fastsimcoal ver 2.8
fsc28

a continuous-time coalescent simulator of genomic diversity under arbitrarily complex evolutionary scenarios

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Manual ver 2.8, September 2023

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2. INTRODUCTION

This manual describes the use of *fastsimcoal28,* shortened to *fsc28,* a program to generate the neutral genomic molecular diversity in current or ancient samples drawn from a population with a complex demographic history. *fsc28* is the fifth version of *fastsimcoal, which was* a completely rewritten version of *simcoal2* (Laval and Excoffier 2004), a coalescent simulation program implementing a generation by generation approach while *fsc28* is based on a much faster continuous time approximation. Despite a completely new coalescent engine, *fsc28* uses exactly the same input files as *simcoal2*, and it produces very similar output files.

fsc28 typically generates many replicates of random outcome of molecular diversity under a user-defined evolutionary scenario. The evolutionary scenario is defined in an input parameter file (extension .par) and the output diversity is written in *arlequin* project files (extension .arp) that can then be processed with *arlequin* or *arlsumstat* (Excoffier and Lischer 2010) to get distributions of various summary statistics. Since ver2.7, *fastsimcoal* can also generates genotype tables. Additional options of *fastsimcoal27* can be specified on the command line (type "*fastsimcoal27 -h*" for help on command line options).

fsc28 can handle very complex evolutionary scenarios including an arbitrary migration matrix between samples, historical events allowing for population resize, populations fusion and fission, admixture events, changes in migration matrix, or changes in population growth rates. The time of sampling can be specified independently for each sample, allowing for serial sampling in the same or in different populations.

Different markers, such as DNA sequences, STRs (microsatellites) or multi-locus allelic data can be generated under a variety of mutation models (e.g. finite- and infinite-site models for DNA sequences, stepwise or generalized stepwise mutation model for STRs data, infinite-allele model for standard multi-allelic data).

fsc28 can simulate data in genomic regions with arbitrary recombination rates, thus allowing for recombination hotspots of different intensities at any position. *fsc27* implements an approximation to the ancestral recombination graph in the form of sequential Markov coalescent allowing it to generate genetic diversity very quickly for >100 Mb genomic segments.

Compiled versions of *fsc28* for Windows, Linux or MacOs X are available on http://cmpg.unibe.ch/software/fastsimcoal28

Since *fsc27* output is meant to be interfaced with *Arlequin* or *arlsumstat*, the reader may also want to get more information on Arlequin on http://cmpg.unibe.ch/software/arlequin35

Since ver 2.1, *fastsimcoal* can be used to estimate demographic parameters from the (joint) SFS, as described in Excoffier et al. (2013)

CITATION

The following citations should be used for *fsc28*:

- Excoffier, L., Dupanloup, I., Huerta-Sánchez, E., and M. Foll (2013) Robust demographic inference from genomic and SNP data. PLOS Genetics 9(10):e1003905.
- Excoffier, L., Marchi, N., Marques, D. A., Gouy, A., Sousa, V. C. (2021) *fastsimcoal2*: demographic inference under complex evolutionary scenarios. Bioinformatics 37: 4882–4885.
- Excoffier, L., Kapopoulou, A., Marchi, N. (2023) Demogenomic inference from spatially and temporally heterogeneous samples. Molecular Ecology Resources https://doi.org/10.1111/1755-0998.13877.

DISCUSSION GROUP

A Google discussion group has been created to discuss any issue related to *fastsimcoal*. It is available on https://groups.google.com/forum/#!forum/fastsimcoal

ACKNOWLEDGEMENTS

Many people have contributed to the development and improvement of *simcoal* and *fastsimcoal*. Historically, John Novembre has contributed to the early development of *simcoal*, and Guillaume Laval has written most routines dealing with recombination and the ancestral recombination graph in *simcoal2*. Mathias Currat has also contributed in improving the earlier coalescent code shared between *simcoal2* and *SPLATCHE*. Thierry Schuepbach contributed to the initial development of the multithreaded version. Some piece of code written by all these persons might still be present in some parts of *fsc28* even though most has been rewritten when implementing the *fastsimcoal2* continuous time coalescent approach, the SMC' recombination framework or the multithreaded version. *fastsimcoal2* has also benefitted from many suggestions from Isabel Alves, Isabelle Duperret, Matthieu Foll, Stephan Peischl, and Benjamin Peter. The use of *.tpl* and *.est* file is largely inspired by the corresponding settings files of *ABCToolBox* developed by Daniel Wegmann, and its syntax also follows developments of Samuel Neuenschwander and Mathias Currat in earlier ABC work. The latest developments of ver 2.6 and onwards have been thoroughly discussed, tested, and debugged by Alexandre Gouy and David Alexander Marques. Sandra Oliveira has also promoted and tested the development of the recoding of admixture tracts in ver 2.8. We would also like to thank all users of *fastsimcoal* who have been reporting bugs directly by email or by using the fastsimcoal Google group list.

3. CHANGES COMPARED TO SIMCOAL2 AND FASTSIMCOAL

FASTSIMCOAL VS. SIMCOAL2

1. Faster continuous-time coalescent simulations
2. Faster recombination model
3. Serial sampling
4. Generation of DNA sequence data under the infinite-site model
5. Sampling of parameter values from prior distributions
6. Computation of population-specific and joint site frequency spectrum
7. Optional output of all trees under the serial Markov coalescent model of recombination
8. RFLP data cannot be simulated anymore

FASTSIMCOAL2 VS. FASTSIMCOAL (JANUARY 2013)

1. Several bug corrections
2. Optional output of all simulated sites (including monomorphic sites)
3. Optional use of a manual seed for the random number generator (***--seed xxx*** command line option)
4. Simulation of ascertained SNP data
5. Generation of the (joint) site frequency spectrum (SFS) from DNA sequence data
6. Generation of multidimensional (>2D) SFS
7. Ability to estimate demographic parameters from the site frequency spectrum inferred from DNA sequences or ascertained SNP chips
8. -s option now requires an additional number specifying the number of SNPs to output

FASTSIMCOAL2.01 VS. FASTSIMCOAL2 (DECEMBER 2013)

1. Several bug corrections
2. User manual update
3. 64 bit version for windows
4. Consolidated *.par* file reading
5. Outputs *.par* file with ML parameters estimation from SFS at the end of the estimation. They are found in file "*<template_file_name>_maxL.par*".

FASTSIMCOAL2.5 VS. FASTSIMCOAL2.1 (JULY 2014)

1. The *fastsimcoal2* program ver2.5 has been renamed ***fsc25*** (shorter name is better)
2. Use of a different random number generator (same seed will produce different results than in *fastsimcoal21*)
3. Code optimization resulting in up to 1-75% speed gain for single threaded version (see benchmark)
4. Multithreading (64 bit only), for more speed gain on a multicore processor desktop machine (see benchmark)
5. Result files for parameter estimation now output in separate result directory
6. More options to generate SNP data
7. New specification for MAF SFS (see below)

8. Added a version for macOS X running in earlier versions (e.g. from 10.6 upwards) (thanks to Iain Mathieson)
9. More tolerant reading of input files (thanks to Allan Strand)
10. Rules in *.est files* can now be used for parameter estimations

FASTSIMCOAL2.5.1 VS. FASTSIMCOAL2.5 (SEPTEMBER 2014)

1. Example files are back in zip files (thanks to Alfredo)
2. Description of the exact format of the multiSFS format has been modified in the manual (thanks to Vitor Sousa and Raphael Leblois)
3. Problem in implementing recombination with multiple runs (option ***-nx*** where x>1) (thanks to Vitor Sousa and Yang)
4. More precision on branch length when outputting tree in NEXUS format (thanks to Shuo Yang)
5. Faster implementation of recombination under the SMC' algorithm and its extension to multiple recombinations between sites

FASTSIMCOAL2.5.2 VS. FASTSIMCOAL2.5.1 (MARCH 2015)

1. Bug corrections:
    - *fsc251* asked for a joint SFS when two populations samples were listed in *.tpl* file but only one contained active lineages. Bug found by Charleston Chiang.
    - TMRCA was not found in case of recombination and demes with some inactive lineages. Bug found by Ryan Bohlender)
    - *fsc251* was not generating output files when path was provided before input file names (*.par* or *.tpl*).Note that *fsc25* should always be run from the directory containing the input files, even though the program can be can be physically located elsewhere. Bug found by Greer Dolby.
    - *fsc251* was not taking into account growth rate changes specified in historical events (bug introduced in 2.5.1, and it was not present in ver 2.5.0).

2. ***-k*** option has no upper limit anymore, and its default value is 100,000.
3. Added new ***-P*** command line option, allowing to get the global pooled SFS obtained by pooling all lineages as if in a single population.
4. Added two new operators in *.est* file for complex parameters: %min% and %max%.
5. Added new functions in *.est* files for complex parameters: abs(), exp(), log(), log10(), pow10().
6. Added a new "bounded" keyword in *.est* file to specify that the upper range of a simple parameter is bounded. Needs to be listed after the "output" or "hide" keywords.
7. Added two new keywords for historical events: "keep" and "nomig".
8. Expected joint SFS is now rescaled such that the sum of sfs entries for polymorphic sites is 1.

FASTSIMCOAL2.5.2.8 VS. FASTSIMCOAL2.5.2 (MAY 2015)

1. Bug corrections:
    - Incorrect simulation of mutations in case of high recombination rates. There was a strong negative correlation between the recombination rate and the number of polymorphic loci, when adjacent sites were the object of recombination. The number of

mutations was underestimated for recombination rates, say >1e-7. This bug affected ALL previous *fsc* releases.
- Possible overestimation of TMRCA and overall tree size in case of recombination. Bug present since early *fsc2* release.
- Crash of *fsc2* in case of very high recombination rate with DNA data.
- Incorrect writing of recombination positions in output *.arp* file when simulating several threads.
- maxObsLhood was not correctly computed when estimation of parameters in a scenario with a single population.
- Change of migration matrix not implemented after first recombination event (thanks to Stefano Mona).
- Computation of MAF SFS incorrect in case of multiple mutations per site (when ***-I*** option not provided and high mutation rates).

2. Speed optimization.
3. Output of random DNA nucleotides instead of N for monomorphic loci with the ***-S*** option.
4. Possibility to run *fsc* without command line option if file "fsc_run.txt" is present and contains run path and command line options in current working directory.

FASTSIMCOAL2.5.2.21 VS. FASTSIMCOAL2.5.2.8 (NOVEMBER 2015)

1. Bug corrections:
   - Non implementation of exponential growth at time zero for the first simulated tree. Initial population size therefore does not change for that tree. Note that specifications of exponential growth rates in historical events are correctly implemented even in the first tree. Exponential growth is then correctly implemented in the next simulated trees (thanks to Anand Bhaskar).
   - Crash in case of very large samples sizes (e.g. 60,000) (thanks to Anand Bhaskar).
   - Incorrect computation of the maxlhood when non integers are used in the observed SFS (thanks to Andi Knautt).
   - Reported expected SFS was that of the last iteration and not that associated to the maxlhood parameter estimates.
   - In case of crash due to bad *.tpl* file, parameters reported in file called *<generic name>_bad.par* were not those leading to the crash.

2. Speed optimization. Up to 30% speed gain.
3. Output of time to MRCA in file *<generic name>_mrca.txt* with new compiler directive ***--recordMRCA***. Beware that this option really slows down computations. Note that we also output the deme in which MRCA occurred.

FASTSIMCOAL2.6 (FSC26) VS. FASTSIMCOAL2.5.2.21 (OCTOBER 2017)

1. Simple implementation of individual inbreeding
   - The average inbreeding coefficient of individuals in a population can now be specified as a third optional parameter in the sample size definition. In this case, the sample age needs to be defined (set to zero in most applications), as:
     <sample size> <sample age> <inbreeding coefficient>
2. Possibility to define initial parameter values for demographic inference

- Option ***-initvalues file.pv*** , where *file.pv* lists initial *non-complex parameter* values to use. This option is mainly useful when computing bootstrap confidence intervals, as it allows one to use less replicates for each bootstrap data set. A **.pv* file is now automatically generated after each parameter estimation by *fsc27.*

3. Computation of MAF 1D and 2D SFS with option ***--foldedSFS*** by simply folding the corresponding unfolded SFS (for compatibility with *angsd,* where the minor allele is computed separately for each SFS).
4. Optional faster but approximate log computations with option ***--logprecision n***, where *n* is a number between 10 and 23 specifying the precision of the computation of logarithms. 23 means full precision and is the default value.
5. Optional parameter optimization without taking singletons into account specified with option ***–nosingleton.***
6. Syntax changes
   - For parameter optimization
     - ***-N*** option has been suppressed, and maximum no. of iteration is now equal to that set by the ***-n*** option.
     - The number of cycles to performed is now fixed and only specified with option ***-L.***
     - The ***-I*** option is now optional and means something different. It is now used to specify the number of cycles where information on monomorphic sites is used. After these initial cycles, likelihood will only be computed (and optimized) on the polymorphic sites. This option needs to be used together with the "*reference"* keyword in the *.est* file (see section on *.est* file).
     - The ***-M*** option is now just a flag mentioning we want to perform parameter estimation from the observed SFS. It should therefore not be followed by any number.
   - Removed ***-D*** option to produce output in dadi format, as this is virtually identical to the multidimensional SFS output, barring the header.
7. Implementation of instantaneous bottleneck using the ***instbot*** keyword at the end of a line in a *.par* or *.tpl* file. One can now define the intensity of a bottleneck at the position of a sink resize. A bottleneck occurs in one generation and its intensity *I* is defined as $1/N_{Bot}$, where N_{Bot} is the haploid size during the bottleneck. It simplifies the writing of complex scenarios where no event was supposed to happen during a bottleneck of a given duration. Note that this option is not implemented in case of recombination yet. A warning message is issued in that case.
8. Bug corrections:
   - Expected marginal SFS were not computed when computing expected SFS with FREQ data.
   - Wrong likelihoods were computed with option ***-0.***
   - No more (hopefully) program crash when using large recombination rates.

FASTSIMCOAL2.7 (FSC27) VS. FASTSIMCOAL2.6 (APRIL 2021)

1. New syntax in the *.est* files. It is now possible to include previously defined simple parameters as search range delimiters. The keyword ***paramInRange*** needs to be specified at the end of lines containing such parameters.
2. New keyword in *.par* or *.tpl* file: ***absoluteResize***. It allows a given sink population to take a new absolute size, independently of its previous size. It eliminates the need to compute this resize as a complex parameter in the *.est* file.

3. The **[RULES]** section has been suppressed from input files. It is simply not read anymore. These rules have become obsolete given the new syntax described in point 1.
4. **SNP** data type is not considered anymore, as they led to biased simulations. Use short segments of DNA and the ***-sX*** option to generate *X* SNPs instead.
5. Simulations of large and sparsely occupied structured populations has been optimized and can be up to 10 times faster than the previous version. There is very little gain for simulations with a small number of migration-connected demes, though.
6. Simulations of large recombining chromosomes have been optimized, when using large values of the ***-k*** options.
7. Generation of genotype table (*.gen* file) as an alternative output to Arlequin (***-G*** option). The additional *-g* option allows one to generate diploid genotypes (coded as 0, 1 or 2) instead of haploid genotypes (coded as 0 or 1).
8. Possibility to "kill" demes, such as to make them inaccessible to migration. Setting a sink deme size to zero (using a sink resize of zero in a historical event) will now prevent further migration to this deme. This is useful as one can keep the same migration matrix after the disappearance of some demes (e.g., due to population fusion backward in time).
9. Comments are now possible at the end of any line of *.est* and *.par* files.
10. Other changes and bug corrections:
    - When a deme size goes to zero (e.g., due to negative growth), a warning is only produced if the deme is occupied (thanks to David Marques for requesting this change).
    - Bug corrected when computing likelihood with ghost populations and a single sampled deme.
    - Corrected bug (found by David Marques) with options ***--noSingleton*** and ***--foldedSFS*** in the presence of ghost populations (the max est lhood was larger than the max obs lhood).
    - Corrected bug occurring when computing the position of the next recombination position in case of very small recombination rates (thanks to Silvert Martin).
    - Corrected important bug (thanks to David Marques) in case of the introduction of population growth at a given point in a population of initial constant size. The population size was adjusted as if there had been growth since generation zero.
    - Corrected bug (thanks to Yu Sugihara) when generating diversity based on random parameters and using ***-Ex*** option when *x* >1.

FASTSIMCOAL2.8 (FSC28) VS. FASTSIMCOAL2.7 (SEPTEMBER 2023)

1. New syntax in the *.tpl* files to deal with sample heterogeneity. We introduce the concept of sfs pools where the sfs of different samples can be computed as a pool. It allows for considering any spatial of temporal heterogeneity. New key word "***sfspool***" in deme size section.
2. Possibility to record the deme of origin of chromosome segments when implementing an admixture even so that it is possible to simulate chromosome painting. New keyword "***recordAdmOrigin***" in historical events
3. New command line options (**-y** and **-z**) to fine tune the parameter estimation procedure
4. Other changes and bug corrections:

    - When simulating several data files with definition files (*.def*) the SFSs are written in different files, either in separate directories with the -j option, or in the same directory without the -j option

- Program was crashing when simulating exponential growth and migration. Bug found by Jason Weir.
- Optimisation of computations when estimating data from multidimensional SFS
- Bad computation of lhood when estimated from the *maxL.par* files as compared to that computed during parameter estimations, in case of population growth. Bug found by Kyle Lewald
- Incorrect simulations from par files when some demes are explicitly killed. Bug found by Kyle Lewald

4. GETTING STARTED

Compiled version of *fastsimcoal2* and example files can be downloaded from http://cmpg.unibe.ch/software/fastsimcoal2.

The archives include an executable version of *fastsimcoal2* for a given platform, the *fastsimcoal2 pdf* manual, as well as examples *.par* files and example template (*.tpl*), distribution (*.est*), and definition files (*.def*) for a variety of simple evolutionary scenarios and several types of markers, with and without recombination. We have also added a few site frequency spectra (SFS) files as well as associated *.tpl* and *.est* files to estimate parameters from the SFS (new to *fastsimcoal2*).

INTALLATION

Unzip the archive file to the directory of your choice. A version of *fsc27* should be present (*fs27.exe* under windows, or *fsc27* under linux of macOS X). On a command line, simply type "`fsc27`" and you will have a list of the different command-line options available to run *fsc27*.

To access *fsc27* from any directory on your hard disk, put the directory on your path under windows, or put *fsc27* in your `~/bin` directory under linux or macOS X.

RUNNING FSC

There are 3 ways to simulate genetic data with *fastsimcoal2:*

1) Simulate data under an evolutionary scenario with parameter values defined in an input parameter file

```
fsc27 -i test.par -n 100
```

fastsimcoal2 will use the scenario and the parameter values defined in the parameter file *test.par* and make 100 simulations under this scenario.

2) Simulate data under an evolutionary scenario with parameter values randomly drawn from priors

```
fsc27 -t test.tpl -n 10 -e test.est -E 100
```

fsc27 will use the scenario defined in the template file *test.tpl* and generate 100 sets of parameter values by randomly drawing these values from the priors defined in the file *test.est*. 10 simulations will be done for each sets of randomly drawn parameter values.

3) Simulate data under an evolutionary scenario with parameter values defined in an external definition file

```
fsc27 -t test.tpl -n 100 -f test.def
```

fsc27 will use the scenario defined in the template file test.tpl and use the parameter values found in the definition file *test.def*. 100 simulations will be done for each set of predefined parameter values.

Additional descriptions of command line options and input file format can be found in the next chapters.

Fastsimcoal2 can also be used to infer demographic history parameters from the SFS (see details in section *Estimation of demographic parameters from the SFS via likelihood maximization*).

5. STRUCTURE OF INPUT FILES AND OUTPUT FILES

A SIMPLE UNSUBDIVIDED POPULATION AND DNA SEQUENCES

Let us consider the case of a single population made up of 20,000 haploid individuals (or 10,000 diploid individuals) where we want to generate diversity along a 10kb DNA sequence, with mutation rate $\mu = 1 \times 10^{-8}$ / bp / gen.

The *fastsimcoal2*-compatible input file *1popDNA.par* describing such a scenario would look like:

1popDNA.par

```
//Number of population samples (demes)
1
//Population effective sizes (number of genes)
20000
//Sample sizes
10
//Growth rates      : negative growth implies population expansion
0
//Number of migration matrices : 0 implies no migration between demes
0
//hist. event: time, source, sink, migrants, new size, new growth rate, migr. matrix
0  historical event
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
1
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 10000 0 1e-8 0.33
```

OUTPUT FILES

ARLEQUIN FILE (*.ARP)

Running *fsc27* with this simple command line

```
fsc27 -i 1popDNA.par -n1
```

generates an *Arlequin* type of file which is located in a directory having the same name as the input file but with the extension *.arp* removed, *1popDNA* in our case:

./1popDNA/1popDNA_1_1.arp

```
[Profile]
       Title="A series of simulated samples"
       NbSamples=1

       GenotypicData=0
       GameticPhase=0
       RecessiveData=0
       DataType=DNA
       LocusSeparator=NONE
       MissingData='?'

[Data]
       [[Samples]]

#Number of independent chromosomes: 1
#Total number of polymorphic sites: 15
# 15 polymorphic positions on chromosome 1
#506, 607, 1092, 2775, 2874, 3919, 4155, 4194, 4314, 5031, 5335, 6311, 8214, 9081,
9423
              SampleName="Sample 1"
              SampleSize=10
              SampleData= {
```

```
1_1    1      AGAGTGCGACAACCT
1_2    1      AGAGTGCGACAACCT
1_3    1      CCACGGCCGCAGAGA
1_4    1      AGAGTGGCATTACCA
1_5    1      AGAGTGCCATTACCA
1_6    1      AGAGTTCCATTACCA
1_7    1      CCACGGCCGCAGAGA
1_8    1      AGAGTGCCATTACCA
1_9    1      AGAGTGCCATTACCA
1_10   1      CCCCGGCCGCAGAGA

}
[[Structure]]

       StructureName="Simulated data"
       NbGroups=1
       Group={
          "Sample 1"
       }
```

New to ver 2.6, one now only outputs polymorphic sites for DNA sequences and the position of these sites is provided as a comment above the sample definition.

To output all sites, irrespective of their polymorphic status, use the ***-S*** command line option.

GENOTYPE TABLE FILE (*.GEN)

New with ver 2.7, the use of the additional command option ***-G*** or ***--indgenot*** as in

```
fsc27 -i 1popDNA.par -n1 -G
```

now also generates a genotype table (*.*gen*) from DNA data (add the ***-x*** option if you do not want to generate the *Arlequin* corresponding file) such as

./1popDNA/1popDNA_1_1.gen

Chrom	Pos	Anc_all	Der_all	A_1_1	A_1_2	A_1_3	A_1_4	A_1_5	A_1_6	A_1_7	A_1_8	A_1_9	A_1_10
1	506	C	A	1	1	0	1	1	1	0	1	1	0
1	607	G	C	0	0	1	0	0	0	1	0	0	1
1	1092	A	C	0	0	0	0	0	0	0	0	0	1
1	2775	C	G	1	1	0	1	1	1	0	1	1	0
1	2874	G	T	1	1	0	1	1	1	0	1	1	0
1	3919	G	T	0	0	0	0	0	1	0	0	0	0
1	4155	C	G	0	0	0	1	0	0	0	0	0	0
1	4194	C	G	1	1	0	0	0	0	0	0	0	0
1	4314	A	G	0	0	1	0	0	0	1	0	0	1
1	5031	C	T	0	0	0	1	1	1	0	1	1	0
1	5335	A	T	0	0	0	1	1	1	0	1	1	0
1	6311	A	G	0	0	1	0	0	0	1	0	0	1
1	8214	C	A	0	0	1	0	0	0	1	0	0	1
1	9081	G	C	1	1	0	1	1	1	0	1	1	0
1	9423	A	T	1	1	0	0	0	0	0	0	0	0

where the genotypes at each polymorphic position are listed on a different line. The header of the file is almost self-explanatory. The first column reports the simulated chromosome, the second column reports the position of the polymorphic position on the chromosome, the 3rd and 4th columns report the state of the ancestral and derived alleles, and the remaining columns report the states of the *n* sampled chromosomes at those positions. The column headers are of the form *A_x_y*, where *A* stands for "*Allele*", *x* indicates the sampled population, and *y* is the index of the sample in the *x*-th population. The alleles are coded as 0 and 1 for the ancestral and derived forms, respectively.

Note that it is also possible to output diploid genotypes if one uses the ***-g*** option, as in

```
fsc27 -i 1popDNA.par -n1 -G -g
```

which generates the following genotype table where genotype column headers are now of the form *G_x_y*, where *G* stands for "*Genotype*", and where genotype entries are coded as 0 (homozygote ancestral), 1 (heterozygote), and 2 (homozygote derived).

./1popDNA/1popDNA_1_1.gen

```
Chrom  Pos   Anc_all  Der_all  G_1_1  G_1_2  G_1_3  G_1_4  G_1_5
1      506   C        A        2      1      2      1      1
1      607   G        C        0      1      0      1      1
1      1092  A        C        0      0      0      0      1
1      2775  C        G        2      1      2      1      1
1      2874  G        T        2      1      2      1      1
1      3919  G        T        0      0      1      0      0
1      4155  C        G        0      1      0      0      0
1      4194  C        G        2      0      0      0      0
1      4314  A        G        0      1      0      1      1
1      5031  C        T        0      1      2      1      1
1      5335  A        T        0      1      2      1      1
1      6311  A        G        0      1      0      1      1
1      8214  C        A        0      1      0      1      1
1      9081  G        C        2      1      2      1      1
1      9423  A        T        2      0      0      0      0
```

MIGRATION

fsc27 can generate data from samples drawn from a subdivided population. For instance, the following input file describes an asymmetric 2-deme island model:

2popSTRmigr.par

```
//Number of population samples (demes)
2 samples to simulate :
//Population effective sizes (number of genes)
1000
1000
//Samples sizes
5
5
//Growth rates      : negative growth implies population expansion
0
0
//Number of migration matrices : 0 implies no migration between demes
1
//migration matrix
0.000 0.005
0.001 0.000
//historical event: time, source, sink, migrants, new size, growth rate, migr. matrix
0  historical event
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
1
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
MICROSAT  10  0.0000  0.0005 0 0
```

The population sizes are of 1000 genes each, and we want to generate samples of size 5 in each population. One migration matrix is defined, listing the migration rates between the two populations. The migration matrix can be asymmetric, and in the case the entry m_{ij} list the **migration rates backward in time** from population *i* to population *j*, *i* representing the row, and *j* the column. The above-mentioned matrix

```
0.000 0.005
0.001 0.000
```

states that, for each generation **backward in time**, any gene from population 0 has probability 0.005 to be sent to population 1, and that a gene from population 1 has a probability 0.001 to move to population 0.

