An introduction to the PopGenome package

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1 Introduction

PopGenome is a new package for population genomic analyses and method development. PopGenome includes, e.g., a wide range of polymorphism, neutrality statistics, and FST estimates; these can be applied to sequence data stored in alignment format, as well as to whole genome SNP data, e.g., from the 1000/1001 Genome projects. The full range of methods can be applied to whole alignments, sets of sub-sequences, and sliding windows based on either nucleotide positions or on SNP counts. PopGenome is also able to handle GFF/GTF annotation files and automatically specifies the SNPs located in, e.g., exon or intron regions. Those subsites can be analyzed together (e.g., all introns together) or each region separately (e.g., one value per intron). The PopGenome framework is linked to Hudson’s MS and Ewing’s MSMS programs for significance tests using coalescent simulations.

The following sections explain how to use the PopGenome package. Detailed information about the functions and their parameters can be found in the PopGenome manual on CRAN.

2 Installing PopGenome

Installing the package via R

```r
> install.packages("PopGenome")
```

Loading the PopGenome package

```r
> library(PopGenome)
```

3 Reading data (alignments)

Reading three alignments in FASTA-format (`4CL1tl.fas`, `C4H1tl.fas` and `CAD1tl.fas`) stored in the folder "FASTA". (An example FASTA-file can be found in the data subdirectory of the PopGenome package on CRAN. Other alignment formats – such as Phylip, MEGA, MAF – are also accepted. Note: valid nucleotides are A,a,C,c,T,t,U,u,G,g,-(gap),N,n(unknown). Internally those nucleotides are re-coded into numerical values:

- `T,U` → 1
- `C` → 2
- `G` → 3
- `A` → 4
- `unknown` → 5
- `−` → 6

```r
> GENOME.class <- readData("FASTA")
```
**GENOME.class** is an object of class **GENOME**. When typing **GENOME.class**, we get some information about the main methods provided by PopGenome and how to access the results. The **GENOME** class is the input for every function printed below.

**Note:** **GENOME.class** is just a variable, you can choose an arbitrary variable name instead.

```r
> GENOME.class
```

---

Modules:

---

| Calculation          | Description                  | Get.the.Result |
|----------------------|------------------------------|----------------|
| 1 readData           | Reading data                 | get.sum.data   |
| 2 neutrality.stats   | Neutrality tests             | get.neutrality |
| 3 linkage.stats      | Linkage disequilibrium       | get.linkage    |
| 4 recomb.stats       | Recombination                | get.recomb     |
| 5 F_ST.stats         | Fixation index               | get.F_ST, get.diversity |
| 6 MKT                 | McDonald-Kreitman test       | get.MKT        |
| 7 detail.stats       | Mixed statistics             | get.detail     |
| 8 MS                  | Coalescent simulation        | @              |
| 9                      |                               |                |
| 10 set.populations   | Defines the populations      |                |
| 11 sliding.window.transform | Sliding window               |                |
| 12 splitting.data    | Splits the data              |                |
| 13 show.slots        | ?provided slots?             |                |
| 14 get.status        | Status of calculations       |                |

The **GENOME** class contains all observed data and statistic values which are presentable in a multi-locus-scale (vector or matrix). Use the function `show.slots(GENOME.class)` to get an overview, or check out the PopGenome manual on CRAN. To access those values we use the `@`-operator.

How many sites were analyzed in each alignment?

```r
> GENOME.class@n.sites
```

| 4CLl1tl.fas | C4Htl.fas | CADtl.fas |
|-------------|----------|----------|
| 2979        | 2620     | 2930     |

```r
> GENOME.class@region.names
```

| [1] "4CLl1tl.fas" "C4Htl.fas" "CADtl.fas"
|:------------------------------------------|

To get some summary information from the alignments, use the `get.sum.data` function. This function extracts the values from the class **GENOME** and puts them into a matrix. We can also look at those values seperately with the `@`-operator (`GENOME.class@n.biallelic.sites`).

```r
> get.sum.data(GENOME.class)
```

