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1 Introduction

This manual has been written as a generic, user-friendly guide to using diveRsity in the R environment. It will outline briefly how to get the latest version of R, how to install the diveRsity package as well as how to install suggested packages. Fully reproducible Worked examples for functions will be provide as a guide to how the package should be implemented. Effort has been made to keep R jargon to a minimum to ensure accessibility for R beginners.

1.1 About R

R is an extremely powerful and popular software for statistical programming. It is very well supported by a dedicated group of people known as the R core development team (R Development Core Team, 2011a), as well as an active community of developers and useRs. More information about R can be found at http://www.r-project.org/about.html.

1.2 About diveRsity

diveRsity is a package containing multiple functions written in the statistical programming environment R. It allows the calculation of both genetic diversity partition statistics (e.g. $G_{ST}$ & $F_{ST}$), genetic differentiation statistics (e.g. $G'_{ST}$ and $D_{Jost}$), and locus informativeness for ancestry assignment (e.g. $I_n$), as well as basic population parameters such as allele frequencies, observed/expected heterozygosity and Hardy-Weinberg tests. The package also provides functions for the calculation of Weir & Cockerham’s 1984 F-statistics and ‘Yardstick’ diversity ratios from Skrbinšek et al., (2012).

In addition to these features, diveRsity also provides users with various options to calculate bootstrapped 95% ci’s both across loci and for pairwise population comparisons. All of these results are returned in convenient formats and can be plotted interactively.

diveRsity was written to ensure that even R beginners can carry out genetic analyses in R without major difficulties. By automatically writing analysis results to file, useRs do not need to understand how to access variables in the R environment, let alone know what a variable is. However, for more experienced useRs, all analysis functions return results variables to the R environment, details of which are provided in the Function usage section below.
1.2.1 How to cite

1.2.2 What’s new?
Versions 1.2.0 and up introduce a complete rewrite of diveRsity v1.0. All subsequent versions have been vectorized in all but the least computationally intensive pieces of code, resulting in much faster execution speed.

Parallel computations are also now available when using the inCalc and divPart functions. These two major changes mostly affect the speed at which the program executes. An additional results object, (i.e. pairwise) is now also returned from the function divPart. This additional functionality now allows useRs to calculate pairwise statistics without having to run the computationally intensive bootstrap algorithm, thus saving time.

As of version 1.2.3, Weir and Cockerham’s (1984) F-statistics are also calculated for global estimates, locus estimates and pairwise population estimates as well as 95% confidence intervals in the function divPart.

The calculation of Weir and Cockerham’s F-statistics increases analysis time by around 0.3 seconds per bootstrap replicate, thus leading to significant increases in overall execution time if a large number of bootstrap iterations are used. For this reason, the calculation of F-statistics has been included as an optional extra through the new argument `WC_Fst`.

 Versions 1.3.0 and up includes additional plotting functions to aid in data visualisation. These new functions are;

**corPlot** - provides useRs with the ability to plot locus $G_{ST}$, $\theta$, $G'_{ST}$ and $D_{Jost}$ against the number of alleles at each locus. This method may be useful to assess whether particular loci might be suitable for the inference of demographic processes (i.e. they are not unduly affected by mutation).

**difPlot** - is a function intended to be used as a data exploration tool. This function plots pairwise estimated statistics, allowing useRs to easily visualise pairwise comparisons of interest (e.g. highly differentiated population pairs).
Version 1.3.2 provides a more flexibility in reading genepop files. It also returns more informative error when genepop files are in the wrong format. This version also fixes a bug in writing results to disk. If `outfile` is set to `NULL` in the functions `divPart` or `inCalc`, no directories will be created.

As of version 1.3.6, a web app version of `diveRsity` is packaged with the R console version. This application can be launched simply by typing:

```
divOnline()
```

An online version of the app is also available at:


This web app allows users to carry out most of the analyses provided by the `divPart` function, with additional plotting options.

Version 1.3.6 also contains a new function allowing the calculation of genetic heterogeneity, using $X^2$ tests. This new function is named `chiCalc`.

Version 1.4.2 introduces a new function, `divBasic`. This function calculates multiple population sample specific parameters including; allelic richness, observed & expected heterozygosity and Hardy-Weinberg equilibrium $\chi^2$ tests. See below for more details on its' usage.

Version 1.4.4 represents a major update of `diveRsity`, introducing two new functions, `fstOnly` and `divRatio`, as well as the deprecation of some old function (`div.part`, `in.calc` & `readGenepop.user`). `fstOnly` calculates only Weir and Cockerham’s 1984, $\theta$ and $F$ for loci, global and pairwise levels. Bootstrapped confidence intervals can also be calculated. The function is intended for use by those with very large data sets as it should be more memory efficient than `divPart` which also calculates these statistics along with a variety of other parameters. The function `divRatio` calculates the allelic richness and expected heterozygosity standardised ratios originally presented by [Skrbinšek et al. 2012](#).
Version 1.4.6 is the first in a series of package releases with developments focusing on the analysis of large SNP data sets. This release contains a new function, bigDivPart, which allows users to calculate the same parameters as divPart for large data sets containing thousands of marker loci. The function only accepts genepop format files currently, but work is ongoing to allow for additional formats. No bootstrapping procedures are yet available for this function. These are also under development. In any case, given the massive computational effort involved in bootstrapping large data sets (e.g., 500 individuals across 10 population samples, genotyped for 100,000 SNP loci), these procedures may have to be restricted to High Performance Computing (HPC) environments.

Version 1.5.0 now contains citation information for the diveRsity package (Keenan et al., 2013). Users can extract bibtex information for diveRsity by typing the following into the R console:

```
citation("diveRsity")
```

Version 1.5.0 also fixes a major bug on Mac systems. Due to unexpected behaviour of the sprintf function, divPart, inCalc and readGenepop were incorrectly coding alleles, thus leading to erroneous parameter calculations.

Version 1.5.3 introduces the new web app, microPlexer. This application is intended to aid researchers wishing to develop microsatellite multiplex system based on locus size ranges and fluorophore tags.

Version 1.5.6 introduces a new function arp2gen, which allows users to convert Arlequin genotype files to genepop files. The function is intended to provide a more integrated experience to users by negating the need to use separate software to carry out file conversions. Although this function only allows .arp to .gen file conversions, other R packages provide additional conversion facilities. Some such packages are; adegenet and PopGenKit.

Version 1.5.7 introduced a new experimental function, divMigrate. The function is based on a new methods presented in (Sundqvist et al., 2013).
for detecting the direction of differentiation, and using this information to infer the relative migration between pairs of populations. While the method performs well for some simple migration scenarios, it is still not clear how generally applicable it is under other scenarios (e.g. the effect of high mutation rates, or recent divergence etc.). For this reason, we recommend caution when applying the method to your own data.

**Version 1.6.0** contains a new function, `fastDivPart`. This function is virtually identical to `divPart`, with some slight changes to output structures and formats. The key differences in these formats are:

1. `$pairwise` in `fastDivPart` only contains the unbiased estimators of $G_{ST}$, $G'_{ST}$, $D_{Jost}$ and $\theta$.
2. The names of the output data structures in `$pairwise`, `$bs_pairwise` and `$meanPairwise` are now `gstEst`, `gstEstHed`, `djostEst` and `thetaWC`, rather than `Gst_est`, `G_hed_st_est`, `D_Jost_est` and `Fst_WC`, respectively.

This new function is optimised to provide faster pairwise calculations, especially where the number of pairwise comparisons is much greater than the number of loci. Some performance tests can be found at: [GitHub](https://github.com/kkeenan02/diveRsity-dev/blob/master/fastDivPart-tests.md)

**IMPORTANT NOTE**

Users are encouraged to use `fastDivCalc` rather than `divPart`, as the latter function will eventually be removed from future releases of `diveRsity`.

**Version 1.6.1** contains a new function, `haploDiv`, for calculating Weir & Cockerham’s $\theta$ from haploid genotype data. The data should be in the two or three digit genepop format. See below for further description of the function.
2 Setup

2.1 Installing R

To use diveRsity you will need to download and install R. It is available at: http://cran.r-project.org/
Simply download the R distribution appropriate for your operating system and install as normal.

2.2 Installing diveRsity

The package diveRsity is currently available on CRAN (The Comprehensive R Archive Network), thus installation is simple. Launch R, and in the console (you will see the > symbol when R is ready for you to type), use the following command:

```
install.packages("diveRsity")
```

The package is updated regularly, both with added functionality and bug fixes. The most up to version of diveRsity can be installed from github using the devtools package:

```
# install and load devtools
install.packages("devtools")

library("devtools")

# download and install diveRsity
install_github(name = "diveRsity", subdir = "kkeenan02")
```
2.3 Installing optional enhancer packages

The dependencies plotrix (Lemon, 2006) and shiny (RStudio & Inc., 2012) will download automatically if you install diveRsity from CRAN. Suggested/optional packages should be installed manually (excluding parallel, which is distributed with R).

Optional packages are:

xlsx — writes results to .xlsx. (Dragulescu, 2012)

sendplot — Plots results to .html files with tool-tip information. (Gaile et al., 2012)

doParallel — Used in parallel computations. (Revolution Analytics, 2012a)

parallel — Used in parallel computations. (R Development Core Team, 2011b)

foreach — Used in parallel computations. (Revolution Analytics, 2012b)

iterators — Used in parallel computations. (Revolution Analytics, 2012c)

Each of these packages can be installed using the below command:

```r
install.packages("package_name")
```

Just replace ‘package_name’ with the name of the package you want to install. See ?install.packages for details.
2.4 Loading diveRsity

To load diveRsity in the current R session, type the following into the console:

```r
library("diveRsity")
```

You will not need to load any of the other dependencies or optional packages as diveRsity will do this as and when it needs to use them. After loading diveRsity into your current R session all of its functions are available for you to use.