Note that if no migration matrix is defined, no migration is assumed between populations.

Coming back to our example input file above, the data to be generated consist here of 10 microsatellite markers that are fully linked on a chromosome, with mutation rate $\mu = 5 \times 10^{-4}$ per generation per locus. A pure stepwise mutation model without range constraints is assumed.

The following command line:

```
fsc27 -i 2popSTRmigr.par -n1
```

produces the following *Arlequin* output:

2popSTRmigr_1_1.arp

```
#Arlequin input file written by the simulation program fastsimcoal.exe

[Profile]
       Title="A series of simulated samples"
       NbSamples=2

       GenotypicData=0
       GameticPhase=0
       RecessiveData=0
       DataType=MICROSAT
       LocusSeparator=WHITESPACE
       MissingData='?'

[Data]
       [[Samples]]

#Number of independent chromosomes: 1
#Polymorphic positions on chromosome 1
#1, 2, 3, 4, 5, 6, 7, 8, 9, 10

              SampleName="Sample 1"
              SampleSize=5
              SampleData= {
1_1    1       500  499  500  500  499  500  499  500  501  503
1_2    1       498  498  501  502  500  501  498  500  499  499
1_3    1       499  498  500  501  500  501  499  501  502  499
1_4    1       498  498  500  502  500  501  498  501  499  500
1_5    1       498  497  500  503  500  500  498  500  500  499

}
              SampleName="Sample 2"
              SampleSize=5
              SampleData= {
2_1    1       500  499  500  500  499  500  499  500  500  503
2_2    1       498  498  500  501  500  501  498  501  499  499
2_3    1       500  499  500  500  499  500  499  500  500  503
2_4    1       501  499  500  500  499  500  499  500  500  503
2_5    1       499  498  500  501  501  501  499  501  501  499

}

[[Structure]]

       StructureName="Simulated data"
       NbGroups=1
       Group={
          "Sample 1"
          "Sample 2"
       }
```

Note that for microsatellite data, the ancestral allele at each locus is arbitrarily set to have 500 repeats, and that different numbers indicate different number of repeats.

HISTORICAL EVENTS

Historical events can be used to:

- Change the size of a given population.
- Change the growth rate of a given population.
- Change the migration matrix to be used between populations.

- Move a fraction of the genes of a given population to another population. This amounts to implementing a (stochastic) admixture or introgression event.
- Move all genes from a population to another population. This amounts to fusing two populations into one, looking backward in time, or implementing a populations' fission looking forward in time.
- One or more of these events at the same time

Note that several events can be defined at different or at the same time in the past. When several events happen at the same time, the order in which they are implemented is that defined in the input file.

Let us have a look at a simple example:

2popSTRdiv.par

```
//Number of population samples (demes)
2 samples to simulate :
//Population effective sizes (number of genes)
1000
1000
//Samples sizes
5
5
//Growth rates      : negative growth implies population expansion
0
0
//Number of migration matrices : 0 implies no migration between demes
2
//migration matrix
0.000 0.0005
0.0001 0.000
//migration matrix
0.000 0.000
0.000 0.000
//historical event: time, source, sink, migrants, new size, growth rate, migr. matrix
2 historical event
1000 0 0 0 1 0 1
10000 1 0 1 10 0 1
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
1
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
MICROSAT  10  0.0000  0.0005 0
```

In this example, we have two migration matrices, and the first migration matrix (migration matrix 0) is used by default from present-day and going backwards in time until it is changed by a historical event. The second matrix (migration matrix 1) has all entries set to zero, and thus specifies an absence of migrations.

The first historical event:

```
1000 0 0 0 1 0 1
```

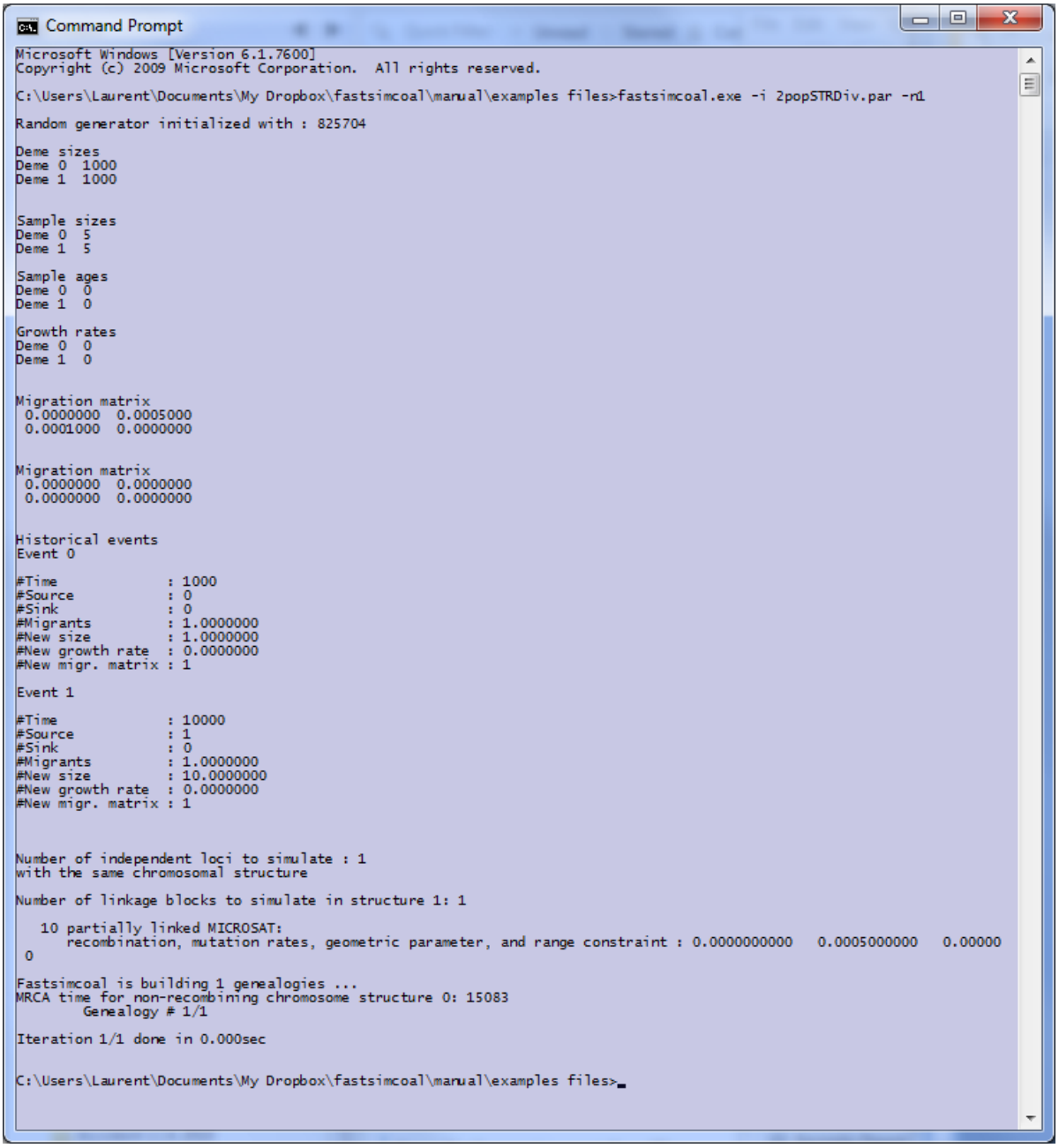
specifies that 1000 generations in the past, the migration matrix will change to migration matrix 1, which is the migration matrix used from 1000 generations onwards (backward in time). In doing that it basically stops all migrations between demes.

The second historical event:

```
10000 1 0 1 10 0 1
```

says that 10,000 generations in the past all the genes from deme 1 move to deme 0, the size of which is resized by a factor 10 (to 10,000 genes).

You can check that these historical events are correctly understood by *fsc27* by looking at the console output, which should look like



Alternatively, the simulations conditions are also output in the file *./2popSTRdiv/2popSTRdiv_1.simparam* together with the *Arlequin* project *2popSTRdiv_1_1.arp*.

SERIAL SAMPLING

In *fsc27,* it is possible to specify at which time sampling was performed in the past. This is simply achieved by adding sampling time after the specification of the sample size. If no time is specified after the sample size, than sampling at present time is assumed, which also ensures compatibility with *simcoal2* input files. The use of this new feature is shown in the simple following input file.

3popDNAserial.par

```
//Number of population samples (demes)
3
//Population effective sizes (number of genes)
1000
0
0
//Sample sizes
5
3 500
2 1000
//Growth rates      : negative growth implies population expansion
0
0
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new size, growth rate, migr. matrix
2  historical event
500 1 0 1 1 0 0
1000 2 0 1 1 0 0
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
1
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 10000 0.00000 0.00000002 0.33
```

In this scenario, 5 sequences were sampled at the present time, 3 sequences 500 generations ago, and 2 sequences 1000 generations ago. As we consider that these 3 samples come from the same population, we simply use 2 historical events to transfer these ancient DNA sequences to deme 0 at the time of their sampling.

The resulting genealogy can be visualized by asking *fsc27* to output coalescent trees. This is done with the ***-T*** option as in the following command:

```
fsc27 -i 1popDNAserial.par -n 2 -T
```

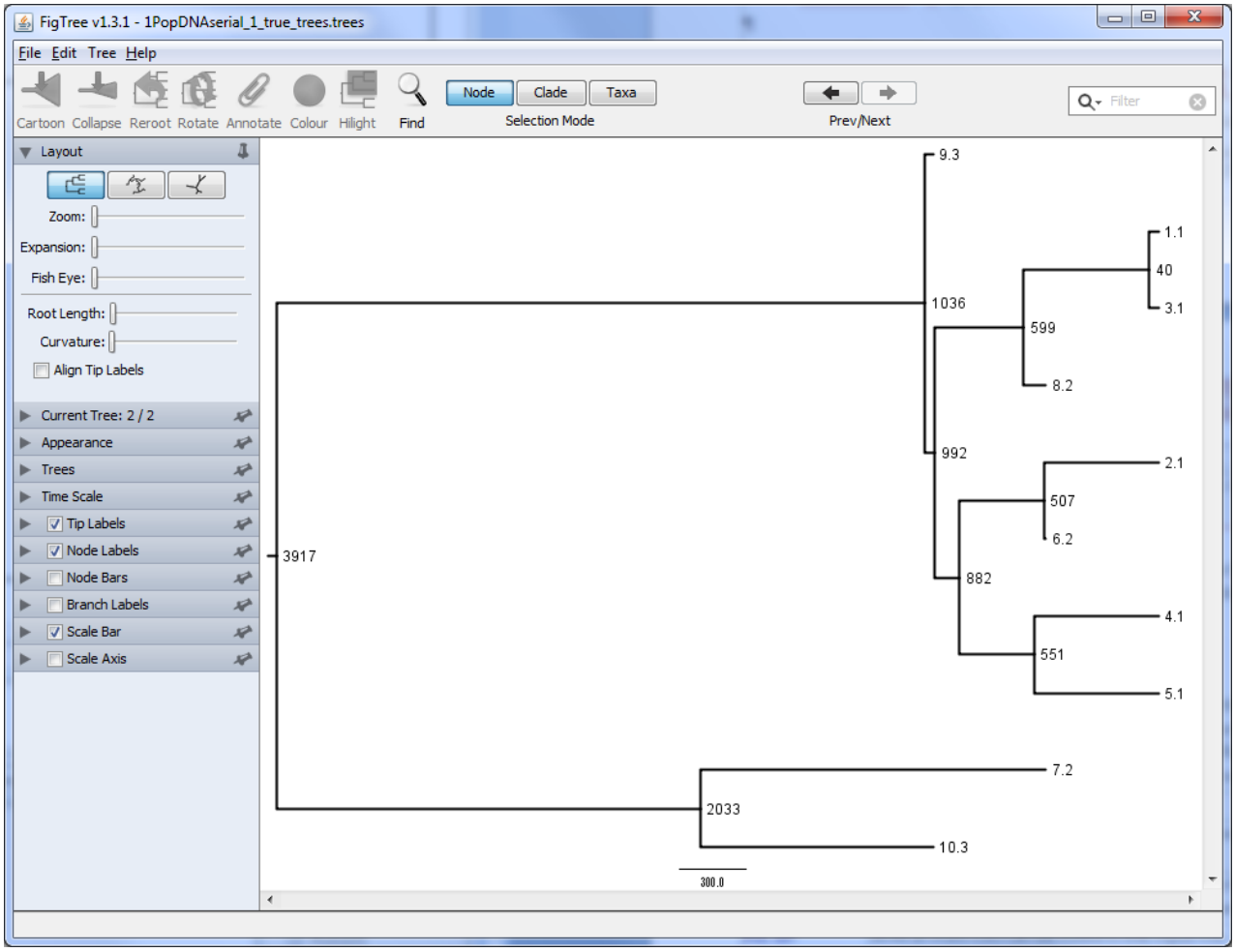
produces tree files in the nexus format, looking like:

./3PopDNAserial/3PopDNAserial_1_true_trees.trees

```
#NEXUS
begin trees;  [Treefile generated by fsc27 .exe (Laurent Excoffier)]

       tree NumGen_tree_1_1_pos_0 = [&U] ((10.3:103, ((4.1:269, 3.1:269):728,
7.2:497):106):1197, ((8.2:580, 9.3:80):852, (6.2:572, (5.1:811, (2.1:55,
1.1:55):756):261):860):368);
       tree NumGen_tree_2_1_pos_0 = [&U] ((9.3:36, (((1.1:40, 3.1:40):559,
8.2:99):393, ((2.1:507, 6.2:7):375, (4.1:551, 5.1:551):331):110):44):2881, (7.2:1533,
10.3:1033):1884);
end;
```

These trees can be conveniently visualized with visualization tools, like *FigTree* (http://tree.bio.ed.ac.uk/software/figtree) freely available for Windows, Linux, or MacOS X.

our tree of 10 DNA sequences looks like:



We indeed see that the first 5 sequences from deme 0 were sampled at time 0, that the 3 sequences from deme 1 were sampled at time 500, and that the 2 sequences of deme 2 were sampled at time 1000.

Note that it may not be a good idea to record trees for long DNA sequences with recombination, as trees files can become extremely large (>100Mbytes for 10Mbase sequences).

Note also that in *fsc27* branch lengths are now expressed in fractions of generations (e.g. 1205.123)

INBREEDING

With version 2.6 of *fastsimcoal*, we introduce the possibility to simulate a simple form of inbreeding, but this *only in absence of recombination*.

The average inbreeding coefficient of a population must be indicated as the third parameter in the sample size section.

For instance, in the following file, we specify that an individual sampled 2000 generations has an inbreeding coefficient of 1/8. This would be similar to the inbreeding coefficient recently estimated for a Neanderthal individual from the Denisova cave in the Altai mountains (Prüfer et al. 2014).

3popDNAInbreeding.par

```
//Number of population samples (demes)
3
//Population effective sizes (number of genes)
10000
10000
0
//Sample sizes, ages and inbreeding
6
3
2 2000 0.125
```

```
//Growth rates      : negative growth implies population expansion
0
0
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new size, new growth rate, migr.
matrix
2  historical event
500 1 0 1 1 0 0
20000 2 0 1 1 0 0
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
1
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 10000 0.00000 0.00000002 0.33
```

Note that this parameter is optional, and the default inbreeding coefficient is zero. However, if it is non-zero, then the optional age of the sample (which can be zero for present samples) needs to be specified as well.

The way *fsc27* simulates inbreeding is very simple and differs from a classical structured coalescent way to simulate inbreeding (see e.g. Nordborg 1997, Nordborg and Donnelly 1997). Since *fsc27* is essentially an haploid coalescent simulator, we assume here that pairs of lineages in a given deme corresponds to a diploid individual, i.e. lineages 1 and 2 in individual 1, lineages 3 and 4 in individual 2… Then, at sampling time, each pair of lineage has a probability equal to the inbreeding coefficient to instantaneously coalesce. If it coalesces, the number of lineages is decremented, else nothing happens. No further round of inbreeding is assumed going backward in time. This procedure is repeated for each independent locus. Note that all individuals of a given deme are supposed to have the same inbreeding coefficient.

SIMULATION OF SEVERAL CHROMOSOMAL SEGMENTS

It is easy to simulate several chromosomal segments with different types of markers and different recombination or mutation rates.

In the following example files, one simulates 2 independent non-recombining chromosome segments with the same structure. Each chromosome is made up of 4 blocks, each time with a different mutation model.

1PopMultiLocus.par

```
//Number of population samples (demes)
1
//Population effective sizes (number of genes)
10000
//Sample sizes
5
//Growth rates      : negative growth implies population expansion
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new size, new growth rate, migr.
matrix
0  historical event0
//Number of independent loci [chromosome]
2 0
//Per chromosome: Number of linkage blocks
3
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 1000 0 0.0000002 0.33
MICROSAT 3 0 0.0005 0 0
STANDARD 2 0 0.001
```

The data section of a typical output of this scenario is:

./1PopMultiLocus/1PopMultiLocus_1_1.arp

```
[Data]
       [[Samples]]

#Number of independent chromosomes: 2
#Polymorphic positions on chromosome 1
#40, 255, 1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008
#Polymorphic positions on chromosome 2
#216, 382, 485, 899, 997, 1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008

              SampleName="Sample 1"
              SampleSize=5
              SampleData= {
1_1    1      CC 000 499  502  499  5  2        AGTGA 100 501  501  503  10  16
1_2    1      CG 011 501  499  500  1  4        CCGCA 011 500  503  498  9  10
1_3    1      GG 000 498  501  500  6  2        ACGCG 001 498  500  498  6  10
1_4    1      GG 100 499  501  500  6  2        ACGCA 001 500  503  499  10  13
1_5    1      CG 000 500  499  501  2  4        ACGCA 001 498  504  498  8  8

}
```

One can also simulate several chromosomes with completely different structures, like:

1PopMultiLocusDiffChrom.par

```
//Number of population samples (demes)
1
//Population effective sizes (number of genes)
10000
//Sample sizes
5
//Growth rates      : negative growth implies population expansion
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new size, new growth rate, migr.
matrix
0  historical event0
//Number of independent loci [chromosome]
2 1
//Per chromosome: Number of linkage blocks
2
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 1000 0 2e-7 0.33
DNA 1000 0 1e-8 0.33
//Per chromosome: Number of linkage blocks
2
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
MICROSAT 3 0 0.0005 0 0
STANDARD 2 0 0.001
```

The difference with the previous example is that we explicitly tell *fsc27* to simulate 2 chromosomes with different structures by stating:

```
//Number of independent loci [chromosome]
2 1
```

The second number (1) indicates we want to describe different chromosomal structures. Now we need to repeat the block definition for the two structures, while a single block definition was used previously and simply repeated for simulating the two identical chromosomes.

The following output is now produced:

./1PopMultiLocusDiffChrom/1PopMultiLocusDiffChrom_1_1.arp

```
[Data]
       [[Samples]]

#Number of independent chromosomes: 2
#Polymorphic positions on chromosome 1
#85, 105, 169, 277, 372, 470, 629, 635, 702, 934, 960, 998, 1001, 1002, 1003
#Polymorphic positions on chromosome 2
#1, 2, 3, 4, 5

              SampleName="Sample 1"
              SampleSize=5
              SampleData= {
1_1    1      ACCGGCCCACTA 000     502  501  500  19  20
1_2    1      TTATTCGTATTG 111     504  500  501  21  25
1_3    1      ACCGGTCCACTA 000     503  500  501  22  23
1_4    1      TTATGCCTCTCG 111     501  499  506  23  24
1_5    1      TTATGCCTATTG 111     500  498  504  23  21

}
```

RECOMBINATION

Like *simcoal2*, *fsc27* can generate molecular diversity along recombining chromosomal segments, and recombination rates between adjacent loci are specified just after the definition of the data type and the number of loci of this type, like in the following example file

1PopDNArec.par

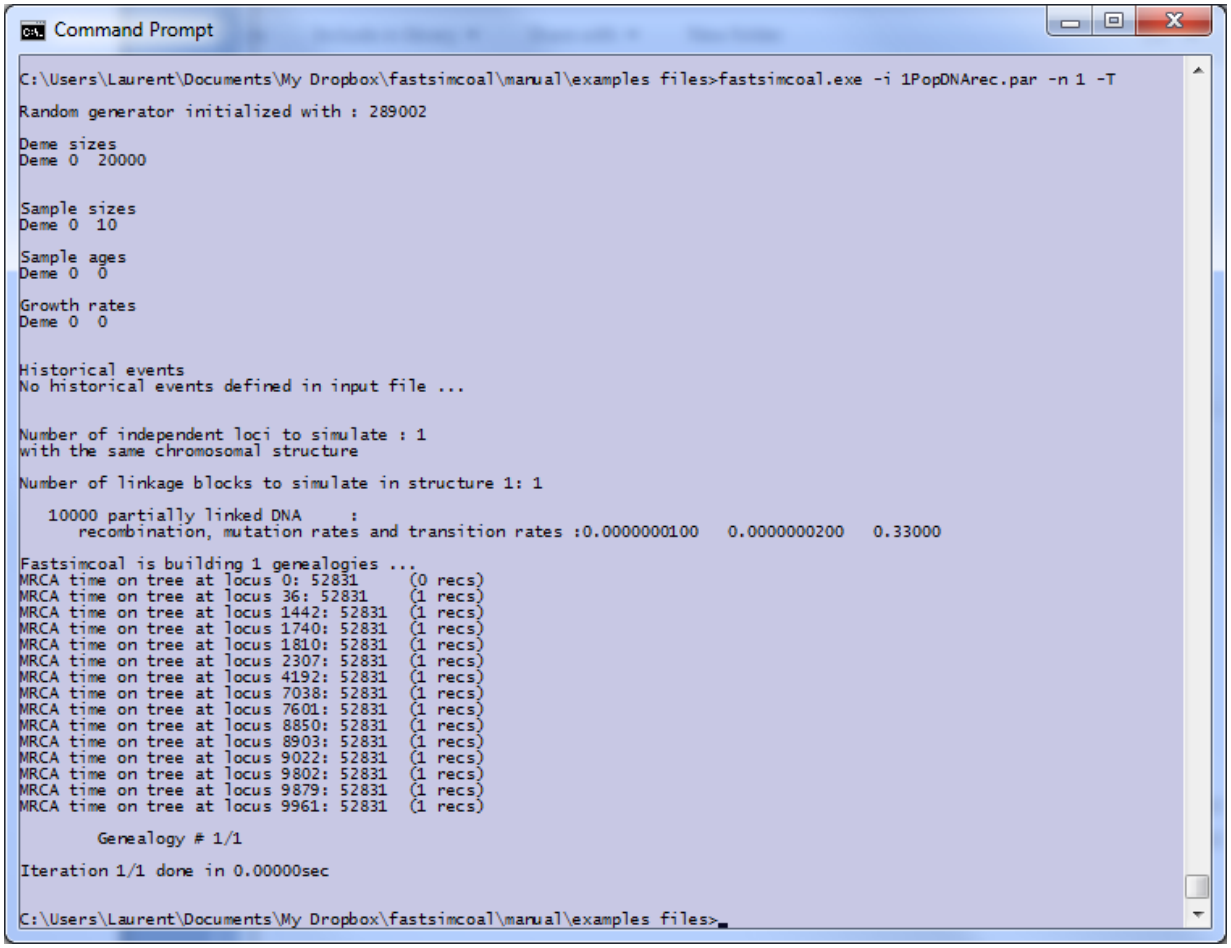
```
//Number of population samples (demes)
1
//Population effective sizes (number of genes)
20000
//Sample sizes
10
//Growth rates      : negative growth implies population expansion
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new size, new growth rate, migr.
matrix
0  historical event
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
1
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 10000 0.00000001 0.00000002 0.33
```

In this case, we want to simulate 10 DNA sequences of 10kb with a recombination rate of $r = 10^{-8}$ and mutation rate $\mu = 2 \times 10^{-8}$ per site per generation.

While the syntax to specify recombination is the same as in *simcoal2*, the underlying simulation model is completely different, as we now use a sequential Markov coalescent model (McVean and Cardin 2005) to simulate recombination. In brief, instead of using the classical ancestral recombination graph for the whole chromosomal segment, we generate a first tree on the left of the segment to be simulated. Then we compute where the next recombination event will occur on

the segment, implement one recombination event randomly on the tree, detach the recombining lineage from the left tree, and leave this recombining lineage evolve until it coalesces with one of the lineages on the left tree, potentially changing the topology and the height of the tree. See the Sequential Markov Coalescent section for more details.