3
The Slot `region.data` contains some detailed (site specific) information that cannot be presented in a multi-locus-scale. `region.data` is another class, and its slots are also accessible with the @ operator. See also the figure in section PopGenome classes.

```r
> GENOME.class@region.data
```

-----

**SLOTS:**

-----

| Slots                 | Description                                         |
|-----------------------|-----------------------------------------------------|
| 1 populations         | Samples of each population (rows)                   |
| 2 populations2        | Samples of each population (names)                  |
| 3 outgroup            | Samples of outgroup                                 |
| 4 transitions         | Biallelic site transitions                          |
| 5 biallelic.matrix    | Biallelic matrix                                    |
| 6 n.singletons        | Number of singletons                                |
| 7 biallelic.sites     | Position of biallelic sites                         |
| 8 reference           | SNP reference                                       |
| 9 n.nucleotides       | Number of nucleotides per sequence (biallelic)      |
| 10 biallelic.compositions | Nucleotides per sequence (biallelic)                  |
| 11 synonymous         | Synonymous biallelic sites                           |
| 12 biallelic.substitutions | Biallelic substitutions                           |
| 13 polyallelic.sites  | Sites with >2 nucleotides                           |
| 14 sites.with.gaps    | Sites with gap positions                            |
| 15 sites.with.unknowns| Sites with unknown positions                        |
| 16 minor.alleles      | Minor alleles                                       |
| 17 codons             | Codons of biallelic substitutions                    |
| 18 IntronSNPS         | SNPs in intron region                               |
| 19 UTRSNPS            | SNPs in UTR region                                  |
| 20 CodingSNPS         | SNPs in coding region                               |
| 21 ExonSNPS           | SNPs in exon region                                 |
| 22 GeneSNPS           | SNPs in gene region                                 |

These are the Slots (class region.data)

The first 10 biallelic positions ([1:10]) of the first alignment ([[1]]):

```r
> GENOME.class@region.data@biallelic.sites[[1]][1:10]

[1] 12 13 31 44 59 101 121 154 165 202
```
Which of those biallelic sites are transitions?

> GENOME.class@region.data@transitions[[1]][1:10]

[1] TRUE TRUE TRUE TRUE TRUE FALSE TRUE FALSE FALSE FALSE

3.1 The slots of the class region.data

- **populations**: 'list' of length n.populations. Contains the row identifiers (biallelic.matrix) of each individual
- **populations2**: list of length n.populations. Contains the character names of each individual
- **outgroup**: contains the row identifiers (biallelic.matrix) of the outgroup individuals
- **transitions**: a boolean vector of length n.snps. **TRUE** if the substitution producing the SNP was a transition
- **biallelic.matrix**: all calculations are based on this matrix. It contains zeros (major alleles) and ones (minor alleles). rows=individuals, columns=SNPs (see `get.biallelic.matrix` in the manual) If the parameter **include.unknown** of the `readData` function is switched to **TRUE**, the unknown nucleotides are **NA** in the biallelic matrix.
- **n.singletons**: vector of length n.individuals. Number of SNPs where the minor allele occurs in exactly one individual.
- **biallelic.sites**: positions of the single nucleotide polymorphisms (SNP)
- **n.nucleotides**: number of valid nucleotides for each individual.
- **biallelic.composition**: the nucleotide distribution for each individual
- **synonymous**: vector of length=n.snps. **TRUE**: synonymous, **FALSE**: non-synonymous, **NA**: non-coding region
- **biallelic.substitutions**: The correspondig nucleotides of the SNPs: first row: minor allele, second row: mayor allele
- **polyallelic.sites**: position of poly-allelic sites (>2 nucleotides)
- **sites.with.gaps**: sites including gaps (those sites are excluded)
- **sites.with.unknowns**: sites with unknown positions (N,n,?). Those sites are included if the parameter **include.unknown** ist **TRUE**
- **minor.alleles**: The minor allele of the SNP represented as a numerical value
- **codons**: a list of length=n.coding.snps. The codon changes are represented as numerical values. This slot is only available for data in alignment format.
For SNP data we provide the function `set.synnonsyn` because of memory issues. See also `get.codons` for detailed information about the codon changes, and `codontable()` to define alternative genetic codes.