For convenient access to usage information on each function, type:

```r
?divPart
?inCalc
?readGenepop
?corPlot
?diffPlot
?chiCalc
?divOnline
?divBasic
?fstOnly
?divRatio
?microPlexer
?arp2gen
?divMigrate
?hapl0Div
?fastDivPart
```

Each of these commands will provide information on function usage. The help pages associated with each function describe in detail how each argument should be passed to the function.
3 Function details

3.1 fastDivPart()

NOTE

This function was previously known as divPart. This name has since been deprecated. Please use fastDivPart instead. Be aware that some of the output structures are different. See below for details.

fastDivPart (diversity partition and differentiation), allows for the calculation of four main diversity partition/differentiation statistics and their respective estimators. The function can be used to explore locus values for the identification of 'outliers' and also to visualise pairwise differentiation between populations. Bootstrapped, bias corrected 95% confidence intervals are calculated also. Results can be optionally plotted for data exploration purposes. The statistics and their basic formulae are as follows:

3.1.1 Standard formulae

\[ G_{ST} \] (Nei, 1973; Nei & Chesser, 1983)

\[
G_{ST} = \frac{D_{ST}}{H_T} \tag{1}
\]

Where \( D_{ST} = H_T - H_S \), \( H_T \) is the total heterozygosity and \( H_S \) is intra-population
heterozygosity.

\[ G'_{ST} \quad (\text{Hedrick, 2005}) \]

\[ G'_{ST} = \frac{G_{ST}}{G_{ST(max)}} \quad (2) \]

Where \( G_{ST} \) is as above, \( G_{ST(max)} = \frac{H_{T(max)} - H_{S}}{H_{T(max)}} \) and \( H_{T(max)} \) calculated as \( H_{T(max)} = \frac{(k-1)+H_{S}}{k} \) and is the maximum possible \( H_T \) value given the observed within sample heterozygosity.

\[ D_{Jost} \quad (\text{Jost, 2008}) \]

\[ D_{Jost} = \left[ \frac{(H_T - H_S)}{(1 - H_S)} \right] \left[ \frac{n}{(n-1)} \right] \quad (3) \]

Where \( H_T \) and \( H_S \) are as defined above, and \( n \) is the number of population samples.
3.1.2 Estimator formulae

The estimators of both $G_{ST}$ and $G'_{ST}$ were calculated by simply substituting the $H_S$ and $H_T$ components of each statistic with their estimators calculated using equations 4 and 5 respectively. $D_{estChao}$ was calculated using the method described in (Chao et al., 2008) (eqn 6 below). The formulae are as follows:

$\hat{H}_S$ (Nei & Chesser, 1983)

$$\hat{H}_S = H_S \left[ \frac{2\hat{N}}{(2\hat{N} - 1)} \right]$$ (4)

Where $H_S$ is the inter-population heterozygosity and $\hat{N}$ is the harmonic mean of sample size across all samples.

$\hat{H}_T$ (Nei & Chesser, 1983)

$$\hat{H}_T = H_T + \left[ \frac{\hat{H}_S}{(2Nn)} \right]$$ (5)

Where $H_T$ is the total heterozygosity, $\hat{H}_S$ is as defined in equation (4), $\hat{N}$ is the harmonic mean of sample sizes and $n$ is the number of population samples.
\[ D_{\text{est}(\text{Chao})} = \left( \frac{1}{A} \right) + \text{var}(D)(\frac{1}{A})^3 \]  

(6)

Where \( A \) is the arithmetic mean of \( D_{\text{Jost}} \) across loci, and \( \text{var}(D) \) is the variance of \( D_{\text{Jost}} \) across loci.

\[ \hat{\theta} = \frac{\hat{\sigma}^2_P}{\hat{\sigma}^2_P + \hat{\sigma}^2_I + \hat{\sigma}^2_G} \]  

(7)

Where \( \hat{\sigma}^2_P \) is the sum of variance components for populations, \( \hat{\sigma}^2_I \) is the sum of variance components for individuals within populations and \( \hat{\sigma}^2_G \) is the sum of variance components for alleles within individuals.

3.1.3 Bootstrapping

The variance of each statistic can be assessed using the bootstrapping method implemented in \texttt{diversity}. 95% confidence intervals are calculated by taking the upper and lower 2.5% sample quantiles from the bootstrapped parameter distribution.

For pairwise calculations carried out by the function, \texttt{fastDivPart}, a bias corrected 95% confidence interval is also calculated. This method accounts for the bias commonly associated with bootstrapping these types of statistics.
The method is basically a technique which re-centers the confidence interval around the initial parameter estimate. Say $\theta$ is our parameter of interest, $\hat{\theta}$ is our parameter estimate, and $\hat{\theta}^*$ is a vector of length $n$, containing estimates of $\hat{\theta}$ for resampled data. The correction is carried out as follows:

$$
\hat{\theta}^{*}_{[BC]} = \hat{\theta}^* - z0
$$

Where $z0 = \frac{\sum \hat{\theta}^*}{n} - \hat{\theta}$.

The 2.5% and 97.5% quantile are then taken from the re-centered vector, $\hat{\theta}^{*}_{[BC]}$.

### 3.2 inCalc()

**NOTE**

This function was previously known as `in.calc`. This name has since been deprecated. Please use `inCalc` instead. `inCalc` allows the calculation of locus informativeness for the inference of ancestry both across all population samples and pairwise comparisons. These parameters can be bootstrapped using the same procedure as above to obtain 95% confidence intervals. The basic equations for both the allele specific and locus specific calculation of $I_n$ are as follows:

$$
I_n(alleles) \quad (Rosenberg \ et \ al., \ 2003)
$$

$$
I_n(Q; J = j) = -p_j \log_e p_j + \sum_{i=1}^{K} \frac{p_{ij}}{K} \log_e p_{ij} \quad (8)
$$
Where $p_j$ is the parametric mean frequency of the $j^{th}$ allele across populations, $\log_e$ is the natural logarithm, $p_{ij}$ is the frequency of the $j^{th}$ allele in the $i^{th}$ population, and $K$ is the number of populations.

$$I_n(\text{locus}) \quad (\text{Rosenberg et al., 2003})$$

$$I_n(Q; J) = \sum_{j=1}^{N} I_n(Q; J = j) \quad (9)$$

Where $N$ is the number of allele at the locus of interest and $I_n(Q; J = j)$ is as in equation 7.

### 3.3 readGenepop()

**NOTE**

This function was previously known as `readGenepop.user`. This name has been deprecated as of version 1.4.4.

Although the `readGenepop` function is used extensively in both `divPart` and `inCalc`, its complexity is well hidden from general users. However, it has been included in `diveRsity` as a usable function for more experienced users, who may find it useful for data exploration and the development of analysis methods. As of version 1.3.0, this function is also implemented for use with the function `corPlot`. The function `readGenepop` returns up to 18 distinct variables (described in detail below), some of which have particularly
complex structures. Although this manual provides basic summaries of each returned variable, for the function to be useful, useRs are advised to explore the individual objects. This can be done using functions such as `str`, `names` and `typeof`.

3.4 corPlot()

**New to v1.3.0**

This function allows useRs to graphically visualise the relationship between locus polymorphism (i.e. Number of alleles) and corresponding $G_{ST}$, $\theta$, $G'_{ST}$ and $D_{Jost}$ values per locus. This information is plotted along with the respective Pearson’s product-moment correlation coefficients for each comparison. This information is intended to help useRs to decide whether it would be appropriate to use their particular loci for the inference of demographic processes (i.e. effective number of migrants per generation). Typically this is done following the relationship between $F_{ST}$ and $Nm$ arising under the finite-island model from the following formula:

$$F_{ST} \approx \frac{1}{4Nm + 1}$$

(10)

Where $F_{ST}$ is the standardised measure of genetic variance among populations (i.e. $G_{ST}$ or $\theta$ in this package), $N$ is the effective number of breeding individuals and $m$ is the migration rate among populations.

The requirement to validate the use of certain marker types to infer demography is particularly important given that such information is often used to inform conservation and management strategies. It has been shown extensively in the literature that the relationship between $F_{ST}$ and $Nm$ in equation 10 breaks down if other evolutionary forces are strong (e.g. [Whitlock & McCauley, 1999]). For example if migration rate ($m$) is not $\gg$ than mutation
rate \( (\mu) \), then \( F_{ST} \neq \frac{1}{4Nm+1} \), and the quantity \( Nm \) cannot be accurately derived.

For marker loci to be useful in the inference of demography, the the effects of such demographic processes must be detectable independently of the affects of processes such as mutation. As demographic processes are expected to have similar effects at all neutral loci, it is reasonable to expect that where mutation/selection/range constraints are having negligible effects on divergence at a particular set of loci, \( F_{ST} \) should be more or less equal across these loci. \texttt{corPlot} allows users to visualise if this is in fact the case. In general, the function will allow users to determine if mutation (assumed to be a major factor contributing to the the number of allele per locus), is having a noticeable effect on \( F_{ST} \) thus rendering them unsuitable for the inference of demography. As \texttt{corPlot} returns correlation plots of \( G_{ST} \), \( \theta \), \( G_{ST}' \) and \( D_{Jost} \) against the number of alleles per locus, users have the additional benefit of assessing the effect of mutation on the differentiation statistics (e.g. \( D_{Jost} \)), which are more sensitive to the effects of mutation.

There is both theoretical and empirical evidence for this approach to assessing of the effects of processes other than migration and drift on divergence at neutral loci. O’Reilly et al (2004) [O’Reilly et al.] [2004], demonstrated from empirical data that \( F_{ST} \) (i.e. \( \theta \), specifically) had an inverse relationship with allelic richness in walleye pollock. Although the authors of this study attributed this observation to homoplasious mutations, the general affect is the same (i.e. mutational processes obscure divergence due to demographic processes). The results from this study can also be interpreted in light of the fact that \( F_{ST} \) has a theoretical maximum value defined as:

\[
F_{st(max)} = \frac{H_{T(max)} - H_S}{H_{T(max)}}
\]  \hspace{1cm} (11)

Where \( H_{T(max)} \) is the maximum possible total heterozygosity given the observed subpopulation heterozygosity, \( H_S \).

Thus, because of the negative dependence of \( F_{ST} \) on heterozygosity, and the positive dependence of heterozygosity on number of alleles, we can predict a
negative relationship between $F_{ST}$ and number of alleles. The thrust of this argument is depicted in the figure below, where we can see the response of both $G_{ST}$ and $D_{Jost}$ to the number of unique alleles at a locus (Following Jost 2008 [Jost 2008]).

The relationship between the number of unique alleles per subpopulation and $G_{ST}$ and $D_{Jost}$

From this figure, it is clear that where the number of alleles at a locus is high (thus heterozygosity is high), $G_{ST}$ is expected to be low. It is important to note that the negative relationship for $G_{ST}$ and positive relationship for $D_{Jost}$, are complex, with multiple contributing factors. Thus this method should not be seen as definitive, but rather as a method to assess whether caution must be exercised when applying a particular marker set to address specific questions in populations of interest.
3.5 difPlot()

New to v1.3.0

difPlot is yet another plotting function introduced to help users easily visualise trends in their analysis results. Modern population genetic studies typically involve large numbers of population samples. It is often useful to know the pairwise relationships between each of these population samples. Due to the relationship between the number of sampled populations and the maximum number of possible pairwise comparisons, shown below, pin-pointing comparisons of interest can be very difficult.
The number of possible pairwise comparisons as a function of the number of sampled populations

To overcome this problem, difPlot plots the pairwise values calculated by the function divPart using a diagonal matrix coupled with a colour gradient used to indicate the magnitude of a particular pairwise value. The function plots the estimated pairwise values for $G_{ST}$, $\theta$, $G_{ST}'$ and $D_{Jost}$. 