In the console, *fsc27* lists the position of each recombination breakpoint and the MRCA of the resulting tree, as in:



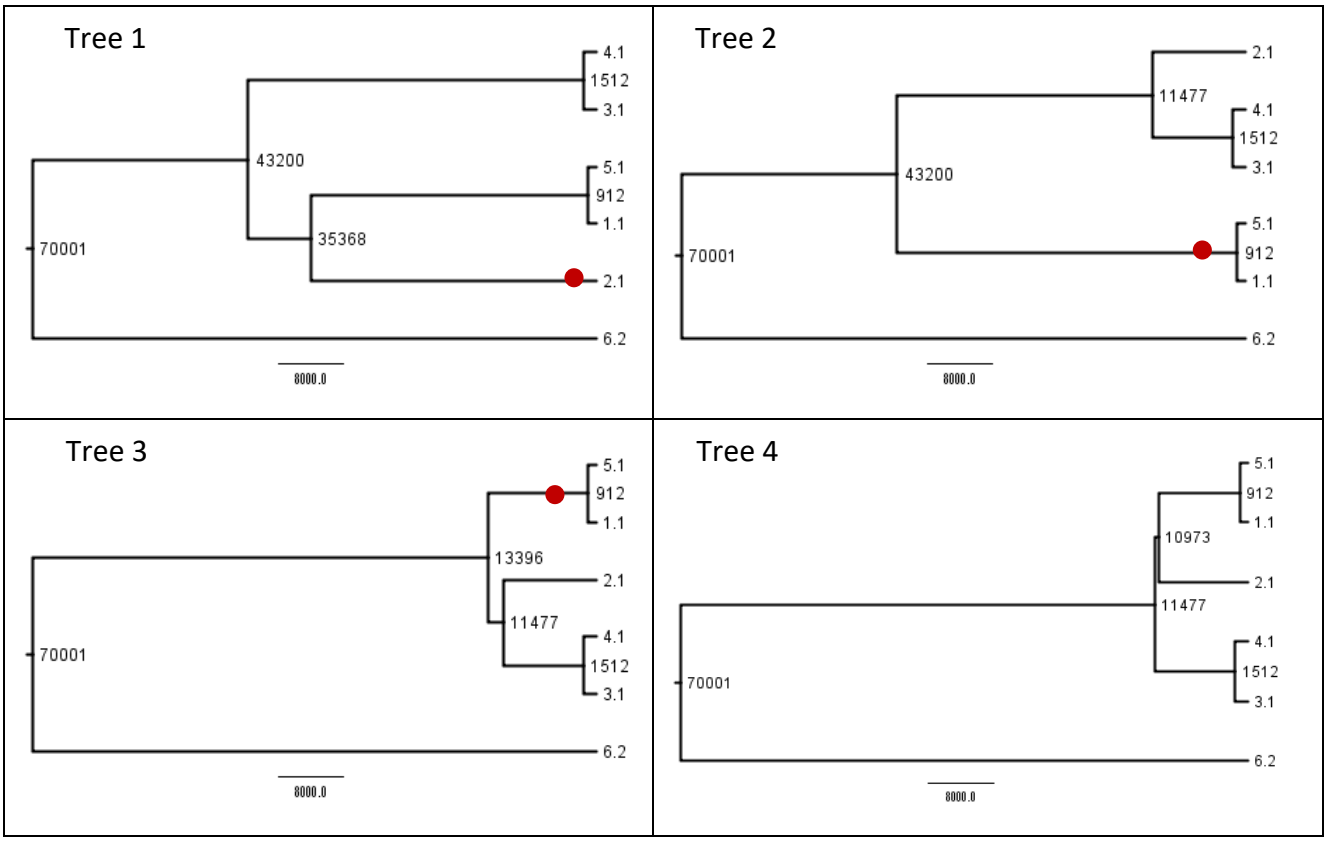
The produced *Arlequin* output file is similar to what would be obtained without recombination, but the nexus tree file now lists all trees produced along the chromosomal segments, as:

./1PopDNArec/1PopDNArec_1_true_trees.trees

```
#NEXUS
begin trees;  [Treefile generated by fastsimcoal.exe (Laurent Excoffier)]

       tree NumGen_tree_1_1_pos_0 = [&U] ((5.1:3556, (4.1:2838, 8.1:2838):718):49275,
((2.1:707, (6.1:384, 7.1:384):323):36435, ((3.1:3209, 9.1:3209):12690, (1.1:148,
10.1:148):15751):21243):15689);
       tree NumGen_tree_1_2_pos_36 = [&U] ((5.1:3556, (4.1:2838, 8.1:2838):718):49275,
((2.1:707, (6.1:384, 7.1:384):323):26269, ((3.1:3209, 9.1:3209):12690, (1.1:148,
10.1:148):15751):11077):25855);
       tree NumGen_tree_1_3_pos_1442 = [&U] ((5.1:3556, (4.1:2838,
8.1:2838):718):49275, (((3.1:3209, 9.1:3209):4915, (2.1:707, (6.1:384,
7.1:384):323):7417):18852, (1.1:148, 10.1:148):26828):25855);
       tree NumGen_tree_1_4_pos_1740 = [&U] ((5.1:3556, (4.1:2838,
8.1:2838):718):49275, (((3.1:3209, 9.1:3209):4915, (2.1:707, (6.1:384,
7.1:384):323):7417):25747, (1.1:148, 10.1:148):33723):18960);
...
Etc...
...
```

```
end;
```

Note that these trees can also all be browsed individually e.g. in *FigTree* to see the changes in topology produced by the recombination events. For instance, using different settings, here are the changes introduced by 3 recombination events having occurred in the subtree connecting nodes 1 to 5 (approximately located by red dots). Note that additional recombination events fell on other branches of the tree but did not lead to observable changes in tree topology.



INPUT FILE SYNTAX

The syntax of the input file is the same as in *simcoal2*, with a few exceptions.

The input file is divided into the following sections that are each time separated by a comment line. The order of these sections is fixed and cannot be changed:

- Number of populations samples
- Deme sizes
- Sample sizes, sampling times and inbreeding
- Growth rates
- Migration matrices
- Historical events
- Genetic information

NUMBER OF POPULATIONS SAMPLES

This section begins with a comment line. On the second line, the first item should be the number of samples (or demes) to simulate. Additional items on the second line are ignored.

```
//Number of population samples (demes)
1
```

or

```
//Number of population samples (demes)
2 samples to simulate
```

DEME SIZES AND SFS POOLS

This section begins with a comment line, and then has as many lines as demes to be simulated, as mentioned in the previous section.

So, if only one sample was defined previously:

```
//Population effective sizes (number of genes)
20000
```

or if, say, three samples were defined previously:

```
//Population effective sizes (number of genes)
20000
10000
100
```

Note that the deme size corresponds here to the number of genes present in a population, which would be the number of individuals for haploid species or two times the number of individuals for diploid species.

SFS POOLS

Since version 2.8, it is possible to assign different populations to different SFS pools, either when simulating SFS, or when estimating parameters from the SFS. This can be useful when individuals are sampled from populations that were structured in the past, or from a relatively broad geographic region including differentiated populations, but also when analyzing ancient genomes where one often faces a potential temporal heterogeneity as fossils from a given archeological site rarely have the same age, i.e. they are often separated by hundreds or thousands of years. The analysis of such heterogeneous samples is therefore equivalent to the analysis of samples drawn from a (very often unknown) structured population. With the new SFS explicit pooling, we can now propose a model for this structure and estimate the parameters of this structure, e.g. by specifying a given age for the samples or by specifying some divergence times between samples collected in a given geographic region.

In the following examples of a *tpl* file, we show how to define these sfs pools.

```
//Parameters for the coalescence simulation program: fastsimcoal2
6 samples to simulate:
//Population effective sizes (number of genes)
NPOP sfspool 0
NPOP sfspool 0
NPOP sfspool 1
NPOP sfspool 1
NCONT sfspool -1
NCONT sfspool -1
//Samples sizes and samples age
2 300
2 350
2 1000
2 1500
0
0
...
```

Population samples are thus assigned to different sfs pools by using the "*sfspool*" keyword, followed by a number specifying to which pool they should be assigned.

In the above example, the first two population samples are assigned to pool 0 and the next two are assigned to pool 1. The next two samples are assigned to pool -1, which tells fastsimcoal not to assign them to any pool, which makes sense since no chromosomes have been sampled in these populations.

Another advantage (and motivation) of this new syntax is the ability to build relatively complex models while keeping the dimensionality of the SFS relatively low. For instance, the SFS of 15 samples of one diploid individual each would have 3^{15} = 14,348,907 entries, whereas the SFS of 3 pools of 5 diploids would have only 11^3 = 1331 entries, which is much more manageable.

Note that fastsimcoal is now using sfs pools by default to build SFSs, but that the syntax is backward compatible so that previous input files not using the *sfspool* keyword will be interpreted by fastsimcoal as if each population sample would be assigned to a different pool.

Therefore, the old syntax

```
//Parameters for the coalescence simulation program: fastsimcoal2
6 samples to simulate:
//Population effective sizes (number of genes)
NPOP
NPOP
NPOP
NPOP
NCONT
NCONT
//Samples sizes and samples age
2 300
2 350
2
2
0
0
...
```

is equivalent to the following explicit syntax:

```
//Parameters for the coalescence simulation program: fastsimcoal2
6 samples to simulate:
//Population effective sizes (number of genes)
NPOP sfspool 0
NPOP sfspool 1
NPOP sfspool 2
NPOP sfspool 3
NCONT sfspool -1
NCONT sfspool -1
//Samples sizes and samples age
2 300
2 350
2
2
0
0
...
```

Hence, deme0 to 3 will be automatically assigned to pools 0, 1, 2 and 3, respectively. Since demes 4 and 5 do not contain any sample, they will not be assigned to any pool. Note however, that the *sfspool* keyword specification needs to be used for none or all demes, but that you cannot specify them for only a subset of demes.

Whereas the SFS pools simplify the SFS, one still needs to specify the demography of each deme. Here is a complete example of how to specify such demography:

```
//Parameters for the coalescence simulation program: fastsimcoal2
6 samples to simulate:
//Population effective sizes (number of genes)
NPOP sfspool 0
NPOP sfspool 0
NPOP sfspool 1
NPOP sfspool 1
NCONT sfspool -1
NCONT sfspool -1
//Samples sizes and samples age
2 300
2 350
2 1000
2 1500
0
0
//Growth rates
0
0
0
0
0
0
//Number of migration matrices: 0 implies no migration between demes
0
//historical events
5
TJOINCONT1 0 4 1 1 0 0
TJOINCONT1 1 4 1 1 0 0
TJOINCONT2 2 5 1 1 0 0
TJOINCONT2 3 5 1 1 0 0
TDIVCONT 4 5 1 20000 0 0 absoluteResize
//Number of independent loci [chromosome]
1 0
//Number of contiguous linkage blocks
1
// data type, no. of loci, per gen. rec. and mut. rates and optional parameters
FREQ  1   0   1.25e-8 OUTEXP
```

Historical events are used here to specify how populations relate to each other. In this simple example, one assumes that populations 0 and 1 join continent 4 at time *TJOINCONT*1 and populations 2 and 3 join continent 5 t time *TJOINCONT*2. Finally, the two continents join an ancestral population of haploid size 20,000 *TDIVCONT* generations ago. Note that the syntax of the historical event is based on the deme numbers and not the pool numbers.

Importantly, with SFS pool the entries of the observed SFs (in *.obs* files) follow the sfspool notation, whereas without sfspool the obs SFS indices follow the deme numbers.

SAMPLES SIZES, SAMPLING TIMES AND INBREEDING

This section begins with a comment line, and then lists, for as many samples as defined in the first section, the haploid size of the sample, the sampling time (the number of generations backward in time when the lineages were sampled), and the average inbreeding level of the samples. If the sampling time and the inbreeding coefficient are omitted, values of zero are assumed by default (present sampling and no inbreeding).

So the following input:

```
//Sample sizes
10
```

is equivalent to

```
//Sample sizes
10 0 0
```

Other possibilities are:

```
//Sample sizes
10 20
100 1000
```

for sampling 10 lineages at generation 20 and 100 lineages at generation 1000 for lineages in deme 1 and 2, respectively.

Also

```
//Sample sizes
5
30
0
```

shows that sample sizes of zero are possible, which just means that no genes were sampled in a given deme at time zero. This additional empty deme may be used in a given evolutionary scenario, for instance as a source (ghost) population for the currently sampled demes.

Finally

```
//Sample sizes
6 0 0.00625
2 1000
2 2000 0.125
```

shows that i) sampling times is compulsory (even if it is null) if inbreeding is specified (0.0625 in deme 0), ii) inbreeding coefficient is optional (assumed zero) when sampling time is specified (at generation 1000 in deme 1).

GROWTH RATE

This section lists the initial growth rates for all population samples.

So the following input:

```
//Growth rates    : negative growth implies population expansion
0
```

means that the sampled deme has a stationary population size. Note that growth rates are measured here backward in time, so that negative growth rates imply a forward population expansion. Generally, if the current population size is N_0, and the growth rate is i then the population size *t* generations ago is given by $N_t = N_0 \, \mathrm{e}^{rt}$.

In the following example, two populations are shrinking backward in time, implying that the sampled demes went through a recent population expansion

```
//Growth rates    : negative growth implies population expansion
-0.001
-0.025
```

Note that the growth rates can be modified at any time by the use of a historical event.

MIGRATION MATRICES

As usual, this section begins with a comment line, and is followed by a line with the number of migration matrices. If there is no migration between demes, then simply enter 0, like

```
//Number of migration matrices : 0 implies no migration between demes
0
```

If we assume that there are two demes connected by migration, then one could enter for instance the following two migration matrices:

```
//Number of migration matrices : 0 implies no migration between demes
2
//migration matrix
0.000 0.0005
0.0001 0.000
//migration matrix
0.000 0.000
0.000 0.000
```

Here the two migration matrices are put below each other separated by a comment line.

The first migration matrix is asymmetric, with deme 0 sending migrant **backward in time** at rate 0.0005 towards deme 1, while deme1 is sending migrants at a lower rate 0.0001 towards deme 0. Therefore, the non-diagonal entries $\{m_{ij}\}$ of each migration matrix represent the probability for any given lineage to move backward in time from deme *i* to deme *j*. The diagonal entries are ignored. In the above example, the second migration matrix implies an absence of migration. One can switch between migration matrices at any time by means of historical events. Note that by default, the first migration matrix is used at the current time and further back in time until a historical event changes the active migration matrix.

HISTORICAL EVENTS

This section begins with a comment line and is followed by a line specifying the number of historical events. Then, each historical event is defined on a different line. Each historical event is defined by 7 numbers:

1) Number of generations *t* before present at which the historical event happened.
2) Source deme (the first listed deme has index 0).
3) Sink deme.
4) Expected proportion of migrants to move from source to sink. Note that this proportion is not fixed, and that it also represents the probability for each lineage in the source deme to migrate to the sink deme.
5) New size for the **sink** deme, relative to its size at generation *t*.
6) New growth rate for the **sink** deme.
7) New migration matrix to be used further back in time.

In the following example, 2 historical events are defined.

```
//historical event: time, source, sink, migrants, new size, growth rate, migr. matrix
2 historical event
1000 0 0 0 1 0 1
10000 1 0 1 10 0 1
```

The first event occurred 1000 generation in the past, and just sets the active migration matrix to matrix 1, whereas matrix 0 had been active until then.

The second event, which occurred 10,000 generations ago, states that all lineages (*i.e.*, a proportion of 1) present in deme 1 will migrate to deme 0. At the same time, the size of deme 1 is increased by a factor 10 and we still use migration matrix 1.

If no historical events are necessary in your simulations just set the number to zero, as

```
//historical event: time, source, sink, migrants, new size, growth rate, migr. matrix
0 historical event
```

keep AND *nomig* KEYWORDS

Since version 2.5.2, it is possible to specify two additional historical events directives to *fastsimcoal*:

- *nomig*: if this keyword is added at the end of a historical event definition, migrations between demes are suppressed until the end of the current coalescent simulation. If next in line historical events were to specify the use of some new migration matrix, this would be ignored by fastsimcoal.
- *keep*: if instead of a given value for a **growth rate** or a **migration matrix** one uses the keyword keep, then the former values of these parameters will be used.

These directives are mostly useful in the context of parameter estimation (in *.tpl* files), when the exact timing of a historical event is determined by some parameter, so that the exact sequence of events is not known a priori. Note that ***nomig*** will prevail over `keep` for the migration matrix if both directives are used for the same historical event.

Example of the use of the ***nomig*** directive:

```
//historical event: time, source, sink, migrants, new size, growth rate, migr. matrix
2 historical event
1000 0 0 0 1 0 0 nomig  //This directives suppresses migrations between demes
10000 1 0 1 10 0 1      //there will be no migration between demes, even if some are
                          specified in migration matrix 1
```

Example of the use of the ***keep*** directive:

```
//historical event: time, source, sink, migrants, new size, growth rate, migr. matrix
3 historical event
200 0 0 0 1 -0.001 0
1000 0 0 0 1 keep 1  //Keep current growth rate (-0.001) (and change migration matrix
10000 1 0 1 1 0 keep //Keep migration matrix 1 (plus fuse deme 1 with deme 0, and
                       stop growth rate)
```

INSTANTANEOUS BOTTLENECKS

Since version 2.6, it is possible to implement **instantaneous bottlenecks** (for non-recombining data only), happening in a single generation. This simplifies considerably the modeling of bottlenecks, which previously required one to specify the bottleneck size and duration, which required the definition of two historical events. In the new implementation, one only needs to specify the bottleneck intensity, normally defined as t / N_{bot}, where *t* is the bottleneck duration and N_{bot} is the bottleneck size. Here since *t* is arbitrarily set to 1, it implies that the bottleneck size is 1/intensity. The new notation needs the keyword ***instbot*** at the end of the line, and to specify the bottleneck intensity instead of the sink new size, as in

```
//hist event: time, source, sink, migrants, bot intensity, growth rate, migr. mat
1 historical event
1000 0 0 0 1 0 0 instbot
```

where the intensity is set to 1, which implies a very strong bottleneck. Note that even though the duration of the bottleneck is of 1 generation, we set all coalescent events to happen exactly at the time of this instantaneous bottleneck. So we may have multiple coalescent at the same time, but we did not implement multiple mergers.

Note also that the size of the population after the bottleneck is assumed to be the same as before the bottleneck. An extra historical event would be needed to change this size to something else, as in

```
//hist event: time, source, sink, migs, bot intensity|new size, growth rate, migr mat
2 historical event
1000 0 0 0 1 0 0 instbot
1000 0 0 0 10 0 0
```

where, with the second historical event, the size of deme 0 is set at generation 1000 to something 10 times larger than it size before generation 1000. Note that the order of historical events is preserved so that the bottleneck will be first implement and the resize will occur afterwards (going backward in time).

CHANGING THE SIZE OF A POPULATION

Since version 2.7, you can also specify that a given sink population takes a new absolute size with the ***absoluteResize*** keyword. In the following example, deme 0 new size is directly set to 100:

```
//hist event: time, source, sink, migrants, bot intensity, growth rate, migr. mat
1 historical event
1000 0 0 0 100 0 0 absoluteResize
```

This new parameterization eliminates the need to use complex parameters to specify the resize necessary to reach a new population size.

KILLING A DEME

Since version 2.7, if you explicitly set a sink deme size to zero, this deme is considered dead (not a ghost), and *it is not accessible to migration anymore further back in time*. This option allows one to keep the same migration matrix after some populations have fused.

The following example file is using a single matrix and demes 1 and 2 are explicitly killed:

3Pop1MigrMat.par

```
//Parameters for the coalescence simulation program : fastsimcoal.exe
3 samples to simulate :
//Population effective sizes (number of genes)
20000
50000
10000
//Samples sizes and samples age
20
20
20
//Growth rates : negative growth implies population expansion
0
0
0
//Number of migration matrices : 0 implies no migration between demes
1
//Migration matrix 0
0.000 0.005 0.005
0.000 0.000 0.000
0.000 0.000 0.000
//historical event: time, source, sink, migrants, new deme size, new growth rate, migration
matrix index
4 historical event
2000 2 0 1 1 0 0
2000 2 2 0 0 0 0 //Deme 2 is killed
3000 1 0 1 1 0 0
3000 1 1 0 0 0 0 //Deme 1 is killed
//Number of independent loci [chromosome]
100000 0
//Per chromosome: Number of contiguous linkage Block: a block is a set of contiguous loci
1
```

```
//per Block:data type, no of loci, per gen recomb. and mut. rates and opt. params
DNA 100 0 2.5e-8 OUTEXP
```

However, not killing these demes explicitly, as in:

```
//Number of migration matrices : 0 implies no migration between demes
1
//Migration matrix 0
0.000 0.005 0.005
0.000 0.000 0.000
0.000 0.000 0.000
//historical event: time, source, sink, migrants, new deme size, new growth rate,
migration matrix index
2 historical event
2000 2 0 1 1 0 0
3000 1 0 1 1 0 0
```

This leads to error messages, as in:

```
# ./fsc270.exe -i 3Pop1MigrMatBad.par -n1 -I -x -d -m -q --seed 1234 -s0 -c12 --multiSFS
fastsimcoal was invoked with the following command line arguments:
D:\Users\Laurent\Dropbox\fastsimcoal\paper Bioinformatics fsc2\testFiles\fsc270.exe -i 3Pop1MigrMatBad.par -n1 -I -x -d -m -q --seed 1234 -s0 -c12 --multiSFS

Random generator seed : 1234

No population growth detected in input file

fastsimcoal2 is building 100000 genealogies ...

Simulating 100000 independent chromosomes using 12 batches and 12 threads.

Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 0
Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 41665
Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 33332
Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 49998
Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 16666
Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 24999
Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 91663
Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 66664
Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 8333
Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 74997
Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 58331
Simulations did not converge. MRCA not found! (2 lineages remaining)
Unable to build coalescent tree for chromosome 83330

Program total execution time: 0.561 seconds
```

Note that the file parameter file *3Pop1MigrMat.par* shown above would be equivalent to a file without deme killing where three matrices are explicitly provided and migration matrices are changed after each deme fusion, as in:

3Pop3MigrMat.par

```
//Parameters for the coalescence simulation program : fastsimcoal.exe
3 samples to simulate :
//Population effective sizes (number of genes)
20000
50000
10000
//Samples sizes and samples age
20
20
20
//Growth rates : negative growth implies population expansion
0
0
0
//Number of migration matrices : 0 implies no migration between demes
3
```

```
//Migration matrix 0
0.000 0.005 0.005
0.000 0.000 0.000
0.000 0.000 0.000
//Migration matrix 1
0.000 0.005 0.000
0.000 0.000 0.000
0.000 0.000 0.000
//Migration matrix 2
0.000 0.000 0.000
0.000 0.000 0.000
0.000 0.000 0.000
//historical event: time, source, sink, migrants, new deme size, new growth rate, migration
matrix index
2 historical event
2000 2 0 1 1 0 1
3000 1 0 1 1 0 2//Number of independent loci [chromosome]
100000 0
//Per chromosome: Number of contiguous linkage Block: a block is a set of contiguous loci
1
//per Block:data type, no of loci, per gen recomb. and mut. rates and opt. params
DNA 100 0 2.5e-8 OUTEXP
```

Chromosome painting: Recording the origin of a chromosome segment after admixture with the recordAdmOrigin KEYWORD

It is now possible to simulate chromosome painting by using the keyword *recordAdmOrigin* when implementing a population admixture with historical events. For instance, if a given population is the product of the hybridization of two parental populations, we can record the origin of chromosome segments (assuming we simulated DNA chromosomes with recombination). To do that, we can use the following historical events:

```
2 historical event
100 2 0 0.2 1 0 0 recordAdmOrigin
100 2 1 1   1 0 0 recordAdmOrigin
```

The origin of the chromosomes will be then output in a file with the extension *.adm*, looking like:

*.adm

beg	a1	a2	a3	a4	a5	a6	a7	a8	a9	a10	a11	a12	a13	a14
0	1	1	0	1	1	1	1	1	0	1	1	0	1	0
326907	1	1	0	1	1	1	1	1	0	1	0	0	1	0
1044589	1	1	0	1	1	1	0	1	0	1	0	0	1	0
1790342	1	1	0	1	1	1	0	1	1	1	0	0	1	0
1903874	1	1	0	1	1	1	0	1	1	1	0	0	1	0
2004810	1	1	1	1	1	1	0	1	1	1	0	0	1	0
2521280	1	1	1	1	1	1	0	1	1	1	0	0	1	0
...														

Here we see for instance that the 3rd admixed chromosome (a3) has an initial segment originating from deme 0 , followed by a new segment originating from deme 1 starting at position 2004810. Contrastingly, chromosome a7 has an initial segment originating from deme 1, followed by a segment from deme 0 starting at position 1044589.

Note that we can simulate more than two possible origins for a given population, like for 3 which is a mixture of 3 demes:

```
3 historical event
100 3 0 0.2 1 0 0 recordAdmOrigin
100 3 1 0.1 1 0 0 recordAdmOrigin
100 3 2 1   1 0 0 recordAdmOrigin
```

GENETIC SETTINGS: CHROMOSOMES, BLOCKS, DATA TYPES, MUTATION, AND RECOMBINATION

GENETIC SETTINGS SUBSECTIONS

The genetic information section has 3 subsections:

1) The number of independent **chromosomes** to be simulated and a flag (0, 1) indicating if the different chromosomes have a different (1) or a similar (0) structure. These chromosomes are assumed to be completely unlinked. If the chromosomes have a different structure, the sub-sections 29 and 3) need to be repeated for each chromosome structure.
2) The number of **blocks** to be simulated per chromosome. By block we mean segments of chromosomes that may differ by the type of markers to be simulated, the recombination rate, or the mutation rate. Two consecutive blocks may be of the same type but just differ by the recombination rate, such as for instance simulate a recombination hot spot. Note that the recombination rate between two blocks is that specified in the first block, which is thus valid both within and between blocks.
3) The properties of genetic data to be simulated per block. In each block the following properties need to be specified, in this order:
   i) **Data type**: DNA, MICROSAT, or STANDARD
   ii) Number of markers with this data type to be simulated. For DNA, this is the sequence length.
   iii) Recombination rate between adjacent markers (between adjacent nucleotides for DNA).
   iv)-vii) Additional data type-specific properties, like the mutation rate per bp and the transition bias for DNA (see list below).

In the example below, we define genetic data to be simulated in two different types of chromosomes:

```
//Number of independent loci [chromosome]
2 1
//Per chromosome: Number of linkage blocks
2
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 1000 0 2e-7 0.33
DNA 1000 0 1e-8 0.33
//Per chromosome: Number of linkage blocks
2
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
MICROSAT 3 0 0.0005 0 0
STANDARD 2 0 0.001
```

On the first type of chromosome, we simulate two DNA sequences of 1000 bp with different mutation rates. On the second type of chromosome, we simulate 3 microsatellites under a pure stepwise mutation model and 2 multi-allelic loci under an infinite-allele model. On each chromosome, all markers are fully linked as we do not assume any recombination.

