Whole genome analyses using PopGenome and VCF files

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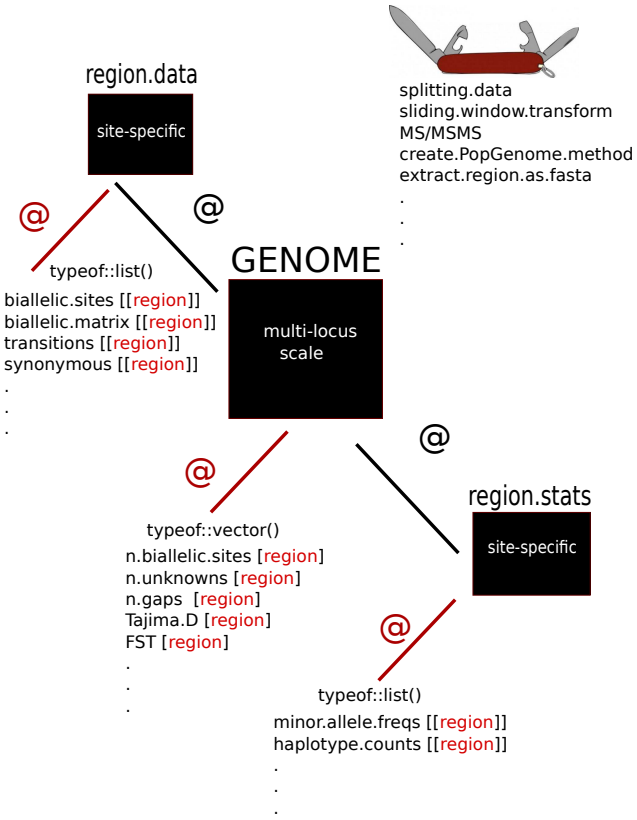
March 15, 2017

Contents

1 PopGenome classes

PopGenome contains mainly three classes. The class `GENOME` and the two sub-classes `region.data` and `region.stats`. The class `GENOME` includes all informations which are representable as matrices and vectors; the two subclasses store informations about each SNP seperately, e.g synonymous and non-synonymous SNP. This kind of data is stored in lists as the values can differ between genomic windows/regions.



2 Reading tabixed VCF files (readVCF)

If one would like to perform population genomic analyses on VCF files storing whole genome SNP data using the PopGenome framework, the function `readVCF()` can be used. This function expects a gzipped VCF file which needs to be tabixed via the program TABIX first. To do so see `http://genome.ucsc.edu/goldenPath/help/vcf.html` and `http://samtools.sourceforge.net/tabix.shtml`. All reading functions provided by PopGenome will create an object of class `GENOME`. This object is then the input for the functions performing statistical tests on this data.

The following parameter can be set:

2.1 filename

Here, the user have to set the path of the gzipped VCF file as a character string like "chr6.vcf.gz". Note, the corresponding *.tbi* file have to be stored in the same folder.

2.2 numcols

This parameter defines the number of SNPs that should be read into the RAM at once while streamline the whole data into the PopGenome framework. In other words, numcols defines the SNP-chunk size. If alot of RAM is available we advise to increase this parameter in order to accelerate computations. On a standard dektop computer (4 GB RAM) a value about 10.000 should be fine when a sample size of 1000 individuals is considered.

2.3 tid

`tid` is the chromosome identifier and have to be defined as a character string like "chr6". If this is not known you can choose any character string (e.g "?"). `readVCF` will print out the available identifier after the function call.

2.4 frompos

Here the genomic position can be set from which the data should start to read in SNP data information. `frompos` have to be a numeric value.

2.5 topos

`topos` defines the genomic position where `readVCF` should stop to read SNP data information. In the same way as `frompos` the parameter have to be set as a numeric value.

2.6 include.unknown

The parameter `include.unknown` can be switched to `TRUE` in order to include missing/unknown gentotypes like *./.* . As a default, sites including missing values are completely deleted and the positions of those sites are stored in the slot `GENOME.class@region.data@unknowns`. How PopGenome does handle SNPs including missing nucleotides is described in the statistics section in this manual.

2.7 samplenames

To read in SNP data from a subset of individuals the parameter `samplenames` requires an character vector including the individual names. To extract the individual names from the VCF file do the following:

```
vcf_handle  <- .Call("VCF_open",filename)
ind         <- .Call("VCF_getSampleNames",vcf_handle)
samplenames <- ind[1:10]
```

In this example we will extract the first 10 individuals from the VCF file.

2.8 approx

If the parameter approx is switched to `TRUE` only SNPs (two variant positions) are considered and a logical OR will be applied to the genotype fields. 0|0 goes to 0, 1|1 to 1, 0|1 to 1 and 1|0 to 1. If this approximation scheme is applicable `approx` should definetly switched to TRUE as the computation speed will significantly be increased.

2.9 out

This parameter is only important if you intend to perform the readVCF in parallel (e.g using the R-package parallel). readVCF writes temporary files on the hard drive while interpreting the data. Thus, the parameter `out` should be set for each parallized job differently. More about using readVCF in parallel is described in the section *Performing readVCF in parallel*.

2.10 parallel

Parallel computation using `mclapply` provided by the R-package parallel. In this case the data is splitted into subregions which are interpreted in parallel and afterwards automatically concatenated via the functions `concatenate.classes` and `concatenate.regions`.

2.11 gffpath

If an GFF file is available it has to be specified via the `gffpath` parameter as a character string. (gffpath="chr6.gff", for instance) Note, the chromosome identifier in the GFF have to be identical to the identifier used in the VCF file.

A typical function call would be:

```
GENOME.class <- readVCF("chr1.vcf.gz",numcols=10000, tid="1",
from=1, to= 10000000, approx=FALSE, out="", parallel=FALSE, gffpath=FALSE)
```

`GENOME.class` is an object of class `"GENOME"`

3 Reading in VCF files via the function readData()

The main input of the readData function is a folder (e.g "VCF") containing the genomic data. It can read in multiple "fairly-sized" VCF files iteratively. In case of VCF files

the parameter `format` have to be set to format="VCF". In contrast to the readVCF function the data does not need to be compressed or tabixed and additionaly supports polyploid (e.g tetraploid) genotypes. However, each VCF file is completely loaded into the RAM and interpreted via efficient C Code which can increase computation performance dramatically, but at the same time is not suitable for single big VCF files. If one would like to performe whole genome analyses via the readData function the user could split a whole genome VCF file into SNP chunks and analyse those chunks seperately or concatenate them afterwards via the function `concatenate.regions`.