$N_{pw} = (1/2n)(n-1)$
3.6 chiCalc

New to v1.3.6

chiCalc allows the calculation of $X^2$ statistics for population genetic heterogeneity. The function contains a unique feature which allows users to exclude particular alleles if they are not observed frequently enough to be considered reliably. This feature allows a more conservative assessment of population genetic structure, but may result in a loss of power to detect actual differences.

3.7 divOnline

New to v1.3.6

divOnline is a simple function which allows users to launch a web app version of the divPart function. This function provides a less flexible but much more user friendly interface for the use of the diveRsity package. The web app was built using the shiny package from RStudio and Inc (RStudio & Inc., 2012).
3.8 fstOnly

New to v1.4.4

This function was written as a more RAM efficient way to calculate Weir & Cockerham’s (1984) $F_{ST}$ and $F_{IT}$, than divPart. The function should be particularly useful to users wishing to analyse large SNP data sets, where negative dependence on heterozygosity is not a concern.

3.9 divRatio

New to v1.4.4

This function introduces a new implementation of the method presented in Skrbinšek et al., (2012), whose paper proposes the use of a well-characterised, ‘yardstick’ population sample to calculate a standardised diversity ratio for less well characterised population samples.

Interested users should see the original paper for a comprehensive description and justification of the method.

3.10 bigDivPart

New to v1.4.6

This function is the first in a series focused in the analysis of data sets containing large number of marker loci (e.g. RAD-seq derived SNPs). The function implements a more memory efficient programming technique (i.e.
extensive use of array data structures) to overcome some of the limitations associated with divPart. bigDivPart allows users to calculate all parameters calculated by divPart except via bootstrapping (e.g. locus and global \( G_{st} \), \( G'_{st} \), \( \theta \) and \( D_{Jost} \) etc.).

3.11 microPlexer

New to v1.5.3

microPlexer is a simple function which allows users to launch a web application designed to aid in the development of microsatellite multiplex systems. The application allows users to flexibly organise microsatellite loci into PCR groups based on two main algorithms. The first, the high-throughput algorithm, aims to group as many loci into as few multiplex groups as possible, while ensuring user defined locus separation limits are observed. The second, the balanced throughput algorithm, organises loci into multiple multiplex groups with, roughly, the same number of loci. This algorithm is useful for minimising the possible risk of primer interactions during PCR. The web app was built using the shiny package from RStudio and Inc (RStudio & Inc. 2012).

3.12 arp2gen

New to v1.5.6 arp2gen is a small function allowing the conversion of Arlequin genotype files to genepop file format.

3.13 divMigrate

New to v1.5.7

divMigrate is an experimental function, allowing users to investigate and
plot migration pattern in microsatellite allele data. See \texttt{?divMigrate} for more details. The paper that the method is derived from is \textit{Sundqvist et al.} [2013].

3.14 haploDiv

\textbf{New to v1.6.1}

\texttt{haploDiv} allows users to calculate \citeauthor{weir1984estimating}'s (1984) $\theta$ from haploid genotype data. These data should be provided in the form of a two digit or three digit genepop file. The function essentially reads the haploid genotypes, \textit{diploidizes} them (e.g. alleles coded as '01' will become '0101'), then the function \texttt{fastDivPart} is used to calculate $F_{ST}$ estimates for various user defined levels. The function provides the facility to write a pairwise $F_{ST}$ matrix to .txt file, as well as pairwise bootstrapped 95\% confidence limits for each pairwise comparison.
4 Function Usage

In this section the arguments and returned values for each function are explained.

4.1 divPart()

The general usage of this function is as follows:

```
divPart(infile = NULL, outfile = NULL, gp = 3, pairwise = FALSE, WC_Fst = FALSE, bs_locus = FALSE, bs_pairwise = FALSE, bootstraps = 0, plot = FALSE, parallel = FALSE)
```

4.1.1 Arguments

- `infile`: Specifies the name of the ‘genepop’ (? ) file from which the statistics are to be calculated. This file can be in either the 3 digit or 2 digit format. The name must be a character string.

- `outfile`: Allows useRs to specify a prefix for an output folder. Name must a character string enclosed in either "" or "".

- `gp`: Specifies the digit format of the `infile`. Either 3 (default) or 2.

- `pairwise`: A logical argument indicating whether pairwise matrices for each relevant statistic should be calculated. This feature can increase computation time for large number of population samples. Calculations will be made in parallel if the argument `parallel = TRUE`
WC_Fst  A logical indication as to whether Weir and Cockerham’s, 1984 F-statistics should be calculated. This option will increase analysis time.

bs_locus  Gives users the option to bootstrap locus statistics. Results will be written to .xlsx workbook by default if the package xlsx is installed, and to a .html file if plot = TRUE. If xlsx is not installed, results will be written to .txt files.

bs_pairwise  Gives users the option to bootstrap statistics across all loci for each pairwise population comparison. Results will be written to a .xlsx file by default if the package xlsx is installed, and to a .html file if plot = TRUE. If xlsx is not installed, results will be written to .txt files.

bootstraps  Determines the number of bootstrap iterations to be carried out. The default value is bootstraps = 0, this is only valid when all bootstrap options are false. There is no upper limit on the number of bootstrap iterations, however very large numbers of bootstrap iterations for pairwise calculations (> 1000) may take a long time to run for large data sets and may also lead to excessive RAM consumption. As an example, a test data set containing over 4000 individuals across 97 population samples typed for 15 microsatellite loci, took 1.5 days to complete on a Windows 7 ultimate 64bit machine with an Intel Core i5-2435M CPU @ 2.40GHz x 4.

plot  Optional interactive .html image files of the plotted bootstrap results for loci if bs_locus = TRUE and pairwise population comparisons if bs_pairwise = TRUE and the package sendplot is installed. The default option is plot = FALSE.

parallel  A logical argument specifying if computations should be run in parallel on all available CPU cores. If parallel = TRUE, batches of jobs will be distributed to all cores resulting in faster completion.
4.1.2 Returned values

Results returned by `divPart` vary depending on the argument options chosen. If the packages `xlsx` and `sendplot` are installed, results will be written to a single `.xlsx` workbook and `.png`/.html files providing `plot = TRUE`. Alternatively, if these packages are unavailable the plot option is no longer available. Results will be written to multiple `.txt` files, the number of which varies between three and five depending on the argument options chosen. An example screenshot of the `.xlsx` output file is shown below:
Returned values cont.

Examples of the interactive plots written, if \texttt{xlsx} is available, are given below. Error bars represent bootstrapped 95\% confidence intervals, and the red dotted lines represent the global statistic values.

Example of bootstrapped locus results plot

Example of bootstrapped...
pairwise results plot
For users wishing to carry out post analysis manipulations, all results from `divPart` are returned to the R environment. Depending on the bootstrap options chosen these results include between three to five of the variables below:

```
$standard

A matrix containing identical data to the `Standard_stats` worksheet in the `.xlsx` workbook or the `Standard-stats[divPart].txt` text file. The last row in this matrix represents statistics calculate across all population samples and loci.
```

<table>
<thead>
<tr>
<th></th>
<th>H_st</th>
<th>D_st</th>
<th>G_st</th>
<th>G_hed_st</th>
<th>D_jost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus1</td>
<td>0.0659</td>
<td>0.0224</td>
<td>0.0328</td>
<td>0.1093</td>
<td>0.0791</td>
</tr>
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<td>0.0058</td>
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<td>0.0070</td>
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<td>0.0721</td>
<td>0.5049</td>
<td>0.4664</td>
</tr>
<tr>
<td>Locus4</td>
<td>0.0882</td>
<td>0.0290</td>
<td>0.0415</td>
<td>0.1429</td>
<td>0.1058</td>
</tr>
<tr>
<td>Locus5</td>
<td>0.2683</td>
<td>0.0266</td>
<td>0.0287</td>
<td>0.3414</td>
<td>0.3219</td>
</tr>
<tr>
<td>Locus6</td>
<td>0.1536</td>
<td>0.0306</td>
<td>0.0368</td>
<td>0.2143</td>
<td>0.1843</td>
</tr>
<tr>
<td>Locus7</td>
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<td>0.0315</td>
<td>0.0935</td>
<td>0.0640</td>
</tr>
<tr>
<td>Locus8</td>
<td>0.0255</td>
<td>0.0192</td>
<td>0.0724</td>
<td>0.1008</td>
<td>0.0306</td>
</tr>
<tr>
<td>Locus9</td>
<td>0.3616</td>
<td>0.0244</td>
<td>0.0255</td>
<td>0.4484</td>
<td>0.4340</td>
</tr>
<tr>
<td>Locus10</td>
<td>0.3585</td>
<td>0.0522</td>
<td>0.0576</td>
<td>0.4630</td>
<td>0.4302</td>
</tr>
<tr>
<td>Global</td>
<td>NA</td>
<td>NA</td>
<td>0.0493</td>
<td>0.2163</td>
<td>0.1757</td>
</tr>
</tbody>
</table>
loci
A list of locus names

H\_st
Between subpopulation heterozygosity per locus

D\_st
Absolute differentiation per locus (Nei 1973)

G\_st
F\_st analogue for multiple alleles per locus (Nei 1973)

G\_hed\_st
Hedrick’s standardized “differentiation” per locus (Hedrick 2005)