**<FEATURE>SNPS**

boolean vector of length=n.snps, TRUE, if the SNP lies in a (coding, exon, intron or UTR) region. This slot will be present after reading data with the corresponding GFF-file.

### 4 Reading data with GFF/GTF information

The GFF folder contains GFF-files for each alignment stored in the FASTA folder. The GFF files should have the same names as the corresponding FASTA files (in this example: `4CL1tl.gff`, `C4Htl.gff` and `CADtl.gff`) to ensure that sequence and annotation are matched correctly.

```r
> GENOME.class <- readData("FASTA", gffpath="GFF")
```

Which of the first 10 SNPs ([1:10]) of the second ([2]) alignment are part of a synonymous mutation?

```r
> GENOME.class@region.data@synonymous[[2]][1:10]
```

```
[1] TRUE TRUE TRUE TRUE TRUE TRUE NA NA NA NA
```

NA values indicate that the sites are not in a coding region

```r
> GENOME.class@region.data@CodingSNPS[[2]][1:10]
```

```
[1] 1413 1428 1446 1455 1482 1488 1744 1756 1798 1802
```

#### 4.1 Splitting the data into subsites

PopGenome can subdivide the data based on features defined in the GFF file. In this example we split the alignment into coding (CDS) regions. The returned value is again an object of class `GENOME`.

```r
> GENOME.class.split <- splitting.data(GENOME.class, subsites="coding")
```

Each region now contains the SNP information of each coding region defined in the GFF files. In case of whole-genome SNP data this mechanism can be very useful. (see manual: `readSNP, readVCF` and section (Reading data (SNP files))

```r
> GENOME.class.split@n.sites
```

```
[1] 1056 413 103 96 785 132 595 92 112 226 438 220
```

```r
> GENOME.class.split <- neutrality.stats(GENOME.class.split)
```

Apply the methods in the neutrality module to all synonymous SNPs in the coding regions.

```r
> GENOME.class.split <- neutrality.stats(GENOME.class.split, subsites="syn")
```

```r
> GENOME.class.split@Tajima.D
```

The function `get.gff.info` provides additional features to extract annotation informations out of a GFF/GTF file.
5 Define the populations

Define two populations as a list.

```r
> GENOME.class <- set.populations(GENOME.class,list(
+   c("CON","KAS-1","RUB-1","PER-1","RI-0","MR-0","TUL-0"),
+   c("MH-0","YO-0","ITA-0","CVI-0","COL-2","LA-0","NC-1")
+ ))
```

Individual names are returned by the function `get.individuals(GENOME.class)`

6 Define an outgroup

If one or more outgroup sequences are defined, PopGenome will only consider SNPs where the outgroup is monomorphic; the monomorphic nucleotide is then automatically defined as the major allele (encoded by 0).

```r
> GENOME.class <- set.outgroup(GENOME.class,c("Alyr-1","Alyr-2"))
```

7 Statistics

The methods and statistical tests provided by PopGenome are listed in the user manual. The corresponding references are in the references section.

7.1 Neutrality statistics

```r
> GENOME.class <- neutrality.stats(GENOME.class)
```

Getting the results from the object of class `GENOME`.

```r
> get.neutrality(GENOME.class)
```

**Neutraliti stats**

|       | pop 1 | pop 2 |
|-------|-------|-------|
| Numeric,27 |       |       |