If GFF files for each VCF file are available they need to be stored in a seperate folder, for instance "GFF". Note, the files in the VCF folder as well as the GFF folder have to be EXACTLY the same names to ensure correct matching. For example, the file "chr1" in the VCF folder corresponds to the GFF file "chr1" in the GFF folder.

```
GENOME.class <- readData("VCF", format="VCF", gffpath="GFF")
```

4 Set the populations

The population can be set via the `set.population` function. This function expects an object of class `GENOME` and the populations defined as a list. Each element of the list contains the individual names as a vector. In addition, the parameter `diploid` have to be switched to TRUE in case of diploid organisms. If no population was defined all individuals are treated as one population. The following function call will generate two populations. The first population contains the individuals a,b and c. The second one d and e.

```
GENOME.class <- set.populations(GENOME.class,
list(c("a","b","c"),c("d","e")), diploid=TRUE)
```

To re-check the setting one can have a look at the slots `GENOME.class@populations` or `GENOME.class@region.data@populations`. The function `get.individuals` prints out the individual names. Note, the populations should set BEFORE you transform or split the data in sub-regions via the functions `sliding.window.transform` or `splitting.data`. When the number of individuals is very high it might be useful to store the individuals for one population in a seperat file in a way that the following line for instance works without problems.

```
pop1 <- as.character(read.table("pop1.txt")[[1]])
```

The native R function `scan` can be also applied.

5 Set the outgroup

For some method modules provided by PopGenome it might be useful to define an outgroup in order to specify the derived allele of each SNP site. To do so, only SNP

sites are considered where the outgroup is monomorph. The monomorphic value is then defined as the non-derived allele. The following call will define the individual z as the outgroup sequence.

```
GENOME.class <- set.outgroup(GENOME.class,c("z"), diploid =TRUE)
```

Note, the population or/and outgroup should be defined BEFORE you transform the data via `sliding.window.transform` or `splitting.data`.

6 Verify synonymous and non-synonymous SNPs

PopGenome is able to verify if an SNP produces a synonymous or non-synonymous codon change. PopGenome will perform the calculation for each SNP seperately with the assumption that the probability to observe two SNPs in the same codon is small. All we need is the reference sequence in fasta format. A typical function call would be the following line:

```
GENOME.class <- set.populations(GENOME.class, ref.chr="chr1.fas")
```

In addition, one can switch on the parameter `save.codons` which will save the codons in the slot `GENOME.class@region.data@codons`. To extract them, or to convert those values into character strings the function `get.codons` can be applied afterwards. Note, this function can only be performed when the data was read in together with the corresponding GFF file, because PopGenome needs to have informations about the coding regions, reading frames and informations about the reading directions. The function should be performed before anything is done via the functions `sliding.window.transform` or `splitting.data`. This function will not work on splitted data.

7 Sliding window analyses

Sliding windows can be generated via the function `sliding.window.transform`. This function transforms the object of class `"GENOME"` in another object of the same class. It can be used to scan only SNPs (`type=1`) or genomic regions (`type=2`). Furthermore one can define window sizes and jump sizes. The windows can be consecutive as well as overlapped.

```
GENOME.class.slide <- sliding.window.transform(GENOME.class,10000,10000, type=2)
```

will scan the data with 10.000 consecutive nucleotide windows. The slot `GENOME.class@regions` will store the genomic regions of each window as a character string. To convert those strings into a genomic numeric position we can apply the following script:

```
genome.pos <- sapply(GENOME.class.slide@region.names, function(x){
split <- strsplit(x," ")[[1]][c(1,3)]
```

```
val   <- mean(as.numeric(split))
return(val)
})

plot(genome.pos, <slide.statistic.values>)
```

This script will return the mean position of each window.

8 Splitting data into subsites (e.g genes)

The `splitting.data` function works very similar to the `sliding.window.transform` function. Via the parameter `positions` one can define genomic or SNP windows using numeric values defined as a list. The following line will split the data into the genomic regions from 10 to 30 and 1000 to 12000.

```
GENOME.class.split <- splitting.data(GENOME.class,
positions=list(c(10:30),c(1000:12000)), type=2)

is(GENOME.class.split)
```

If a GFF file was specified as part of the `readVCF` function, PopGenome automatically can split the data into exon, gene, coding and intron regions. Note, those features must be annotated in the corresponding GFF file. The following line of code splits the data into genes.

```
genes <- splitting.data(GENOME.class, subsites="gene")
is(genes)
```

The slot `GENOME.class@regions` will store the genomic regions of each window as a character string. Note, the user might be interested in other features which are not labeled as exon, intron, gene or CDS. In this case the `get_gff_info` can be used. More about this function the section *Look up information stored in GFF files.*

The function `get.feature.names` might be a useful method to extract additional informations (like gene names) from the given `GENOME` object. The returned character string will exactly match the data stored in the slot `genes@region.names`.

9 Splitting data into GFF-attributes

The function `split_data_into_GFF_attributes` allows the user to split the data into user-defined subsites based on the attributes stored in a GFF file (last column). The following commands split the Human chromosome 1 variant data into genes.

```
GENOME.class <- readVCF("chr1.vcf.gz",10000,"1",1,100000)
GENOME.class.split <-

split_data_into_GFF_attributes(GENOME.class,"GRCh37.73.gtf", "1", "gene_name")
GENOME.class.split@region.names
GENOME.class.split@feature.names
```

Note, the data should also be read in with the corresponding GFF file (readVCF) before splitting the data if one would like to verify syn/nonsyn sites via the function `set.synnonsyn()`.