In the next example, we want to simulate two chromosomes with the same structure, and we indicate this with the 0 flag after the number of independent chromosomes to simulate.

```
//Number of independent loci [chromosome]
2 0
//Per chromosome: Number of linkage blocks
2
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 1000 0 2e-7 0.33
DNA 1000 0 1e-8 0.33
```

Note that if a sequence of DNA is found monomorphic in a given population sample (due to a too short tree or a too small mutation rate), a question mark '`?`' will be output for each individual sequence.

SPECIFIC PARAMETERS FOR DIFFERENT DATA TYPES

The following optional parameters are required for the different types of markers.

	Extra parameters		
Data types[1]	**4th parameter**	**5th parameter**	**6th parameter**
DNA[2]	Mutation rate per bp	Transition rate (fraction of substitutions that are transitions). A value of 0.33 implies no transition bias.	
MICROSAT	Mutation rate per locus.	Value of the geometric parameter for a Generalized Stepwise Mutation (GSM) model. This value represents the proportion of mutations that will change the allele size by more than one step. Values between 0 and 1 are required. A value of 0 is for a strict Stepwise Mutation Model (SMM).	Range constraint (number of different alleles allowed). A value of 0 means no range constraint
STANDARD	Mutation rate per marker. An infinite allele model is assumed.		
FREQ	OUTEXP Output of expected site frequency spectrum for estimated parameters of the model		

[1] Note that since ver. 2.7, SNP data type is not supported anymore.

[2] Note that by default, *fsc27* uses a finite site model, implying that short DNA sequences simulated with a high mutation rate can be the target of multiple hits. The command-line option **`-I`** ensures that an infinite-site model is used.

EXAMPLES:

1. 1 Mb DNA sequence with recombination rate of 1×10^{-8} and mutation rate of 2×10^{-8}, no transition bias

```
DNA 1000000 1E-8 2E-8 0.33
```

2. 10 kb DNA sequence without recombination and mutation rate of 2×10^{-6}, high transition bias

```
DNA 10000 0 2E-6 0.95
```

3. 20 MICROSAT with recombination rate of 1×10^{-5} between adjacent markers, a mutation rate of 5×10^{-4} per marker, a geometric GSM parameter of 0.1, and a maximum allele size range of 30.

```
MICROSAT 20 0.00001 5E-4 0.1 30
```

Note that with such a high recombination rate, more than 1 recombination event can occur between adjacent markers, which is adequately taken into account by our modified serial Markov coalescent model (see the description of the SMC' below).

4. 20 STANDARD fully linked markers with a mutation rate of 5×10^{-6} per marker.

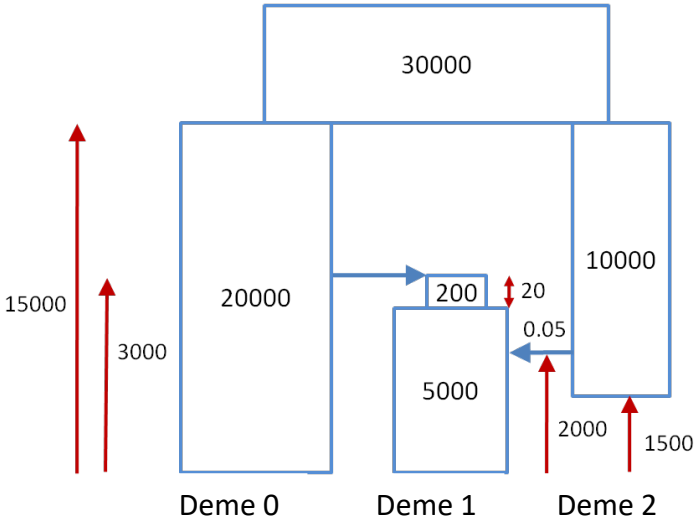
```
STANDARD 20 0 0.000005
```

Note finally that a given chromosome can be simulated for different types of markers, e.g. with a mixture of DNA sequences and MICROSAT markers:

```
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
3
//per Block: data type, num loci, rec. rate + optional parameters

DNA 1000 0 2e-7 0.33
MICROSAT 1 2E-7 5E-4 0 0
DNA 1000 0 1e-8 0.33
```

A RELATIVELY COMPLEX EXAMPLE WITH 3 POPULATIONS, SERIAL SAMPLING, BOTTLENECK, AND INTROGRESSION

Let's assume we are interested in the following relatively complex demographic scenario involving 3 demes (populations).



This scenario assumes that genes from deme 2 were sampled 1500 generations ago, and that 5% of the genes present in deme 1 2000 generations ago actually came from deme 2 (which implies an introgression or admixture event). It also assumes that deme 1 originated from deme 0 3000 generations ago and went through a bottleneck size of 200 (haploid) individuals during 20 generations before recovering a size of 5000 (haploid) individuals. Finally, deme 0 and deme 2 diverged 15000 generations ago from an ancestral population of size 30000, to form two populations of size 20000 and 10000 corresponding to deme 0 and deme 2, respectively.

We can simulate the evolution of 6 10Mb DNA sequences for deme2, and 20 10Mb sequences in both deme 0 and deme 1 under this scenario with the following parameter file *3popDNASFS.par*:

3popDNASFS.par

```
//Number of population samples (demes)
3
//Population effective sizes (number of genes)
20000
5000
10000
//Sample sizes
20
20
6 1500
//Growth rates: negative growth implies population expansion
0
0
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new size, new growth rate, migr. matrix
4  historical event
2000 1 2 0.05 1 0 0
2980 1 1 0 0.04 0 0
3000 1 0 1 1 0 0
15000 0 2 1 3 0 0
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
1
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 10000000 0.00000001 0.00000002 0.33
```

6. SAMPLING PARAMETER VALUES FROM SOME PRIOR DISTRIBUTIONS OR RANGES

When used as the simulation program for ABC estimation, *fsc27* has a built-in procedure to sample parameters from prior distributions. The principle is very much the same as that implemented in *ABCToolBox* (Wegmann et al. 2010), using a template file (**.tpl*) where parameters to be sampled are input as keyword, and an estimation file (**.est*) where parameter distributions are fully specified. Such a way to simulate data is invoked e.g. with the following command line arguments

```
fsc27 -t 1popDNArand.tpl -n10 -e 1popDNArand.est -E 1000
```

which tells *fsc27* to use the template file *1popDNArand.tpl* and the estimation *1popDNArand.est* to generate 10 simulations for each of the 1000 sets of randomly drawn parameter values.

Note that when *fsc27* is used to estimate parameters from some observed SFS, the intervals defined in the *.est* files are not considered as priors but as search ranges.

TEMPLATE FILE

An example template file reads as follows:

1popDNArand.tpl

```
//Number of population samples (demes)
1
//Population effective sizes (number of genes)
NPOP
//Sample sizes
10
//Growth rates      : negative growth implies population expansion
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new size, new growth rate, migr.
matrix
1  historical event
TEXP 0 0 0 RESIZE 0 0
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
1
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 10000 0.00000 MUTRATE 0.33
```

As can be seen above, a template file has exactly the same structure as a parameter file, but some keywords (NPOP, TEXP, RESIZE, and MUTRATE) are present instead of actual parameter values.

Those keywords will be substituted with actual parameter values by *fsc27,* if the distributions of these parameters are found in the estimation file.

CAUTION

Keyword for parameter need to be a *unique* string of characters. By unique, we mean that a keyword cannot be part of another keyword. Note also that *fsc27* is not case sensitive, and therefore NPOP and nPop are identical for *fsc27*. So, for instance, NPOP and NPOP1 are incompatible as NPOP is included into NPOP1, and if NPOP is defined before NPOP1 in the *.est* file, the value of NPOP (say 100) will also be inserted into NPOP1, resulting of a value of 1001 for NPOP1. Such problems are difficult to spot, and often go un-noticed and result in bad estimations. It

has been suggested by some users that parameter keywords could be ended by a "$" sign, which is a good idea. In that case NPOP and NPOP1 would become NPOP$ and NPOP1$, which are now compatible. Any other ending character (other than "\") would be okay, as well.

ESTIMATION FILE

An estimation file matching the template file *1popDNArand.tpl* would look as follows

1popDNArand.est

```
// Priors and rules file
// *********************

[PARAMETERS]
//#isInt? #name  #dist. #min   #max
//all N are in number of haploid individuals
1  NPOP     logunif     100  100000   output bounded
1  TEXP     logunif     100  5000     output
0  RESIZE   logunif     1e-3   1000   output
0  MUTRATE  unif        1e-7   1e-9   output

[COMPLEX PARAMETERS]

1  ANCSIZE  =  NPOP*RESIZE    output
0  2N       =  2*NPOP         hide
0  THETA    =  2N*MUTRATE     output
```

The estimation file is divided into two main sections

1. The [**PARAMETERS**] section
2. The [**COMPLEX PARAMETERS**] section

Note that since ver 2.7, the former [**RULES**] section has been removed.

PARAMETERS SECTION

The [**PARAMETERS**] section lists the prior distributions of simple parameters.
Each parameter can be an *integer* or a *float*, as specified by a first indicator variable.
Each parameter can either be uniformly or log-uniformly distributed between a minimum and a maximum value that need to be specified.

Since ver. 2.7, it is possible to include the name of a previously defined simple parameter as a lower and/or upper bound for the search range.

COMPLEX PARAMETERS SECTION

The [**COMPLEX PARAMETERS**] lists parameters that are obtained as simple operations between any 2 simple or complex parameters or between a parameter and a scalar.
The following simple operations are possible between two parameters, or between a parameter and a scalar:

"+", "-", "*", and "/"

Since ver. 2.5.2, the following functions are possible on a single parameter or on a scalar:

log(), log10(), exp(), abs(), pow10()

Since ver. 2.5.2, the following functions are possible on a single parameter or between a parameter and a scalar:

%min%, %max%

Since ver 2.7, the following function can also be used:

<*condition*> ? <*if true*>: <*if false*>

ADDITIONAL SYNTAX

As seen in the example `1popDNArand.est` above, comments can be included in the *.est* file as double slash, as in C++.

Simple or complex parameters can then be output or hidden in output files by using the keywords "output" or "hide", respectively.

The **%min%** and **%max%** functions are a bit special here as they should be used like **operators**.

Example:

```
[PARAMETERS]
1 N1  100    100000     output
1 N2  100    100000     output

[COMPLEX PARAMETERS]
1  NMAX       = N1 %max% N2    output
```

The function **<condition> ? <if true>: <if false>** also requires some explanations, and it can be easily understood by looking at the following example, where *MigrMat* is set to 1 if *TDIV1>TDIV2* and to 2 otherwise.

```
[PARAMETERS]
1 TDIV1  100    100000     output
1 TDIV2  100    100000     output

[COMPLEX PARAMETERS]
1  MigrMat       = TDIV1>TDIV2 ? 1 : 2    hide
```

This can be useful if patterns of migrations might differ depending on which population separates first from an ancestral population.

Valid tested conditions are:

- *X>Y* *(X strictly largen than Y)*
- *X<Y* *(Xstrictly smaller than Y)*
- *X!=Y* *(X not equal to Y)*

Note that the equality condition *X==Y* is not implemented as it is equivalent to *X!=Y* with an inversion of the result of the tested condition.

BOUNDED PARAMETERS

Since version 2.5.2, one can also specify that the range of a simple parameter is bounded (during parameter estimation) by using the keyword "***bounded***" after keywords ***output*** or ***hide***. Note that

this option only applies to est files used for parameter estimation and not when simply generating random samples (as in ABC) where ranges are bounded prior ranges.

Example:

```
[PARAMETERS]
0 MIGR_RATE  0    0.1     output    bounded
```

This will prevent the `MIGR_RATE` parameter to grow beyond 0.1

PARAMETERS WITH A RANGE DEPENDING ON OTHER PARAMETERS

Since ver 2.7, it is possible to specify that a given parameter is bounded by other parameters, which need to have been defined previously. Note that one needs to add the additional keyword ***paramInRange*** to tell *fsc* that these parameters have a variable boundary depending on another parameter. Note also that such parameters are automatically bounded upwards.

Example:

```
[PARAMETERS]
1 TDIV1  100    100000     output
1 TDIV2  TDIV1  100000     output paramInRange
1 TBOT   TDIV1  TDIV2      output paramInRange
```

In this example, the time TBOT of a bottleneck is set to occur between two divergence times TDIV1 and TDIV2, and additionally TDIV2 is declared as being larger than TDIV1. It shows that this new syntax eliminates the need to define rules as in previous versions.

This new syntax also considerably simplifies the specification of complex scenarios and makes input files much more readable. For instance, the same parameters would have been defined previously by using the following simple and complex parameters

Example 2:

```
[PARAMETERS]
1 TDIV1  100    100000     output
1 TPLUS  1      100000     hide
0 relTB  0      1          hide bounded

[COMPLEX PARAMETERS]
1 TDIV2    = TDIV1 + TPLUS    output
1 DiffTDIV = TDIV2 - TDIV1    hide
1 TdiffBot = DiffTDIV* relTB  hide
1 TBOT     = TDIV1 + TdiffBot output
```

PARAMETER NAMING CAUTION

Equations of complex parameters should not include any variable that contain the name of a function, i.e. log, log10, abs, pow10, min, or max (all in small caps). To avoid this problem, **it might be better to use capital letters for parameter names**.

For instance, the following complex parameter NPOP1 will be incorrectly estimated

```
0  Nlog    = log(N2)    output
1  NPOP1   = exp(Nlog)  output   //Wrong: Nlog conflicts with log function
```

But it will be correctly estimated in this case

```
0  NLOG    = log(N2)    output
1  NPOP1   = exp(NLOG)  output
```

As already mentioned for *.tpl* files, it might be good to use a special character at the end of a parameter name, like a $ sign, as in

```
0  NPOP$    10 100000    output
1  NPOP2$   10 100000    output
```

In this way, the parameters NPOP$ and NPOP2$ are clearly different from each other.

OUTPUT OF SAMPLED PARAMETERS

The sampled parameters are then output in a file having the same name as the template *.tpl* file, but with the *.params* extension. Typing the following command-line

```
fsc27 -t 1popDNArand.tpl -n 10 -e 1popDNArand.est -E10
```

will draw 10 sets of parameters according to the distributions found in file *1popDNArand.est*. It will then replace the corresponding keywords found in *1popDNArand.tpl* by the values just drawn, and write a *.par* file in the output directory as well as the randomly drawn values in the *.params* file, which should look like:

./1popDNArand/1popDNArand.params

NPOP	TEXP	RESIZE	MUTRATE	ANCSIZE	THETA
299	396	4.1771490	9.76393e-08	1248	5.83883e-05
202	561	28.9307465	9.35616e-09	5844	3.77989e-06
4311	1062	0.0630165	8.50824e-08	271	7.33581e-04
30824	580	0.0046161	7.65865e-08	142	0.0047214
669	188	46.5000659	7.59988e-08	31108	1.01686e-04
118	2132	63.0435917	9.61273e-08	7439	2.26861e-05
49854	722	28.3119282	2.93767e-08	1411462	0.0029291
45727	2870	2.7773714	3.18337e-08	127000	0.0029113
9719	575	15.5669955	6.23843e-08	151295	0.0012126
2021	888	0.0652933	1.12363e-08	131	4.54173e-05

Note that the complex parameter 2N was not output here as it was tagged has hidden in the *.est* file.

Also, the command line "*fsc27 -t 1popDNArand.tpl -n 10 -e 1popDNArand.est -E10*" will generate a total of 100 *.arp* file (10 simulations for each of the 10 sets of randomly drawn parameters), to be found in the directory *./1popDNArand*.

USING PREDEFINED VALUES FOR A PARTICULAR EVOLUTIONARY MODEL

fsc27 allows you to simulate data under a given evolutionary scenario with fixed and predefined values of the model parameters. This is an alternative to the generation of random parameter values seen just above, and it uses pretty much the same syntax on the command line.

For instance,

```
fsc27 -t 1popDNArand.tpl -n 5 -f 1popDNA.def
```

will replace the keywords found in the *.tpl* file by the values listed in the definition file `1popDNA.def` , and it will perform five simulation for each set of defined values. Note that the *.tpl* file mentioned above was used in the previous section, and we show below the content and structure of a definition file.

DEFINITION FILE

A *.def* file needs to have a first header line listing the **keywords** associated to each parameter. The keywords present in the template *.tpl* file must **all** be listed in the *.def* file, but there can be more keywords listed in the *.def* file than those listed in the *.tpl* file. These additional parameters will simply not be used.

The following lines then list the values of each parameter. For each parameter set, a *.par* file will be created by replacing the keywords in the *.tpl* file by the corresponding parameter values. In the example below, we list just 10 sets of parameter values.

1popDNA.def

NPOP	TEXP	RESIZE	MUTRATE	ANCSIZE	THETA
299	396	4.1771490	9.76393e-08	1248	5.83883e-05
202	561	28.9307465	9.35616e-09	5844	3.77989e-06
4311	1062	0.0630165	8.50824e-08	271	7.33581e-04
30824	580	0.0046161	7.65865e-08	142	0.0047214
669	188	46.5000659	7.59988e-08	31108	1.01686e-04
118	2132	63.0435917	9.61273e-08	7439	2.26861e-05
49854	722	28.3119282	2.93767e-08	1411462	0.0029291
45727	2870	2.7773714	3.18337e-08	127000	0.0029113
9719	575	15.5669955	6.23843e-08	151295	0.0012126
2021	888	0.0652933	1.12363e-08	131	4.54173e-05

Note that this *.def* file corresponds to the *params* file we had just generated from the *.est* file in the previous section.

The output files (*.par* and *.arp* files) will be placed in a directory with the same name as the *.tpl* file but without the *.tpl* extension.

7. ESTIMATING PARAMETERS FROM THE SITE FREQUENCY SPECTRUM

fsc27 implements a new way to estimate demographic parameters from the site frequency spectrum (SFS) computed from DNA sequence data or from ascertained SNP chips (see methodological section "Estimation of demographic parameters from the SFS"). In short, *fsc27* simulates the expected SFS under a given set of parameters and computes their (composite) likelihood. It uses a robust maximization procedure to find those parameters maximizing the composite likelihood.

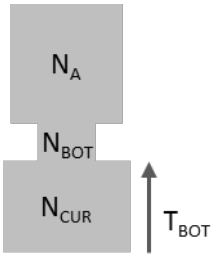
In order to do this, you need the following three input files:

1. A file or a series of files containing the **observed (joint) SFS**.
2. **A template file** (***.tpl***, see Section on "Sampling parameter values from some prior distributions") specifying the evolutionary model to be studied, with a similar structure as conventional *fsc27* parameter (*.par*) files. In this file, the parameters to be estimated are simply replaced by keywords.
3. **An estimation file** (***.est***, see chapter "Sampling parameter values from some prior distributions"), which specifies the list of the search ranges for those parameters defined by their keywords corresponding to those listed in the *.tpl* file.

NOTE:When estimating demographic parameters from the SFS, all estimates are scaled relative to a fixed mutation rate (or to a fixed effective size), and it is therefore not possible to infer simultaneously the effective sizes and the mutation rates.

EXAMPLE OF THE ESTIMATION OF A BOTTLENECK DEMOGRAPHIC HISTORY

Let's have a look at a simple example, where we want to estimate the parameters of a model where a population went through a recent bottleneck as shown in the figure below:



Note that more complex examples are provided in the Appendix section "Example files for the estimation of demography from the (joint) SFS".

OBSERVED SFS

Assume that we have the following observed SFS collected from 20Mb of DNA

1PopBot20Mb_DAFpop0.obs

```
1 observations
d0_0      d0_1  d0_2  d0_3  d0_4  d0_5  d0_6  d0_7  d0_8  d0_9  d0_10 d0_11 d0_12 d0_13 d0_14 d0_15 d0_16 d0_17 d0_18 d0_19 d0_20
19960052  9331  3572  2530  2221  2059  1963  1952  1730  1682  1572  1520  1426  1453  1335  1179  1195  1069  1129  1030  0
```

TEMPLATE FILE

Let's now define the following template file that describes the demographic model and the parameters of interests, which is similar to a *.par* file, but where parameters are replaced by keywords.

1PopBot20Mb.tpl

```
//Number of population samples (demes)
1
//Population effective sizes (number of genes)
NCUR
//Sample sizes
20
//Growth rates : negative growth implies population expansion
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new size, new growth rate, migr. matrix
2 historical event
TBOT 0 0 0 RESBOT 0 0
TENDBOT 0 0 0 RESENDBOT 0 0
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
1
//per Block: data type, num loci, rec. rate and mut rate + optional parameters

FREQ 1 0 2.5e-8
```

Here note the FREQ data type, telling *fsc27* that we want to estimate site **frequency** spectra with simulations.

ESTIMATION FILE

The search range of the different parameters to estimate are defined in the following *.est* file:

1PopBot20Mb.est

```
// Priors and rules file
// *********************
[PARAMETERS]
//#isInt? #name #dist.#min #max
//all Ns are in number of haploid individuals
1 NCUR unif 10 100000 output
1 NANC unif 10 100000 output
1 NBOT unif 10 100000 output
1 TBOT unif 10 10000 output

[COMPLEX PARAMETERS]
0 RESBOT = NBOT/NCUR hide
0 RESENDBOT = NANC/NBOT hide
1 TENDBOT = TBOT+100 hide
```

IMPORTANT NOTES

Even though the syntax of these *.est* files is the same than that used when Sampling parameter values from prior distributions (see section on " Estimation file" above), some important differences exist:

1. The parameter range and distributions are not priors but rather search ranges.
2. The lower range limit is an absolute minimum, whereas the upper range is only used as a maximum for choosing a random initial value for this parameter. There is actually no upper limit to the search range, as this limit can grow by 30% after each cycle if we have reached the predefined upper bound. Note that this growth can be prevented by using the ***bounded*** keyword.

REFERENCE PARAMETER DURING PARAMETER OPTIMIZATION

Since version 2.6, one can specify that a given parameter is a reference parameter, by adding the "***reference***" keyword at the end of the line, as in

```
[PARAMETERS]
0 NANC  1e3    1e6     output bounded reference
```

This keyword will be used in conjunction of the ***–l*** xxx command line option, which specifies that during xxx initial ECM cycles, both monomorphic and polymorphic sites will be used to compute the likelihood. After these xxx initial cycles, the likelihood will only be computed (and maximized) based on the polymorphic sites, using the currently estimated reference parameter as fixed, to scale all other parameters. The choice of this (unique) reference parameter is thus important and should be one that affects the total number of polymorphic sites, like the size of an ancestral population, or a divergence time.

PARAMETER RESCALING

At the end of the computations, if one is using an *xxx* value of the ***-l*** parameter that is different from the *yyy* value of the ***-L*** parameter, a scaling parameters *r* defined as $r = S_{obs} / (\mu T_{tot})$ is then computed and output in the maxlhood result file. Here, S_{obs} is the total number of polymorphic sites, and T_{tot} is the estimated average total length of the genealogy under the maximum-likelihood parameter values. This *r* parameter should then be used to rescale population sizes *N* and divergence times *T* as *rN* and *rT*, and migration rates as *m/r* (such that *Nm* products remain unchanged). Note that admixture rates should not be rescaled.

COMMAND LINE

Then parameter estimation can be simply initiated by launching the following command line:

```
./ fsc27 -t 1PopBot20Mb.tpl -n 100000 -d -e 1PopBot20Mb.est -M -L 40 -q
```

This command line tells *fsc27* that the model is defined in the file *1PopBot20Mb.tpl* (***-t***), that the search range of the parameters to be estimated are in the file *1PopBot20Mb.est* (***-e***), that (***-n***) 100,000 simulations need to be done to estimate the expected derived (***-d***) SFS, that (***-L***) 40 ECM cycles, respectively, will be performed for estimating the parameters (***-M***), and that a minimum console output (quiet mode ***-q***) is required.

Since ver 2.5.2.8, it is possible to write the command line in a file called "**fsc_run.txt**". If this file is present in the current working directory, and if *fsc27* is launched without any argument, the command line found in the file "fsc_run.txt" will be executed.

Note that command line notation for *fsc27* has changed as compared to previous versions. The main changes are:

- ***-M*** option is only need to specify that parameter estimation my Maximum likelihood needs to be performed. It does not need any additional parameter.
- ***-l*** option is now optional. It used to specify a minimum number of cycles to be performed. Now we do a **fixed number of cycles** specified with option ***-L***. However, it can be specified in connection with the use of the **reference** keyword in *.est* files, such as to specify the number

of ECM cycles for which the likelihood of the model will be evaluated on both monomorphic and polymorphic sites. In absence of this ***–l*** option, the likelihood is estimated on both monomorphic and polymorphic sites unless the ***-0*** option is used.

