))
observed

## [1] 0.4180619
```

```
set.seed(9999)
bd.test <- replicate(999, mean(bruvo.dist(shufflepop(nan1, method = 2), replen = reps)))
```

You could use this method to replicate the resampling 999 times and then create a histogram to visualize a distribution of what would happen under different assumptions of panmixia.

```
hist(bd.test, xlab = "Bruvo's Distance", main = "Average Bruvo's distance over 999 randomizations")
abline(v = observed, col = "red")
legend('topleft', legend="observed", col="red", lty = 1)
```



2.5 Removing uninformative loci {Cut It Out!}

Phylogenetically uninformative loci are those that have only one sample differentiating from the rest. This can lead to biased results when using multilocus analyses such as the index of association (Brown *et al.* , 1980; Smith *et al.* , 1993). These nuisance loci can be removed with the following function.

2.5.1 Function: informloci

Default Command:

```
informloci(pop, cutoff = 2/nInd(pop), MAF = 0.01, quiet = FALSE)
```

- `pop` - a `genind` object.
- `cutoff` - this represents the minimum fraction of individuals needed for a locus to be considered informative. The default is set to $2/n$ with n being the number of individuals in the data set (represented by the *adegenet* function `nInd`). Essentially, this means that any locus with fewer than 2 observations differing will be removed. The user can also specify a fraction of observations for the cutoff (eg. 0.05).
- `quiet` - if `TRUE`, nothing will be printed to the screen, if `FALSE` (default), the cutoff value in percentage and number of individuals will be printed as well as the names of the uninfomrative loci found.

Here's a quick example using the H3N2 virus SNP data set from section ON SUBSETTING YOUR DATA. We will only retain loci that have a minor allele frequency of $\geq 5\%$

```
H.five <- informloci(H3N2, cutoff = 0.05)
```

Now what happens when you have all informative loci. We'll use the nancycats data set, which has microsatellite loci. It is important to note that this is searching for loci with a specified genotype frequency as fixed heterozygous sites are also uninformative:

```
data(nancycats)
naninform <- informloci(nancycats, cutoff = 0.05)
```

3 Multilocus Genotype Analysis

In populations with mixed sexual and clonal reproduction, it common to have multiple samples from the same population that have the same set of alleles at all loci. Here, we introduce tools for tracking MLGs within and across populations in GENIND objects from the *adegenet* package. We will be using the H3N2 data set containing SNP data from isolates of the H3N2 virus from 2002 to 2006. Note that genclone objects are optimal for these analyses. For a more in-depth document on methods for multilocus genotypes in *poppr*, see the "Multilocus Genotype Analysis" vignette by typing

```
vignette("mlg", package = "poppr")
```

3.1 How many multilocus genotypes are in our data set? {Just a peek}

Counting the number of MLGs in a population is the first step for these analyses as they allow us to see how many clones exist. With the GENCLONE object, This information is already displayed when we view the object.

```
H3N2
```

```
## /// GENIND OBJECT /////////
##
##  // 1,903 individuals; 125 loci; 334 alleles; size: 3.9 Mb
##
##  // Basic content
##    @tab:  1903 x 334 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 2-4)
##    @loc.fac: locus factor for the 334 columns of @tab
##    @all.names: list of allele names for each locus
##    @ploidy: ploidy of each individual  (range: 1-1)
##    @type:  codom
##    @call: .local(x = x, i = i, j = j, drop = drop)
##
##  // Optional content
##    @pop: population of each individual (group size range: 1-646)
##    @strata: a data frame with 17 columns ( accession, length, host, segment, subtype, country, ... )
##    @other: a list containing: x  xy  epid
```

If we need to store the number of MLGs as a variable, we can simply run the `mlg` command.

```
H3N2_mlg <- mlg(H3N2)
```

```
## ############################
## # Number of Individuals:  1903
## # Number of MLG:  752
## ############################
```

```
H3N2_mlg
```

```
## [1] 752
```

Since the number of individuals exceeds the number of multilocus genotypes, we conclude that this data set contains clones. Let's examine what populations these clones belong to.

3.2 MLGs across populations {clone-ing around}

Since you have the ability to define hierarchical levels of your data set freely, it is quite possible to see some of the same MLGs across different populations. Tracking them by hand can be a nightmare with large data sets. Luckily, `mlg.crosspop` has you covered in that regard.

3.2.1 Function: mlg.crosspop

Analyze the MLGs that cross populations within your data set. This has three output modes. The default one gives a list of MLGs, and for each MLG, it gives a named numeric vector indicating the abundance of that MLG in each population. Alternate outputs are described with `indexreturn` and `df`.

Default Command:

```
mlg.crosspop(gid, strata = NULL, sublist = "ALL", blacklist = NULL,
    mlgsub = NULL, indexreturn = FALSE, df = FALSE, quiet = FALSE)
```

- `pop` - a `genind` object.
- `sublist` - Populations to include (Defaults to "ALL"). see `popsub`.
- `blacklist` - Populations to exclude. see `popsub`.
- `mlgsub` - see `mlg.table`. Only analyze specified MLGs. The vector for this flag can be produced by this function as you will see later in this vignette.
- `indexreturn` - return a vector of indices of MLGs. (You can use these in the `mlgsub` flag, or you can use them to subset the columns of an MLG table).
- `df` - return a data frame containing the MLGs, the populations they cross, and the number of copies you find in each population. This is useful for making graphs in *ggplot2*.
- `quiet` - `TRUE` or `FALSE`. Should the populations be printed to screen as they are processed? (will print nothing if `indexreturn` is `TRUE`)

We can see what MLGs cross different populations and then give a vector that shows how many populations each one of those MLGs crosses.