10 Statistics

PopGenome provides a wide range of methods which can also be applied to transformed `GENOME` class objects (e.g subregions like genes or diverse genomic windows). We have pooled those statistics into modules. However, specific statistics can be switched off to increase computational power. In some cases also slots in the class `GENOME.class@region.stats` are filled (see the PopGenome manual). The main modules are described in the following subsections. The statistics and methods for each module as well as the corresponding references are listed in the CRAN manual !

10.1 Neutrality statistics

In the PopGenome manual, available on CRAN, one can find the statistics which are included in this module. Note, some of those will need an outgroup. When an outgroup is specified the Tajima's D, for instance will only be applied on sites where the outgroup is monomorph and the non-derived allele is specified as the monomorphic nucleotide given in the outroup sequence. We also provide efficient compiled C implementations which will be applied when the parameter `FAST` is set to `TRUE`. This will speed up calculations but might be a bit unstable in some cases. A typical function call would be:

```
GENOME.class <- neutrality.stats(GENOME.class, FAST=TRUE)
get.neutrality(GENOME.class)[[1]]
```

[[1]] will extract the results of the first population. Also try to use `GENOME.class@Tajima.D`,for instance, which will give you a population and statistic specific view on the data.

10.2 FST measurenments

This module provides a wide range of FST as well as diversity measurenments. There exists two main classes. First, calculations which are either based on haplotypes `mode=¨haplotype¨` or second, the sequence based methods focussing on nucleotides `mode="nucleotide"`. Note, be careful with haplotype based methods if missing data is included as in this module those sites will be excluded from the analyses. If fixation indices should be calculated the user have to define more than one population via `set.populations`, in cases

where only one population is defined the module will calculate the within diversities for this single population. Please also have look at the module `F_ST.stats.2`.

```
GENOME.class <- F_ST.stats(GENOME.class)
get.F_ST(GENOME.class)[[1]]
GENOME.class@nucleotide.F_ST
```

Note, the nucleotide diversities `GENOME.class@nuc.diversity.within` have to normalized/devided by the total number of nucleotides considered in a given window/region !

10.3 Diversities

We have implemented some within diversity measurenments like pi in the module `diversity.stats`. In principle this can also be done via `F_ST.stats` but this will slightly slow down data analyses if one would like to perform only diversities within the populations.

```
GENOME.class <- diversity.stats(GENOME.class)
get.diversity(GENOME.class)
GENOME.class@nuc.diversity.within
```

10.4 Linkage disequilibrium

The main module for linkage disequilibrium statistics is the module `linkage.stats`. Moreover, the module `R2.stats` is designed for fast compution of the correlation coefficient r^2.

10.5 Site frequency spectrum (SFS)

We include the SFS calculation together with some other calculations in the module `detail.stats`. If an outgroup is defined only sites where the outgroup is monorphic are considered.

10.6 Mcdonald-Kreitman test

PopGenome enables to perform the Mcdonald-Kreitman test on SNP data. Our algorithm assumes that the probability that a SNP occurs in the same codon is quite low. Thus, PopGenome treats each SNP independently and verifies if the Codon change is synonymous or non-synonymous with respect to the reference genome. Before the MKT test can be performed we have to set the syn/non-syn SNPs via the function `set.synnonsyn`. The outgroup can be defined as a population as the MKT module performs the statistic on ALL pairwise population comparisons.

A typical function call would be:

```
GENOME.class <- set.synnonsyn(GENOME.class, ref.chr="twoL.fas")
GENOME.class <- set.populations(GENOME.class,list(c(...),c(...)), diploid=TRUE)
```

```
# twoL.fas is the reference chromosome the data has been mapped
# against to create the VCF file
GENOME.class <- MKT(GENOME.class)
get.MKT(GENOME.class)
```

Note, when more than two populations are defined `get.MKT(GENOME.class)` will return a list. To access the results from the second region/window we have to do:

```
get.MKT(GENOME.class)[[2]]
```

See also the *example* section.

11 The slot region.data

During the reading process PopGenome will store some SNP specific information in the slot `GENOME.class@region.data`. This slot will for example store the genomic position of each SNP `GENOME.class@region.data@biallelic.sites`. In general, all informations here are stored as numeric vectors of length = n.biallelic.sites. Just typing `GENOME.class@region.data` will print a summary of the available slots. When multiple files have been read in the slots of the object of class `region.data` are organized as lists. Each element of the list is accessible via `[[region.id]]`, where `region.id` is the identifier of the file of interest. The corresponding information is stored in the slot `GENOME.class@region.names`. In case of transformed GENOME objects e.g performed by `sliding.window.transform` [[region.id]] will be the identifier for the window of interest.

12 The slot region.stats

In some cases a multi-locus-scale representation of the statistic values is not possible and we were forced to organize those values as a list. In the slot `GEOME.class@region.stats` for example we can find the slot `haplotype.counts` which contains the haplotype distribution of each population. Here, the haplotypes regarding the whole population (whole data set) was specified (n.haplotypes=n.columns). Each row corresponds to one population and the sum of each line is the sample size of each population. Obviously, the dimension of this matrix can differ between regions/windows. As described in the previous section specific files or regions/windows are accessible via `[[region.id]]`.

13 How PopGenome handles missing data

VCFs often contain gentypes with missing nucleotides like *./.*. When the parameter `include.unknown=TRUE` was set, those positions are included and stored as `NaNs` in the biallelic.matrix (see `get.biallelic.matrix`). However, haplotype based methods should be not applied to those sites as it can lead to misleading results. The following methods should be performed with caution:

- `F_ST.stats(...,mode="haplotype")` can be applied, but this module will automatically remove SNPs containing missing data
- `diversity.stats`: pi and haplotype diversity should not be used

In case of site by site calculations as provided by the module `F_ST.stats(..., mode="nucleotide")` everthing should work fine. PopGenome calculates the site specific diversity as follows:

```
# Lets assume we have an biallelic vector b
b <- c(1,0,NaN,0)
# The nucleotide diversity is then all pairwise
# comparisons exluding those which would compare a value
# with a NaN entry

1 vs 0    -> mismatch
1 vs NaN  -> not count
1 vs 0    -> mismatch
0 vs NaN  -> not count
0 vs 0    -> match
Nan vs 0  -> not count

We have 3 valid comparisons and 2 mismatches.
So, the average nucleotide diversity is 2/3.

# The minor allele frequency of this vector would be 1/5
# as NaN is excluded from the sample
```

Also lingage disequilibrium measurenments will only compare nucleotide pairs without any `NaN` entry. For example:

```
SNP1  0 NaN 1 0
SNP2  0 1   1 0
```

Those two sites are completely identical in the PopGenome framework.