D\_jost
Jost’s true allelic differentiation per locus (Jost 2008)
$estimate$ A matrix containing identical data to the Estimated_stats worksheet in the .xlsx workbook or the Estimated-stats[divPart].txt text file. The last row in this matrix represents statistics calculate across all population samples and loci.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Harmonic_N</th>
<th>H_st_est</th>
<th>D_st_est</th>
<th>G_st_est</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus1</td>
<td>43.1218</td>
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<td>0.0160</td>
<td>0.0234</td>
</tr>
<tr>
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<td>43.5209</td>
<td>-0.0038</td>
<td>-0.0019</td>
<td>-0.0038</td>
</tr>
<tr>
<td>Locus3</td>
<td>43.6403</td>
<td>0.3610</td>
<td>0.0566</td>
<td>0.0629</td>
</tr>
<tr>
<td>Locus4</td>
<td>43.4476</td>
<td>0.0700</td>
<td>0.0225</td>
<td>0.0321</td>
</tr>
<tr>
<td>Locus5</td>
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<td>0.1998</td>
<td>0.0177</td>
<td>0.0191</td>
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<td>0.0274</td>
</tr>
<tr>
<td>Locus7</td>
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<td>0.0382</td>
<td>0.0142</td>
<td>0.0221</td>
</tr>
<tr>
<td>Locus8</td>
<td>43.2566</td>
<td>0.0224</td>
<td>0.0168</td>
<td>0.0632</td>
</tr>
<tr>
<td>Locus9</td>
<td>43.0673</td>
<td>0.2703</td>
<td>0.0153</td>
<td>0.0160</td>
</tr>
<tr>
<td>Locus10</td>
<td>43.2469</td>
<td>0.3237</td>
<td>0.0439</td>
<td>0.0483</td>
</tr>
<tr>
<td>Global</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.0397</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Locus</th>
<th>G_hed_st_est</th>
<th>D_Jost_est</th>
<th>Fst_WC</th>
<th>Fit_WC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus1</td>
<td>0.0799</td>
<td>0.0578</td>
<td>0.0257</td>
<td>0.0610</td>
</tr>
<tr>
<td>Locus2</td>
<td>-0.0084</td>
<td>-0.0046</td>
<td>-0.0042</td>
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<tr>
<td>Locus3</td>
<td>0.4688</td>
<td>0.4332</td>
<td>0.0745</td>
<td>0.0991</td>
</tr>
<tr>
<td>Locus4</td>
<td>0.1134</td>
<td>0.0840</td>
<td>0.0357</td>
<td>0.0555</td>
</tr>
<tr>
<td>Locus5</td>
<td>0.2542</td>
<td>0.2397</td>
<td>0.0222</td>
<td>0.0749</td>
</tr>
<tr>
<td>Locus6</td>
<td>0.1675</td>
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<td>0.0300</td>
<td>0.2250</td>
</tr>
<tr>
<td>Locus7</td>
<td>0.0670</td>
<td>0.0459</td>
<td>0.0258</td>
<td>0.0427</td>
</tr>
<tr>
<td>Locus8</td>
<td>0.0884</td>
<td>0.0268</td>
<td>0.0689</td>
<td>0.2529</td>
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<tr>
<td>Locus9</td>
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<td>0.3244</td>
<td>0.0189</td>
<td>0.0588</td>
</tr>
<tr>
<td>Locus10</td>
<td>0.4181</td>
<td>0.3885</td>
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<tr>
<td>Global</td>
<td>0.1806</td>
<td>0.1462</td>
<td>0.0456</td>
<td>0.1081</td>
</tr>
</tbody>
</table>

**loci**
A list of locus names
Harmonic_N
Harmonic mean number of individuals typed per locus

H_st_est
Estimator of between subpopulation heterozygosity (Nei & Chesser, 1983)

D_st_est
Estimator of absolute differentiation (Nei & Chesser, 1983)

G_st_est
Nearly unbiased estimator of G_st (Nei & Chesser, 1983)

G_hed_st_est
Estimator of Hedrick’s G’_st (Hedrick, 2005)

D_Jost_est
Estimator of Jost’s D (Jost, 2008)

Fis_WC
Weir and Cockerham’s inbreeding coefficient estimator (Weir & Cockerham, 1984)

Fst_WC
Weir and Cockerham’s fixation index estimator (Weir & Cockerham, 1984)

Fit_WC
Weir and Cockerham’s overall fixation index estimator (Weir & Cockerham, 1984)
Returned values cont.

$pairwise 

A list of six (WC_Fst = FALSE) nine (WC_Fst = TRUE) matrices containing pairwise diversity statistics without bootstrapped confidence intervals.

```r
## [1] Gst
## pop1, pop2, pop3, pop4,
## pop1, NA NA NA NA
## pop2, 0.0077 NA NA NA
## pop3, 0.0401 0.0351 NA NA
## pop4, 0.0349 0.0307 0.009 NA
## [1] G_hed_st
## pop1, pop2, pop3, pop4,
## pop1, NA NA NA NA
## pop2, 0.0486 NA NA NA
## pop3, 0.2562 0.2293 NA NA
## pop4, 0.2271 0.2041 0.0606 NA
## [1] D_Jost
## pop1, pop2, pop3, pop4,
## pop1, NA NA NA NA
## pop2, 0.0409 NA NA NA
## pop3, 0.2254 0.2011 NA NA
## pop4, 0.1989 0.1790 0.0519 NA
## [1] Gst_est
## pop1, pop2, pop3, pop4,
## pop1, NA NA NA NA
## pop2, 0.0019 NA NA NA
## pop3, 0.0341 0.0287 NA NA
## pop4, 0.0296 0.0251 0.0032 NA
## [1] G_hed_st_est
## pop1, pop2, pop3, pop4,
## pop1, NA NA NA NA
## pop2, 0.0124 NA NA NA
## pop3, 0.2264 0.1954 NA NA
## pop4, 0.1992 0.1732 0.0224 NA
## [1] D_Jost_est
## pop1, pop2, pop3, pop4,
## pop1, NA NA NA NA
## pop2, 0.0027 NA NA NA
## pop3, 0.1803 0.1579 NA NA
```
<table>
<thead>
<tr>
<th>Pop</th>
<th>Fst_WC</th>
<th>Fit_WC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pop4</td>
<td>0.1484 0.1325 0.0102</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>[1] Fst_WC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pop1</td>
<td>pop2, pop3, pop4,</td>
<td>pop1, pop2, pop3, pop4,</td>
</tr>
<tr>
<td></td>
<td>NA NA NA NA</td>
<td>NA NA NA NA</td>
</tr>
<tr>
<td>pop2</td>
<td>0.0027 NA NA NA</td>
<td>NA NA</td>
</tr>
<tr>
<td></td>
<td>pop3, 0.0647 0.0543</td>
<td>pop3, 0.1323 0.1331</td>
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<tr>
<td></td>
<td>NA NA</td>
<td>NA NA</td>
</tr>
<tr>
<td>pop3</td>
<td>pop4, 0.0563 0.0478 0.0057</td>
<td>pop4, 0.1233 0.1245 0.0689</td>
</tr>
<tr>
<td></td>
<td>NA NA</td>
<td>NA NA</td>
</tr>
<tr>
<td>pop4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Returned values cont.

$bs_locus
A list containing six (WC_Fst = FALSE) - nine (WC_Fst = TRUE) matrices of locus values for each estimated statistic, along with their respective 95% confidence interval.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Lower_CI</th>
<th>Upper_CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus1</td>
<td>0.0342</td>
<td>0.0237</td>
<td>0.0464</td>
</tr>
<tr>
<td>Locus2</td>
<td>0.0153</td>
<td>0.0067</td>
<td>0.0282</td>
</tr>
<tr>
<td>Locus3</td>
<td>0.0824</td>
<td>0.0726</td>
<td>0.0925</td>
</tr>
<tr>
<td>global</td>
<td>0.0587</td>
<td>0.0579</td>
<td>0.0602</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Lower_CI</td>
<td>Upper_CI</td>
</tr>
<tr>
<td>Locus1</td>
<td>0.1156</td>
<td>0.0837</td>
<td>0.1559</td>
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<tr>
<td></td>
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<td>Upper_CI</td>
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<td>0.2005</td>
<td>0.2059</td>
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<tr>
<td></td>
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<td>Upper_CI</td>
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<td>0.0371</td>
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<td>0.0507</td>
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<td></td>
<td>Mean</td>
<td>Lower_CI</td>
<td>Upper_CI</td>
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<td></td>
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<td>Upper_CI</td>
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<td>0.0940</td>
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<tr>
<td>----------------</td>
<td>----------</td>
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<td>----------</td>
</tr>
<tr>
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<table>
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<tr>
<th></th>
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<th>Upper_CI</th>
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<tbody>
<tr>
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<td>0.1020</td>
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</table>
$bs_pairwise$  A list containing six (WC_Fst = FALSE) - nine (WC_Fst = TRUE) matrices of pairwise values for each estimated statistic, along with their respective 95% confidence interval.

<table>
<thead>
<tr>
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<tbody>
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<td></td>
<td></td>
</tr>
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<td>0.0121</td>
<td>0.0163</td>
</tr>
<tr>
<td>pop1, vs. pop3</td>
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<td>0.0388</td>
<td>0.0434</td>
</tr>
<tr>
<td>pop1, vs. pop4</td>
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<td>0.0390</td>
<td>0.0421</td>
</tr>
<tr>
<td>pop5, vs. pop6</td>
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<td>0.0325</td>
<td>0.0360</td>
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<tr>
<td>G_hed_st</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pop1, vs. pop2</td>
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<td>0.0733</td>
<td>0.0967</td>
</tr>
<tr>
<td>pop1, vs. pop3</td>
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<td>0.2722</td>
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<tr>
<td>pop1, vs. pop4</td>
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<td>0.2653</td>
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<td>pop5, vs. pop6</td>
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</tr>
<tr>
<td>D_Jost</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pop1, vs. pop2</td>
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<td>0.0620</td>
<td>0.0816</td>
</tr>
<tr>
<td>pop1, vs. pop3</td>
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<td>0.2100</td>
<td>0.2394</td>
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<td>pop1, vs. pop4</td>
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<td>0.2198</td>
<td>0.2331</td>
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<tr>
<td>pop5, vs. pop6</td>
<td>0.1984</td>
<td>0.1878</td>
<td>0.2105</td>
</tr>
<tr>
<td>Gst_est</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pop1, vs. pop2</td>
<td>0.0079</td>
<td>0.0061</td>
<td>0.0104</td>
</tr>
<tr>
<td>pop1, vs. pop3</td>
<td>0.0354</td>
<td>0.0327</td>
<td>0.0375</td>
</tr>
<tr>
<td>pop1, vs. pop4</td>
<td>0.0354</td>
<td>0.0336</td>
<td>0.0369</td>
</tr>
<tr>
<td>pop5, vs. pop6</td>
<td>0.0276</td>
<td>0.0264</td>
<td>0.0297</td>
</tr>
<tr>
<td>G_hed_st_est</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pop1, vs. pop2</td>
<td>0.0495</td>
<td>0.0383</td>
<td>0.0639</td>
</tr>
<tr>
<td>pop1, vs. pop3</td>
<td>0.2282</td>
<td>0.2107</td>
<td>0.2442</td>
</tr>
<tr>
<td>pop1, vs. pop4</td>
<td>0.2308</td>
<td>0.2229</td>
<td>0.2404</td>
</tr>
<tr>
<td>pop5, vs. pop6</td>
<td>0.1916</td>
<td>0.1812</td>
<td>0.2056</td>
</tr>
<tr>
<td>D_Jost_est</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pop1, vs. pop2</td>
<td>0.0301</td>
<td>0.0186</td>
<td>0.0489</td>
</tr>
<tr>
<td>pop1, vs. pop3</td>
<td>0.1902</td>
<td>0.1835</td>
<td>0.2004</td>
</tr>
<tr>
<td>pop1, vs. pop4</td>
<td>0.1815</td>
<td>0.1800</td>
<td>0.1837</td>
</tr>
</tbody>
</table>
## pop5, vs. pop6, 0.1517 0.1325 0.1695
## [1] Fst_WC
## Mean Lower_CI Upper_CI
## pop1, vs. pop2, 0.0147 0.0111 0.0194
## pop1, vs. pop3, 0.0673 0.0623 0.0712
## pop1, vs. pop4, 0.0674 0.0643 0.0703
## pop5, vs. pop6, 0.0529 0.0506 0.0571
## [1] Fit_WC
## Mean Lower_CI Upper_CI
## pop1, vs. pop2, 0.1039 0.0919 0.1189
## pop1, vs. pop3, 0.1205 0.1109 0.1258
## pop1, vs. pop4, 0.1104 0.1050 0.1203
## pop5, vs. pop6, 0.0751 0.0664 0.0863
4.2 \texttt{inCalc()}