```
setPop(H3N2) <- ~country
v.dup <- mlg.crosspop(H3N2, quiet=TRUE)
```

Here is a snippet of what the output looks like when `quiet` is `FALSE`. It will print out the MLG name, the total number of individuals that make up that MLG, and the populations where that MLG can be found.

```
## MLG.3: (12 inds) USA Denmark
## MLG.9: (16 inds) Japan USA Finland Denmark
## MLG.31: (9 inds) Japan Canada
## MLG.75: (23 inds) Japan USA Finland Norway Denmark Austria Russia Ireland
```

```
## MLG.80: (2 inds) USA Denmark
## MLG.86: (7 inds) Denmark Austria
## MLG.95: (2 inds) USA Bangladesh
## MLG.97: (8 inds) USA Austria Bangladesh Romania
## MLG.104: (3 inds) USA France
## MLG.110: (16 inds) Japan USA China
```

The output of this function is a list of MLGs, each containing a vector indicating the number of copies in each population. We'll count the number of populations each MLG crosses using the function `sapply` to loop over the data with the function `length`.

```
head(v.dup)

## $MLG.3
##     USA Denmark
##       4       8
##
## $MLG.9
##   Japan     USA Finland Denmark
##       1      13       1       1
##
## $MLG.31
##  Japan Canada
##      2      7
##
## $MLG.75
##   Japan     USA Finland  Norway Denmark Austria  Russia Ireland
##       2       8       2       1       6       2       1       1
##
## $MLG.80
##     USA Denmark
##       1       1
##
## $MLG.86
## Denmark Austria
##       3       4

v.num <- sapply(v.dup, length) # count the number of populations each MLG crosses.
head(v.num)

##  MLG.3  MLG.9 MLG.31 MLG.75 MLG.80 MLG.86
##      2      4      2      8      2      2
```

3.3 Producing MLG tables and graphs {bringing something to the table}

We can also create a table of MLGs per population as well as bar graphs to give us a visual representation of the data. This is achieved through the function `mlg.table`

3.3.1 Function: mlg.table

This function will produce a matrix containing counts of MLGs (columns) per population (rows). If there are not populations defined in your data set, a vector will be produced instead.

Default Command:

```
mlg.table(gid, strata = NULL, sublist = "ALL", blacklist = NULL,
    mlgsub = NULL, bar = TRUE, plot = TRUE, total = FALSE, quiet = FALSE)
```

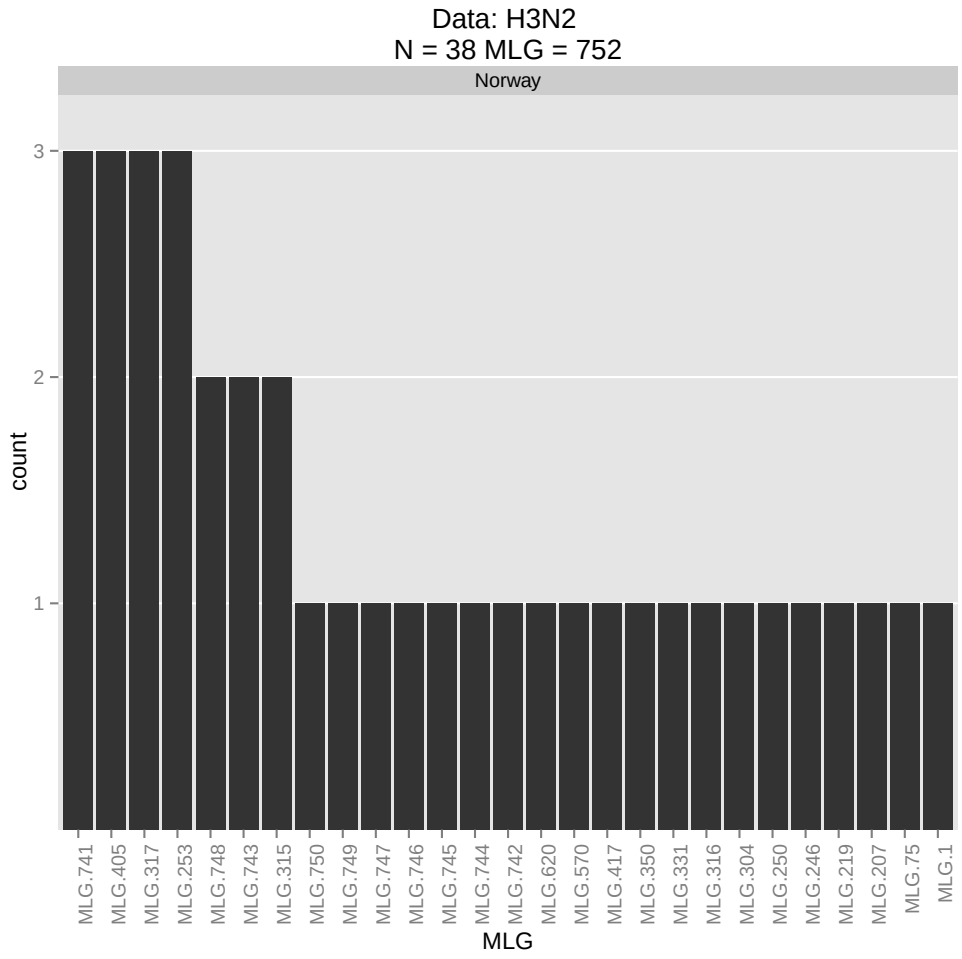
- `pop` - a `genind` object.
- `sublist` - Populations to include (Defaults to "ALL"). see `popsub`.
- `blacklist` - Populations to exclude. see `popsub`.
- `mlgsub` - a vector containing the indices of MLGs you wish to subset your table with.
- `plot` - `TRUE` or `FALSE`. If `TRUE`, a bar plot will be printed for each population with more than one individual.
- `total` - When set to `TRUE`, the pooled data set will be added to the table. Defaults to `FALSE`.
- `quiet` - Defaults to `FALSE`: population names will be printed to the console as they are processed.