14 Look up information stored in GFF files

The function `get_gff_info` is a flexible tool to extract some informations out of a GFF file.

14.1 Extract feature positions

To extract the genomic positions of a feature of interest one can use the following line:

```
gene.positions <- get_gff_info(gff.file="twoL.gff", chr="2L", feature="gene")
is(gene.positions)
```

`gene.positions` is a list containing the genomic positions for each gene annotated in the GFF file. This list can be parsed to the function `splitting.data` in order to scan the data by genes.

```
GENOME.class.split <- splitting.data(GENOME.class, positions=gene.positions, type = 2)
```

We have to set `type=2` as `gene.positions` contains genomic positions. The following line will extract the corresponding gene IDS.

```
gene.ids <- get_gff_info(gff.file="twoL.gff", chr="2L", extract.gene.names=TRUE )
```

Note, in principle this can also be done via `readVCF(...,gffpath="twoL.gff")` and `splitting.data(..., subsites="gene")`. But, in this case genes which are annotated before the first SNP and those genes after the last SNP are not considered. In this case the SNP data is always the reference and it might be difficult to map the `gene.ids` to the regions specified by PopGenome.

14.2 Extract INFO fields

Lets assume we have scaned the data with windows and detect interesting values in the 5th window containing 8 SNPs. To extract the INFO field of each SNP in this region we could use the following line:

```
GENOME.class <- readVCF(...)
GENOME.class.slide <- sliding.window.transform(...)
get_gff_info(GENOME.class.slide, position= 5, gff.file="twoL.gff", chr="2L")
```

This function call would print the INFO field information found in the GFF for each SNP (in total 8) of window 5.

15 Examples

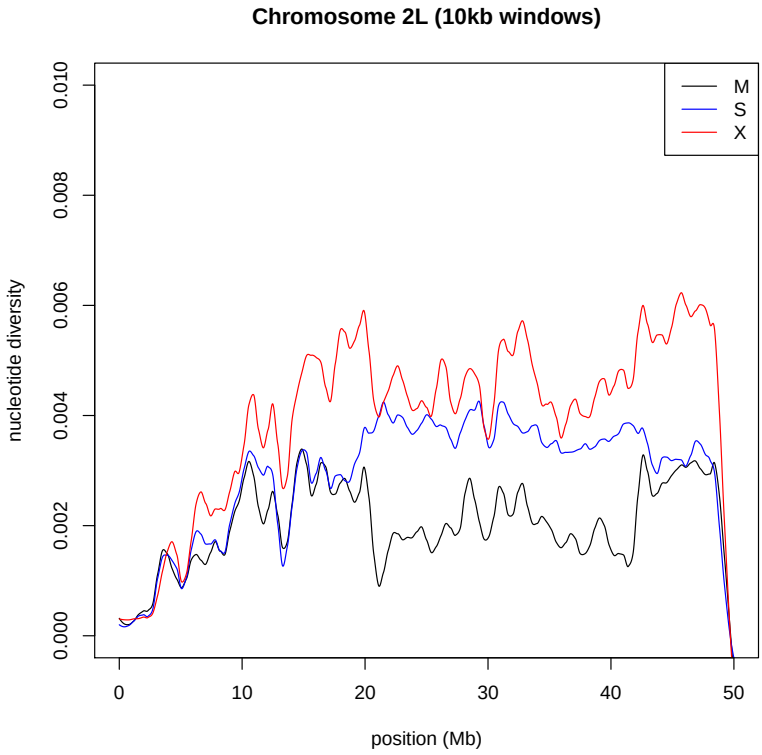
```
# Reading in the data via readVCF

GENOME.class <- readVCF("AGC_refHC_bialSNP_AC2_2DPGQ.2L_V2.CHRcode2.vcf.gz",
10000,"2",1,50000000,include.unknown=TRUE)

GENOME.class@n.biallelic.sites
[1] 1740885
```

```
# Set the populations (in this example: 3 populations)
# population M: 11 individuals (8 from cameroon, 3 from burkina)
# population S: 15 samples (4 from burkina, 8 from cameroon, 3 from tanzania)
# population X: 12 arabiensis individuals (4 tanzania, 4 burkina, 4 cameroon)

M <- c("X4631","X4634","X4691","X4697","X5090","X5107",
 "X5108","X5113","A7.4","C27.2","C27.3")

S <- c("X40.2","X44.4","X45.3","X4696","X4698","X4700","X4701",
 "X5091","X5093","X5095","X5109","M20.7","TZ102","TZ65","TZ67")

X <- c("SRS408146","SRS408148","SRS408154","SRS408183","SRS408970","SRS408984",
 "SRS408985","SRS408987","SRS408989","SRS408990","SRS408991","SRS408993")


GENOME.class <- set.populations(GENOME.class,list(M,S,X), diploid=TRUE)
```