The general usage of this function is as follows:

\begin{verbatim}
\texttt{inCalc(infile, outfile = NULL, gp = 3, bs_locus = FALSE,}
  \hspace{1cm} \texttt{bs_pairwise = FALSE, bootstraps = 0, plot = FALSE,}
  \hspace{1cm} \texttt{parallel = FALSE)}
\end{verbatim}

4.2.1 Arguments

\begin{itemize}
  \item \texttt{infile} Specifying the name of the \textit{genepop} (? file from which the statistics are to be calculated. This file can be in either the 3-digit or 2-digit format. The name must be a character string.
  \item \texttt{outfile} Allows users to specify a suffix for output folder and files. Name must be a character string enclosed in either “” or “”.
  \item \texttt{gp} Specifies the digit format of the \texttt{infile}. Either 3 (default) or 2.
  \item \texttt{bs_locus} Gives users the option to bootstrap locus statistics. Results will be written to .xlsx file by default if the package \texttt{xlsx} is installed, and to a .png file if \texttt{plot=TRUE}. If \texttt{xlsx} is not installed, results will be written to .txt files.
  \item \texttt{bs_pairwise} Gives users the option to bootstrap statistics across all loci for each pairwise population comparison. Results will be written to a .xlsx file by default if the package \texttt{xlsx} is installed. If \texttt{xlsx} is not installed, results will be written to .txt files.
\end{itemize}
Arguments cont.

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>bootstraps</td>
<td>Determines the number of bootstrap iterations to be carried out. The default value is <code>bootstraps = 0</code>, this is only valid when all bootstrap options are false. There is no upper limit on the number of bootstrap iterations, however very large numbers of bootstrap iterations for pairwise calculations (&gt;1000) may take a long time to run for large data sets.</td>
</tr>
<tr>
<td>plot</td>
<td>Optional <code>.png</code> image file of the plotted bootstrap results for locus $L_n$ if <code>bs_locus = TRUE</code>. The default option is <code>plot = FALSE</code>.</td>
</tr>
<tr>
<td>parallel</td>
<td>A logical argument specifying if computations should be run in parallel on all available CPU cores. If <code>parallel = TRUE</code>, batches of jobs will be distributed to all cores resulting in faster completion.</td>
</tr>
</tbody>
</table>
4.2.2 Returned values

Values returned from \texttt{inCalc} are a single .\texttt{xlsx} workbook (if the package \texttt{xlsx} is installed), containing between one to three worksheets, (\texttt{In\_allele\_stats} by default or separate .\texttt{txt} files (if \texttt{xlsx} is unavailable). If \texttt{plot = TRUE} an additional .\texttt{png} plot file will be written. An example of a .\texttt{xlsx} workbook and a .\texttt{png} plot are given below:

**Example of bootstrapped locus $I_n$ results**

![Example of bootstrapped locus $I_n$ results](image)
Example of bootstrapped locus $I_n$ results plot
Returned values cont.

For users wishing to carry out post analysis manipulations, all results from \texttt{inCalc} are returned to the \texttt{R} environment. Depending on the bootstrap options chosen these results include between one to three of the variables below:

\begin{verbatim}
Allele_In   A character matrix of allelic \(I_a\) values per locus along with locus sums.
\end{verbatim}

\begin{verbatim}
## Allele.1 Allele.2 Allele.3 Allele.4
## Locus1 0.0036 0.0036 0.0144 0.004
## Locus2 0.0095 0.0015 0.0013
## Locus3 0.0473 0.0029 0.0053 0.0135
## Locus4 0.0111 0.0029 0.0042 0.0045
## Locus5 0.0394 0.0379 0.0181 0.005
## Locus6 0.077 0.0131 0.0046 0.0087
## Locus7 0.0157 0.0469 0.0054 0.0048
## Locus8 0.0107 0.0075 0.0069 0.0054
## Locus9 0.0038 0.0232 0.0091 0.0326
## Locus10 0.0178 0.0581
## Allele.5 Sum
## Locus1 0.0178 0.0581
## Locus2 0.0123
## Locus3 0.027 0.4482
## Locus4 0.0109 0.08
## Locus5 0.0044 0.3983
## Locus6 0.0352 0.2839
## Locus7 0.0166 0.1068
## Locus8 0.0728
## Locus9 0.0081 0.4571
## Locus10 0.0295 0.4799
\end{verbatim}
Each row of this results matrix represents each locus in the `infile`. Each column represents the allele specific \( I_n \) per locus except the last column, which contains the sum of allele \( I_n \) for each locus.

**l_bootstrap**  
A character matrix of locus \( I_n \) values as well as 95% confidence intervals, calculated from bootstraps (Manly, 1997). Returned when `bs_locus = TRUE`.

<table>
<thead>
<tr>
<th></th>
<th>In</th>
<th>Lower_95CI</th>
<th>Upper_95CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>##</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>##</td>
<td>Locus1</td>
<td>0.0627</td>
<td>0.0434</td>
</tr>
<tr>
<td>##</td>
<td>Locus2</td>
<td>0.0176</td>
<td>0.0092</td>
</tr>
<tr>
<td>##</td>
<td>Locus3</td>
<td>0.4878</td>
<td>0.4797</td>
</tr>
<tr>
<td>##</td>
<td>Locus4</td>
<td>0.0870</td>
<td>0.0675</td>
</tr>
<tr>
<td>##</td>
<td>Locus5</td>
<td>0.4702</td>
<td>0.4545</td>
</tr>
<tr>
<td>##</td>
<td>Locus6</td>
<td>0.3215</td>
<td>0.2931</td>
</tr>
<tr>
<td>##</td>
<td>Locus7</td>
<td>0.1148</td>
<td>0.1033</td>
</tr>
<tr>
<td>##</td>
<td>Locus8</td>
<td>0.0891</td>
<td>0.0706</td>
</tr>
<tr>
<td>##</td>
<td>Locus9</td>
<td>0.5489</td>
<td>0.5307</td>
</tr>
<tr>
<td>##</td>
<td>Locus10</td>
<td>0.5286</td>
<td>0.5136</td>
</tr>
</tbody>
</table>

Each row in this matrix represents each locus. The first column is the locus sum \( I_n \) as in the final column in `Allele_In`. The second and third columns represent the lower and upper confidence intervals per locus respectively.
**PW_bootstrap**  A list of matrices for each pairwise population comparison of bootstrapped pairwise locus $I_n$ values.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Lower_95CI</th>
<th>Upper_95CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus1</td>
<td>0.0337</td>
<td>0.0381</td>
</tr>
<tr>
<td>Locus2</td>
<td>0.0210</td>
<td>0.0314</td>
</tr>
<tr>
<td>Locus3</td>
<td>0.1081</td>
<td>0.1131</td>
</tr>
<tr>
<td>Locus4</td>
<td>0.0600</td>
<td>0.0832</td>
</tr>
<tr>
<td>Locus5</td>
<td>0.1298</td>
<td>0.1449</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Locus</th>
<th>Lower_95CI</th>
<th>Upper_95CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus1</td>
<td>0.0340</td>
<td>0.0408</td>
</tr>
<tr>
<td>Locus2</td>
<td>0.0156</td>
<td>0.0330</td>
</tr>
<tr>
<td>Locus3</td>
<td>0.3344</td>
<td>0.3437</td>
</tr>
<tr>
<td>Locus4</td>
<td>0.1161</td>
<td>0.1617</td>
</tr>
<tr>
<td>Locus5</td>
<td>0.2973</td>
<td>0.3292</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Locus</th>
<th>Lower_95CI</th>
<th>Upper_95CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus1</td>
<td>0.0277</td>
<td>0.0380</td>
</tr>
<tr>
<td>Locus2</td>
<td>0.0084</td>
<td>0.0122</td>
</tr>
<tr>
<td>Locus3</td>
<td>0.3277</td>
<td>0.3316</td>
</tr>
<tr>
<td>Locus4</td>
<td>0.0500</td>
<td>0.0816</td>
</tr>
<tr>
<td>Locus5</td>
<td>0.3122</td>
<td>0.3289</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Locus</th>
<th>Lower_95CI</th>
<th>Upper_95CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus1</td>
<td>0.0697</td>
<td>0.0715</td>
</tr>
<tr>
<td>Locus2</td>
<td>0.0117</td>
<td>0.0133</td>
</tr>
<tr>
<td>Locus3</td>
<td>0.4010</td>
<td>0.4228</td>
</tr>
<tr>
<td>Locus4</td>
<td>0.0731</td>
<td>0.1109</td>
</tr>
<tr>
<td>Locus5</td>
<td>0.2869</td>
<td>0.3562</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Locus</th>
<th>Lower_95CI</th>
<th>Upper_95CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus1</td>
<td>0.0286</td>
<td>0.0422</td>
</tr>
<tr>
<td>Locus2</td>
<td>0.0163</td>
<td>0.0280</td>
</tr>
<tr>
<td>Locus3</td>
<td>0.3524</td>
<td>0.3747</td>
</tr>
<tr>
<td>Locus4</td>
<td>0.0380</td>
<td>0.0446</td>
</tr>
<tr>
<td>Locus5</td>
<td>0.2543</td>
<td>0.2764</td>
</tr>
</tbody>
</table>
4.3 readGenepop()

The general usage of readGenepop is:

\[
\text{readGenepop}(\text{infile} = \text{NULL}, \text{gp} = 3, \text{bootstrap} = \text{FALSE})
\]

4.3.1 Arguments

infile Specifying the name of the ‘genepop’ file from which the statistics are to be calculated. This file can be in either the 3 digit of 2 digit format. The name must be a character string.

gp Specifies the digit format of the infile. Either 3 (default) or 2.

bootstrap A logical argument indicating whether the infile should be sampled with replacement and all returned parameters calculated from this bootstrapped data.