Let’s look at the first population [[1]].

```r
> get.neutrality(GENOME.class)[[1]]
```

|                | Tajima.D | n.segregating.sites | Rozas.R_2 | Fu.Li.F | Fu.Li.D |
|----------------|----------|----------------------|-----------|---------|---------|
| 4CL1tl.fas     | -1.1791799 | 16                   | NA        | -0.9247377 | -1.1331823 |
| C4Htl.fas      | 0.6987394  | 17                   | NA        | 0.6742617  | 0.4167836  |
| CADtl.fas      | 0.5503743  | 14                   | NA        | 0.4458431  | 0.1590690  |

```

|                | Fu.F_S | Fay.Wu.H | Zeng.E | Strobeck.S |
|----------------|--------|----------|--------|------------|
| 4CL1tl.fas     | NA     | NaN      | NaN    | NA         |
| C4Htl.fas      | NA     | NaN      | NaN    | NA         |
| CADtl.fas      | NA     | NaN      | NaN    | NA         |

The **NA** values indicate that the statistics could not be calculated. This can have several reasons.
• the statistic needs an outgroup
• the statistic was not switched on
• there are no SNPs in the entire region

In each module you can switch on/off statistics (to accelerate calculations), and you can define an outgroup. Check out the PopGenome manual on CRAN for details. PopGenome also provides a population specific view of each statistics.

```r
> GENOME.class@Tajima.D

|             | pop 1          | pop 2          |
|-------------|----------------|----------------|
| 4CL1tl.fas  | -1.1791799     | -0.0702101     |
| C4Htl.fas   | 0.6987394      | 1.1819777      |
| CADtl.fas   | 0.5503743      | 0.2682897      |
```

If we have read in the data together with the corresponding GFF files, PopGenome can also analyse subsites such as `exon`, `coding`, `utr` or `intron regions`.

```r
> GENOME.class <- neutrality.stats(GENOME.class, subsites="coding")

> GENOME.class@Tajima.D

|             | pop 1          | pop 2          |
|-------------|----------------|----------------|
| 4CL1tl.fas  | -1.023785      | 0.2626617      |
| C4Htl.fas   | 1.013372       | 1.9121846      |
| CADtl.fas   | 1.981520       | 1.5191652      |
```

We can also analyse each subsite-region seperately by splitting the data as described in section 2.1.

```r
> GENOME.class.split <- splitting.data(GENOME.class, subsites="coding")

> GENOME.class.split <- neutrality.stats(GENOME.class.split)

> GENOME.class.split@Tajima.D

|             | pop 1          | pop 2          |
|-------------|----------------|----------------|
| 240 - 1295 | -0.2749244     | -0.3186974     |
| 1890 - 2302| -1.0062306     | 0.7546749      |
| 2679 - 2781| -1.0062306     | 0.5590170      |
| 2884 - 2979| -1.0062306     | NaN            |
| 3465 - 4249| NA             | NA             |
| 4337 - 4468| NaN            | NaN            |
| 4696 - 5290| -1.6097384     | 2.1259529      |
| 6181 - 6272| NaN            | NaN            |
| 6412 - 6523| NaN            | NaN            |
| 7320 - 7545| 0.2390231      | 1.8112198      |
| 7643 - 8080| -0.3018700     | 1.1684289      |
| 8176 - 8395| NaN            | NaN            |
```

The `splitting.data` function transforms the class into another object of class `GENOME`. Thus, we can apply all methods easily to the transformed class `GENOME.class.split`. Let’s, for example, analyse all non-synonymous SNPs in the coding regions.
The PopGenome framework provides several modules to calculate statistics. All methods will work in the same way as the `neutrality.stats()` function described above. The input is always an object of class `GENOME`.

### 7.2 The slot `region.stats`

The slot `region.stats` includes some site-specific statistics or values that cannot be shown in a multi-locus-scale. See also the section PopGenome classes.