```
v.tab <- mlg.table(H3N2, plot = TRUE)
v.tab[1:10, 1:10] # Showing the first 10 columns and rows of the table.
```

```
##             MLG.1 MLG.2 MLG.3 MLG.4 MLG.5 MLG.6 MLG.7 MLG.8 MLG.9 MLG.10
## Japan           0     0     0     0     0     0     1     2     1      0
## USA             0     2     4     1     1     0     0     0    13      0
## Finland         0     0     0     0     0     0     0     0     1      0
## China           0     0     0     0     0     0     0     0     0      0
## South Korea     0     0     0     0     0     1     0     0     0      0
## Norway          1     0     0     0     0     0     0     0     0      0
## Taiwan          0     0     0     0     0     0     0     0     0      0
## France          0     0     0     0     0     0     0     0     0      0
## Latvia          0     0     0     0     0     0     0     0     0      0
## Netherlands     0     0     0     0     0     0     0     0     0      0
```

Figure 4: An example of a bar-chart produced by `mlg.table`. Note that this data set would produce several such charts but only the chart for Norway is shown here.

```
mlg.table(H3N2, sublist = "Norway", plot = TRUE)
```



The MLG table is not limited to use with *poppr*. In fact, one of the main advantages of `mlg.table` is that it allows easy access to diversity functions present in the package *vegan* (Oksanen *et al.* , 2012). One example is to create a rarefaction curve for each population in your data set giving the number of expected MLGs for a given sample size. For more information, type `help("diversity", package="vegan")` in your R console.

For the sake of this example, instead of drawing a curve for each of the 37 countries represented in this sample, let's set the hierarchical level to year.

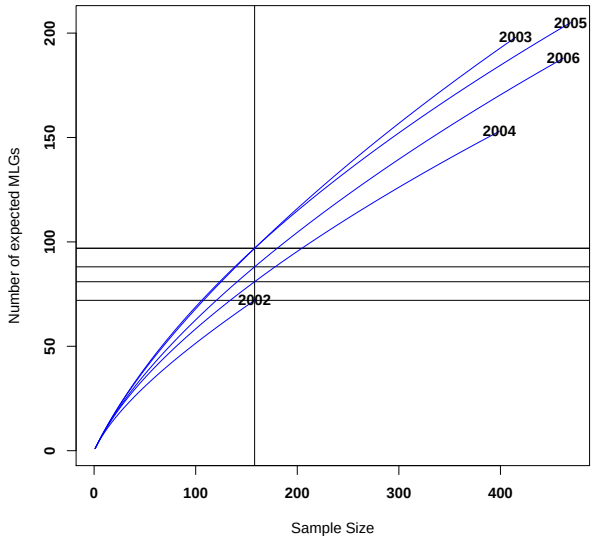
```
setPop(H3N2) <- ~year
summary(H3N2) # Check the data to make sure it's correct.
```

```
##
##  # Total number of genotypes:  1903
```

```
##
##  # Population sample sizes:
## 2002 2003 2004 2005 2006
##  158  415  399  469  462
##
##  # Number of alleles per locus:
##   6  17  39  42  45  51  60  72  73  90 108 123 129 134 145 148 149 157 168 171 177 225
##   3   3   4   2   4   2   3   2   4   3   4   2   4   3   2   2   3   3   2   2   3   3
## 233 243 247 262 267 280 303 313 317 327 334 345 351 357 376 382 384 391 396 399 412 418
##   3   2   2   2   2   2   2   2   2   2   2   4   4   3   3   3   4   2   2   2   4   3
## 424 425 429 430 433 434 435 451 463 464 468 470 476 483 490 517 529 546 555 557 561 562
##   2   3   4   2   3   2   3   2   2   2   4   2   2   2   2   2   2   2   4   4   4   3
## 564 566 576 577 578 582 592 594 595 597 600 602 604 612 627 642 647 648 654 658 663 664
##   3   2   3   4   3   2   3   3   3   3   2   3   2   4   2   3   2   2   3   3   3   3
## 666 667 673 674 676 679 681 685 717 763 806 807 824 837 882 897 906 910 915 929 933 936
##   2   2   2   2   3   2   3   2   3   2   3   2   3   3   2   2   2   3   2   2   2   3
## 939 940 957 961 962 963 966 967 969 973 975 977 978 979 980
##   3   3   2   2   3   3   3   3   4   2   3   3   4   3   2
##
##  # Number of alleles per population:
## 2002 2003 2004 2005 2006
##  203  255  232  262  240
##
##  # Percentage of missing data:
## [1] 2.363426
##
##  # Observed heterozygosity:
## [1] 0
##
##  # Expected heterozygosity:
## [1] 0
```