4.3.2 Returned values

nops The number of population samples in infile.

nloci The number of loci in infile.

pop_alleles A list of two matrices per population. Each matrix per population contains haploid allele designations.

pop_list A list of matrices (n = nops) containing the diploid genotypes of individuals per locus.

loci_names A character vector containing the names of loci from infile.
pop_pos  A numeric vector or the row index locations of the first individual per population in infile.

pop_sizes  A numeric vector of length npops containing the number of individuals per population sample in infile.

allele_names  A list of npops lists containing nloci character vectors of alleles names per locus. Useful for identifying unique alleles.

all_alleles  A list of nloci character vectors of all alleles observed across all population samples in infile.

allele_freq  A list containing nloci matrices containing allele frequencies per alleles per population sample.

raw_data  An unaltered data frame of infile.

loci_harm_N  A numeric vector of length nloci, containing the harmonic mean number of individuals genotyped per locus.

n_harmonic  A numeric value representing the harmonic mean of npops.

pop_names  A character vector containing a six character population sample name for each population in infile (the first six characters of the first individual).

indtyp  A list of length nloci containing character vectors of length npops, indicating the number of individuals per population sample typed at each locus.

nalleles  A vector of the total number of alleles observed at each locus.

bs_file  A dataframe/genpop object of bootstrapped data. Returned if bootstrap = TRUE.

obs_all...  A list of matrices of the observed number of allele occurrences per population.
4.4  corPlot()

The general usage of corPlot is:

\texttt{corPlot(x,y)}

4.4.1  Arguments

\begin{itemize}
\item \texttt{x} \hspace{1cm} The object returned by the function \texttt{readGenepop}.
\item \texttt{y} \hspace{1cm} The object returned by the function \texttt{divPart}.
\end{itemize}

4.4.2  Returned values

\begin{itemize}
\item \texttt{plot} \hspace{1cm} A console plot is automatically created using this functions. As the plot is intended for exploratory purposes, it is not written to file. UseR\texttt{s} can save the lot manually if required. below is an example of the returned plot.
\end{itemize}
Returned plot from the function corPlot

The plot depicts the relationship between the estimated statistics calculated by divPart and the number of alleles per locus. Lines represent the line of best fit. Pearson’s product-moment correlation coefficient is also provided.
4.5  difPlot()

The general usage of difPlot is:

```r
difPlot(x, outfile = NULL, interactive = FALSE)
```

4.5.1 Arguments

- **x**: The object returned by the function `divPart`.
- **outfile**: A folder name or directory indicating where interactive plots should be written. It is advisable, though not essential that this argument be set to the same `outfile` argument as for `divPart`. This argument is only valid when `interactive = TRUE`. If no argument is given for `outfile`, while `interactive = TRUE`, plot files will be written to the working directory. Folder name should be given as a character string.
- **interactive**: A logical argument indicating whether users would like to plot their results to interactive `.html` files produced by `sendplot`. `TRUE` indicates that results should be written to file, whereas `FALSE` indicates that results should be plotted to the R graphics device.

4.5.2 Returned values

- **Plot**: Depending on the argument given for `interactive`, either a single plot will be passed to the R graphic device (i.e. when `interactive = FALSE`) or 3-4 `.html` files will be written to a user defined location.
Returned plot from the function difPlot when interactive = FALSE
One of the returned plots from the function difPlot when interactive—TRUE

As can be seen, the plots produced when interactive = TRUE are much more useful than when interactive = FALSE, due to useRs ability to identify population comparisons of interest. These plots contain tool-tip information, courtesy of the sendplot package.
4.6 chiCalc

The general usage of chiCalc is:

\[
\text{chiCalc}(\text{infile} = \text{NULL}, \text{outfile} = \text{NULL}, \text{gp} = 3, \text{minFreq} = \text{NULL})
\]

4.6.1 Arguments

infile   Specifying the name of the ‘\textit{genepop}’ (?) file from which the statistics are to be calculated. This file can be in either the 3 digit or 2 digit format. The name must be a character string.

outfile  A character string specifying the name given to an output file, containing analysis results. If this argument is passed as \text{NULL}, no file will be written.

gp       Specifies the digit format of the \text{infile}. Either 3 (default) or 2.

minFreq  A threshold minimum value or vector of values, below which alleles are not included in the analysis.

4.6.2 Returned values

chi table A character matrix containing locus chi-square values, degrees of freedom, p.values and significance indicators, as well as overall values.
4.7 divOnline

The general usage of divOnline is:

```r
divOnline()
```

By executing the above command, a web browser (system default) will open with the divOnline application running. Users can read file from their system into the app and choose many of the analysis options. Most analysis results can be downloaded to .txt files.

4.8 fstOnly

The general usage of fstOnly is:

```r
fstOnly(infile = NULL, outfile = NULL, gp = 3, bs_locus = FALSE,
        bs_pairwise = FALSE, bootstraps = 0, parallel = FALSE)
```

4.8.1 Arguments

- **infile**: Specifying the name of the ‘genepop’ (?) file from which the statistics are to be calculated. This file can be in either the 3 digit or 2 digit format. The name must be a character string.

- **outfile**: Allows users to specify a suffix for output folder and files. Name must a character string enclosed in either “” or “”.

- **gp**: Specifies the digit format of the infile. Either 3 (default) or 2.
**bs_locus**  Gives useRs the option to bootstrap locus statistics. Results will be written to .xlsx file by default if the package xlsx is installed, and to a .png file if plot=TRUE. If xlsx is not installed, results will be written to .txt files.

**bs_pairwise**  Gives useRs the option to bootstrap statistics across all loci for each pairwise population comparison. Results will be written to a .xlsx file by default if the package xlsx is installed. If xlsx is not installed, results will be written to .txt files.

**bootstraps**  Determines the number of bootstrap iterations to be carried out. The default value is bootstraps = 0, this is only valid when all bootstrap options are false. There is no upper limit on the number of bootstrap iterations, however very large numbers of bootstrap iterations for pairwise calculations (> 1000) may take a long time to run for large data sets.

**parallel**  A logical argument specifying if computations should be run in parallel on all available CPU cores. If parallel = TRUE, batches of jobs will be distributed to all cores resulting in faster completion.

### 4.8.2 Returned values

**locus**  A list contain two matrices, $F_{ST}$ and $F_{IT}$. Each matrix contains the actual calculated statistic along with their respective 95% confidence intervals per locus, as well as a global estimate across all population samples and loci. This result is only returned if bs_locus = TRUE.

**pairwise**  A list contain two matrices, $F_{ST}$ and $F_{IT}$. Each matrix contain the actual and respective 95% confidence intervals across loci for each pairwise population comparison. This result is only returned when bs_pairwise = TRUE.
4.9 divRatio

The general usage of divRatio is:

```r
divRatio(infile = NULL, outfile = NULL, gp = 3, pop_stats = NULL, refPos = NULL, bootstraps = 1000, parallel = FALSE)
```

4.9.1 Arguments

infile A character string argument specifying the name of either a 3 digit or 2 digit genepop file containing the raw genotypes of at least the reference population sample.

outfile A character string specifying a prefix name for an automatically generated results folder, to which results file will be written.

gp Specifies the digit format of the infile. Either 3 (default) or 2.

pop_stats A character string indicating the name of the population statistics data frame file. This argument is required if only raw data for the reference population are give in infile. The data frame should be structured in a specific way. An example can be seen by typing data(pop_stats) into the console. The validloci column is only required if mean allelic richness and expected heterozygosity for populations of interest have been calculated from loci for which data is not present in the reference population. This column should contain a single character string of common loci between each population sample and the reference population sample.

refPos A numeric argument specifying the position of the reference population in infile. The argument is only valid when raw genotype data has been provided for the reference population sample and all other populations of interest and pop_stats is NULL.
bootstraps Specifies the number of times the reference population should be resampled when calculating the sample size standardised allelic richness and expected heterozygosity for calculating the diversity ratios. The larger the number of bootstraps the longer the analysis will take to run. As an indication of runtime, running `divRatio` on the `Big_data` data set (type `?Big_data` for details), takes 10min 42s on a Toshiba Satellite R830 with 6GB RAM, and an Intel Core i5 - 2435M CPU running Linux.

parallel A logical argument indicating whether the analysis should make use of all available cores on the users system.

4.9.2 Returned values

All results will be written to a user defined folder, providing an argument is passed for 'outfile'. Results will be written to `.xlsx` files if the package `xlsx` and its dependencies are installed, or a `.txt` file otherwise.

A data frame containing the following variables is also returned to the R console:

- **pop** The names of each population of interest, including the reference population.
- **n** The sample size of each population
- **alr** Mean allelic richness across loci
- **alrSE** The standard error of the allelic richness across loci
- **He** Mean expected heterozygosity across loci
- **HeSE** Standard error of expected heterozygosity across loci
- **alrRatio** The ratio of the allelic richness of the subject population sample and the sample size standardised reference population allelic richness
The standard error of divisions for the allelic richness ratio

The ratio of expected heterozygosity between the standardised reference population sample and subject population samples

The standard error of divisions for the expected heterozygosity ratio

4.10 bigDivPart()

The general usage of this function is as follows:

```r
bigDivPart(infile = NULL, outfile = NULL, WC_Fst = FALSE, format = NULL)
```

4.10.1 Arguments

**infile** Specifies the name of the ‘genepop’ (?) file from which the statistics are to be calculated. This file can be in either the 3 digit or 2 digit format. The name must be a character string.

**outfile** Allows users to specify a prefix for an output folder. Name must be a character string enclosed in either " or ".

**WC_Fst** A logical indication as to whether Weir and Cockerham’s, 1984 F-statistics should be calculated. This option will increase analysis time.

**format** A character string specifying the preferred output format for calculated results. The arguments txt or xlsx are valid when outfile is not NULL.
4.10.2 Returned values

**standard**  
See divPart description for details.

**estimates**  
See divPart description for details.

4.11 microPlexer

The general usage of this function is as follows:

```
microPlexer()
```

By typing the above command into the R console, a web application will be launched in the system’s default internet browser. At initiation, the application appears as the figure below:
After uploading input data and making the desired parameter selections, multiplex group plots will be displayed as below. These plots can be downloaded to a single .PDF workbook for further inspection.