15.1 Sliding windows

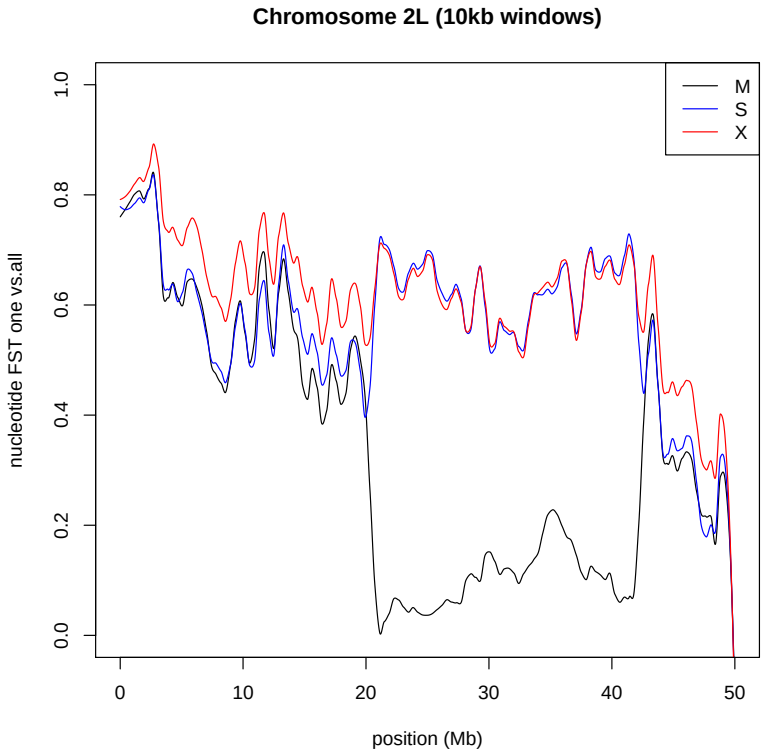
```
# split the data in 10kb consecutive windows
slide <- sliding.window.transform(GENOME.class,10000,10000, type=2)

# total number of windows
length(slide@region.names)
[1] 5000

# Statistics
slide <- diversity.stats(slide)

nucdiv <- slide@nuc.diversity.within
# the values have to be normalized by the number of nucleotides in each window
nucdiv <- nucdiv/5000
head(nucdiv)

            pop 1        pop 2        pop 3
[1,] 0.0006600000 0.0005838095 0.0003312447
[2,] 0.0001200000 0.0002071429 0.0004343523
[3,] 0.0003666667 0.0001666667 0.0002505013
[4,] 0.0000000000 0.0000000000 0.0003287257
[5,] 0.0002200000 0.0001666667 0.0005232758
[6,] 0.0006600000 0.0002357143 0.0001756433
```

```
# Generate output
# Smoothing lines via spline interpolation

ids <- 1:5000
loess.nucdiv1 <- loess(nucdiv[,1] ~ ids, span=0.05)
loess.nucdiv2 <- loess(nucdiv[,2] ~ ids, span=0.05)
loess.nucdiv3 <- loess(nucdiv[,3] ~ ids, span=0.05)

plot(predict(loess.nucdiv1), type = "l", xaxt="n", xlab="position (Mb)",
ylab="nucleotide diversity", main = "Chromosome 2L (10kb windows)", ylim=c(0,0.01))

lines(predict(loess.nucdiv2), col="blue")

lines(predict(loess.nucdiv3), col="red")

axis(1,c(1,1000,2000,3000,4000,5000),
c("0","10","20","30","40","50"))

# create the legend
legend("topright",c("M","S","X"),col=c("black","blue","red"), lty=c(1,1,1))
```




```
slide <- F_ST.stats(slide, mode="nucleotide")

# Lets have a look at the pairwise nucleotide FST

 pairwise.FST <- t(slide@nuc.F_ST.pairwise)
 head(pairwise.FST)

    pop1/pop2 pop1/pop3 pop2/pop3
[1,]  0.0115421 0.9153987 0.9306777
[2,] -0.6357143 0.9860194 0.9878351
[3,]  0.2098765 0.9771882 0.9847311
[4,]        NaN 0.5434366 0.5434366
[5,]  0.1407407 0.8785497 0.8807703
[6,]  0.1667774 0.9795149 0.9899651

# Here i used the function t() to transpose the matrix
# To extract the data for the pop1/pop3 comparison
# we can use the following function call

 head(pairwise.FST[,"pop1/pop3"])
```

```
[1] 0.9153987 0.9860194 0.9771882 0.5434366 0.8785497 0.9795149

# or

pairwise.FST[,2]


# Lets plot some data for the nucleotide FST  ONE vs. ALL slot

 head(slide@nuc.F_ST.vs.all)

        pop 1     pop 2     pop 3
[1,] 0.8277415 0.8506838 0.9234930
[2,] 0.9778805 0.9817008 0.9870558
[3,] 0.9585102 0.9660492 0.9809781
[4,] 0.5434366 0.5434366 0.5434366
[5,] 0.8280148 0.8273743 0.8796289
[6,] 0.9586470 0.9689294 0.9847528
```