```
library("vegan")
H.year <- mlg.table(H3N2, plot = FALSE)
rarecurve(H.year, ylab="Number of expected MLGs", sample=min(rowSums(H.year)),
          border = NA, fill = NA, font = 2, cex = 1, col = "blue")
```

Figure 5: An example of a rarefaction curve produced using a MLG table.



The minimum value from the *base* function `rowSums()` of the table represents the minimum common sample size of all populations defined in the table. Setting the "sample" flag draws the horizontal and vertical lines you see on the graph. The intersections of these lines correspond to the numbers you would find if you ran the function `poppr` on this data set (under the column "`eMLG`").

3.4 Combining MLG functions {getting into the mix}

Alone, the different functionalities are neat. Combined, we can create interesting data sets. Let's say we wanted to know which MLGs were duplicated across the regions of the United Kingdom, Germany, Netherlands, and Norway. All we have to do is use the `sublist` flag in the function:

```
setPop(H3N2) <- ~country
UGNN.list <- c("United Kingdom", "Germany", "Netherlands", "Norway")
UGNN <- mlg.crosspop(H3N2, sublist=UGNN.list, indexreturn=TRUE)
```

OK, the output tells us that there are three MLGs that are crossing between these populations, but we do not know how many are in each. We can easily find that out if we subset our original table, `v.tab`.

```
UGNN # Note that we have three numbers here. This will index the columns for us.

## [1] 315 317 620

UGNN.list # And let's not forget that we have the population names.

## [1] "United Kingdom" "Germany"        "Netherlands"    "Norway"

v.tab[UGNN.list, UGNN]

##                MLG.315 MLG.317 MLG.620
## United Kingdom       1       0       0
## Germany              0       1       1
## Netherlands          0       0       0
## Norway               2       3       1
```

Now we can see that Norway has a higher incidence of nearly all of these MLGs. We can investigate the incidence of these MLGs throughout our data set. One thing that the GENCLONE object keeps track of is a single vector defining the unique multilocus genotypes within the data. These are represented as integers and can be accessed with `mlg.vector`. This is useful for finding MLGs that correspond to certain individuals or populations. Let's use `mlg.vector` to find individuals corresponding to the MLGs. First we'll investigate what the output of this function looks like.

```
v.vec <- mlg.vector(H3N2)
str(v.vec) # Analyze the structure.

##  int [1:1903] 605 605 672 675 674 673 670 671 670 678 ...
```

The integers produced are the MLG assigment of each individual in the same order as the data set. This means that the first two individuals have the exact same set of alleles at each locus, so they have the same MLG: 605. If we look at the number of unique integers in the vector, it corresponds to the number of observed multilocus genotypes:

```
length(unique(v.vec)) # count the number of MLGs

## [1] 752

H3N2 # equal to the first number in this output.

## /// GENIND OBJECT /////////
##
##  // 1,903 individuals; 125 loci; 334 alleles; size: 3.9 Mb
##
##  // Basic content
##    @tab:  1903 x 334 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 2-4)
##    @loc.fac: locus factor for the 334 columns of @tab
##    @all.names: list of allele names for each locus
##    @ploidy: ploidy of each individual  (range: 1-1)
##    @type:  codom
##    @call: .local(x = x, i = i, j = j, drop = drop)
##
##  // Optional content
##    @pop: population of each individual (group size range: 1-646)
##    @strata: a data frame with 17 columns ( accession, length, host, segment, subtype, country, ... )
##    @other: a list containing: x  xy  epid
```

We will take `UGNN` (MLGs crossing UK, Germany, Netherlands, and Norway) and compare its elements to the MLG vector (`v.vec`) to see where else they occur.

```
UGNN # Show what we are looking for

## [1] 315 317 620

UGNN_match <- v.vec %in% UGNN
table(UGNN_match) # How many individuals matched to those three MLGs?

## UGNN_match
## FALSE  TRUE
##  1881    22
```

22 individuals matched to those three MLGs. We can use this vector to show us the 22 individuals.

```
indNames(H3N2)[UGNN_match]
```

```
##       0329       0330       0331       0332       0341       0342       0345       0556
## "CY026119" "CY026120" "CY026121" "CY026122" "CY026131" "CY026132" "CY026135" "EU502462"
##       0557       0558       0870       0974       1112       1113       1114       1122
## "EU502463" "EU502464" "EU501513" "AB243868" "DQ883618" "DQ883619" "DQ883620" "DQ883628"
##       1193       1209       1210       1281       1288       1426
## "EU501609" "EU501642" "EU501643" "EU501735" "EU501742" "EU502513"
```

Note that there is an alternative way to list individuals matching specific MLGs using the function `mlg.id`. This function will return a list where each element represents a unique MLG. You can use this data to find out which individuals correspond to specific MLGs. Each element in the list is named with the MLG, but the index does not necessarily match up, so it is important to convert your query MLGs to strings:

```
H3N2.id <- mlg.id(H3N2)
H3N2.id[as.character(UGNN)]
```

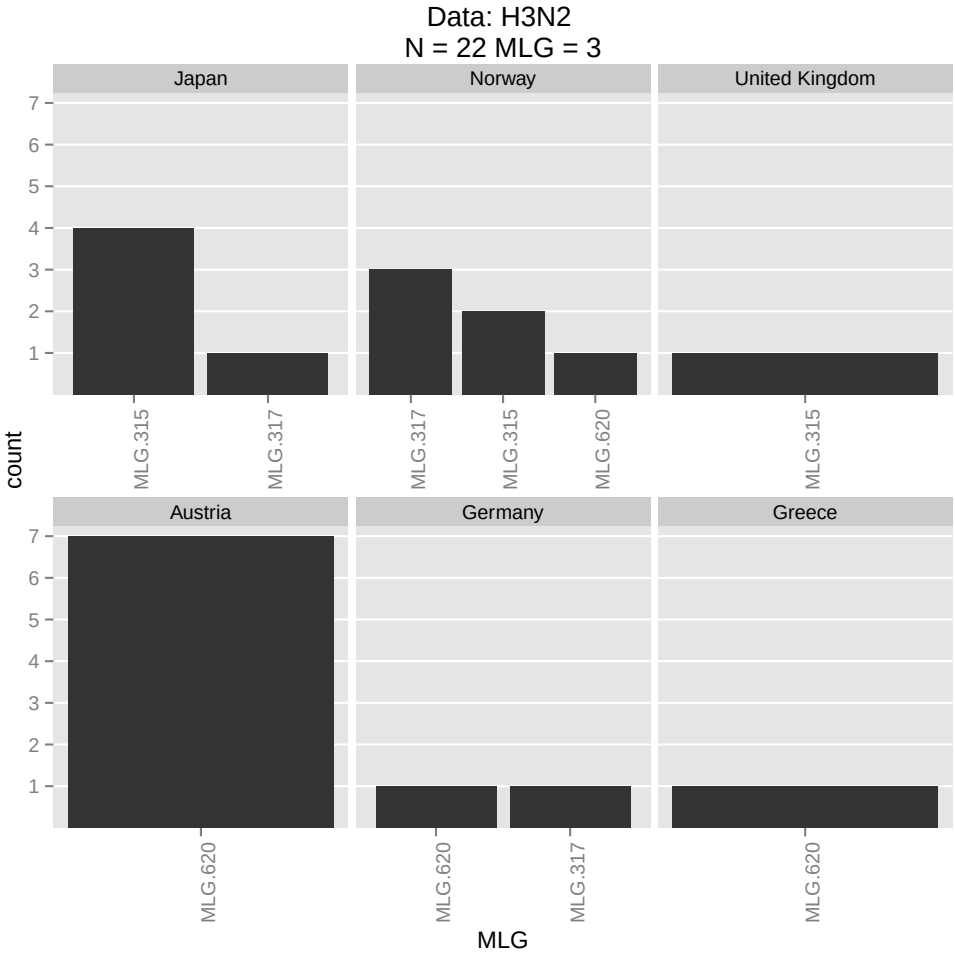
```
## $`315`
##       0974       1112       1122       1209       1210       1281       1288
## "AB243868" "DQ883618" "DQ883628" "EU501642" "EU501643" "EU501735" "EU501742"
##
## $`317`
##       0870       1113       1114       1193       1426
## "EU501513" "DQ883619" "DQ883620" "EU501609" "EU502513"
##
## $`620`
##       0329       0330       0331       0332       0341       0342       0345       0556
## "CY026119" "CY026120" "CY026121" "CY026122" "CY026131" "CY026132" "CY026135" "EU502462"
##       0557       0558
## "EU502463" "EU502464"
```

We can also use the vector of MLGs to subset `mlg.table` with the `mlgsub` flag.

