An introduction to the PopGenome package

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Contents

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 seperately (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

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

Loading the PopGenome package

```
> library(PopGenome)
```

3 Reading data (alignments)

Reading three alignments in FASTA-format (*4CL1tl.fas, C4Htl.fas and CADtl.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:

- $T, U \rightarrow 1$
- $C \rightarrow 2$
- $G \rightarrow 3$
- $A \rightarrow 4$
- $unknown \rightarrow 5$

- $- \rightarrow 6$

```
> 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.

```
> 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 class `GENOME` 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 ?

```
> GENOME.class@n.sites

4CL1tl.fas  C4Htl.fas  CADtl.fas
      2979       2620       2930
```

```
> GENOME.class@region.names

[1] "4CL1tl.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`).

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

```
           n.sites n.biallelic.sites n.gaps n.unknowns n.valid.sites
4CL1tl.fas    2979               176    617          0          2362
C4Htl.fas     2620                84   1454          0          1161
CADtl.fas     2930               197    740          0          2189
           n.polyallelic.sites trans.transv.ratio
4CL1tl.fas                   0           1.120482
C4Htl.fas                    5           1.470588
CADtl.fas                    1           0.970000
```

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 accessable with the @ operator. See also the figure in section `PopGenome classes`.

```
> 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
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]]`):

```
> 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. 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 (without any extensions like .fas or .gff) as the corresponding FASTA files (in this example: *4CL1tl, C4Htl and CADtl*) to ensure that sequence and annotation are matched correctly.

```
> 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?

```
> 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

```
> 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`.

```
> 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)*))

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

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

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

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

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

> 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 poulations as a list.

```
> 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 ore 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).

```
> 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

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

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

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

```
      neutrality stats
pop 1 Numeric,27
pop 2 Numeric,27
```

Let's look at the first population `[[1]]`.

```
> 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  0.6742517  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.

```
> 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`.

```
> 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.

```
> 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.

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

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`.

```
> GENOME.class@region.stats

-----
SLOTS:
-----
                   Slots                     Description  Module
1   nucleotide.diversity           Nucleotide diversity     FST
2    haplotype.diversity            Haplotype diversity     FST
3       haplotype.counts         Haplotype distribution     FST
4     minor.allele.freqs       Minor allele frequencies  Detail
5 linkage.disequilibrium         Linkage disequilibrium Linkage
6    biallelic.structure Shared and fixed polymorphisms  Detail

---------------
These are the Slots (class region.data)

> GENOME.class  <- F_ST.stats(GENOME.class)
or
> GENOME.class  <- diversity.stats(GENOME.class)

> 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@n.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.indivuals. Number of times the sequence of a specific indivual 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 measurenments 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 seperately.

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

8.1 Scanning the whole data

```
> 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]]

               Wall.B    Wall.Q   Rozas.ZA    Rozas.ZZ  Kelly.Z_nS
1 - 50 :    0.6666667 0.7500000 0.66666667  0.29166667 0.375000000
51 - 100 :        NaN       NaN 0.00000000  0.00000000 0.000000000
101 - 150 : 0.0000000 0.0000000 0.01851852 -0.05266204 0.071180556
151 - 200 : 0.6250000 0.6666667 0.37847222  0.10206619 0.276406036
201 - 250 : 0.5833333 0.6923077 5.40972222  1.05354208 4.356180145
251 - 300 : 0.0000000 0.0000000 0.01388889 -0.17860000 0.192488889
301 - 350 : 0.0000000 0.0000000 0.01388889  0.00462963 0.009259259
351 - 400 : 0.4000000 0.5000000 3.95688889  2.19704321 1.759845679
401 - 450 : 0.5000000 0.6000000 1.81250000  1.31916667 0.493333333
```

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`.

```
> 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

```
> 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   0.75 0.66666667  0.29166667 0.37500000
2:4CL1tl.fas       NaN    NaN 0.00000000  0.00000000 0.00000000
3:4CL1tl.fas 0.0000000   0.00 0.01851852 -0.05266204 0.07118056
4:C4Htl.fas  0.6666667   0.80 0.54086420 -0.09315802 0.63402222
5:CADtl.fas  0.0000000   0.00 2.09259259 -0.04456019 2.13715278
6:CADtl.fas  0.0000000   0.00 0.01388889 -1.37808642 1.39197531
7:CADtl.fas  0.5000000   0.60 0.88888889 -0.27527778 1.16416667
```

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)

```
# 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 alle exons of chromosome 1 and only calculate the diversity of the nonsynonymous sites, do the following:

```
# 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 statistics are compared to the simulated values. You have to specify the θ 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`.