15.2 Splitting data into genes

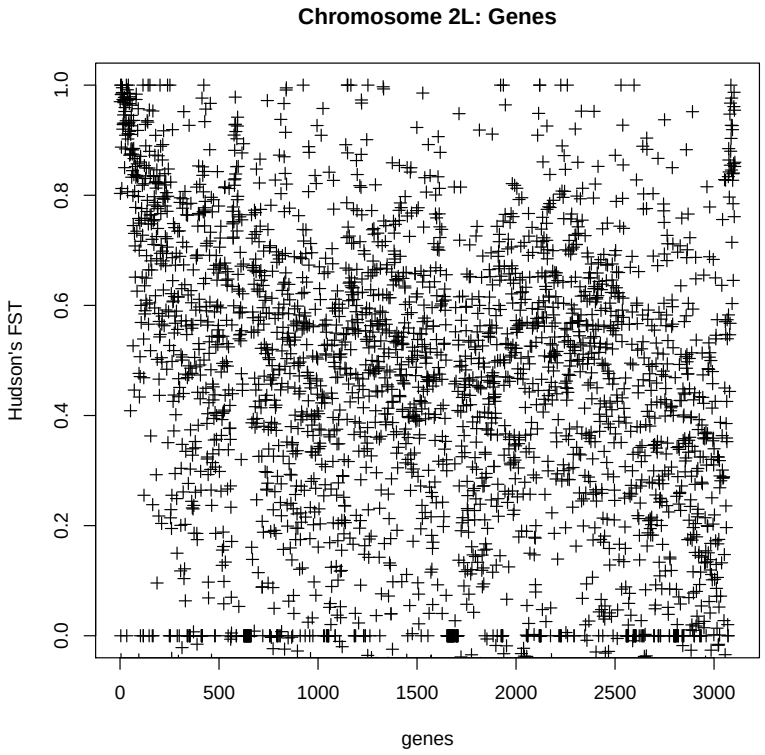
If one would like to use informations about subsites like exon or coding regions the data should be read in with the corresponding GFF file. However, we are still working on a `set_gff_info` function in order to provide an meachanism which allows the user to set the infomrations stored in a GFF file afterwards. Also have a look at the `get_gff_info` function, which can extract positions based on feature identifier. The returned list containing numeric vetors can be parsed to the `splitting.data` function defined as the parameter `positions` and `type=2`. An short example is also given in this section.

```
# Reading in the data with the corresponding GFF file

GENOME.class <- readVCF("AGC_refHC_bialSNP_AC2_2DPGQ.2L_V2.CHRcode2.vcf.gz",
 10000,"2",1,50000000, include.unknown=TRUE, gffpath="twoL.gff")

GENOME.class <- set.populations(GENOME.class,list(M,S,X), diploid=TRUE)

genes  <- splitting.data(GENOME.class, subsites="gene")

# An alternative approach would be, if the data was not
# read in with a GFF file

genePos  <- get_gff_info(gff.file="twoL.gff",chr="2L", feature="gene")

genes  <- splitting.data(GENOME.class, positions=genePos, type=2)
# --------------------------------

length(genes@region.names)
[1]  3105

genes <- F_ST.stats(genes, mode="nucleotide")

plot(genes@nucleotide.F_ST, ylim=c(0,1), xlab="genes", ylab="Hudson's FST",pch=3)

# Get the region/window ids with max FST values

 maxFSTgenes <- which(genes@nucleotide.F_ST==1)
 [1]    3    8   32   42  116  142  151  202  240  252  423  925 1148 1150 1166
 [16] 1252 1922 1936 2119 2120 2226 2259 2530 2596 3085

 genes@region.names[maxFSTgenes]

 [1] "207894 - 210460"     "493039 - 493543"     "2482553 - 2483310"
 [4] "2714472 - 2719933"   "3574266 - 3575387"   "3839485 - 3840411"
 [7] "4066369 - 4068651"   "4830049 - 4830309"   "5862276 - 5863688"
```

```
[10] "6099934 - 6100005"   "10058609 - 10058681" "17320615 - 17321803"
[13] "20649734 - 20650289" "20669715 - 20671003" "21351719 - 21351782"
[16] "23189286 - 23189439" "34125951 - 34126410" "34152345 - 34152618"
[19] "37757406 - 37757494" "37757408 - 37757496" "39200923 - 39201294"
[22] "39359058 - 39359155" "43600970 - 43601852" "44412534 - 44412731"
[25] "49171517 - 49173289"

 # Looking up the gene ID
 head(get_gff_info(genes, position=3, chr="2L", gff.file="twoL.gff")[[1]])

                                208143                                    208162
"ID=AGAP004679;biotype=protein_coding" "ID=AGAP004679;biotype=protein_coding"
                                208164                                    208170
"ID=AGAP004679;biotype=protein_coding" "ID=AGAP004679;biotype=protein_coding"
                                208176                                    208178
"ID=AGAP004679;biotype=protein_coding" "ID=AGAP004679;biotype=protein_coding"
```

Here, for each SNP in the region 3 of the object of class GENOME (genes) the INFO field is printed.