```
mlg.table(H3N2, mlgsub = UGNN)
```

That showed us exactly which populations these three MLGs came from in our data set.

Figure 6: All populations that contain these MLGs

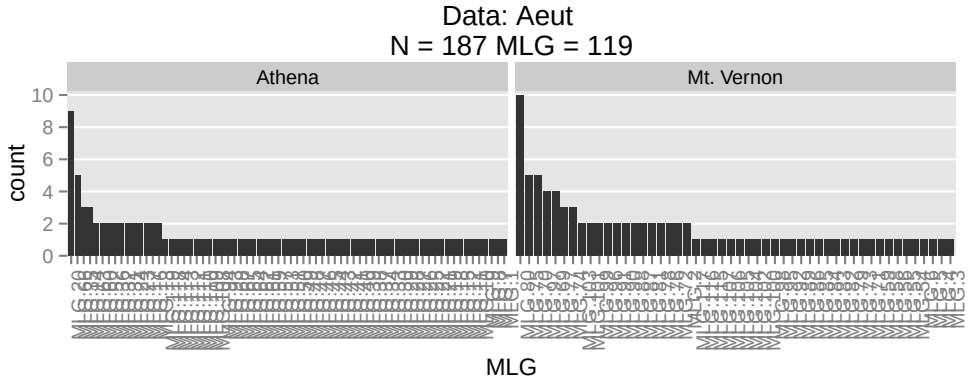


4 Appendix

4.1 Manipulating Graphics

Poppr utilizes *ggplot2* to produce many of its graphs. One advantage it gives the user is the ability to manipulate these graphs. With base R graphs, the only manipulation that can be performed is by adding elements to the graph. It is a static image. The ggplot graphs are actually represented as objects in your R environment. We can use the function `last_plot()` from *ggplot2* to be able to grab the plot that was plotted last in our window. Let's illustrate this using a MLG bar graph from the Aeut data set.

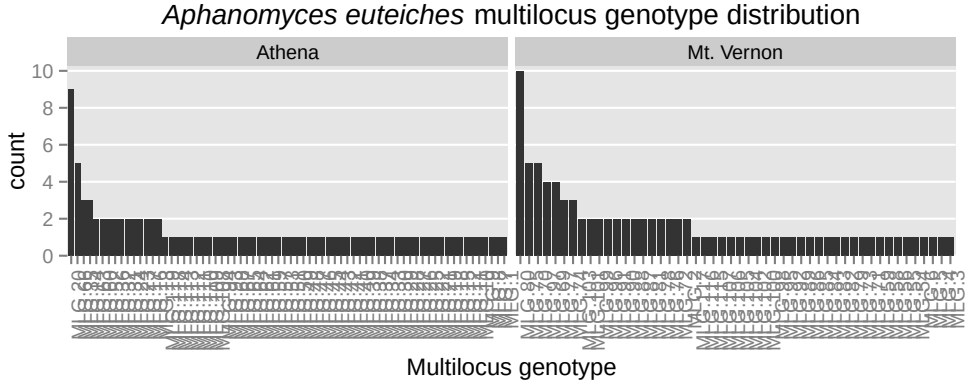
```
library("poppr")
library("ggplot2")
data(Aeut)
Aeut.tab <- mlg.table(Aeut)
```




```
p <- last_plot()
```

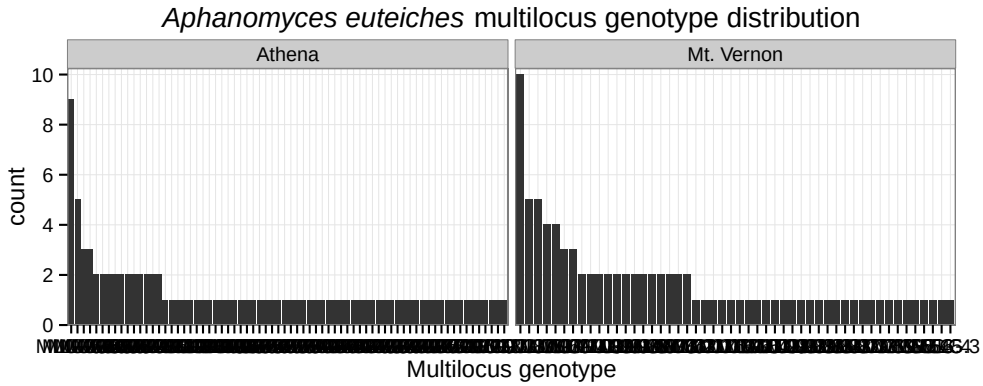
We've captured our plot using `last_plot()` and now we can manipulate it. One common need is to change the title. We can easily do that with the function `ggtitle()`. Let's say we wanted to label it "*Aphanomyces euteiches* multilocus genotype distribution". We would use `ggtitle("Aphanomyces euteiches multilocus genotype distribution")`. Unfortunately, we need italics for a latin binomial. One way to acheive this is by using the `expression()` function and declaring which text needs to be italicized.

```
myTitle <- expression(paste(italic("Aphanomyces euteiches"), " multilocus genotype distribution"))
(pt <- p +
   ggtitle(myTitle) +
   xlab("Multilocus genotype")) # We can label the x axis, too
```



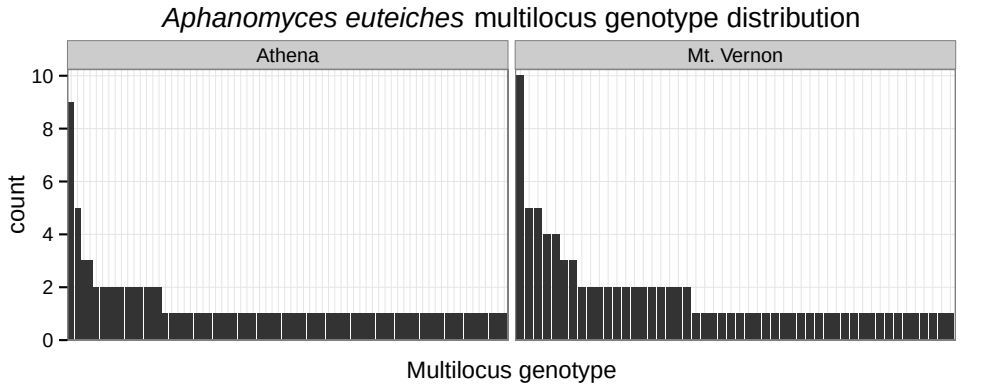
Let's say we wanted to remove the grey background. We could use the `theme()` function to do this, or we could use a theme already implemented in ggplot2 called `theme_bw()`.