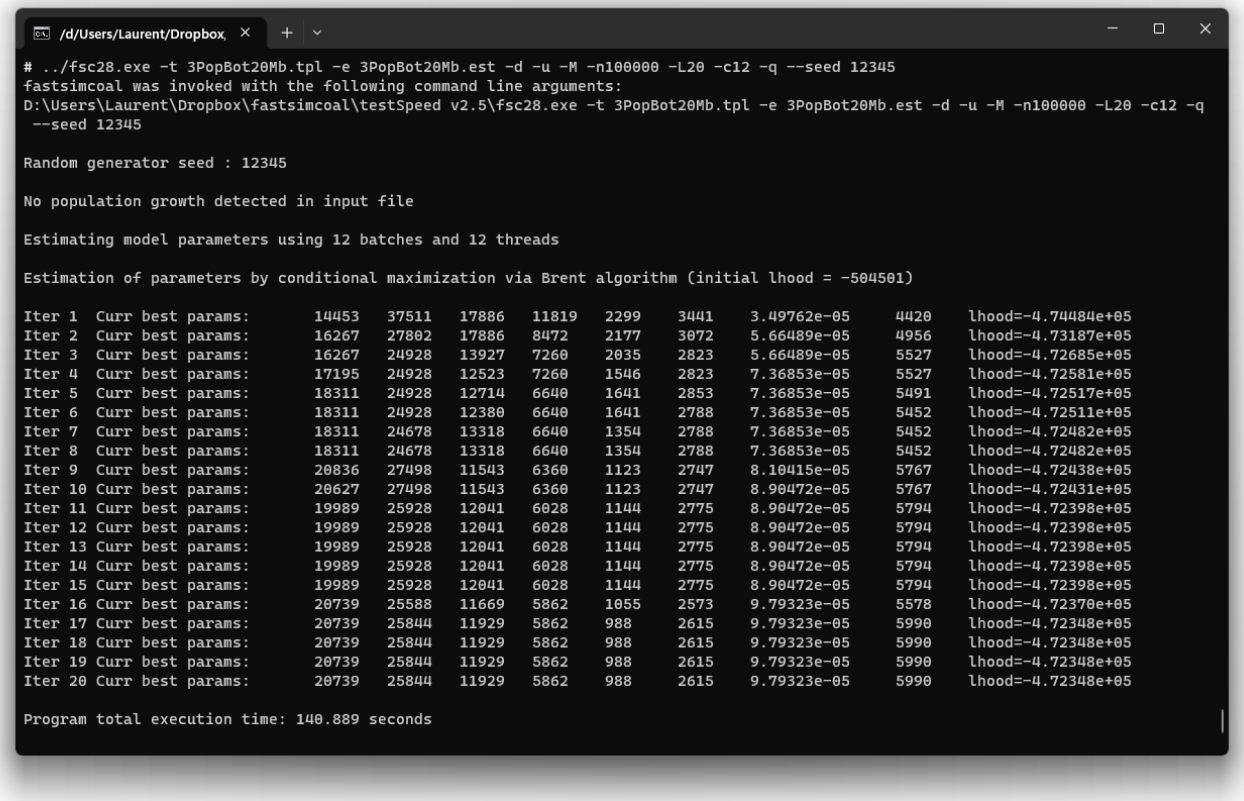
- ***-N*** option has been suppressed since ver 2.6. It used to specify a maximum number of coalescent simulations to perform to estimate the expected SFS. Now we use a **fixed number of simulations**, simply specified by option ***-n***.

FINE TUNING PARAMETER ESTIMATION

Since version 2.8, parameter estimation can be fined tuned by preventing that the search get stuck in some suboptimal parameter space using the **-y** or **--resetParam** command line options. Indeed, when we launch a given parameter estimation as:

```
../fsc28.exe -t 3PopBot20Mb.tpl -e 3PopBot20Mb.est -d -u -M -n100000 -L20 -c12 -q --seed 12345
```

We see that the likelihood cannot be improved for 5 ECM cycle after iteration 11.



The command line option **-y xxx** or **--resetParam xxx** can be used, where xxx specifies a number of cycles that did not lead to an improvement of the likelihood. After these non-improving cycles, one comes back to the parameter values prevailing after the last improvement and the following ECM cycles start from there.

For instance, with the command line

```
../fsc28.exe -t 3PopBot20Mb.tpl -e 3PopBot20Mb.est -d -u -M -n100000 -L20 -c12 -q --seed 12345 -y 4
```

We obtain the following run

```
/d/Users/Laurent/Dropbox,
# ../fsc28.exe -t 3PopBot20Mb.tpl -e 3PopBot20Mb.est -d -u -M -n100000 -L20 -c12 -q --seed 12345 -y 4
fastsimcoal was invoked with the following command line arguments:
D:\Users\Laurent\Dropbox\fastsimcoal\testSpeed v2.5\fsc28.exe -t 3PopBot20Mb.tpl -e 3PopBot20Mb.est -d -u -M -n100000 -L20 -c12 -q
 --seed 12345 -y 4

Random generator seed : 12345

No population growth detected in input file

Estimating model parameters using 12 batches and 12 threads

Estimation of parameters by conditional maximization via Brent algorithm (initial lhood = -504501)

Iter 1  Curr best params:       14453   37511   17886   11819   2299    3441    3.49762e-05     4420    lhood=-4.74484e+05
Iter 2  Curr best params:       16267   27802   17886   8472    2177    3072    5.66489e-05     4956    lhood=-4.73187e+05
Iter 3  Curr best params:       16267   24928   13927   7260    2035    2823    5.66489e-05     5527    lhood=-4.72685e+05
Iter 4  Curr best params:       17195   24928   12523   7260    1546    2823    7.36853e-05     5527    lhood=-4.72581e+05
Iter 5  Curr best params:       18311   24928   12714   6640    1641    2853    7.36853e-05     5491    lhood=-4.72517e+05
Iter 6  Curr best params:       18311   24928   12380   6640    1641    2788    7.36853e-05     5452    lhood=-4.72511e+05
Iter 7  Curr best params:       18311   24678   13318   6640    1354    2788    7.36853e-05     5452    lhood=-4.72482e+05
Iter 8  Curr best params:       18311   24678   13318   6640    1354    2788    7.36853e-05     5452    lhood=-4.72482e+05
Iter 9  Curr best params:       20836   27498   11543   6360    1123    2747    8.10415e-05     5767    lhood=-4.72438e+05
Iter 10 Curr best params:       20627   27498   11543   6360    1123    2747    8.90472e-05     5767    lhood=-4.72431e+05
Iter 11 Curr best params:       19989   25928   12041   6028    1144    2775    8.90472e-05     5794    lhood=-4.72398e+05
Iter 12 Curr best params:       19989   25928   12041   6028    1144    2775    8.90472e-05     5794    lhood=-4.72398e+05
Iter 13 Curr best params:       19989   25928   12041   6028    1144    2775    8.90472e-05     5794    lhood=-4.72398e+05
Iter 14 Curr best params:       19989   25928   12041   6028    1144    2775    8.90472e-05     5794    lhood=-4.72398e+05
Iter 15 Curr best params:       19989   25928   12041   6028    1144    2775    8.90472e-05     5794    lhood=-4.72398e+05

***  Restoring parameters to current best values since there was no improvement during 4 cycles ... ***
Iter 16 Curr best params:       19989   25669   12041   6028    1144    2775    8.90472e-05     5794    lhood=-4.72390e+05
Iter 17 Curr best params:       19638   25412   12161   6147    1248    2614    8.11178e-05     5038    lhood=-4.72383e+05
Iter 18 Curr best params:       21401   24850   11699   6147    1029    2605    7.38257e-05     5615    lhood=-4.72373e+05
Iter 19 Curr best params:       21401   25098   11699   6147    987     2543    8.11943e-05     5553    lhood=-4.72328e+05
Iter 20 Curr best params:       21401   25098   11699   6147    987     2543    8.11943e-05     5553    lhood=-4.72328e+05

Program total execution time: 142.502 seconds
```

which leads to a final likelihood that is slightly better than in the original one.

Note however, that we have no guarantee that a better final likelihood wil be obtained by reinitializing the parameter estimation after some non improved cycles. Our experience tells us that there are a few runs where the likelihood does not improve after a few initial cycles, so that having an option **-y 3** or **-y5** might be useful, especially when one has chosen to perform quite a large number of cycles, e.g. **-L30** or higher.

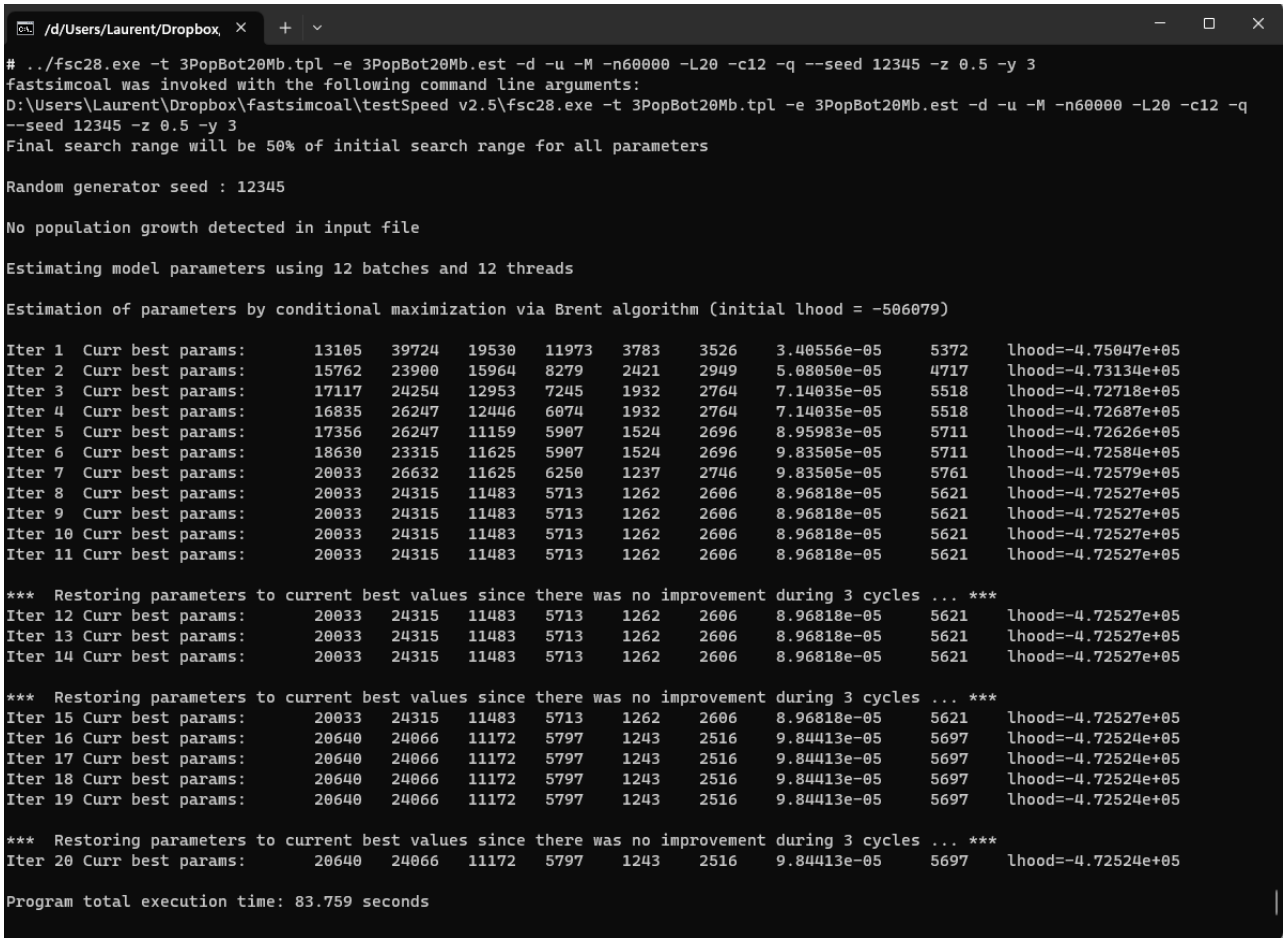
Another command line option (**-z** or **--finalRange**) can be used to modify the width of the initial search range. For instance, a conventional run (with less simulations than the former one) would look like

```
/d/Users/Laurent/Dropbox,
# ../fsc28.exe -t 3PopBot20Mb.tpl -e 3PopBot20Mb.est -d -u -M -n60000 -L20 -c12 -q --seed 12345
fastsimcoal was invoked with the following command line arguments:
D:\Users\Laurent\Dropbox\fastsimcoal\testSpeed v2.5\fsc28.exe -t 3PopBot20Mb.tpl -e 3PopBot20Mb.est -d -u -M -n60000 -L20 -c12 -q
--seed 12345

Random generator seed : 12345

No population growth detected in input file

Estimating model parameters using 12 batches and 12 threads

Estimation of parameters by conditional maximization via Brent algorithm (initial lhood = -506079)

Iter 1  Curr best params:       13105   39724   19530   11973   3783    3526    3.40556e-05     5372    lhood=-4.75047e+05
Iter 2  Curr best params:       14701   24437   17389   8766    2001    3077    5.03698e-05     5016    lhood=-4.73351e+05
Iter 3  Curr best params:       17608   22052   14442   7402    1860    2900    6.14950e-05     4839    lhood=-4.72851e+05
Iter 4  Curr best params:       17802   25805   15027   6548    1860    2900    6.14950e-05     4839    lhood=-4.72804e+05
Iter 5  Curr best params:       18732   24483   13620   6239    1114    2664    7.17148e-05     5820    lhood=-4.72634e+05
Iter 6  Curr best params:       20816   23521   11679   6239    1114    2664    7.17148e-05     5901    lhood=-4.72544e+05
Iter 7  Curr best params:       20816   23521   11679   6239    1114    2664    7.17148e-05     5901    lhood=-4.72544e+05
Iter 8  Curr best params:       20816   23521   11679   6239    1114    2664    7.17148e-05     5901    lhood=-4.72544e+05
Iter 9  Curr best params:       20816   23521   11679   6239    1114    2664    7.17148e-05     5901    lhood=-4.72544e+05
Iter 10 Curr best params:       19743   25388   11710   6159    1263    2677    7.89702e-05     5364    lhood=-4.72536e+05
Iter 11 Curr best params:       19743   25388   11710   6159    1263    2677    7.89702e-05     5364    lhood=-4.72536e+05
Iter 12 Curr best params:       19743   25388   11710   6159    1263    2677    7.89702e-05     5364    lhood=-4.72536e+05
Iter 13 Curr best params:       19743   25388   11710   6159    1263    2677    7.89702e-05     5364    lhood=-4.72536e+05
Iter 14 Curr best params:       19743   25388   11710   6159    1263    2677    7.89702e-05     5364    lhood=-4.72536e+05
Iter 15 Curr best params:       19743   25388   11710   6159    1263    2677    7.89702e-05     5364    lhood=-4.72536e+05
Iter 16 Curr best params:       19743   25388   11710   6159    1263    2677    7.89702e-05     5364    lhood=-4.72536e+05
Iter 17 Curr best params:       21181   24957   11738   6321    885     2733    7.90448e-05     6022    lhood=-4.72536e+05
Iter 18 Curr best params:       21181   24957   11738   6321    885     2733    7.90448e-05     6022    lhood=-4.72536e+05
Iter 19 Curr best params:       22307   25464   12075   6525    926     2684    7.90448e-05     5771    lhood=-4.72535e+05
Iter 20 Curr best params:       21190   24905   12075   6525    939     2711    7.90448e-05     5798    lhood=-4.72534e+05

Program total execution time: 90.571 seconds
```

And the following command line with the option **-z 0.5** specifies that the final search range will be only 50% of the initial one.

```
../fsc28.exe -t 3PopBot20Mb.tpl -e 3PopBot20Mb.est -d -u -M -n60000 -L20 -c12 -q --seed 12345 -z0.5 -y 3
```

leads to



which is a slight improvement of the final likelihood.

Note that one cannot specify a final search range that is smaller than 1% of the initial one. If a smaller value is mentioned then the minimum **-z** value of 0.01 wil be used.

RUNNING FASTSIMCOAL WITH OPTIONS SPECIFIED IN THE FILE "FSC_RUN.TXT"

It is possible to run *fsc* without command line option if the file "fsc_run.txt" is present and contains run path and command line options in the current working directory. The file "fsc_run.txt" should have just two lines.

The first line should have the path to the input files. The second line should have all command line options

e.g.:

fsc_run.txt

```
D:\Users\Laurent\Dropbox\fastsimcoal\IM20Mb
-t IM20Mb.tpl -e IM20Mb.est -M -n100000 -L20 -c6 -d -q
```

OUTPUT FILES

The results are then output in a "<template_generic_name>.bestlhoods" file, containing the estimated ML parameter values:

1PopExpInst20Mb.bestlhoods

```
NPOP    NANC    TEXP     MaxEstLhood     MaxObsLhood
502642  542     5070      -90349.486      -90348.375
```

In addition to estimated ML parameter values, the file reports the estimated log likelihood, as well as the maximum log likelihood given the observed SFS (MaxObsLhood). MaxObsLhood is obtained by using the observed SFS as the expected SFS when computing the likelihood, i.e., returning the value of the likelihood if there was a perfect fit between the expected and observed SFS.

fsc27 produces additional output files:

- "<template_generic_name>.brent_lhoods" containing the parameter values at different stages of the optimization procedure, after each parameter update of the ECM cycles.
- "<template_generic_name>.last_lhoods" containing the parameter values obtained at the last update of all ECM cycles (which might not be the ML parameter values).
- "<template_generic_name>_maxL.par", which is a *.par* files where the estimated parameters have been replaced by their ML values. This file can be directly used to generate data sets corresponding to the estimated parameter values, for instance for parametric bootstrap confidence interval estimation. In that case you need to replace the FREQ keyword by DNA, to generate new SFS from the max lhood parameters.
- "<template_generic_name>.pv", which is a file containing the maximum likelihood estimates of all simple parameters defined in the *.est* file. This file can then be used to make new parameter estimations with initial values of simple parameters equal to those specified to those in the *pv* file instead of starting from random values (see option ***–initValues***)
- A file or multiple files containing the expected (joint) SFS for the ML parameters. These files have the same name as the *.obs files containing the observed SFS, but they have the *.txt extension.

OBSERVED SFS FILE NAMES

Note that the **name of the observed SFS file was not specified on the command line**. This is because it is assumed to have the **same name as the prefix of the template file** (here 1PopBot20Mb) and a given suffix, which exact definition depends on the number of population samples and on the type of SFS.

Note also that all the observed SFS files **should only contain a single observed SFS**.

ONE OBSERVED SAMPLE

If there is a single observed sample in the model, the suffix will be:

- **_DAFpop0.obs** if it is a file listing the derived allele SFS (unfolded spectrum)
- **_MAFpop0.obs** if it is a file listing the minor allele SFS (folded spectrum)

TWO OBSERVED SAMPLES

If there are two observed samples in the model (0 and 1), one would need a file with the following suffix

- **_jointDAFpop1_0.obs** if it is a file listing the derived allele SFS (unfolded spectrum)
- **_jointMAFpop1_0.obs** if it is a file listing the minor allele SFS (folded spectrum)

MORE THAN TWO OBSERVED SAMPLES

If there are more than two observed samples in the model (say 0, 1, and 2), one would need three separate files with the following suffix

- **_jointDAFpop1_0.obs, _jointDAFpop2_1.obs, _jointDAFpop2_0.obs**

For the folded spectrum, the name would begin by **_jointMAF**

MULTIDIMENSIONAL SFS

It is also possible to tell *fsc27* to use another format for observed SFS using the command line ***multiSFS***. In that case, *fsc27* expects the observed SFS to be in a single file, even when more than one population sample is specified, with the following suffix:

- **_DSFS.obs**

See the Appendix section "Multidimensional site frequency spectrum" for the exact format of this type of files.

ASCERTAINED SFS FILES

Obs files containing ascertained SFS have a suffix "*-ascx.obs*" instead of simply "*.obs*" a, where x is the ascertainment panel size (i.e. x=2 for the Affymetrix Human Origins chip panels).

8. APPENDIX

COMMAND-LINE OPTIONS

As we have already seen, *fsc27* include several command-line flags and options allowing you to parameterize simulations. These options can be listed by simply typing:

```
fsc27 or fsc27 -h
```

We detail the command line options in more detail below. You can use a one-letter (case sensitive) shortcut after a single dash or a longer multi-letter full option after two dashes. The value required after "some options can be separated or not from the option by a white space. For instance, `"-n1"` and "`-n 1`" are both valid.

Shortcut (-)	Full (--)	Description
`-h`	`--help`	Prints a list of all available options
`-i`	`--ifile`	Name of parameter file. Example: `-i 1popDNA.par`
`-n`	`--numsims`	Number of simulations to perform per parameter file or sets of parameter (see *-e* option) Example: `-n 1000`
`-t`	`--tplfile test.tpl`	Name of template parameter file. The template file must have the extension *.tpl* Example: `-t 1popDNArand.tpl`
`-f`	`--dfile test.def`	Name of a parameter definition file. This file includes a list of sets of parameter values to be substituted in the *.tpl* file. Listed parameter values are substituted in the template file to produce a valid parameter file (*.par*). This is an alternative to the random drawing of parameter values from distributions defined in the *.est* file. Example: `-f 1popDNArand.def`
`-F`	`--dFile test.def`	Same as *-f* option, but only it only uses the simple parameters defined in *.est* file. Complex parameters are recomputed from the simple parameters. Note that this option must be used together with both the *-t* and the *-e* options. The *.est* file is here necessary to define the relationships between the simple parameters. Example: `-F 1popDNArand.def`
`-e`	`--efile test.est`	Name of parameter prior definition file. Parameters are drawn from specified distributions and then substituted into template file. The file must have the extension *.est* . A template file must be provided for this option to work. Example: `-e 1popDNArand.est`
`-E`	`--numest`	Number of sets of parameters to draw from the prior distributions defined in the *.est* file. Parameter can be omitted for FREQ data type. Example: `-e 1popDNArand.est -E 100`

`-g`	`--genotypic`	Generates *Arlequin* projects outputs (*.*arp*) in genotypic (diploid) format. Without this option, *.arp* file are in the haploid format.
`-p`	`--phased`	Specifies that phase is known in *Arlequin .arp* output files. Without this options phase is assumed unknown.
`-s`	`--dnatosnp 2000`	Output DNA as SNP data, with a given maximum number to output (use 0 to output all SNPs in the DNA sequence(s)).
`-S`	`--allsites`	Output the whole DNA sequence, including all monomorphic sites.
`-I`	`--inf`	Generates DNA mutations according to an infinite site (IS) mutation model. Under this model, each mutation is supposed to occur at a different but random site on the DNA sequence. Under the IS model, if different mutations are allocated to the same DNA sequence position, they are generated independently, but marked to have occurred at the same position in the *Arlequin* output *.arp* file.
`-d`	`--dsfs`	Computes the site frequency spectrum (SFS) for the **derived** alleles for each population sample and for all pairs of samples (joint 2D SFS). Note that this is only done on DNA data. If your input file is about DNA sequences, then use the *-s* option to convert DNA to SNP data.
`-m`	`--msfs`	Computes the site frequency spectrum (SFS) for the **minor** allele for each population sample, for all pairs of samples (joint SFS), and for all populations samples pooled (global SFS). Note that this is only done on DNA sequences, with the use the *-s* option to convert DNA to SNP data (0 and 1).
`-j`	`--jobs`	output one simulated or bootstrapped SFS per file in a separate directory for easier analysis (requires *-d* or *-m* and *-s0* options)
`-b`	`--nonparboot 10`	number of bootstraps to perform on polymorphic sites to extract SFS (should be used in addition to *-s0* and *-j* options)
`-H`	`--header`	Generates a header in the site frequency spectrum (SFS) files
`-q`	`--quiet`	Outputs minimal messages to the console instead of detailed messages
`-T`	`--tree`	Output coalescent tree for each non-recombining segment in nexus format. With recombination, a tree is output for each of the segment between recombination breakpoints.
`-k`	`--keep 100000`	Number of simulated polymorphic sites to keep in memory before writing them to temporary files. If this option is not specified, a default value of 200,000 used. This option is mostly useful when generating relatively long DNA sequences, with potentially tens of thousands of polymorphic sites. In that case, extended k to a large value will allow you to keep all genetic information in memory and slightly speed up computations. Note that setting up this *k* value to a very large number may imply prohibitive memory requirement for *fsc27,* especially if you have large sample sizes i.e. thousands of individuals). In that case, it is preferable to adjust *k*, to prevent *fsc27* to use all your computer memory. In general, the default value of *k*= 200000 gives very good results and does not need to be changed. Note that larger values could be used when estimating the DSFS or MSFS from large DNA sequence data. Example: `-k 10000`

-r	--seed	Seed for random number generator (positive integer <= 1E6)
-x	--noarloutput	Does not generate Arlequin output files
-G	--indgenot	Generates an individual genotype table.
-M	--maxlhood	Perform parameter estimation by maximum composite likelihood from the SFS
-L	--numloops 20	Number of loops (ECM cycles) to be performed when estimating parameters from SFS. Default is 20. Example: `-L 30`
-l	-- minnumloops 20	number of loops (ECM cycles) for which the lhood is computed on both monomorphic and polymorphic sites. Example: `-l 10`
-C	--minSFSCount 1	Minimum observed SFS entry count taken into account for parameter estimation (default = 1)") Example: `-C 5`
-0	--removeZeroSFS	Does not take into account monomorphic sites in observed SFS for parameter inference. Parameters are estimated only from the SFS and mutation rate is ignored. This option requires that one parameter be fixed in the *.est* file.
-a	--ascDeme 0	Deme id where ascertainment is performed when simulating ascertained SNPs. Do not use this option for simulating unascertained SNP. This ascertainment applies specifically to DNA sequences. Example: `-a 0`
-A	--ascSize 2	Number of ascertained chromosomes used to validate SNPs. Minimum value is 2. Default = 2. Example: `-A 2`
-u	--multiSFS	Generates or use multidimensional SFS. It implies a different format than when 1D or 2D SFS are used, even though this option can also be used when modeling only 1 or 2 populations.
-w	--brentol 0.01	Tolerance level for Brent optimization Default = 0.01. Smaller value imply more precise estimations, but require more computation time (min;max) = (1e-1;1e-5)
-c	--cores 1	Number of openMP threads to be used for simulation of independent chromosomes and for parameter estimation (default=1, max=numBatches, use 0 to let openMP choose optimal value). Note that this option is only active for 64 bit version of the program. Example: `-c0`
-B	--numBatches 12	Maximum number of batches for multi-threaded runs (default=12). For multithreaded runs, the simulations are divided into a specified number of batches, each executed in a given thread. Best performance is achieved when the number of batches is equal to the number of cores available. Note that simulations and estimation will be the same if different numbers of threads are used with the same number of batches. Different results will be obtained with the same seed for different no. of batches, even if the same number of cores is used. Example: `-c4 -B4`

-P	--pooledsfs	computes pooled SFS over all samples Assumes -d or -m , but not -u (--multiSFS) flag activated
	--recordMRCA	records tMRCAs for each non recombining segment and outputs results in file *<generic name>_mrca.txt*. **Beware: huge slowdown of computing time**
	--foldedSFS	computes the 1D and 2D MAF SFS by simply folding the DAF SFS. This option makes folded spectra compatible with those estimated by *angsd*.
	--log precision 23	precision for computation of logs of random numbers. Default value is 23 (full precision). Min value is 10 and max value is 23. Recommended value is 18
	--initValues my.pv	specifies a file (*.pv) containing initial parameter values for parameter optimization. This is especially useful to reduce the number of runs necessary to estimate parameters when estimating confidence intervals by bootstrap.
	--nosingleton	Ignores singletons in likelihood computation. This is especially useful when one has low confidence in singletons, e.g. due to low coverage. Note that only SNPs that are singletons overall samples are ignored, not those that are singletons in a given population but that are also present in other populations.
-y	--resetParam 3	Number of unsuccessful cycles before resetting parameters to current max lhood values default is zero, implying no resetting
-z	--finalRange 0.01	Proportion of the initial search range remaining in the last cycle (default is 1)

MULTITHREADING

New to fastsimcoal ver 2.5, multithreading is introduced via the use of openMP threads. This has required considerable changes in most C++ classes to make them thread safe. It has also required the introduction of a new thread-safe way to generate random numbers, so that simulations or parameter estimations would give identical results independently of the number of threads used.