```r
> GENOME.class@region.stats
```

SLOTS:

| Slotes                              | Description                   | Module    |
|-------------------------------------|-------------------------------|-----------|
| nucleotide.diversity               | Nucleotide diversity         | FST       |
| haplotype.diversity                | Haplotype diversity          | FST       |
| haplotype.counts                   | Haplotype distribution       | FST       |
| minor.allele.freqs                 | Minor allele frequencies     | Detail    |
| linkage.disequilibrium             | Linkage disequilibrium       | Linkage   |
| biallelic.structure               | Shared and fixed polymorphisms | Detail    |

These are the Slots (class region.data)

```r
> GENOME.class <- F_ST.stats(GENOME.class)
```

or

```r
> GENOME.class <- diversity.stats(GENOME.class)
```

```r
> GENOME.class@region.stats@nucleotide.diversity
```

```
[[1]]

| pop 1 | pop 2 |
|-------|-------|
| pop 1 | 5.142857 | NA |
| pop 2 | 6.163265 | 5.238095 |

[[2]]

| pop 1 | pop 2 |
|-------|-------|
| pop 1 | 7.809524 | NA |
| pop 2 | 8.816327 | 4 |

[[3]]

| pop 1 | pop 2 |
|-------|-------|
| pop 1 | 6.285714 | NA |
| pop 2 | 5.836735 | 4.285714 |
```

**nucleotide.diversity**

The nucleotide diversity (average pairwise nucleotide differences) within and between the populations. Have to be divided by the slot `GENOME.class@m.sites`
to obtain diversity per site (see also `diversity.stats`).

**haplotype.diversity**
The haplotype diversity (average pairwise haplotype differences) within and between the populations. (see also: `diversity.stats`)

**haplotype.counts**
A vector of length=n.individuals. Number of times the sequence of a specific individual appears in the whole population

**minor.allele.freqs**
The minor allele (0) frequencies for each SNP calculated with the function `detail.stats`.

**linkage.disequilibrium**
The function `linkage.stats(...)`, `detail=TRUE`) calculates some linkage disequilibrium measurements for each pair of SNPs ($r^2$, $D'$...). See also: `R2.stats`

**biallelic.structure**
Can be calculated with the function `detail.stats(GENOME.class, biallelic.structure=TRUE)`. To extract the results use the function `get.detail(GENOME.class, biallelic.structure=TRUE)`
The returned values (for each SNP) are described in the user manual.

### 8 Sliding Window Analyses

The function `sliding.window.transform()` transforms an object of class `GENOME` into another object of class `GENOME`, where now regions correspond to individual windows. This mechanism enables the user to apply all methods that exist in the PopGenome framework.

PopGenome tries to concatenate the data if the parameter `whole.data` is set to `TRUE`. This mechanism enables the user to work with very large datasets, which can be split into smaller chunks that are stored in the input folder. PopGenome is able to concatenate these chunks for analysis. Functions like `readVCF` and `readSNP` will do this automatically (see also `concatenate.regions`) If `whole.data=FALSE`, the regions are scanned separately.

**type=1**: Define windows based on SNP counts
**type=2**: Define windows based on nucleotide counts

#### 8.1 Scanning the whole data

```r
> GENOME.class.slide <- sliding.window.transform(GENOME.class, width=50, +        jump=50, type=1, whole.data=TRUE)
> GENOME.class.slide@region.names
[1] "1 - 50 :" "51 - 100 :" "101 - 150 :" "151 - 200 :" "201 - 250 :"
[6] "251 - 300 :" "301 - 350 :" "351 - 400 :" "401 - 450 :"
> GENOME.class.slide <- linkage.stats(GENOME.class.slide)
> get.linkage(GENOME.class.slide)[[1]]
```
The slot `GENOME.class.slide@region.names` can be used to generate the positions on the x-axis for, e.g., a plot along the chromosome. See also the function `PopGplot`.