The third gene corresponds to the ID:AGAP004679

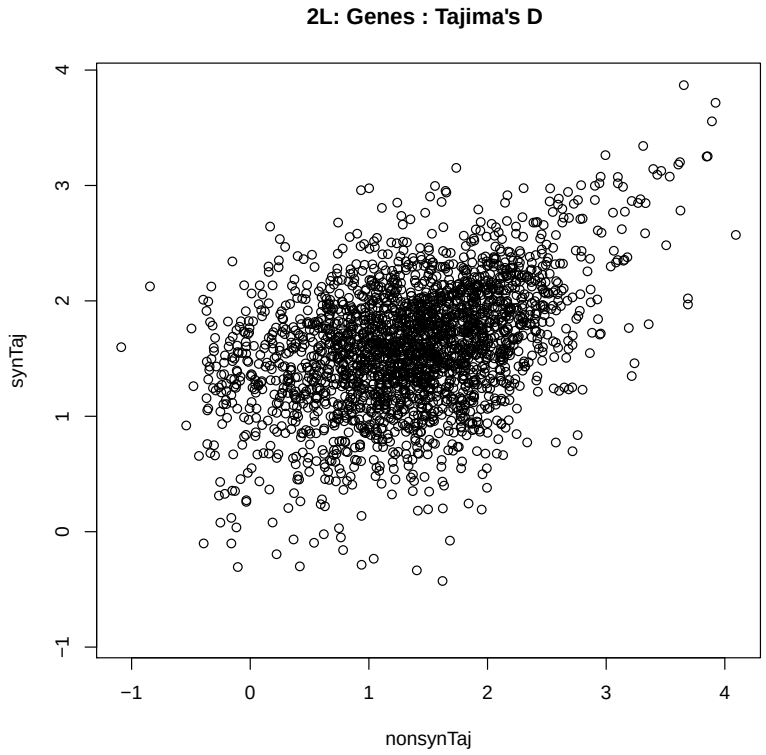


15.3 Synonymous and Non-synonymous SNPs

As long as a `set.gff` function is not implemented we have to read in the data with the corresponding GFF file in order to verify syn & nonsyn SNPs afterwards.

```
# Reading in the data with the corresponding GFF file

GENOME.class <- readVCF("AGC_refHC_bialSNP_AC2_2DPGQ.2L_V2.CHRcode2.vcf.gz",
 10000,"2",1,50000000, include.unknown=TRUE, gffpath="twoL.gff")

# Set syn & nonsyn SNPs: The results are stored
# in the slot GENOME.class@region.data@synonymous
# The input of the set.synnonsyn function is an object of
# class GENOME and a reference chromosome in FASTA format.

GENOME.class <- set.synnonsyn(GENOME.class, ref.chr="twoL.fas")

# number of synonymous changes
sum(GENOME.class@region.data@synonymous[[1]]==1, na.rm=TRUE)
[1] 74195
```

```
# number of non-synonymous changes
sum(GENOME.class@region.data@synonymous[[1]]==0, na.rm=TRUE)
[1] 28726
# Here, we have to define the parameter na.rm=TRUE because NaN values in this slot
# indicate that the observed SNP is in a non-coding region

# We now could split the data into gene regions again
genes <- splitting.data(GENOME.class, subsites="gene")

# Now we perform The Tajima's D statistic on the whole data set and
# consider only nonsyn SNPs in each gene/region.

genes <- neutrality.stats(genes, subsites="nonsyn", FAST=TRUE)

nonsynTaj <- genes@Tajima.D

# The same now for synonymous SNPs
genes <- neutrality.stats(genes, subsites="syn", FAST=TRUE)

synTaj <- genes@Tajima.D

# To have a look at the differences of syn and nonsyn Tajima D values in each gene we
# could do the following plot:

plot(nonsynTaj, synTaj, main="2L: Genes : Tajima's D ")
```



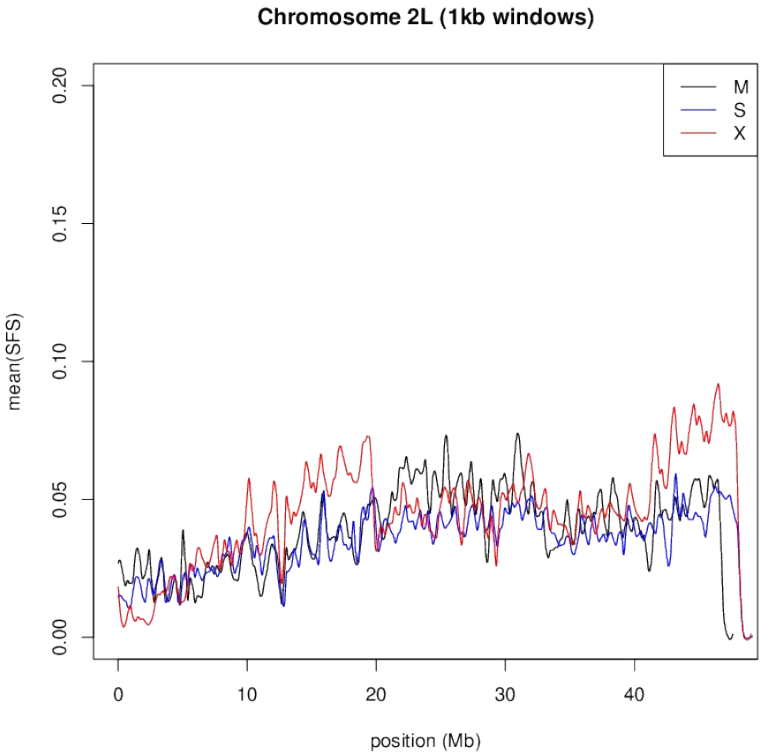
15.4 Site frequency spectrum (SFS)

The SFS is the default calculation in the module `detail.stats` and can be computed quite fast. In this example we will perform the computation on sliding windows. For each window we will take the mean of the corresponding SFS values to plot a figure with smoothed lines.

```
# Reading in the data

GENOME.class <- readVCF("AGC_refHC_bialSNP_AC2_2DPGQ.2L_V2.CHRcode2.vcf.gz",
15000,"2",1,50000000, include.unknown=TRUE)

# set the populations (M,S,X as defined above)
GENOME.class <- set.populations(GENOME.class,list(M,S,X), diploid=TRUE)

# check if the settings worked correctly
GENOME.class@populations


# slide the object in 1 kb windows
```

```
slide <- sliding.window.transform(GENOME.class,1000,1000,type=2)

# calculate SFS: The results are stored in the slot
# slide@region.stats@minor.allele.freqs

slide <- detail.stats(slide)

# Lets have a look at the second window
slide@region.stats@minor.allele.freqs[[2]]

      [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10] [,11] [,12]     [,13]
pop 1    0  0.0    0    0    0    0    0    0    0     0     0 0.250 0.5000000
pop 2    0  0.0    0    0    0    0    0    0    0     0     0 0.125 0.1666667
pop 3    0  0.5    0    0    0    0    0    0    0     0     0   NaN       NaN

# There are 13 SNPs in this window. NaN indicates that only unknown positions
# where detected in pop 3 for SNP 12 and 13.
# As we read in more samples then defined individuals in our example,
# 0 indicates that no minor allele is present at the given SNP.

# calculate the mean SFS for each window and each population
# To extrect the results we could use the following script:

SFSmeanPop1 <-  sapply(slide@region.stats@minor.allele.freqs, function(x){
if(length(x)==0){return(0)}
return(mean(x[1,], na.rm=TRUE))
})

SFSmeanPop2 <-  sapply(slide@region.stats@minor.allele.freqs, function(x){
if(length(x)==0){return(0)}
return(mean(x[2,], na.rm=TRUE))
})

SFSmeanPop3 <-  sapply(slide@region.stats@minor.allele.freqs, function(x){
if(length(x)==0){return(0)}
return(mean(x[3,], na.rm=TRUE))
})

# Now, lets smooth the lines and plot the results

ids <- 1:length(slide@region.names)

loess.SFSmeanPop1  <- loess(SFSmeanPop1~ids, span=0.02)
loess.SFSmeanPop2  <- loess(SFSmeanPop2~ids, span=0.02)
```

```
loess.SFSmeanPop3  <- loess(SFSmeanPop3~ids, span=0.02)

plot(predict(loess.SFSmeanPop1), type = "l", xaxt="n", xlab="position (Mb)",
ylab="mean(SFS)", main = "Chromosome 2L (1kb windows)", ylim=c(0,0.2))

lines(predict(loess.SFSmeanPop2), col="blue")

lines(predict(loess.SFSmeanPop3), col="red")

axis(1,c(1,10000,20000,30000,40000,50000),
c("0","10","20","30","40","50"))

# create the legend
legend("topright",c("M","S","X"),col=c("black","blue","red"), lty=c(1,1,1))
```