4.12 arp2gen

The general usage of this function is as follows:

arp2gen(infile)

Where infile is a character string pointing to an Arlequin genotype file. The infile argument can simply be the file name if the file is located in the current working directory, otherwise an absolute/relative path must be given.
4.13 divMigrate

The general usage of this function is as follows:

```r
divMigrate(infile = NULL, stat = c("gst", "d_jost"))
```

Where `infile` is either an file name or a path to a file. The file should be in either the Arlequin genotype file format (.arp) or the genepop file format used in other diveRsity functions. The argument `stat` determines which statistic ($G_{ST}$ or $D_{Jost}$) should be used to calculate the relative migration matrix.

4.14 haploDiv

The general usage of this function is as follows:

```r
haploDiv(infile = NULL, outfile = NULL, pairwise = FALSE, bootstraps = 0)
```

Where `infile` is the file name or directory location of a haploid genotype genepop file, `outfile` is the prefix name to be used for output .txt files, `pairwise` indicates whether a pairwise matrix of Weir & Cockerham’s $\theta$ should be calculated, and `bootstraps` indicates whether bootstrapped 95% confidence limits should be estimated for each pairwise comparison.

Details

If the `outfile` argument is `NULL`, no results will be written to file. All results are returned to the R workspace. To calculate 95% confidence limits, `bootstraps` must be $> 0$, and `pairwise` must be `TRUE`. 
5 Examples

In this section worked examples of each of the three functions documented above are given. The examples will employ the test data set distributed with diveRsity, Test_data. Care has been take to ensure that examples can be used independently, thus some processes are repeated for each function examples, such as loading Test_data into the R session.

N.B. All examples assume that you have already downloaded, installed and loaded diveRsity.

5.1 divPart

This example is specific to the function divPart. It has been written to demonstrate way in which the function may be used. It has not been written as an exhaustive demonstration.

5.1.1 Setting your working directory

In any R session it is sensible to have a folder on your system where any output files etc. are to be written. When using diveRsity, it is recommended that you set your working directory to the location of your input file.

To set your working directory, use:

```
setwd("mypath")
```

Simply replace ‘mypath’ with your actual file path. Make sure to use ‘/’ or ‘\’ to separate directory levels (e.g. c:/Users/Kevin/etc., or c:\Users \Kevin \etc.). R does not recognise the ‘\’ symbol for pathways.
5.1.2 Loading Test_data

Test_data is only required for these examples. UseRs should replace the argument ‘infile = Test_data’ with ‘infile = "myfilename"’ when wishing to analyse their own data set.

```
data(Test_data, package = "diveRsity")
```

This command loads Test_data into the current R session.
5.1.3 Running divPart

To run divPart, where locus bootstrap and pairwise bootstrap results are returned without plotting, use the following:

```r
div_results <- divPart(infile = Test_data, outfile = "Test", 
                       gp = 3, pairwise = TRUE, 
                       WC_Fst = TRUE, bs_locus = TRUE, 
                       bs_pairwise = TRUE, bootstraps = 100, 
                       plot = FALSE, parallel = TRUE)
```

N.B. in this example `bootstraps = 100` to reduce the time taken to run the example.

When the analysis has finished a folder named `Test-[diversity]` should be written to your working directory. This folder will contain either a single `.xlsx` workbook named ‘[divPart].xlsx’ (if xlsx is installed), or four `.txt` files named, ‘Standard-stats[divPart].txt’, ‘Estimated-stats[divPart].txt’, ‘Locus-bootstrap[divPart].txt’ and ‘Pairwise-bootstrap[divPart].txt’ if it is not.

5.1.4 Accessing your results within the R session

All of the results written to file are also assigned to the variable `test_results`. To access these results it is useful to understand the structure of the objects `test_results` contains. Although the objects have been described in the Returned values section for divPart, a further visual description will be provided here.

Using the following will show you the names of all objects within `test_results`:

```r
names(div_results)
```

## [1] "standard"  "estimate"  "pairwise"
## [4] "meanPairwise" "bs_locus"  "bs_pairwise"
To access an object within `test_results` you can use the extract operator `$`. For example, if you want to know what type of object `bs_locus` is, use:

```r
typeof(div_results$bs_locus)
```

```r
## [1] "list"
```

From the **Returned values** section for `divPart`, it is known that `bs_locus` is indeed a list containing six matrices. This object can be explored further using:

```r
names(div_results$bs_locus)
```

```r
## [1] "Gst" "G_hed_st" "D_Jost"
## [4] "Gst_est" "G_hed_st_est" "D_Jost_est"
## [7] "Fst_WC" "Fit_WC"
```

Each of the named objects within `test_results$bs_locus` are known to be matrices from above. This means that we can use matrix indexing to access any of the information within any of the matrices. In **R**, to access a specific value within a matrix, we only need to know the row and column that the value is in. If we wanted to access a value that lies in the 5th row and the 1st column the following command could be used:

```r
mymatrix[5, 1]
```

The first digit within the `[ ]` (i.e. before the `,`) in **R** always refers to the **row** location of a value and the second to the **column** location.

It is possible to access more than one value in a matrix using indexing. If we wanted to look at the first 10 rows of `test_results$bs_locus$Gst`, we would use the following code.
By leaving the column index blank (i.e. no numbers after the ‘,’), all columns are returned. Similarly, if we wanted to view all values in the first column of `test_results$bs_locus$Gst`, we would use:

```
div_results$bs_locus$Gst[1:10, ]
```

By leaving the column index blank (i.e. no numbers after the ‘,’), all columns are returned. Similarly, if we wanted to view all values in the first column of `test_results$bs_locus$Gst`, we would use:

```
div_results$bs_locus$Gst[,1]
```

The other values returned by `divPart` can be accessed in a similar fashion. When you understand how to access the results within R, many post-analysis processes can be used such as correlations, regressions and plotting.

### 5.2 inCalc

This example is specific to the function `inCalc`. It has been written to demonstrate way in which the function may be used. It has not been written as an exhaustive demonstration.
5.2.1 Setting your working directory

In any R session it is sensible to have a folder on your system where any output files etc. are to be written. When using diveRsity, it is recommended that you set your working directory to the location of your input file.

To set your working directory, use:

```r
setwd("mypath")
```

Simply replace ‘mypath’ with your actual file path. Make sure to use ‘/’ or ‘\’ to separate directory levels (e.g. c:/Users/Kevin/etc., or c:\Users\Kevin\etc.). R does not recognise the ‘\’ symbol for pathways.

5.2.2 Loading Test_data

Test_data is only required for these examples. UseRs should replace the argument ‘infile = Test_data’ with ‘infile = "myfilename"’ when wishing to analyse their own data set.

```r
data(Test_data, package = "diveRsity")
```

This command loads Test_data into the current R session.

5.2.3 Running inCalc

To run inCalc, where locus bootstrap and pairwise bootstrap results are returned without plotting, use the following:
```r
in_results <- inCalc (infile = Test_data, outfile = "Test",
                     gp = 3, bs_locus = TRUE,
                     bs_pairwise = TRUE, bootstraps = 100,
                     plot = FALSE, parallel = TRUE)
```

N.B. in this example `bootstraps` = 100 to reduce the time taken to run the example.
When the analysis has finished a folder named `Test-[diversity]` should be written to your working directory. This folder will contain either a single `.xlsx` workbook named `'/\text{.xlsx}'` (if `xlsx` is installed), or three `.txt` files named, `Allele-In[inCalc].txt`, `Overall-bootstrap[inCalc].txt` and `Pairwise-bootstrap[inCalc].txt` if it is not.

### 5.2.4 Accessing your results within the R session

All of the results written to file are also assigned to the variable `test_results`. To access these results it is useful to understand the structure of the objects `test_results` contains. Although the objects have been described in the Returned values section for `inCalc`, a further visual description will be provided here.

Using the following will show you the names of all objects within `test_results`:

```r
names(in_results)
```

```r
## [1] "Allele_In"   "l_bootstrap" "PW_bootstrap"
```

To access an object within `test_results` you can use the extract operator `$`. For example, if you want to know what type of object `PW_bootstrap` is, use:

```r
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```
typeof(in_results$PW_bootstrap)

## [1] "list"

From the Returned values section for inCalc, it is known that PW_bootstrap is indeed a list of matrices of bootstrapped locus results for each pairwise comparison. To find the names of the matrices within PW_bootstraps, use:

names(in_results$PW_bootstrap)

## [1] "pop1, vs. pop2,"  "pop1, vs. pop3,"  "pop1, vs. pop4,"  "pop1, vs. pop5,"  "pop1, vs. pop6,"  "pop2, vs. pop3,"  "pop2, vs. pop4,"  "pop2, vs. pop5,"  "pop2, vs. pop6,"  "pop3, vs. pop4,"  "pop3, vs. pop5,"  "pop3, vs. pop6,"  "pop4, vs. pop5,"  "pop4, vs. pop6,"  "pop5, vs. pop6,

From this we see that PW_bootstrap contains 15 matrices for each of the 15 possible pairwise comparisons from the six population samples in Test_data. We can explore any of these matrices using matrix indexing. In R, to access a specific value within a matrix, we only need to know the row and column that the value is in (i.e. its index). If we wanted to access a value that lies in the 5th row and the 1st column the following command could be used:

mymatrix[5, 1]

The first digit within the '[ ]' (i.e. before the ',' in R always refers to the row location of a value and the second to the column location. To look at the first 3 rows of the comparison between pop1 and pop2 in PW_bootstrap, we would use the following code.
By leaving the column index blank (i.e. no numbers after the ','), all columns are returned. Similarly, if we wanted to view all values in the first column of
\[\text{test_results}\$\text{PW\_bootstrap}[\"\text{pop1, vs. pop2,}\]]\], we would use:

\[\text{in\_results}\$\text{PW\_bootstrap}[\"\text{pop1, vs. pop2,}\]][1:,]

The other values returned by \text{inCalc} can be accessed in a similar fashion. When you understand how to access the results within \text{R}, many post-analysis processes can be used such as correlations, regressions and plotting.

### 5.3 \textbf{readGenepop}

This example is specific to the function \text{readGenepop}. It has been written to demonstrate way in the which the function may be used. It has not been written as an exhaustive demonstration.

#### 5.3.1 Setting your working directory

In any \text{R} session it is sensible to have a folder on your system where any output files etc. are to be written. When using \text{diveRsity}, it is recommended that you set your working directory to the location of your input file. To set your working directory, use:
Simply replace ‘mypath’ with your actual file path. Make sure to use ‘/’ or ‘\’ to separate directory levels (e.g. c:/Users/Kevin/etc., or c:\\Users\\Kevin\\etc.). R does not recognise the ‘\’ symbol for pathways.

### 5.3.2 Loading Test_data

*Test_data* is only required for these examples. UseRs should replace the argument ‘*infile = Test_data*’ with ‘*infile = "myfilename"*’ when wishing to analyse their own data set.