Multithreading has been implemented at two levels: when simulating multiple chromosome segments and when estimating model parameters from the SFS. It is thus not implemented when generating a single large recombining segment, as there is no way to split this simulation into independent units. Due to openMP limitations, it is also not implemented in the windows 32bit version.

In summary, a given workload is divided into a predefined number of batches that are processed by independent threads, and results are then collected once all threads are finished. The default number of batches is 12, and the default number of threads is 1. The number of threads can be specified by the command line option ***-c***, and the number of batches by the option ***-B***.

In the figures below, we report the computing time of two tasks as a function of the number of batches and of the number of threads used. Note that the computations were done on a quad core system with hyperthreading activated, resulting in 8 potential independent threads visible to win8.1.

The biggest observed computing gain is to pass from 1 to 2 threads, and then there is a clear diminishing return in speed gain. The figures also show that the highest speed is obtained when the number of batches is equal to the number of threads. In that case the highest performance should be obtained when the number of threads is equal to the number of available cores (8 in our case), but in practice we have a slightly better performance when using 6 threads and 12 batches (as the same threads are each used twice). Actually, the best performance we obtained overall was when

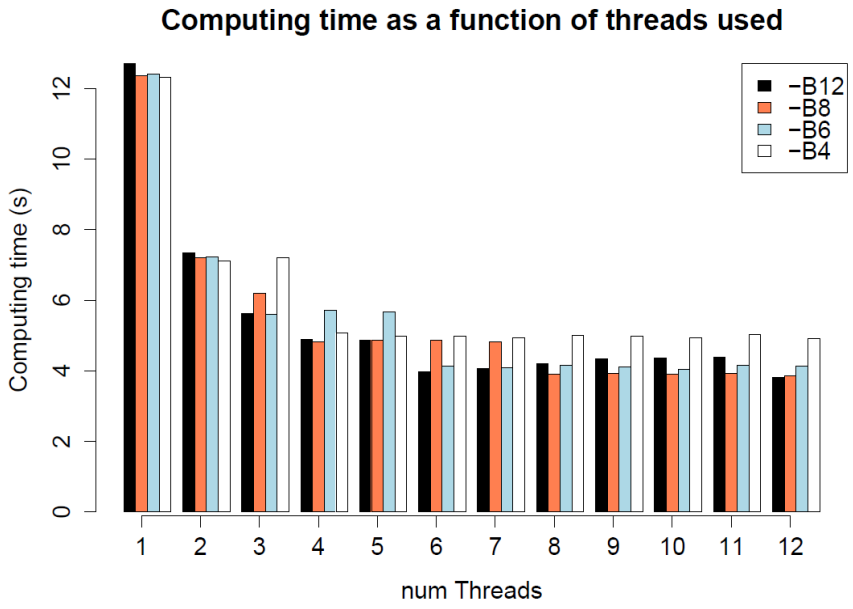
using 8 cores and 16 batches (not shown). Note that you can use the option ***-c0*** to let openMP decide on the maximum number of cores to use if you do not know this for your machine. What is also clear is that performance does not change much once the number of threads exceeds the number of batches. In summary, the use of 6-8 threads on most modern desktops should give a good performance increase.

When performing computations on a linux cluster and admitting that one needs to replicate runs from different starting points (min=50) to get the global maximum likelihood, then best performance should be obtained by using a single thread and using one batch (***-B1***). As the speed gain should be exactly equal to the number of available cores.

The bash file (*fsc_threads_speed_test.sh*) used to compare computing times is available in the "example files" directory, and can be used on other systems to find best combinations of ***-c*** and ***-B*** options.

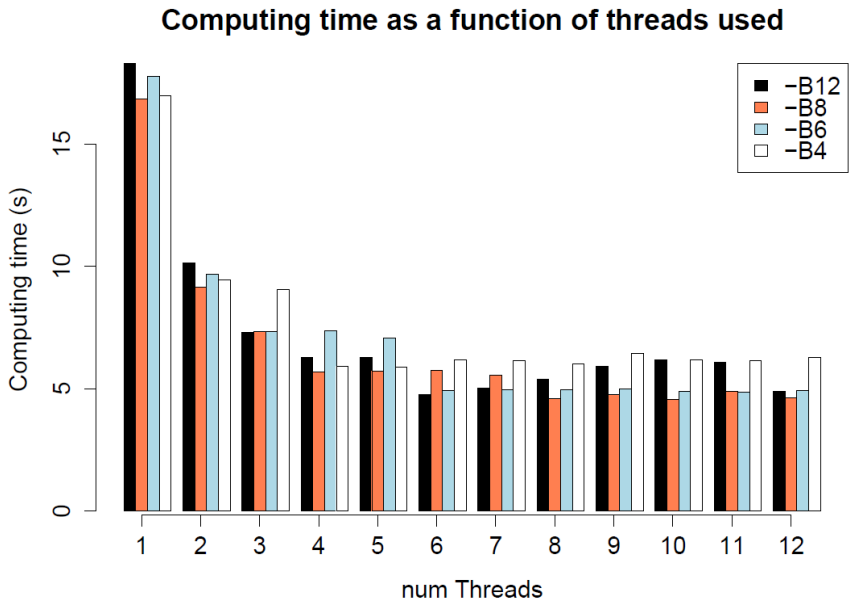
./$fsc25 -i 1PopDNAnoRec10Mb.par -n10 -l -x --seed 1234 - 10 simulations of 100,000 segments of 100 bp

Additional options: -c1 to -c12 and -B4 to -B12



./$fsc25 -t 1PopExpInst20Mb.tpl -e 1PopExpInst20Mb.est -d -M0.001 -n200000 -N200000 -l -l5 -L5 --seed 1234 -q

Additional options: -c1 to -c12 and -B4 to -B12



SEQUENTIAL MARKOV COALESCENT APPROXIMATION

In this section, we briefly describe the principle of the sequential Markov Coalescent model that is used to approximate the classical ancestral recombination graph (ARG). For more details, please read the original publications (McVean and Cardin 2005, Marjoram and Wall 2006, Chen et al. 2009).

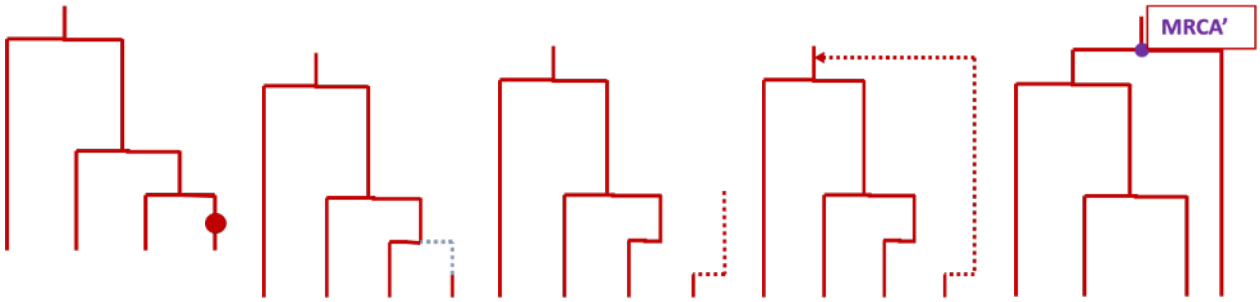
In brief, McVean and Cardin (2005) proposed to approximate the coalescent with recombination over a chromosomal segment based on the ARG by simulating a series of trees that differ each by single recombination events, starting on the left of the segment and moving to the right of the segment.

They thus proposed the following SMC algorithm to simulate coalescent with recombination along a DNA segment of length L:

1) Simulate a normal coalescent tree without recombination on the left-hand side of the segment and set $x = 0$. This initial tree has a total size of $T = T_0$ generations.
2) Select the position of the next recombination event on the segment by drawing a random number $y \sim \text{Exp}(rT)$, where Exp is an exponential distribution, and *r* is the recombination rate per generation between adjacent base pairs.
3) If $x + y < L$, select a position on the current tree where to implement a recombination event, by drawing a uniform number in $1..T$.
4) Detach the branch below the recombining event from the tree, and color in blue the branch between the recombination event and the next interior node.
5) Erase the blue branch.
6) Let the recombining lineage coalesce with the other lineages attached to the tree, and thus reattach the detached tree to the left tree.
7) Record the total length of the new tree T' and set $T = T'$.
8) Let $x += y$
9) Repeat steps 2)-8) until $x + y \geq L$.

The steps 3 to 6 of the SMC algorithm are illustrated below

Illustration of the SMC algorithm



A recombination event ● occurs on a tree

The (blue) branch above the recombination event is erased

The recombining lineage is free to evolve backward in time …

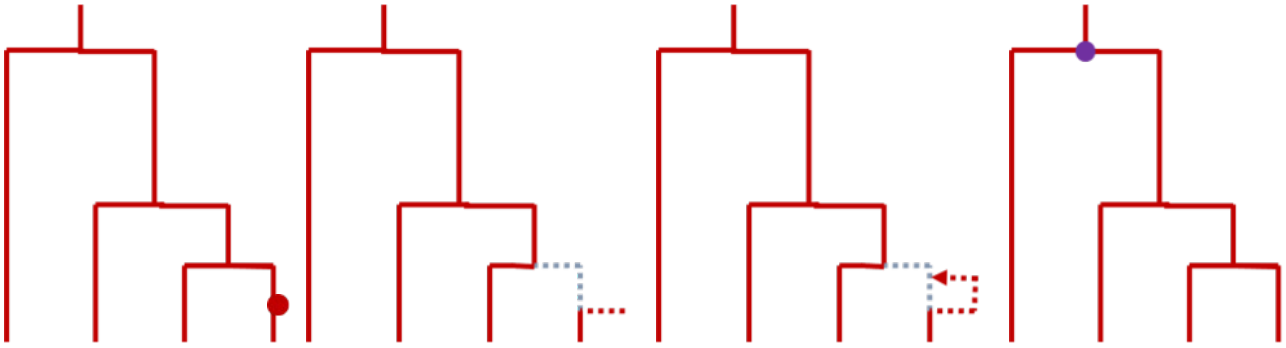
… until it coalesces with one of the lineages on the left tree …

… potentially changing the tree topology and the MRCA of the right tree

Marjoram and Wall (2006) proposed a slight modification of the SMC algorithm consisting in removing step 5 of the SMC algorithm above, and thus keeping the blue branch, and thus leaving the possibility to the recombining lineage to coalesce with it. The blue branches are only erased at the end of the simulation. This SMC' algorithm has been shown to give patterns of linkage disequilibrium more similar to those obtained by *ms* (Marjoram and Wall 2006), and it is therefore this algorithm that is implemented in *fsc27* .

With SMC', the recombining lineage can indeed coalesce with the blue lineages, and thus lead to a tree identical to the left tree, reflecting the fact that in the ancestral recombination graph, coalescent event can also occur between unsampled lineages.

Illustration of the SMC' algorithm



A recombination event ● occurs on a tree

The blue branch above the recombination event is kept

The recombining lineage is free to evolve backward in time, and can coalesce with the blue branch …

… leading to a tree identical to that on the left.

SITE FREQUENCY SPECTRUM

fsc27 can generate the site frequency spectrum for the derived allele (the unfolded site frequency spectrum) of the simulated data sets with the ***-s*** and ***-d*** command-line options as follows in the case of the relatively complex 3-population scenario described above:

```
fsc27 -i 3PopDNASFS.par -x -s0 -d -n10 -q
```

The ***-s*** option is necessary, here because the SFS can only be computed on SNP data, and the ***-s*** options tells *fsc27* to treat DNA as SNP data (where the 0 allele is ancestral). Note that the ***-x*** option

is there to prevent the output of *Arlequin* input files, which would be quite large in this case (about 2.8 Mb for each simulation, with >50,000 polymorphic sites).

fsc27 will then generate for each simulation, the population-specific derived site frequency spectrum, all the pairwise joint frequency spectra.

For instance, the 10 SFS of deme 0 look like:

`./3PopDNASFS/3PopDNASFS_DAFpop0.obs`

10 observations

d0_0	d0_1	d0_2	d0_3	d0_4	d0_5	d0_6	d0_7	d0_8	d0_9	d0_10	d0_11	d0_12	d0_13	d0_14	d0_15	d0_16	d0_17	d0_18	d0_19	d0_20
9964672	8054	4046	3032	2190	2118	1548	1431	1358	1138	1096	1010	1020	845	688	803	673	743	668	614	2253
9964237	8407	4203	2833	2675	1904	1587	1443	1241	1093	1073	923	933	917	855	752	837	590	637	618	2242
9963855	8449	4104	3165	2700	2045	1662	1634	1267	1067	904	897	923	930	844	845	808	696	490	556	2159
9964355	8292	4251	3142	2248	1741	1552	1325	1359	1290	1147	1068	938	851	846	768	632	747	626	728	2094
9963918	8582	4285	3110	2391	2081	1506	1475	1279	1119	967	986	929	841	763	797	841	752	651	687	2040
9964418	8409	4483	2844	2241	1896	1580	1407	1307	1109	978	1015	857	821	720	777	697	599	654	647	2541
9965301	7894	4487	3061	2212	1719	1562	1384	1154	1059	962	992	800	799	785	673	715	664	700	571	2506
9963601	8491	4372	3120	2547	2090	1525	1450	1336	1164	1018	877	945	844	741	849	808	768	705	591	2158
9965241	7855	4273	2918	2294	1960	1503	1362	1103	1023	996	965	793	870	810	863	632	804	571	679	2485
9964845	8306	4674	2928	2205	1828	1519	1414	1142	1203	1062	1010	900	882	785	656	654	581	581	552	2273

Whereas this is the derived SFS, it must be noted that the derived allele can have here a frequency of 20/20 since we use here a finite site model allowing more than one mutation per site. So by chance two mutations might have occurred in the two descending branches stemming from the MRCA node and lead to the same derived allele, implying that the derived allele frequency will be 20. To prevent this, use the ***-I*** option that generates SNPs under an infinite site model. Note that we also output here the SFS entry 0/0, which just lists the number of monomorphic sites in deme 0.

In the following table we show the first two simulated joint SFS between deme 0 and deme 2

`./3PopDNASFS/3PopDNASFS_DAFpop0.obs`

10 observations

	d0_0	d0_1	d0_2	d0_3	d0_4	d0_5	d0_6	d0_7	d0_8	d0_9	d0_10	d0_11	d0_12	d0_13	d0_14	d0_15	d0_16	d0_17	d0_18	d0_19	d0_20
d2_0	9952704	7659	3669	2686	1832	1709	1214	1048	960	811	729	689	689	537	389	453	403	386	317	285	1329
d2_1	3558	32	27	25	22	38	19	25	36	21	19	22	21	35	22	23	24	27	22	24	141
d2_2	2359	38	38	29	46	14	28	36	36	26	31	23	33	23	32	23	8	10	28	32	177
d2_3	1341	37	58	29	31	63	27	38	37	28	15	17	31	27	16	38	23	21	43	23	139
d2_4	1059	38	28	8	24	27	43	34	36	38	43	21	25	26	21	13	46	24	17	22	159
d2_5	830	26	15	29	19	33	34	33	28	16	12	19	16	17	12	16	16	15	13	25	171
d2_6	2821	224	211	226	216	234	183	217	225	198	247	219	205	180	196	237	153	260	228	203	137
d2_0	9951575	8022	3824	2506	2278	1569	1249	1100	911	788	696	572	573	525	483	389	404	316	353	287	1160
d2_1	4443	50	28	38	28	44	38	26	25	34	57	40	42	42	32	35	30	21	28	24	183
d2_2	2278	33	45	42	59	30	25	41	29	25	26	17	42	36	34	26	29	26	24	27	205
d2_3	1422	56	19	14	20	19	16	26	26	36	12	25	27	26	37	24	16	8	16	27	170
d2_4	1185	30	29	36	34	44	39	38	35	27	43	35	19	28	27	52	55	24	33	15	220
d2_5	926	30	44	37	41	33	18	39	42	34	65	23	44	35	26	25	21	31	23	25	217
d2_6	2408	186	214	160	215	165	202	173	173	149	174	211	186	225	216	201	282	164	160	213	87

The other SFS are then just listed below in the file `./3PopDNASFS/3PopDNASFS_DAFpop0.obs`

Again, the SFS entries (n_2,j) and (i,n_0) are also listed here as some derived alleles can be fixed in either or both populations if the defining mutation has occurred on some lineages leading exclusively to genes sampled in the third population. Finally, note that SFS cannot be computed on DNA sequences presenting more than a few 10s of thousand polymorphic sites, due to prohibitive memory requirements, and the need to write temporary files with intermediate results. But for the 3-population model defined above, this would imply the simulation of sequences larger than about 200Mb. However, you can increase the number of polymorphic sites to keep in memory with the ***-k*** command line option. If your computer has enough memory, then you can probably generate and compute the SFS on a very large data set with >1 million SNPs.

It can be important to realize that the computation of SFS can perfectly be done on simulated data obtained for random values of the parameters, with the use of *.tpl* and *.est* files, thus allowing one to get the empirical distribution of SFS under a given model. These SFS could then be perfectly used as summary statistics to estimate parameters from an observed SFS under an ABC framework or for model choice (see below), but they can also be used to infer parameters via likelihood maximization (as discussed below).

Minor allele SFS for multiple populations have now a modified format. Whereas the Derived allele SFS is straightforward to compute, the Minor allele SFS is not when several populations are involved. The observed (and the expected) MAF SFS are now computed given the following rules.

a) The minor allele is identified after computing the global frequency of the two alleles over all samples.
b) For each site where the minor allele frequency (MAF) is equal to the major allele frequency, we cannot decide which allele is the minor allele, and thus, the observed SFS entry of each of the two possible minor allele should be updated by 0.5.

The program *angsd* (Korneliussen et al. 2014) (available here) allows one to estimate the 1D, 2D and higher dimension SFS from low coverage data. When estimating the folded spectrum for more than 1D SFS, it identifies the minor allele based on the used samples. So for instance, for different pairs of populations the minor alleles of 2DSFS might be different at the same SNP position. This contrasts with *fastsimcoal*, which assumes that the minor allele is identified globally, even when estimating parameters from multiple pairs of 2D SFS. To ensure compatibility with angsd, we have introduced in ver 2.6 the new command line ***--foldedSFS***, which simply folds the derived (unfolded) 2D SFS for all pairs of populations.

For instance, assuming we want to generate data under the following scenario:

./3PopDNASFSsmall.par

```
//Number of population samples (demes)
3
//Population effective sizes (number of genes)
20000
5000
10000
//Sample sizes
2
3
6 1500
//Growth rates: negative growth implies population expansion
0
0
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new size, new growth rate, migr. matrix
4  historical event
2000 1 2 0.05 1 0 0
2980 1 1 0 0.04 0 0
3000 1 0 1 1 0 0
15000 0 2 1 3 0 0
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
1
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 10000000 0.00000001 0.00000002 0.33
```

The following command

```
fsc27 -i 3PopDNASFSsmall.par -x -s0 -m -n1 --seed 1234 -q
```

will lead to the three joint (2D) SFS

./3PopDNASFSsmall/

3PopDNASFSsmall_jointMAFpop1_0.obs

```
1 observations
         d0_0     d0_1 d0_2
d1_0     9976100  6690 1715
d1_1     1816     807  470
d1_2     2153     922  791
d1_3     1977     1864 4695
```

3PopDNASFSsmall_jointMAFpop2_0.obs

```
1 observations
         d0_0     d0_1 d0_2
d2_0     9969578  9179 7181
d2_1     4999     408  235
d2_2     2712     316  127
d2_3     2111     185  128
d2_4     1420     195  0
d2_5     1226     0    0
d2_6     0        0    0
```

3PopDNASFSsmall_jointMAFpop2_1.obs

```
1 observations
         d1_0     d1_1 d1_2 d1_3
d2_0     9971883  2621 3282 8152
d2_1     4917     127  292  306
d2_2     2728     161  188  78
d2_3     2238     82   104  0
d2_4     1513     102  0    0
d2_5     1226     0    0    0
d2_6     0        0    0    0
```

whereas the command

```
fsc27 -i 3PopDNASFSsmall.par -x -s0 -m -n1 --seed 1234 -q --foldedSFS
```

will lead to the three joint (2D) SFS

./3PopDNASFSsmall/

3PopDNASFSsmall_jointMAFpop1_0.obs

1 observations

	d0_0	d0_1	d0_2
d1_0	9980795	8554	1794.5
d1_1	2607	873	0
d1_2	1794.5	0	0
d1_3	0	0	0

3PopDNASFSsmall_jointMAFpop2_0.obs

1 observations

	d0_0	d0_1	d0_2
d2_0	9969578	9179	7181
d2_1	4999	408	1461
d2_2	2712	511	292
d2_3	2239	185	0
d2_4	1255	0	0
d2_5	0	0	0
d2_6	0	0	0

3PopDNASFSsmall_jointMAFpop2_1.obs

1 observations

	d1_0	d1_1	d1_2	d1_3
d2_0	9971883	2621	3282	8152
d2_1	4917	127	292	259
d2_2	2728	161	132	0
d2_3	2238	259	0	0
d2_4	1232	0	0	0
d2_5	0	0	0	0
d2_6	0	0	0	0

which are simply 2D SFS folded independently.

MULTIDIMENSIONAL SITE FREQUENCY SPECTRUM

With the option ***--multiSFS***, you have the option to generate multidimensional site frequency spectrum.

For example if you use the following *.par* file:

./3PopDNASFSsmall2.par

```
//Number of population samples (demes)
3
//Population effective sizes (number of genes)
20000
5000
10000
//Sample sizes
2
3
6 1500
//Growth rates: negative growth implies population expansion
0
0
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new size, new growth rate, migr. matrix
4  historical event
2000 1 2 0.05 1 0 0
2800 1 1 0 0.04 0 0
3000 1 0 1 1 0 0
15000 0 2 1 3 0 0
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of linkage blocks
1
//per Block: data type, num loci, rec. rate and mut rate + optional parameters
DNA 10000000 0.00000001 0.00000002 0.33
```

to produce multidimensional SFS with the following command line

```
fsc27 -i 3PopDNASFSsmall.par -x -s0 -d -n1 --multiSFS --seed 1234 -q
```

you will generate the following observed SFS:

./3PopDNASFSsmall/3PopDNASFSsmall2_DSFS.obs

1 observations. No. of demes and sample sizes are on next line

3 2 3 6

9964343	4506	2046	1320	1154	873	3229	1872	50	30	15	29
41	190	982	22	17	20	20	36	179	1936	86	66
63	43	28	371	5506	68	54	73	91	110	618	350
16	22	15	31	7	92	415	13	26	7	19	16
74	1666	86	85	87	42	60	604	1184	41	48	53
55	94	294	286	23	3	4	7	17	58	298	19
12	2	14	14	97	2494	190	188	203	225	255	2

Note that the second line gives the number of populations and the sample sizes in each deme. Note also that the whole **3D SFS is normally produced on a single line** (line 3), but it was split here on 7 lines for better visualization.

The entries on the SFS above correspond to the number of sites having the following derived allele frequencies

3rd line: (0,0,0), (0,0,1), (0,0,2), (0,0,3), (0,0,4), (0,0,5), (0,0,6), (0,1,0), (0,1,1), (0,1,2), (0,1,3), (0,1,4)

4th line: (0,1,5), (0,1,6), (0,2,0), (0,2,1), (0,2,2), (0,2,3), (0,2,4), (0,2,5), (0,2,6), (0,3,0), (0,3,1), (0,3,2)

5th line: (0,3,3), (0,3,4), (0,3,5), (0,3,6), (1,0,0), (1,0,1), (1,0,2), (1,0,3), (1,0,4), (1,0,5), (1,0,6), (1,1,0)

…

9th line: (2,2,2), (2,2,3), (2,2,4), (2,2,5), (2,2,6), (2,3,0), (2,3,1), (2,3,2), (2,3,3), (2,3,4), (2,3,5), (2,3,6)

Note as well that the entries of the matrix are in the form (x_0, x_1, x_2), where x_0, x_1, and x_2 are the derived allele frequencies in demes 0, 1, and 2, respectively. We thus start iterating allele frequencies in demes 2, then in deme 1 and finally in deme 0, which might be counter-intuitive, but this format has been used in *dadi* before, and we kept it for maintaining compatibility.