```
> 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

```
> 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"
```

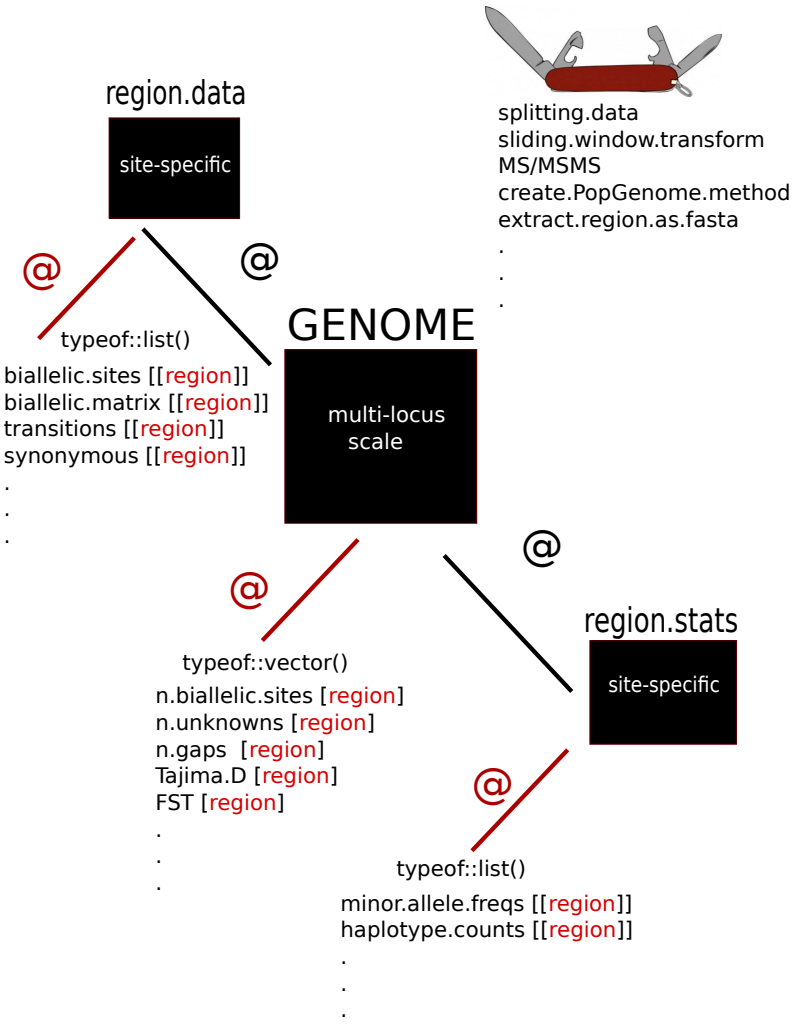
10.1 The function `readMS`

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

```
> GENOME.class <- readMS(file="...")
```

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 How PopGenome does handle missing data.

To include unknown positions (e.g. ./.,N, ?) the parameter `include.unknown` have to be set in the corresponding reading-functions. PopGenome will code those positions into `NaN` in the biallelic.matrix (`get.biallelic.matrix()`). In case of nucleotide diversity measurenments and statistics which can be calculated site by site (e.g. neutrality.stats) PopGenome will ignore the missing positions and will apply the algorithms to the valid nucleotides. Lets consider the following bi-allelic vector:

```
bvector <- c(0,1,NaN,0)
```

To calculate the average nucleotide diversity PopGenome will do the following:

```
ones <- sum(bvector==1, na.rm=TRUE)
zeros <- sum(bvector==0, na.rm=TRUE)
sample.size <- ones + zeros
n.comparisons <- (sample.size*(sample.size-1))/2
nuc.diversity <- (ones * zeros)/n.comparisons
```

In case of haplotype based methods (e.g haplotype FST) sites including unknown positions are completely deleted.

12.2 Synonymous & Non-Synonymous Sites

PopGenome will consider every single nucleotide polymorphism (SNP) seperately 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 neccessary, 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`.

```
> 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

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

The function have to be `codontable()`