```r
data(Test_data, package = "diveRsity")
```

This command loads *Test_data* into the current R session.

### 5.3.3 Running readGenepop

To run *readGenepop* without producing a bootstrap file, use:

```r
gp_res <- readGenepop(infile = Test_data, gp = 3, bootstrap = FALSE)
```

### 5.3.4 Accessing your results within the R session

The *readGenepop* function does not write anything to file. Instead results are only returned to the R environment.
To explore what these results are, use:

```r	names(gp_res)
```

```
## [1] "npops" "nloci"
## [3] "pop_alleles" "pop_list"
## [5] "loci_names" "pop_pos"
## [7] "pop_sizes" "allele_names"
## [9] "all_alleles" "allele_freq"
## [11] "raw_data" "loci_harm_N"
## [13] "n_harmonic" "pop_names"
## [15] "indtyp" "nalleles"
## [17] "obs_allele_num"
```

For a description of each of these objects see section 4.3.2.

### 5.3.5 Applications for readGenepop

readGenepop is not like the other two function in that the results returned have no particularly informative format. Instead the results are the building blocks to developing other analysis methods for useR who may not have the necessary programming skills to extract such information from genetic data. In this section two examples of applications of readGenepop are provided. UseRs are encouraged to use the function to develop their own methods.

`Ad hoc` investigation of locus mutation model

Understanding the likely mutation model a particular microsatellite locus follows is important for a range of analyses which make explicit assumptions. One way to ensure your data does not violate these assumption is to visualise
the allele distribution at loci and assess whether the pattern fits the expectation of a given model.

`readGenepop` returns an object `pop_alleles` which contains `nops x 2` matrices. Each matrix contains a haploid genotype per individual per locus, and every two matrices correspond to a single population sample. For example matrices 1 and 2 correspond to population sample 1, matrices 3 and 4 correspond to population sample 2 and so on. Using this object, it is possible to plot the allele size distribution to assess it allele fragments fit say the single step mutation model (SSM).

```r
locus18_pop1 <- c(gp_res$pop_alleles[[1]][[1]][,18],
                   gp_res$pop_alleles[[2]][[1]][,18])
# sort alleles by size
allele_sort <- order(locus18_pop1, decreasing = FALSE)
# plot
plot(locus18_pop1[allele_sort], ylab = "allele size", col="blue", pch = 16)
```
From this figure we could conclude that locus 18 in population 1 is likely to follow SSM given that allele size increases in a generally regular fashion. Any gaps are also a multiple of the repeat motif length.

Although this example is basic and does not have a rigorous statistical basis, the value of such data exploration is clear. Indeed, useRs with suitable know-how could likely easily develop statistically valid model tests for this particular example.
5.3.6 A hypothetical example

This example is for illustrative purposes. Say for some reason, we were interested in assessing the sampling properties of the number of alleles at a particular locus, readGenepop is ideal to do this. We will use Test_data for this example and the number of bootstrap iterations will be 1000. We know that Test_data contains 37 loci so we will have to be able to count the number of alleles for each of these in each bootstrap iteration.

The code

```r
# Define a results matrix with 37 columns (loci) and 1000 rows (bootstraps) to record allele number per locus
num_all <- matrix(rep(0,(37*10)), ncol = 37)

# Now using readGenepop we can fill the matrix
bs<-10
for(i in 1:bs){
  # first produce a bootstrap file
  x <- readGenepop(infile = Test_data, gp = 3, bootstrap = TRUE)
  # Now record the number of alleles at each locus
  num_all[i, ] <- x$nalleles
}

# Now we can use this data to calculate the mean number of alleles per locus as well at their 95% confidence intervals
mean_num <- colMeans(num_all)
lower<-vector()
upper<-vector()
for(i in 1:ncol(num_all)){
  lower[i] = mean_num[i] - 1.96*sqrt(mean_num[i]/bs)
  upper[i] = mean_num[i] + 1.96*sqrt(mean_num[i]/bs)
}
```

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lower[i] <- mean_num[i] - (1.96 * sd(num_all[,i]))
upper[i] <- mean_num[i] + (1.96 * sd(num_all[,i]))

# Now we can create a data frame of these results
bs_res <- data.frame(mean_num, lower, upper)

bs_res[1:10,]

\[
\begin{array}{ccc}
\text{##} & \text{mean_num} & \text{lower} & \text{upper} \\
\text{## 1} & 6.0 & 4.693333 & 7.306667 \\
\text{## 2} & 3.0 & 3.000000 & 3.000000 \\
\text{## 3} & 17.9 & 17.280194 & 18.519806 \\
\text{## 4} & 7.5 & 5.382958 & 9.617042 \\
\text{## 5} & 35.0 & 32.933979 & 37.066021 \\
\text{## 6} & 14.0 & 14.000000 & 14.000000 \\
\text{## 7} & 8.6 & 7.587860 & 9.612140 \\
\text{## 8} & 4.0 & 4.000000 & 4.000000 \\
\text{## 9} & 41.3 & 39.440581 & 43.159419 \\
\text{## 10} & 32.8 & 30.998884 & 34.601116
\end{array}
\]

This is perhaps not the most efficient way to do this kind of analysis but it
does make it more accessible to non-programmers.
5.4 Running divPart in batch (using parallel)

Application of batch analyses

Often, for a number of reasons, it may be necessary to analyse many separate genepop file. Simulation studies and Approximate Bayesian Computation (ABC) are two examples of where this is commonly done. Below is a hypothetical example, demonstrating how this could be done using diveRsity

The hypothetical experiment

Imagine that we are interested in measuring some group of parameters for a group of populations simulated under various evolutionary models. In this example we would like to estimate global $G_{st}$, $\theta$, $G'_{st}$ and $D_{Jost}$ for a group of population samples evolved under 10 distinct models. However, because we are interested in comparing the distributions of each of these parameters under each of the 10 models, we have replicated each simulation 1000 times (giving a total of 10,000 genepop files to be analysed). Helpfully, our 1000 genepop files per evolutionary model have been organised into 10 separate folders (1 per model). The directory tree might look something like this:
The workflow to analyse these files in parallel (say on 10 CPUs) is as follows:

- Determine the names and locations of all files to be analysed
- Set up the CPU cluster for all R processes (using the doParallel & parallel packages).
- Pipe these file names to the divPart function and return the parameters required (i.e. global $G_{st}$, $\theta$, $G'_{st}$ & $D_{Jost}$).
- Return the results in a convenient format to allow for down stream investigations.

The annotated code is below:
# load the diveRsity package
library("diveRsity")

# We can specify the names of our simulation folders in two ways
# manually
fold_names <- paste("sim", 1:10, sep = "")

or

# automatically (when there is only a single level below the 
# top directory)
fold_names <- list.dirs(full.names = TRUE, recursive = FALSE)

# Now we can determine the names of all genepop files in each folder

file_names <- lapply(fold_names, function(x){
  files <- dir(path = paste(x, "/", sep = ""), pattern = "*.gen",
               full.names = TRUE)
  return(files)
})

# file_names will be a list of length 10. Each element will contain
# the names of all .gen files within the respective simulation folder

# Before we are ready to run the main analyses, we should set up
# the parallel environment

# load the doParallel package
library("doParallel")

# set up a cluster of 10 CPUs (one for each batch of files)
c1 <- makeCluster(10)
# Export the 'divPart' function to the cluster cores
clusterExport(c1, "divPart", envir = environment())

# Now we can run the main analyses

results <- parLapply(c1, file_names, function(x){
  sim_res <- sapply(x, function(y){
    out <- divPart(infile = y, gp = 3, WC_Fst = TRUE)
    return(out$estimate[nrow(out$estimate), 4:7])
  })
  return(t(sim_res)) # transpose sim_res
})
# This will generate a list (of length 10), with each element
# containing a matrix of 1000 rows (1 per file) and 4 columns
# (1 for each diversity statistic)

# Example of output for simulation 1
# G_st_est  G_hed_st_est  D_Jost_est  Fst_WC
# 0.3905    0.8938  0.8256  0.4010
# 0.5519    0.8719  0.6986  0.6031
# 0.5924    0.8880  0.7092  0.6096
# ...
# ...
# ...

# these results could then be piped to further analyses or
# visualisation tools
References


6 Reproducibility

## R version 3.3.3 (2017-03-06)
## Platform: x86_64-apple-darwin16.4.0 (64-bit)
## Running under: macOS Sierra 10.12.3

## attached base packages:
## [1] stats  graphics  grDevices  utils
## [5] datasets  methods  base

## other attached packages:
## [1] diveRsity_1.9.89 knitr_1.15.1

## loaded via a namespace (and not attached):
## [1] splines_3.3.3  ellipse_0.3-8
## [3] network_1.13.0  gtools_3.5.0
## [5] Formula_1.2-1  shiny_1.0.0
## [7] assertthat_0.1  highr_0.6
## [9] stats4_3.3.3  latticeExtra_0.6-28
## [11] d3Network_0.5.2.1  pbivnorm_0.6.0
## [13] backports_1.0.4  lattice_0.20-34
## [15] quadprog_1.5-5  digest_0.6.11
## [17] RColorBrewer_1.1-2  checkmate_1.8.2
## [19] ggm_2.3  minqa_1.2.4
## [21] colorspace_1.3-2  httpuv_1.3.3
## [23] htmltools_0.3.5  Matrix_1.2-8
## [25] plyr_1.8.4  psych_1.6.12
## [27] xtable_1.8-2  corpcor_1.6.8
## [29] scales_0.4.1  glasso_1.8
## [31] ena_2.4  whisker_0.3-2
## [33] jpeg_0.1-8  fdrtool_1.2.15
## [35] lme4_1.1-12  huge_1.2.7
## [37] arm_1.9-3  htmlTable_1.8
## [39] tibble_1.2  ggplot2_2.2.1
## [41] nnet_7.3-12  lazyeval_0.2.0.9000
## [43] mnormt_1.5-5  mime_0.5
## [45] survival_2.40-1  magrittr_1.5
## [47] statnet.common_3.3.0  evaluate_0.10
## [49] nlme_3.1-131  MASS_7.3-45
## [51] foreign_0.8-67  tools_3.3.3
## [53] data.table_1.10.0  stringr_1.1.0
## [55] munsell_0.4.3  cluster_2.0.5
7 Acknowledgements

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