This single file captures all the possible allelic configurations in these three samples, which are only marginalized in the 3 joint SFS that would be produced by the command

```
fsc27 -i 3PopDNASFSsmall.par -x -s0 -d -n1 -seed 1234 -q
```

which generates the three joint SFS files

./3PopDNASFSsmall/

3PopDNASFSsmall_jointDAFpop1_0.obs

1 observations

	d0_0	d0_1	d0_2
d1_0	9976936	6160	2036
d1_1	2866	644	519
d1_2	1435	650	618
d1_3	2409	2469	3258

3PopDNASFSsmall_jointDAFpop2_0.obs

1 observations

	d0_0	d0_1	d0_2
d2_0	9969356	7535	4048
d2_1	4210	218	299
d2_2	2435	157	307
d2_3	1735	247	325
d2_4	1162	149	354
d2_5	1106	226	465
d2_6	3642	1391	633

3PopDNASFSsmall_jointDAFpop2_1.obs

1 observations

	d1_0	d1_1	d1_2	d1_3
d2_0	9970975	2918	1926	5120
d2_1	4196	85	55	391
d2_2	2369	124	76	330
d2_3	1742	124	64	377
d2_4	1155	93	89	328
d2_5	1157	103	40	497
d2_6	3538	582	453	1093

Either multiple joint 2DSFS can be used as input for parameter inference from SFS, and not that the resulting likelihood will differ according to the format we choose, as it will be computed in different ways (see equations 4 and 7 below). The input file format is automatically adjusted with the presence or absence of the ***--multiSFS*** option. Note however that when more than three populations are used most of the entries of the multidimensional SFS are empty, which may cause estimation problems due to imprecisions in the estimation the expected probabilities of these entries. See section on parameter inference "Composite likelihoods".

GENERATING SFS IN SINGLE FILES

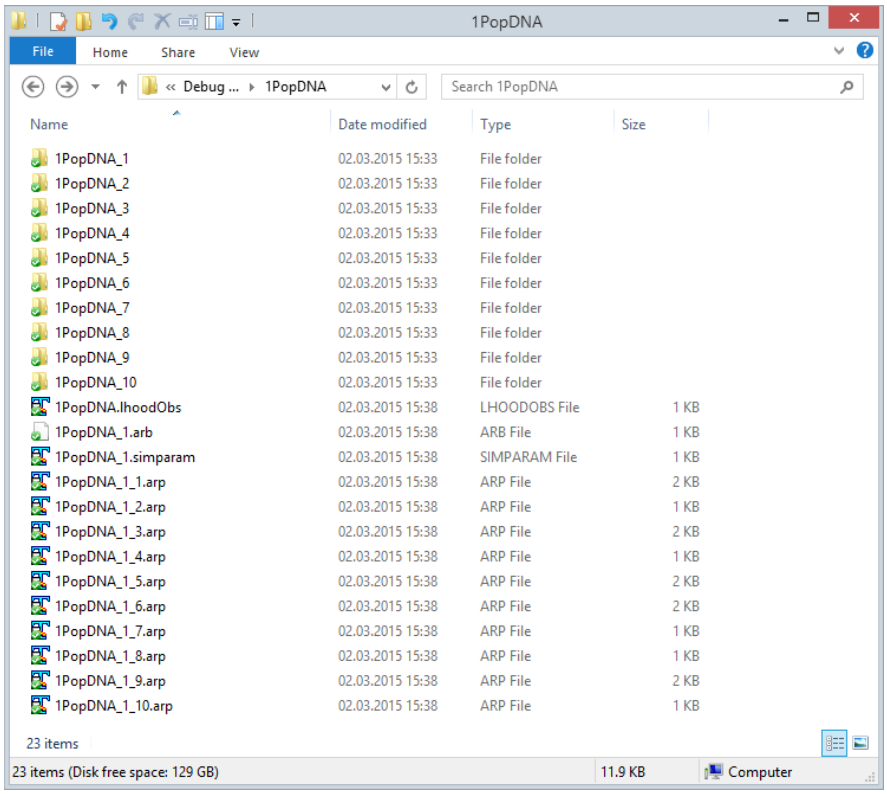
It is possible to ask *fastsimcoal* to put the computed SFS in separate directories, for later easier analyses, for instance for the computation of bootstrap confidence intervals after some parameter estimation. This is done with the `-j` option, which should be associated with the ***-m*** or ***-d*** and ***-s0*** options

For instance, the command:

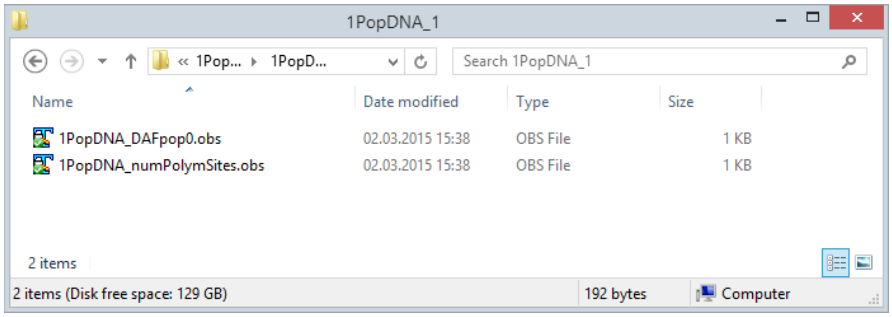
```
./fsc27 -i 1PopDNA.par -n10 -q -j -s0 -d
```

will generate 10 *.arp* files, and will compute their associated derived allele SFS, and each SFS file will be put in a different directory, for easier parallel analysis on a cluster later on.

The result 1PopDNA directory will look like:



and each subdiretory will contain a separate SFS file, like:



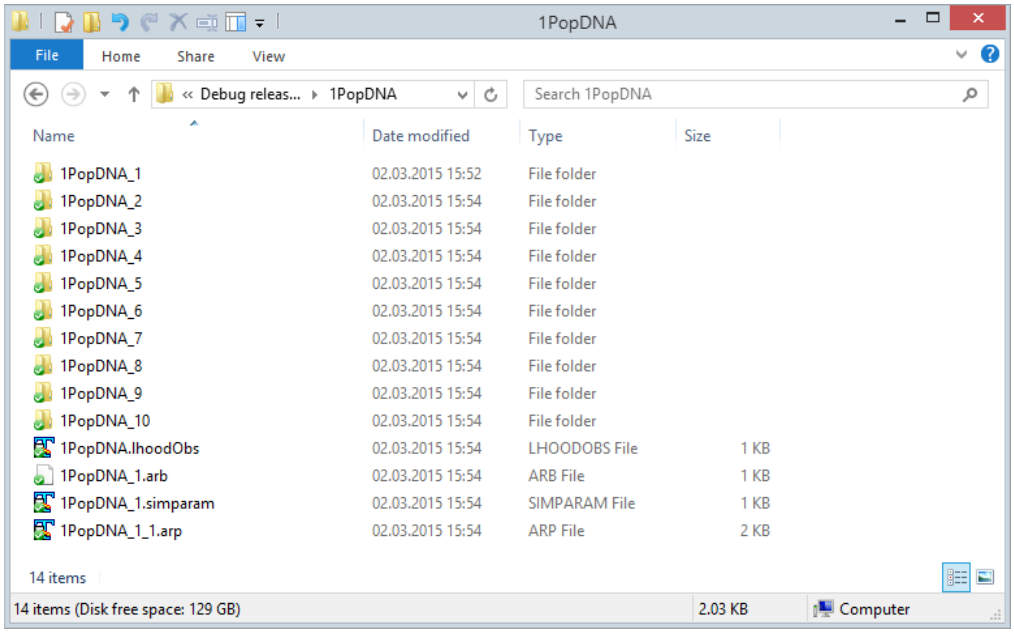
GENERATING NON-PARAMETRIC BOOTSTRAPED SFS

In addition to the generation of a given number of simulated SFS, it is possible to output, for each generated SFS, several bootstrapped SFS, where polymorphic sites are drawn with replacement from those obtained in the simulations.

This is obtained by using the ***-b*** option, like:

```
./fsc27 -i 1PopDNA.par -n1 -q -j -s0 -d -b9
```

In that case it will generate a single *.arp* file, but 10 directories. The first directory "`1PopDNA_1`" will contain the SFS computed on the original data, whereas the 9 others will contain the SFS computed on the bootstrapped data.



CAUTION AND USE OF BLOCK-BOOTSTRAP WITH REAL DATA

With real data, non-parametric bootstraps are usually obtained by resampling observed sites and then recomputing the SFS. If you have some linkage disequilibrium in your data, then it might be a good idea to implement a block-bootstrap strategy, where, instead of bootstrapping single sites, you would be bootstrapping whole chromosome segments, which would include several sites at a time. The size of the segments should be chosen such as that LD between endpoints would be almost nil.

GENERATING PARAMETRIC BOOTSTRAPS SFS

I describe below how to generate parametric bootstraps to get confidence intervals after parameter estimation.

As mentioned above, *fsc* creates a file with the extension **_maxL.par* in the result folder. This *.par* file is created from the maximum likelihood parameters estimated during the optimization and estimation procedure. This file shown below can be used to perform parametric bootstrap.

1PopExpInst20Mb_maxL.par

```
//Parameters for the coalescence simulation program : fsc27.exe
1 samples to simulate :
//Population effective sizes (number of genes)
505946
//Samples sizes and samples age
10
//Growth rates: negative growth implies population expansion
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new deme size, new growth rate,
migration matrix index
1     historical event
5079 0 0 0 1.03173066e-003 0 0
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of contiguous linkage Block: a block is a set of contiguous
loci
1
//per Block:data type, number of loci, per generation recombination and mutation rates and optional parameters
FREQ  1    0    2.5e-8 OUTEXP
```

This file needs to be modified to generate DNA sequence data (here 200,000 non-recombining segments of 100 bp) as

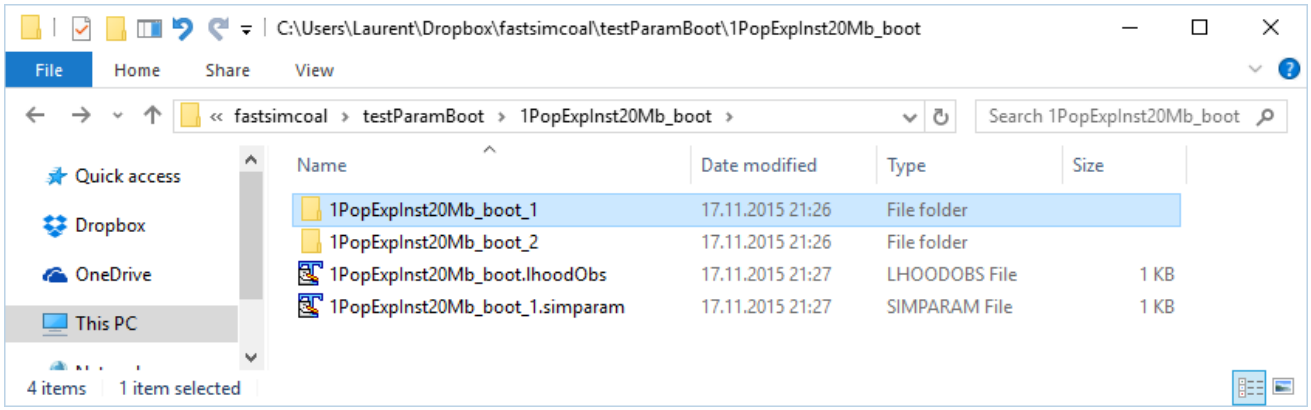
1PopExpInst20Mb_boot.par

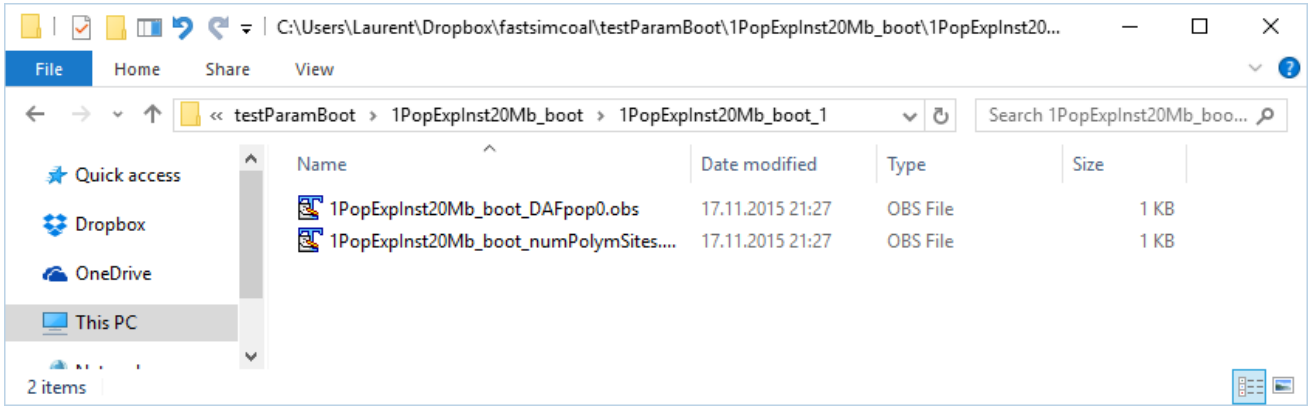
```
//Parameters for the coalescence simulation program : fsc27.exe
1 samples to simulate :
//Population effective sizes (number of genes)
505946
//Samples sizes and samples age
10
//Growth rates: negative growth implies population expansion
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new deme size, new growth rate,
migration matrix index
1     historical event
5079 0 0 0 1.03173066e-003 0 0
//Number of independent loci [chromosome]
200000 0
//Per chromosome: Number of contiguous linkage Block: a block is a set of contiguous
loci
1
//per Block:data type, number of loci, per generation recombination and mutation rates and optional parameters
DNA  100    0    2.5e-8 OUTEXP
```

Then running the following command:

```
./fsc27 -i 1PopExpInst20Mb_boot.par -n2 -j -d -s0 -x -I -q
```

will generate 2 SFS in two separate subdirectories in the result directory *1PopExpInst20Mb_boot*





With this procedure one can easily generate more than 2 SFS for these sets of parameters (say 100) and then estimate parameters from these pseudo-observed data sets using the same *.tpl* and *.est* files as those used to get the **_maxL.par* file.

SPECIFYING INITIAL VALUES FOR BOOTSTRAP PARAMETER ESTIMATIONS

Since one would assume that estimated values obtained from data sets generated by parametric or non-parametric procedures should be close to those initially estimated, one could start parameter estimation from those values. Since version 2.6, this is now possible by using the command line option ***-initvalues*** followed by the name of a file (with extension *.pv* usually) that lists the initial values of all simple parameters that are defined in an *.est* file.

So after the initial estimation of the parameters of a model using original data, e.g. based on the following *.est* file

1PopExpInst20Mb.est

```
// Search ranges and rules file
// ****************************

[PARAMETERS]
//#isInt? #name   #dist.#min  #max
//all Ns are in number of haploid individuals
1  NPOP       logunif  1000   1e7   output
1  NANC       logunif  10     1e5   output
1  TEXP       unif     10     1e5   output

[COMPLEX PARAMETERS]

0  RESIZE    = NANC/NPOP      hide
```

fsc27 produces a file with the extension *.pv*, which contains all the values of the simple parameters that were just estimated

1PopExpInst20Mb.pv

```
NPOP      NANC   TEXP
500764    522    5044
```

This file can then be used when estimating parameter from a bootstrap file under the same model using the command line option ***-initvalues*** as e.g.

```
./fsc27 -t 1PopExpInst20Mb_boot.tpl -e 1PopExpInst20Mb_boot.est -n100000 -d -M
       -L30 -initvalues 1PopExpInst20Mb.pv -c8 -B8 -q
```

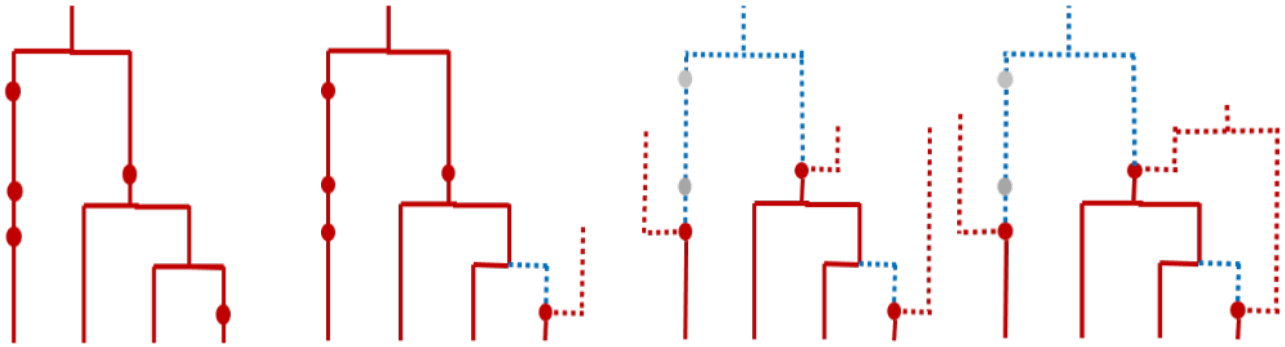
EXTENSION OF THE SMC' ALGORITHM TO MULTIPLE RECOMBINATION EVENTS

In order to simulate markers located at fixed recombination distances on the chromosome, we have extended the SMC' algorithm to allow for multiple recombination events between these markers.

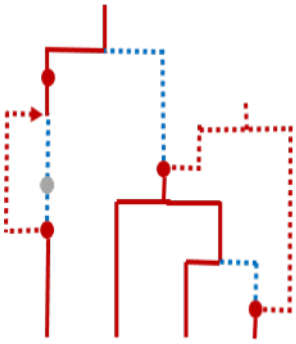
The new algorithm is as follows:

1) Simulate a normal coalescent tree without recombination on the left marker. This initial tree has a total size of $T = T_0$ generations.
2) Draw the number *z* of recombination events to occur between the two markers distant of *r* recombination units as $z \sim \text{Poisson}\,(\lambda = rT)$.
3) Draw *z* random numbers uniformly distributed between 1 and *T* to locate the positions of the *z* recombination events on the tree.
4) Locate the position of the most recent recombination event, and color in blue the branch above this event. All recombination events occurring on a blue branch are temporarily deactivated.
5) Detach the recombination event, and let it evolve until the time of the next active recombination event.
6) If the recombining lineage coalesces with a blue lineage, the lineage is colored back in red and the recombination events above this coalescent event become active again.
7) The next active recombination event is implemented, and we have a new recombining lineage free to evolve and to coalesce either with lineages on the tree or with other detached recombining lineages.
8) Steps 6-7 are repeated until only one lineage remains.
9) Remove all remaining blue lineages to keep a fully red tree.

This algorithm is illustrated below

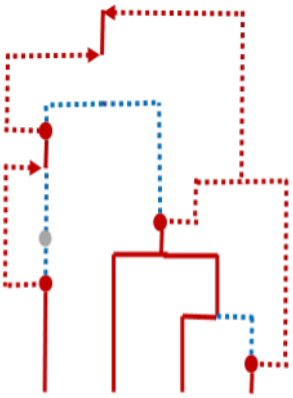


The *z* recombination events are randomly positioned on the tree



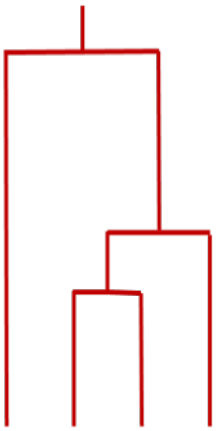
A recombinant lineage coalesces with a blue branch, which reactivates the recombination events above it.

The first recombinant lineage is detached and evolves freely backward in time



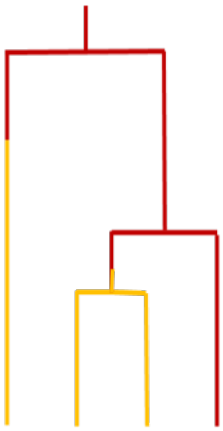
The final coalescent event is reached.

The recombination event on blue branches is deactivated (grayed)



All blue branches are erased to produce the final tree for the right marker.

Two recombinant lineages can coalesce



The shared lineages between the two trees are shown in yellow.

INTEGRATION INTO APPROXIMATE BAYESIAN COMPUTATIONS (ABC)

fsc27 can easily be integrated into an ABC framework (see e.g. Beaumont et al. 2002, Csillery et al. 2010), using for instance the *ABCToolBox* framework (Wegmann et al. 2010), which was specially developed to handle simcoal2, and which should be therefore able to accommodate *fsc27* without modification.

ABCToolBox is available on https://bitbucket.org/wegmannlab/abctoolbox/wiki/Home and its manual on

http://cmpg.unibe.ch/software/ABCtoolbox/ABCtoolbox_manual.pdf .

An alternative, would be to use *fsc27* built-in parameter sampler to generate *.params* files and *Arlequin .arp* files , which could then be processed by *arlsumstat* (available on http://cmpg.unibe.ch/software/arlequin35/) to produce summary statistics. A file combining these parameters and their associated summary statistics could then be used for ABC estimation, using different software, like

- ABCest3 (http://cmpg.unibe.ch/software/ABC/) made by L. Excoffier
- ABCReg (Thornton 2009) https://github.com/molpopgen/ABCreg
- ABCEstimator in ABCToolBox (Wegmann et al. 2010) http://cmpg.unibe.ch/software/ABCtoolbox/

As mentioned above, an alternative use of *fsc27* is to use the site frequency spectrum to estimate parameters, which is explained in the next section.

ESTIMATION OF DEMOGRAPHIC PARAMETERS FROM THE SFS VIA LIKELIHOOD MAXIMIZATION

We provide below some theoretical foundations for the estimation of demographic parameters from observed (joint) SFS. See Excoffier et al. (2013) for more details.

SIMULATION-BASED LIKELIHOODS

Nielsen (2000) has shown that one could estimate the likelihood of a demographic model $L(X,\theta)$, where *X* is the site frequency spectrum, on the basis of coalescent simulations. This is because the probability p_i of a given derived allele frequency *i* is simply a ratio of branch lengths of the coalescent tree expected under model θ as (Nielsen 2000):

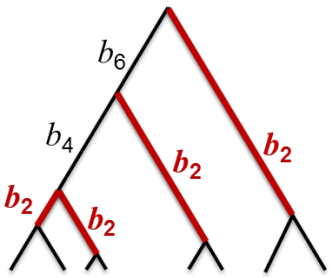
$$p_i = E(t_i \mid \theta) / E(T \mid \theta), \quad (1)$$

where t_i is the total length of a set $B_i = \{b_{ij}\}$ of branches directly leading to *i* terminal nodes, and *T* is the total tree length. This probability can then be estimated with arbitrary precision on the basis of Z simulations as

$$\hat{p}_i = \sum_{k}^{Z} \sum_{j \in B_i} b_{ijk} \Big/ \sum_{k}^{Z} T_k . \quad (2)$$

where b_{ijk} is the length of the *j*-th compatible branch in simulation *k* (see Figure below). Note that the estimator shown in eq. 2 implicitly weights simulations according to the probability that a mutation occurs on the simulated tree. This approach is appropriate for analyzing SNPs extracted from DNA sequence data as it is unlikely that SNPs will occur in genomic regions with shallow trees. Note that an estimator of the form $\hat{p}_i = \sum_k^Z \sum_{j \in B_i} b_{ijk} / T_k$ (as used by Garrigan (2009) to estimate the expected SFS) would give each tree the same weight and would thus give an excessive weight to genomic regions with shallow coalescent trees, which can be a problem for recently bottlenecked populations. If some simulated entries of the SFS were zero (because $\sum_k^Z \sum_{j \in B_i} b_{ijk} = 0$), $\hat{p}_i$ was set to an arbitrarily small values (as in Garrigan 2009) chosen here as $\min(\hat{p}_j \mid \hat{p}_j > 0)/100$.

A) No ascertainment



COMPOSITE LIKELIHOODS

Probabilities inferred from the simulations and eq. (2)can then be used to compute the composite likelihood of a given model as (Adams and Hudson 2004)

$$CL = \Pr(X \mid \theta) \propto P_0^{L-S} (1-P_0)^S \prod_{i=1}^{n-1} \hat{p}_i^{\,m_i} . \quad (3)$$

where $X = \{m_1, \ldots, m_{n-1}\}$ is the SFS in a single population sample of size *n*, *S* is the number of polymorphic sites, *L* is the length of the studied sequence, and P_0 is the probability of no mutation on the tree, obtained as $P_0 = e^{-\mu T}$ assuming a Poisson distribution of mutations occurring at rate μ.

This formulation can be extended for the joint SFS of two populations as

$$CL_{12} \propto P_0^{L-S}(1-P_0)^S \prod_{i=0}^{n_1}\prod_{j=0}^{n_2} \hat{p}_{ij}^{\,m_{ij}}\,, \tag{4}$$

and one can define a *v*-dimensional SFS for more than two (*v*) populations as

$$CL_{1\ldots v} \propto P_0^{L-S}(1-P_0)^S \prod_{i_1}^{n_1}\prod_{i_2}^{n_2}\ldots\prod_{i_v}^{n_v} \hat{p}_{\Phi}^{\,m_{\Phi}} \tag{5}$$

where $\Phi = i_1\, i_2 \ldots i_{v-1}\, i_v$ is a composite index. However, when the number of populations in the model is larger than 2 and samples sizes are relatively large, the number of entries in the v-dimensional SFS can be huge, implying that most entries of the observed SFS will be either zero or a very small number and that the expected values for these low-count entries will be difficult to estimate precisely. In that case, we have chosen to estimate the *v*-dimensional $CL_{1\ldots v}$ by collapsing all entries with observed SFS less than a predefined threshold ε as

$$CL_{1\ldots v} \propto P_0^{L-S}(1-P_0)^S \left(\prod_{obsSFS_i \geq \varepsilon} \hat{p}_i^{\,m_i}\right)\left(\sum_{1<obsSFS_j<\varepsilon} \hat{p}_j\right)^{\sum_{1<obsSFS_j<\varepsilon} m_j}. \tag{6}$$

When *v*>4, this approach will also prove computationally difficult, and in that case we have chosen to compute a composite composite-likelihood (*C2L*) obtained by multiplying all pairwise CL's, as

$$C2L_{1\ldots v} \propto \prod_{i<j} CL_{ij}\,, \tag{7}$$

where CL_{ij} is given by eq. (4).