```
(ptt <- pt + theme_bw())
```



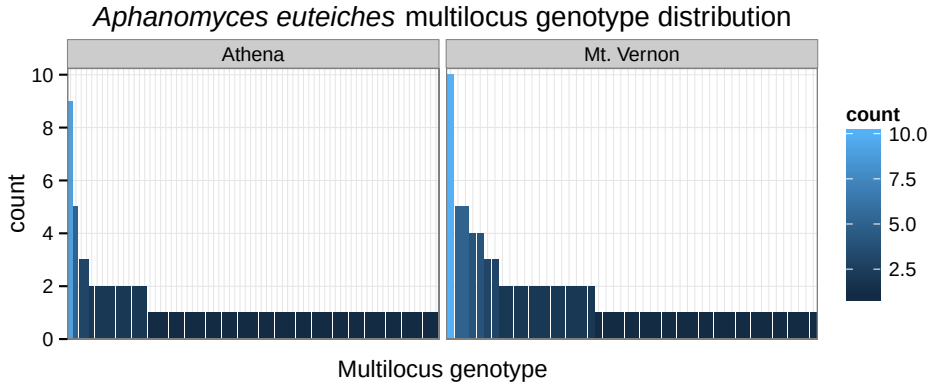
Uh-oh. The x axis labels are now horizontal when they should be vertical. Since it's the overall distribution we're interested in, we don't really need them anyways. We can remove them with `axis.text.x` and `axis.ticks.x`.

```
(ptta <- ptt + theme(axis.text.x = element_blank(), axis.ticks.x = element_blank()))
```



And, if for some bizzarre reason, you liked the color gradient in *poppr* version 1, you can get that back by adding the *fill* aesthetic:

```
(ptttaf <- ptta + aes(fill = count))
```



This allows you to produce publication quality graphs directly in R. Please see Hadley Wickham's *ggplot2* package for more details (Wickham, 2009). Note that if you don't like using *ggplot2*, you can access the data in the ggplot2 object and plot the data yourself:

```
head(p$data)
```

```
## Source: local data frame [6 x 4]
##
##   Population    MLG count fac
## 1     Athena  MLG.1     1 120
## 2     Athena  MLG.7     1 119
## 3     Athena  MLG.8     1 118
## 4     Athena  MLG.9     1 117
## 5     Athena MLG.10     1 116
## 6     Athena MLG.11     1 115
```

4.2 Exporting Graphics

R has the ability to produce nice graphics from most any type of data, but to get these graphics into a report, presentation, or manuscript can be a bit challenging. It's no secret that the R Documentation pages are a little difficult to interpret, so I will give the reader here a short example on how to export graphics from R. Note that any code here that will produce images will also be present in other places in this vignette. The default installation of the R GUI is quite minimal, and for an easy way to manage your plots and code, I strongly encourage the user to use Rstudio http://www.rstudio.com/.

4.2.1 Basics

Before you export graphics, you have to ask yourself what they will be used for. If you want to use the graphic for a website, you might want to opt for a low-resolution image so that it can load quickly. With printing, you'll want to make sure that you have a scalable or at least a very high resolution image. Here, I will give some general guidelines for graphics (note that these are merely suggestions, not defined rules).

- **What you see is not always what you get** I have often seen presentations where the colors were too light or posters with painfully pixellated graphs. Think about what you are going to be using a graphic for and how it will appear to the intended audience given the media type.
- $\geq$ **300 dpi unless its for a web page** For any sort of printed material that requires a raster based image, 300dpi (dots per inch) is the absolute minimum resolution you should use. For simple black and white line images, 1200dpi is better. This will leave you with crisp, professional looking images.
- **If possible, save to SVG, then rasterize** Raster images (bmp, png, jpg, etc...) are based off of the number of pixels or dots per inch it takes to render the image. This means that the raster image is more or less a very fine mosaic. Vector images (SVG) are built upon several interconnected polygons, arcs, and lines that scale relative to one another to create your graphic. With vector graphics, you can produce a plot and scale it to the size of a building if you wanted to. When you save to an SVG file first, you can also manipulate it in programs such as Adobe Illustrator or Inkscape.
- **Before saving, make sure the units and dimensions are correct** Unless you really wanted to save a graph that's over 6 feet wide.

4.2.2 Image Editors

Often times, fine details such as labels on networks need to be tweaked by hand. Luckily, there are a wide variety of programs that can help you do that. Here is a short list of image editors (both free and for a price) that you can use to edit your graphics.

- Bitmap based editors (for jpeg, bmp, png, etc...)

The GIMP	Free, cross-platform. http://www.gimp.org
Paint.net	Free, Windows only. http://www.getpaint.net
Adobe Photoshop	Pricey, Windows and Mac. http://www.adobe.com/products/photoshop.html

- Scalable Vector Graphics based editors (for svg, pdf)

Inkscape	Free, cross-platform http://inkscape.org
Adobe Illustrator	Pricey, Windows and Mac. http://www.adobe.com/products/illustrator.html

4.2.3 Exporting ggplot2 graphics

ggplot2 is a fantastic package that *poppr* uses to produce graphs for the `mlg.table`, `poppr`, and `ia` functions. Saving a plot with *ggplot2* is performed with one command after your plot has rendered:

```
data(nancycats) # Load the data set.
poppr(nancycats, sublist=5, sample=999) # Produce a single plot.
ggsave("nancy5.pdf")
```

Note that you can name the file anything, and `ggsave` will save it in that format for you. The details are in the documentation and you can access it by typing `help("ggsave")` in your R console. The important things to note are that you can set a `width`, `height`, and `unit`. The only downside to this function is that you can only save one plot at a time. If you want to be able to save multiple plots, read on to the next section.

4.2.4 Exporting any graphics

Some of the functions that *poppr* offers will give you multiple plots, and if you want to save them all, using `ggsave` will require a lot of tedious typing and clicking. Luckily, R has Functions that will save any plot you generate in nearly any image format you want. You can save in raster images such as png, bpm, and jpeg. You can also save in vector based images such as svg, pdf, and postscript. The important thing to remember is that when you are saving in a raster format, the default units of measurement are "pixels", but you can change that by specifying your unit of choice and a resolution.

For raster images and svg files, you can only save your plots in multiple files, but pdf and postscript plots can be saved in one file as multiple pages. All of these functions have the same basic form. You call the function to specify the file type you want (eg. `pdf("myfile.pdf")`), create any graphs that you want to create, and then make sure to close the session with the function `dev.off()`. Let's give an example saving to pdf and png files.

```
data(H3N2)
pop(H3N2) <- H3N2$other$x$country
####
png("H3N2_barchart%02d.png", width = 14, height = 14, units = "in", res = 300)
H.tab <- mlg.table(H3N2)
dev.off()
####
```

Since this data set is made up of 30 populations with more than 1 individual, this will save 30 files to your working directory named "H3N2_barchart01.png...H3N2_barchart30.png". The way R knows how to number these files is because of the `%02d` part of the command. That's telling R to use a number that is two digits long in place of that expression. All of these files will be 14x14" and will have a resolution of 300 dots per inch. If you wanted to do the same thing, but place them all in one file, you should use the pdf option.

```
pdf("H3N2_barcharts.png", width = 14, height = 14, compress = FALSE)
H.tab <- mlg.table(H3N2)
dev.off()
```

Remember, it is important not to forget to type `dev.off()` when you are done making graphs. Note that I did not have to specify a resolution for this image since it is based off of vector graphics.

4.3 Table of Functions

Below is a table of functions found in *poppr*. These functions are linked within the document. If a function name is blue, simply click on it to go to its definition and description.

Table 10: Functions available in *poppr*

Function	Description
IMPORT/EXPORT	
`getfile`	Provides a quick GUI to grab files for import
`read.genalex`	Read *GenAlEx* formatted csv files to a genind object
`genind2genalex`	Converts genind objects to *GenAlEx* formatted csv files
`as.genclone`	Converts genind objects to genclone objects
MANIPULATION	
`setPop`	Set the population using defined hierarchies
`splitStrata`	Split a concatenated hierarchy imported as a population
`strata`	Define a population hierarchy of a genclone object
`strata`	Extract the hierarchy data frame
`addStrata`	Add a vector or data frame to an existing hierarchy
`nameStrata`	Rename a population hierarchy
`missingno`	Handles missing data
`clonecorrect`	Clone censors at a specified population hierarchy
`informloci`	Detects and removes phylogenetically uninformative loci
`popsub`	Subsets genind objects by population
`shufflepop`	Shuffles genotypes at each locus using four different shuffling algorithms
`splitcombine*`	Manipulates population hierarchy *Deprecated
`recode_polyploids`	recode polyploid data sets with missing alleles imported as "0"
DISTANCES	
`bruvo.dist`	Bruvo's distance
`diss.dist`	Absolute genetic distance (see `provesti.dist`)
`nei.dist`	Nei's 1978 genetic distance
`rogers.dist`	Rogers' euclidean distance
`reynolds.dist`	Reynolds' coancestry distance
`edwards.dist`	Edwards' angular distance
`provesti.dist`	Provesti's absolute genetic distance
BOOTSTRAPPING	
`aboot`	Creates a bootstrapped dendrogram for any distance measure
`bruvo.boot`	Produces dendrograms with bootstrap support based on Bruvo's distance
ANALYSIS	

Continued on next page...

Table 10 – continued from previous page

Function	Description
poppr.amova	Analysis of Molecular Variance (as implemented in ade4)
ia	Calculates the index of association
mlg	Calculates the number of multilocus genotypes
mlg.crosspop	Finds all multilocus genotypes that cross populations
mlg.table	Returns a table of populations by multilocus genotypes
mlg.vector	Returns a vector of a numeric multilocus genotype assignment for each individual
mlg.id	Identifies individuals associated with each MLG
poppr	Returns a diversity table by population
poppr.all	Returns a diversity table by population for all compatible files specified
private_alleles	Tabulates the occurrences of alleles that only occur in one population
locus_table	Creates a table of summary statistics per locus
VISUALIZATION	
plot_poppr_msn	Plots minimum spanning networks produced in poppr with scale bar and legend
greycurve	Helper to determine the appropriate parameters for adjusting the grey level for msn functions
bruvo.msn	Produces minimum spanning networks based off Bruvo's distance colored by population
poppr.msn	Produces a minimum spanning network for any pairwise distance matrix related to the data
info_table	Creates a heatmap representing missing data or observed ploidy
genotype_curve	Creates a series of boxplots demonstrating how many loci are needed to represent the diversity of your data.

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