```r
> xaxis <- strsplit(GENOME.class.slide@region.names, split=" ; ")
> xaxis <- sapply(GENOME.class.slide@region.names, function(x){
  return(mean(as.numeric(x)))
})
> plot(xaxis, GENOME.class.slide@Wall.B)
```

### 8.2 Scanning the regions separately

```r
> GENOME.class.slide <- sliding.window.transform(GENOME.class, width=50, +     jump=50, type=1, whole.data=FALSE)
> GENOME.class.slide@region.names
[1] "1:4CL1tl.fas" "2:4CL1tl.fas" "3:4CL1tl.fas" "4:C4Htl.fas" "5:CADtl.fas"
[6] "6:CADtl.fas" "7:CADtl.fas"
> GENOME.class.slide <- linkage.stats(GENOME.class.slide)
> get.linkage(GENOME.class.slide)[[1]]

| Wall.B | Wall.Q | Rozas.ZA | Rozas.ZZ | Kelly.Z_nS |
|--------|--------|----------|----------|------------|
| 1:4CL1tl.fas | 0.6666667 | 0.7500000 | 0.6666667 | 0.2916667 | 0.37500000 |
| 2:4CL1tl.fas | NaN | NaN | 0.0000000 | 0.0000000 | 0.0000000 |
| 3:4CL1tl.fas | 0.0000000 | 0.00 | 0.0138889 | -0.0526620 | 0.0711806 |
| 4:C4Htl.fas | 0.6666667 | 0.80 | 0.5408642 | -0.0931580 | 0.6340222 |
| 5:CADtl.fas | 0.0000000 | 0.00 | 2.0925925 | -0.0445601 | 2.1371528 |
| 6:CADtl.fas | 0.0000000 | 0.00 | 0.0138889 | -1.3780864 | 1.3919753 |
| 7:CADtl.fas | 0.5000000 | 0.60 | 0.8888889 | -0.2752778 | 1.1641667 |
```

### 9 Reading data (SNP files)

PopGenome can handle SNP data formats such as VCF (1000 human genomes project), HapMap, and .SNP (1001 Arabidopsis genomes project). VCF files can be read in with the function `readData(format="VCF")`. Just as with alignments, the VCF files have to be stored in a folder that is given as an input.
parameter. To study whole genomes, VCFs can be split into chunks (by position), which should be numbered consecutively and stored in the same folder. PopGenome can concatenate them afterwards internally. Alternatively, use the function `readVCF`, which can read in a tabix-indexed VCF-file like those published from the 1000 Genome project. `readVCF` supports fast access of defined subregions of the file and automatically splits the data into chunks in cases when the region of interest is too big to fit into the available computer memory (RAM).

The function `readSNP` reads data published from the 1001 Genomes project (Arabidopsis), where the `quality-variant.txt` files, which include variant calls from every single individual, have to be stored together in one folder. The `readData` function can also read HapMap data. (`readData(format="HapMap")`) example files can be found in the subdirectory `data` of the PopGenome package.

### 9.1 Example

Reading data from the 1001 Genomes project (Arabidopsis)

```r
# reading chromosome 1
> GENOME.class <- readSNP("Arabidopsis", CHR=1)
# scan the data with consecutive windows
# window size: 1000 nucleotides (type=2)
# jump size: 1000 nucleotides (type=2)
> GENOME.class.slide <- sliding.window.transform(GENOME.class,1000,1000,type=2)
# calculate diversity statistics for all individuals
> GENOME.class.slide <- diversity.stats(GENOME.class.slide)
# Get the results ([[1]], because only one pop is defined)
> get.diversity(GENOME.class.slide)[[1]]
# alternative: directly access the nucleotide diversity
> plot(GENOME.class.slide@nuc.diversity.within)
```

`readSNP` and `readVCF` also accept a GFF-file as an input. To scan all exons of chromosome 1 and only calculate the diversity of the nonsynonymous sites, do the following:

```r
# read chromosome 1 with the corresponding GFF-file
> GENOME.class <- readSNP("Arabidopsis", CHR=1, gffpath="Ara.gff")
# verify the nonsyn/syn SNPs (we need the reference sequence as a FASTA file!)
> GENOME.class <- set.synnonsyn(GENOME.class, ref.chr="chr1.fas")
# split the data into exon regions
> GENOME.class.exons <- splitting.data(GENOME.class, subsites="exon")
# calculate the nonsynonymous diversities
> GENOME.class.exons <- diversity.stats(GENOME.class.exons, subsites="nonsyn")
```

We can split the data into genes, exons, introns, UTRs, and coding regions if these features are annotated in the GFF file. See also `get.gff.info` in the manual.

### 10 Coalescent simulation

PopGenome supports the Coalescent simulation program `MS` from Richard Hudson, as well as the `MSMS` simulation tool from Greg Ewing. The observed statis-
tics are compared to the simulated values. You have to specify the $\theta$ value and the PopGenome module you want to apply to the simulated data. An new object of class `cs.stats` will be created. The main input is an object of class `GENOME`.

```r
> MS.class <- MS(GENOME.class, thetaID="Tajima", neutrality=TRUE)
> MS.class
-----
SLOTS:
-----
Slots Description
1 prob.less Prob. that sim.val <= obs.val P(sim <= obs)
2 prob.equal Prob. that sim.val = obs.val P(sim = obs)
3 valid.iter number of valid iter. for each test and loci
4 obs.val obs.values for each test
5 n.loci number of loci considered
6 n.iter number of iterations for each loci
7 average average values of each statistic (across all loci)
8 variance variance values of each statistic (across all loci)
9 locus list of loc.stats objects, (detail stats for each locus)
```

Lets look at the data of the first region

```r
> MS.class@locus[[1]]

Length Class Mode
1 loc.stats S4
-----
SLOTS:
-----
Slots Description
1 n.sam number of samples for each iteration
2 n.iter number of iteration
3 theta mutation parameter
4 obs.val vector with observed values for each test
5 positions position of each polymorphic site
6 trees if printtree=1, gene tree in Newick format
7 seeds random numbers used to generate samples
8 halplotypes haplotypes in each iteration
9 stats variety of test stats compiled a matrix
10 loc.prob.less Prob. that simulated val. <= to observed val. P(Sim <= Obs)
11 loc.prob.equal Prob. that simulated val = to observed val. P(Sim = Obs)
12 loc.valid.iter number of valid iteration for each test
13 quantiles 13 quantiles for each test
```

[1] "These are the Slots"
10.1 The function \texttt{readMS}

Reading data produced from the coalescent simulation programs MS (Hudson) and MSMS (Ewing).

\begin{verbatim}
> GENOME.class <- readMS(file="...")
\end{verbatim}

After reading the output file of the coalescent simulations, the full range of methods can be applied to this data.
11 PopGenome classes

12 PopGenome internals

12.1 Synonymous & Non-Synonymous Sites

PopGenome will consider every single nucleotide polymorphism (SNP) separately and verify if the SNP is part of a synonymous or nonsynonymous change. When there is an unknown or gap position in the corresponding codon (nucleotide-triplet) of a specific individual, PopGenome will ignore those sequences and will try to find a valid codon and will interpret this change. If there is one non-synonymous change, PopGenome will set this SNP as a non-synonymous SNP, even when there are additional synonymous changes. However, the slot `GENOME.class@region.data@codons` includes all codon changes, and the function `get.codons` will also give more information. If necessary, the user can redefine the synonymous/non-synonymous changes by manipulating the
GENOME.class@region.data@synonymous slot or define subpositions of interest with the splitting.data function.
When typing codontable in R, the codon table is printed, where the rows of the second matrix of the list corresponds to the numerical values of the slot GENOME.class@region.data@codons.

```r
> codonTable <- codontable()
> codonTable[[2]]
```

The first matrix of this list (codonTable[[1]]) codes the corresponding Proteins of the nucleotide Triplets. PopGenome will always use the first row of this matrix (standard code) to interpret whether a change is synonymous or nonsynonymous. Here you can change the coding in the first row and load your own file in the R-environment.

```r
# change the file codontable.R
> library(PopGenome)
> source("./codontable.R")
```

The function have to be codontable()