15.5 Composite Likelihood Ratio (CLR) test from Nielsen

```
# Lets assume we have an object of class GENOME (GENOME.class) and the corresponding
# populations M,S,X are already set.
```

```
# Lets apply the CLR test on genes and define a global set of the SFS
# based on all Coding SNPs

# extract the genomic positions of the genes out of the corresponding GFF file
genes <- get_gff_info(gff.file="twoL.gff",chr="2L", feature="genes")

# splitting the data into gene regions
# type=2 have to be set because the values are genomic positions
split <- splitting.data(GENOME.class, positions=genes, type=2)

# calculate the minor allele frequencies
split <- detail.stats(split)

# extract the minor allele frequencies from all gene regions for each population
freqM <- sapply(split@region.stats@minor.allele.freqs,function(x){return(x[1,])})
freqS <- sapply(split@region.stats@minor.allele.freqs,function(x){return(x[2,])})
freqX <- sapply(split@region.stats@minor.allele.freqs,function(x){return(x[3,])})

# Create the global frequencies table which are neccessary for the CLR test.
freqM <- table(unlist(freqM))
freqS <- table(unlist(freqS))
freqX <- table(unlist(freqX))

# Now, lets calculate the CLR test using the module sweeps.stats

split <- sweeps.stats(split, freq.table=list(freqM,freqS,freqX))

head(split@CLR)

     pop 1     pop 2     pop 3
[1,] 126.53772 187.70825 316.40969
[2,] 10.93362  13.37492  34.85426
[3,] 22.07749  25.43857  42.07533
[4,]       NA        NA        NA
[5,] 10.93362  13.11267  16.18083
[6,] 254.16225 303.04791 352.00897
```

15.6 Mcdonald-Kreitman test

```
# Read in the data with the corresponding GFF file
GENOME.class <- readVCF("twoL.vcf.gz",15000,"2L",1,50000000,
include.unknown=TRUE, gffpath="twoL.gff")
```

```
# Verify the syn/non-syn SNPs
GENOME.class <- set.synnonsyn(GENOME.class, ref.chr="twoL.fas")
# Set the populations
GENOME.class <- set.populations(GENOME.class, list(M,S,X), diploid=TRUE)
# Splitting the data into genes
split <- splitting.data(GENOME.class, subsites="gene")

# Peform the MKT
split <- MKT(split)

# To look at gene 9 we can do the following:
split@MKT[[9]]
       P_nonsyn P_syn D_nonsyn D_syn neutrality.index alpha
pop1/pop2        3     2        0     0              NaN   NaN
pop1/pop3        7     2        5     4              2.8  -1.8
pop2/pop3        6     2        5     4              2.4  -1.4

# or alternatively
get.MKT(split)[[9]]
```

16 Graphical output: R package ggplot2

16.1 Creating data.frames

Lets assume we have transformed the data into sliding windows or specific regions and already performed the Fixation index FST. To create an compact data representation of the most informative values we could do the following:

```
# Extracts the information stored in the slot region.names
# and converts the strings to numeric values (start position of the
# region and end position)


from.pos   <- sapply(split@region.names,function(x)
{return(as.numeric(strsplit(x," ")[[1]][1]))})

to.pos     <- sapply(split@region.names,function(x)
{return(as.numeric(strsplit(x," ")[[1]][3]))})

# Lets concatenate the values into a matrix
DATA <- cbind(from.pos, to.pos, split@nucleotide.F_ST)
```

```
# Converting into a data.frame
DATA <- as.data.frame(DATA)
```

The native R function `sapply` is a very performant approach to extract values also stored in the `region.data` or `region.stats` slots where data is mostly organized as lists.

17 Performing readVCF in parallel

To accelerate computations for the `readVCF` function the mechanism provided by the function `mclapply` from the package `parallel` might be a good option. `readVCF` can be applied to different regions of the VCF file so that we can contribute the reading process on different nodes. The returned object is a list of classes from the type `GENOME`. Note, the ff-package which is used for storing whole genome variation data is limited by `n.individuals * n.snps <= Maschine$integer.max`. If the data is bigger than that the `bigmemory` package will be applied. The corresponding access function are much slower than those provided by the ff-package. As an example we read in data via `readVCF` from the region 1-10.000.000, but parallize the reading process on two nodes. (1-5.000.000 and 5.000.001-10.000.000).

```
# Loading the R-package parallel
require(parallel)

# Lets define the two regions

cregions    <- character(2)
cregions[1] <- "1-5000000"
cregions[2] <- "5000001-10000000"


GENOME.classes <- parallel::mclapply(as.list(cregions),

function(x){

From    <- as.numeric(strsplit(x,"-")[[1]][1])
To      <- as.numeric(strsplit(x,"-")[[1]][2])

        return(readVCF(
filename="AGC_refHC_bialSNP_AC2_2DPGQ.2L_V2.CHRcode2.vcf.gz",
numcols=1000, tid="2", frompos=From, topos=To, samplenames=NA,
gffpath=FALSE, include.unknown=TRUE,
approx=FALSE, out=x, parallel=FALSE))
},

mc.cores = 2, mc.silent = TRUE, mc.preschedule = TRUE)
```

```
> GENOME.classes[[1]]@region.names
[1] "2 : 1 - 5000000"
> GENOME.classes[[2]]@region.names
[1] "2 : 5000001 - 10000000"

The splitted classes can now be used seperately.

slide <- sliding.window.transform(GENOME.classes[[1]],1000,1000)
slide <- diversity.stats(slide)

Also we can concatenate those classes:
GENOME.class <- concatenate.classes(GENOME.classes)
GENOME.class <- concatenate.regions(GENOME.class)
```

18 Pre-filtering VCF files

18.1 VCF tools

18.2 WhopGenome