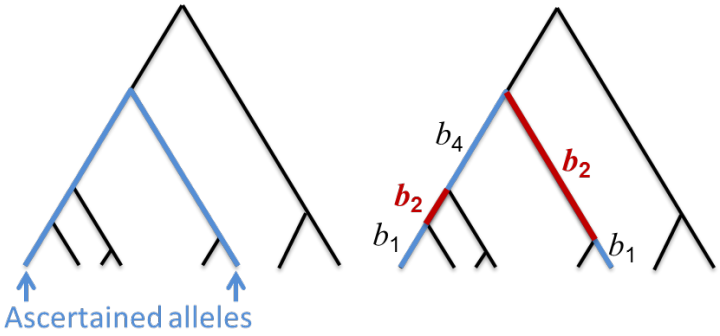
MAXIMIZING THE LIKELIHOOD

As the likelihood is obtained by simulations, which incurs some approximation, we cannot use optimization methods based on partial derivatives. Even though other methods would be possible, we have chosen to use a conditional maximization algorithm (ECM, Meng and Rubin 1993), which is an extension of the EM algorithm where each parameter of the model is maximized in turn, keeping the other parameters at their last estimated value. The maximization of each parameter was done using Brent's (1973, Chapter 5) algorithm, which is a root-finding algorithm using a combination of bisection, secant and inverse quadratic interpolation (see e.g. Press et al. 2007). We start with initial random parameter values and perform a series of ECM optimization cycles until estimated values stabilize or until we have reached a specified maximum number of ECM cycles (usually 20-40). We advise the use of at least 100,000 coalescent simulations for the estimation of the expected SFS for a given set of demographic parameters.

ESTIMATING DEMOGRAPHIC PARAMETERS FROM SNPS WITH KNOWN ASCERTAINMENT

Recently, Affymetrix developed a new SNP array including ~629,000 SNPs with known ascertainment scheme for population inference (Axiom® Genome-Wide Human Origins 1 Array, http://www.affymetrix.com/support/technical/byproduct.affx?product=Axiom_GW_HuOrigin) (Lu et al. 2011). This array, abbreviated hereafter GWHO, is made up of SNPs defined in 13 discovery panels. In the first 12 panels, SNPs have been identified by comparing the two chromosomes of an individual from a known population, further quality checks and validation on a large population

sample (Lu et al. 2011). The 13th panel contains SNPs that are polymorphic when comparing the Denisovan sequence and a random San chromosome. Raw genotypes from 943 unrelated individuals from more than 50 worldwide populations are freely available on ftp://ftp.cephb.fr/hgdp_supp10/.



The ascertainment scheme of this array is simple and homogeneous over a given panel. However, the SFS inferred from this array is biased as only mutations that occur in the ancestry of the two compared chromosomes will be considered (see Figure above). Nielsen et al. (2004) have shown how to correct the expected SFS within a given population under such a simple ascertainment scheme. One could thus be tempted to use their method to unbias the SFS, and then use this unbiased SFS for parameter inference, but this correction method will not work for joint SFS inferred in more than one population. Thus, rather than unbiasing the SFS, we have chosen to infer demographic parameters directly from the ascertained (joint) SFS. It implies we need to model the ascertainment scheme in the coalescent simulations such as to infer the expected ascertained SFS for a given demography. In order to estimate the SFS when SNPs are defined as being sites heterozygous in a given individual, we use the following procedure: 1) we perform conventional coalescent simulations under a given demography, 2) we choose two lineages at random in the ascertained population, 3) we identify the subtree relating the chosen lineages to their most recent common ancestor (MRCA) (highlighted in blue in Figure above), 4) we update the numerator in eq. (2) by summing up branch lengths of the blue subtree that are ancestral to i_1 lineages in population 1, i_2 lineages in population 2, …, i_v lineages in population v, 5) The denominator of eq. (2) is updated by summing up the total length of the blue subtree. Parameter optimization is then performed similarly to the unascertained case.

RUNNING FSC27 ON A CLUSTER

For faster computations *fsc27* can be launched on a Linux cluster.

SIMULATION OF GENETIC DIVERSITY

As an example, the following bash file `fs_scratch.sh` can be submitted on a cluster running the freely available Sun Grid Engine (SGE, http://gridengine.sunsource.net/).

fs_scratch.sh

```
#!/bin/bash

#get local directory
directory=`pwd`
```

```
#$ -cwd
# specify resources needed
#$ -l h_cpu=48:00:00
# using a scratch directory, reserving disk space and enough files for simulations
#$ -l scratch=1,scratch_size=100M,scratch_files=5k
#$ -N fs_run
#$ -o fs.out
#$ -e fs.err
#$ -m a
#$ -q all.q

#Copying all files to scratch directory
cp * $TMP
cd $TMP

#Running fsc27
./ fsc27 -t 1PopDNArand.tpl -n 1 -e 1PopDNArand.est -E 1000 -q

#copying results from scratch to original directory
cp * $directory
cd $directory
```

The simple `qsub` command can be used to launch this bash file on a queue as

```
qsub fs_scratch.sh
```

Note that in *fs_scratch.sh*, *fsc27* is using a scratch directory to write its output. This scratch directory is a temporary directory created by SGE on the node's hard disk, so that *fsc27* does not use the net to transfer files from the node to the master during simulations, which usually increases speed.

To use the scratch file, one needs to specify how much RAM is needed on the disk (*scratch_size*), and how many files will be created (*scratch_files*). These parameters need to be carefully adjusted. Very long DNA sequences may require several Gb per simulations! Also keep in mind that when using *.tpl* and *.est* files, (***-n*** × ***-E***) .arp files will be created (*1* × *1000* in `fs_scratch.sh`), as well as ***-E*** *.par* files, and ***-E*** *.arb* files. In addition, when very long DNA sequences are generated, with many polymorphic sites, *fsc27* creates temporary files in a *./garbage* directory, which is then deleted after the *.arp* file has been written. In the garbage directory, *fsc27* will create as many files as the total number of sampled genes (sum of sample sizes) defined in the *.par* or the *.tpl* file.

Finally, do not forget to make *fsc27* and your bash file executable on your Linux cluster. This is usually done with the *chmod* command as:

```
chmod +x fsc27
chmod +x fs_scratch.sh
```

The use of a scratch is not compulsory and a simpler bash file without use of a scratch directory would simply look like:

fs.sh

```
#!/bin/bash
#$ -cwd
# specify resources needed
#$ -l h_cpu=48:00:00
#$ -N fs_run
#$ -o fs.out
#$ -e fs.err
#$ -m a
#$ -q all.q

#Running fsc27
./ fsc27 -t 1PopDNArand.tpl -n 1 -e 1PopDNArand.est -E 1000 -q
```

Note that a bash file can be created on the fly and submitted from within another bash file. Here is an example, which will submit 10 instances of `fs.sh` on 10 different nodes, each one making 1000 simulations from different random parameter values .

```
./launch_fs.sh 10 1PopDNArand 10 1
```

launch_fs.sh

```
#!/bin/bash

#Laurent Excoffier December 2010
#
# The script will launch several instances of fsc27

#Creating a shortcut for fsc27
fs= fsc27

if [ $# -ne 4 ]; then
   echo "Expecting the following values on the command line, in that order"
   echo "   Number of instances to run"
   echo "   Generic name of template file"
   echo "   Number of random parameter to draw"
   echo "   Number of simulations per sets of parameter values"
else
   #Using values from the command line
   numInstances=$1
   genericName=$2
   numEstimates=$3
   numSimsPerEst=$4
   echo "numInstances=$numInstances"
   echo "genericName=$genericName"
   echo "numEstimates=$numEstimates"
   echo "numSimsPerEst=$numSimsPerEst"
fi

#Directory for job console outputs
msgs=consoleOutputs
mkdir $msgs 2>/dev/null

echo "Launching ${numInstances} instances of $fs"
let COUNT=numInstances
instancesLaunched=0
while [ $COUNT -gt 0 ]; do
  curInst=fs_job${COUNT}.sh
  newDir=${genericName}_res${COUNT}
  mkdir ${newDir} 2>/dev/null
  cp ./$fs ${newDir}/.
  cp ./${genericName}.est ${newDir}/.
  cp ./${genericName}.tpl ${newDir}/.
  cp ./$fs  ${newDir}/.
  cd  ${newDir}
  let instancesLaunched=instancesLaunched+1
  if [ -e ./$fs ] ; then
    (
    echo "#!/bin/bash"
    echo "#$ -cwd"
    echo "# specify resources needed"
    echo "#$ -l h_cpu=48:00:00"
    echo "#$ -N fs${COUNT}_run"
    echo "#$ -o ../$msgs/fs${COUNT}.out"
    echo "#$ -e ../$msgs/fs${COUNT}.err"
    echo "#$ -m a"
    echo "#$ -q all.q"
    echo ""
    echo "chmod +x ./$fs"
    echo "./$fs -t ${genericName}.tpl -n${numSimsPerEst} -e ${genericName}.est -E${numEstimates} -q"
    echo "rm ./$fs"
    ) > $curInst
    chmod +x $curInst
    qsub ${curInst}
  else
    echo "File $fs not found. Aborting instance $instancesLaunched"
  fi
  cd ..
  let COUNT=COUNT-1
done
```

ESTIMATION OF DEMOGRAPHIC PARAMETERS FROM THE SFS

Since the estimation of demographic parameters from the (joint) SFS requires the maximization of the likelihood of a model, it is necessary to repeat this estimation process several times and to get the global maximum likelihood solution. This is best done by launching several estimations from the same data set on a cluster.

The following bash file does just that, assuming that the observed data (*.*obs* files) are in a directory called *1PopBot20Mb.*

It will launch 50 instances of *fsc27* in 50 different directories (run1 to run50).

This bash file is quite generic and could be easily modified for any other demographic model, as well as for estimations from ascertainment SNP chip (if with Ascertainment=1)

Again it assumes that there the Sun Grid Engine running on a cluster, and that there is a queue called "*all.q*" where jobs are going to be submitted.

launchFastSimCoal_1PopBot20Mb.sh

```
#!/bin/bash

#Laurent Excoffier February 2013
#
# The script will launch several jobs of fsc27 to estimate
# demographic parameters from the SFS, using a each time using a
# conditional maximization (ECM) of the parameter likelihood
# This should run on any kind of SFS files generated by fsc27
#
# It assumes the following structure of the observed sfs files:
#     scriptDir
#     |
#     |- - - *.est file
#     |- - - *.tpl file
#     |- - - fsc27
#     |- - - targetDir
#                 |
#                 - *.obs files
#

fsc= fsc27
jobcount=0
msgs=conOutputs

#-------- Number of different runs per data set ------
numRuns=50
runBase=1
#-----------------------------

mkdir $msgs 2>/dev/null

#-------- Default run values ------
numSims=100000                  #-n command line option
numCycles=40                    #-L command line option
minValidSFSEntry=1                 #-C command line option

#-------- Ascertainment ------
withAscertainment=0
ascPop=0                           #-a command line option
ascSize=2                          #-A command line option
#-----------------------------
useMonoSites=""
#useMonoSites="-0"                 #-0 command line option
#----------multiSF------------
multiSFS=""
#multiSFS="--multiSFS"             #--multiSFS command line option
#-----------------------------

#-------- Generic Name ------
genericName=1PopBot20Mb
tplGenericName=1PopBot20Mb
estGenericName=1PopBot20Mb
#-----------------------------

for dirs in $genericName
do
      #Check that dirs is a directory
      if [ -d "$dirs" ]; then
            cd $dirs
```

```
                echo "Main directory : $dirs"
                estFile=$estGenericName.est
                tplFile=$tplGenericName.tpl
                for (( runsDone=$runBase; runsDone<=$numRuns; runsDone++ ))
                do
                      runDir="run$runsDone"
                      mkdir $runDir 2>/dev/null
                      echo "-------------------------------------------------------------------------"
                      echo ""
                      echo "Currrent file: $subDirs $runDir"
                      echo ""
                      cd $runDir
                      #Copying necessary files
                      cp ../../$fsc .
                      cp ../../$tplFile .
                      cp ../../$estFile .
                      cp ../*.obs .
                      #Renaming files for consistency
                      mv $tplFile ${genericName}.tpl
                      mv $estFile ${genericName}.est
                      let jobcount=jobcount+1
                      jobName=${genericName}${jobcount}.sh
                      #Creating bash file on the fly
                      (
                      echo "#!/bin/bash"
                      echo ""
                      echo "#$ -cwd"
                      echo ""
                      echo "# specify resources needed"
                      echo "#$ -l h_cpu=500:00:00"
                      echo ""
                      echo "#$ -N j1P_${jobcount}"
                      echo "#$ -o ../../../$msgs/1P_$runsDone.out"
                      echo "#$ -e ../../../$msgs/1P_$runsDone.err"
                      echo "#$ -m a"
                      echo "#$ -q all.q"
                      echo ""
                      echo "#chmod +x ./$fsc"
                      echo ""
                      echo "#Computing likelihood of the parameters using the ECM-Brent algorithm"
                      echo "echo \"\""
                      if [ $withAscertainment -eq 1 ] ; then
                            echo "./$fsc -t ${genericName}.tpl -n $numSims -d -e
                ${genericName}.est -M $stopCrit -L $numCycles
      -a${ascPop} -A${ascSize} -q ${useMonoSites} ${multiSFS}"
                      else
                            echo "./$fsc -t ${genericName}.tpl -n $numSims -d -e
                ${genericName}.est -M $stopCrit -L $numCycles -q
      ${useMonoSites} ${multiSFS}"
                      fi
                      echo ""
                      echo "echo \"\""
                      echo "rm ./$fsc"
                      echo "echo \"Job $jobcount terminated\""
                      ) > $jobName
                      chmod +x $jobName
                      echo "Bash file $jobName created"
                      qsub ./${jobName}
                      #./${jobName}
                      cd .. #$runDir
                done
           cd .. #dirs
      fi
done
```

COMPARATIVE SPEED TESTS: *FASTSIMCOAL* VS. *MS* AND *MACS*

We report below speed tests comparing *fastsimcoal* to *ms* and *MaCS* running all on a Linux cluster made up of 2.6GHz AMD Opterons with 4 GB of RAM and 74 GB HD.

DATA SETS

The following test data sets were used in our comparisons.

	No. of populations	Diploid population size	Migration rate (gen^{-1})	Mutation rate ($gen^{-1} \times bp^{-1}$)	Recombination rate ($gen^{-1} \times bp^{-1}$)	Sample size per population (no. of genes)
1popNoRec	1	12500		2×10^{-8}	0	2000
1popSmallSample	1	12500		2×10^{-8}	1.2×10^{-8}	20
1popLargeSample	1	12500		2×10^{-8}	1.2×10^{-8}	2000
2popNoRec	2	6250	0.001	2×10^{-8}	0	1000
2popSmallSample	2	6250	0.001	2×10^{-8}	1.2×10^{-8}	10
2popLargeSample	2	6250	0.001	2×10^{-8}	1.2×10^{-8}	1000

The population, mutation and recombination parameters correspond to those used by Chen *et al*. (2009), in their comparison of *ms* to *MaCS* in the one population case.

RESULTS

n=2000, no recombination (CPU times in seconds)

Data set	No. of replicates	Sequence length	Program		
			ms	*MaCS*	*fastsimcoal*
1popNoRec	1000	1 Mb	1.1	11.1	9.5
	100	10 Mb	9.6	107.0	72.9
	100	100 Mb	147.9	1319.5	1038.1
2popNoRec	1000	1 Mb	1.2	12.5	9.3
	100	10 Mb	8.9	128.1	71.5
	100	100 Mb	161.2	1513.2	1099.9

Without recombination, *ms* is much faster than the two other programs based on the SMC' approximation, and *fastsimcoal* becomes increasingly faster than *MaCS* with larger recombination rates and with migration.

n=20, recombination (CPU times in seconds)

Data set	No. of replicates	Sequence length	Program		
			ms	*MaCS*	*fastsimcoal*
1popSmallSample	1000	1 Mb	0.344	0.242	0.095
	100	10 Mb	159.246	2.618	0.460
	100	100 Mb	x	26.124	4.364
2popSmallSample	1000	1 Mb	0.378	0.907	0.152
	100	10 Mb	165.507	9.094	1.080
	100	100 Mb	x	97.876	10.559

x : *ms* crashed

For small sample sizes (total *n*=20) and with recombination, the SMC' based programs are becoming much faster than *ms*, which fails to run for 100Mb sequences. For such small sample sizes, *fastsimcoal* is 2.5 to 9.3 times faster than *MaCS*. For *MaCS* and *fastsimcoal*, computing time increases approximately linearly with sequence length, as expected.

n=2000, recombination (CPU times in seconds)

Data set	No. of replicates	Sequence length	Program		
			ms	***MaCS***	***fastsimcoal***
1popLargeSample	1000	1 Mb	3.7	28.1	25.2
	100	10 Mb	x	327.5	235.7
	100	100 Mb	x	3700.8	2635.4
2popLargeSample	1000	1 Mb	3.9	33.3	25.9
	100	10 Mb	x	393.5	240.6
	100	100 Mb	x	4311.1	2684.7

x : *ms* crashed

For large sample sizes (total *n*=2000), *ms* is actually faster than the two other programs for a "small" sequence of 1Mb, but failed to run successfully for longer sequences. For these large sample sizes, *fastsimcoal* is 1.2 to 1.8 times faster than *MaCs*. *fastsimcoal* computing time still increases approximately linearly with sequence length, which is not the case of *MaCS*, which becomes slightly penalized by larger sequences. Note however, that for 1Mb and 10Mb, we used *fastsimcoal* ***-k*** options allowing it to keep all simulated sites in memory before writing them to the output file, which was not possible for 100Mb sequences, which would use up too much memory.

COMPARATIVE SPEED TESTS: FSC21 VS. FSC25

We report here some comparative speed test done under Win8.1, using the 64-bit version of *fastsimcoal21* and *fsc25*. The tests were performed on a DELL computer equipped with an Intel i7-3770 processor at 3.4 Ghz (quadcore).

	Programs				
		fsc25 (1 thread)		fsc25 (8 threads)	
Data set	**fsc21**	**-c1 -B1**	**gain**	**-c8 -B8**	**gain**
DNA sequence					
constant population of 10,000 diploids, n=100)					
10 x 100000 x 100 bp no rec	18.08 s	12.65 s	1.43	3.85 s	4.70
10 x 1000000 x 100 bp no rec	26.04 s	22.97 s	1.13	8.50 s	3.06
10 x 10 Mb with rec	10.47 s	10.34 s	1.01	10.37 s	1.01
1 x 100 Mb with rec	13.59 s	12.88 s	1.06	13.62 s	1.00
FREQ (parameter estimation)					
1PopExpInst20Mb -n100000 -L5	14.53 s	8.14 s	1.79	2.53 s	5.74
2PopDiv20Mb -n100000 -L5	27.77 s	20.91 s	1.33	4.91 s	5.66
2PopDivMigr20Mb -n100000 -L5	48.94 s	35.54 s	1.38	8.45 s	5.79
3PopExpBotm -n10000 -L5	200.48 s	167.66 s	1.20	35.63 s	5.63
3PopExpBotm mSFS -n10000 -L5	123.39 s	111.96 s	1.10	24.00 s	5.14
10Pop2ContIsl 1 -n1000 -L5	191.11 s	129.35 s	1.48	32.54 s	5.87
10Pop2ContIsl 2 -n1000 -L5	182.18 s	121.07 s	1.50	32.38 s	5.63

Optimizations have not been performed in case of recombination, and in that case *fsc25* performs in a way similar to *fsc21*. In absence of recombination, and with a single thread activated (as on a cluster), speed gain can be of up to 43% for DNA sequence simulation, and up to 80% for parameter estimation. When using the multithreaded option (***-c0*** using all available computing threads, 8 in the present case), the speed gain is 3 to 4x for DNA sequence simulation, and > 5x for parameter estimation. Again, multithreading was not implemented for runs with recombination, but there is no penalty activating this option.

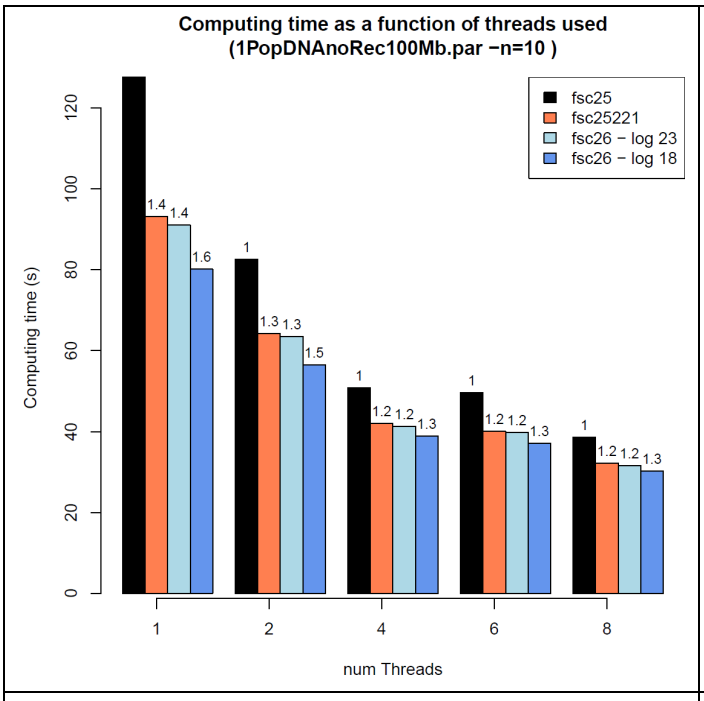
Note that the command line options are not optimal for parameter estimation, but they were just chosen for achieving reasonable computing times for comparative purposes.

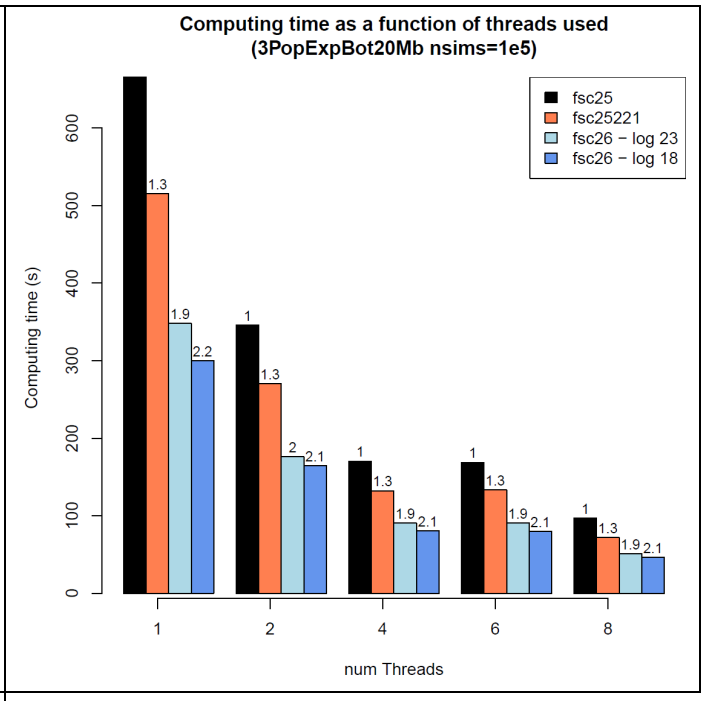
The bash file "*fsc_speed_test.sh*" and all necessary files to perform these tests can be found in the "example file" directory.

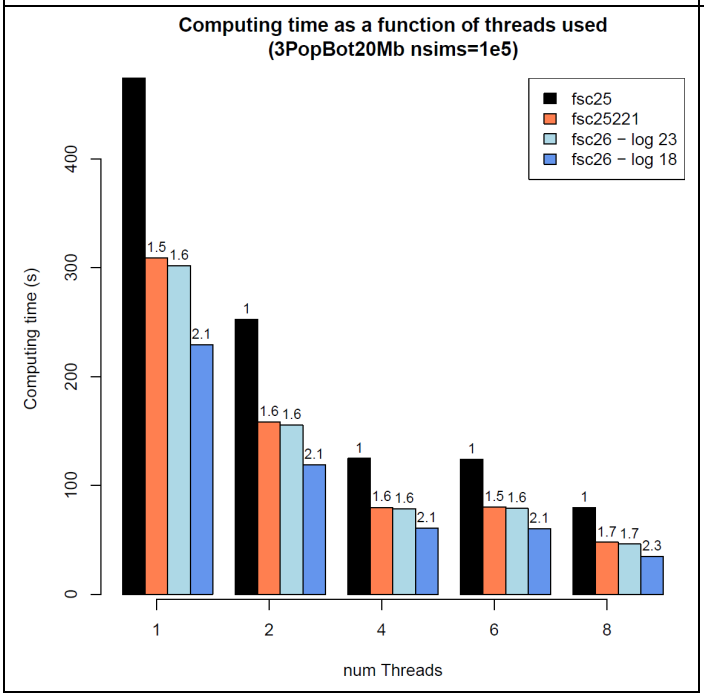
COMPARATIVE SPEED TESTS: FSC25 VS. FSC25.2.21 VS. FSC26

We have compared the speed of *fsc26* with two previous version of the program: *fsc25* released on July 2014, *fsc25.2.21* released in November 2015.

Computations were done on a DELL Precision 5810 with 16 Gb RAM and two quad-processors Intel Xeon 1680 v3 at 3.20 Ghz.







Computation times for the different programs executed with different number of threads (x-axis). Speed gain compared to fsc25 are reported on top of each bar.

- *With 1PopDNAnoRec100Mb.par, one simulates 10 times 100Mb of non recombining DNA*
- *With 3PopExpBot20Mb, one estimates the demography of 3 populations where two of them had an exponential growth (see below), which makes extensive use of logarithms*
- *3PopBot20Mb, same things as 3PopExpBot20Mb but without exponential growth*

Command lines:

For fsc25, fsc25221, and fsc27

```
./ fsc -i 1PopDNANoRec100Mb.par -n10 -I -x --seed 1234 --logprecision xx -q -cyy -B8
```

For fsc25 and fsc25221

```
./ fsc -t 3PopExpBot20Mb.tpl -e 3PopExpBot20Mb.est -d -M 0.001 -n100000 -N100000 -l5 -L5 -
          -seed 1234 --multiSFS -q -cyy -B8
```

```
./ fsc -t 3PopBot20Mb.tpl -e 3PopBot20Mb.est -d -M 0.001 -n100000 -N100000 -l5 -L5 --seed
          1234 --multiSFS -q -cyy -B8
```

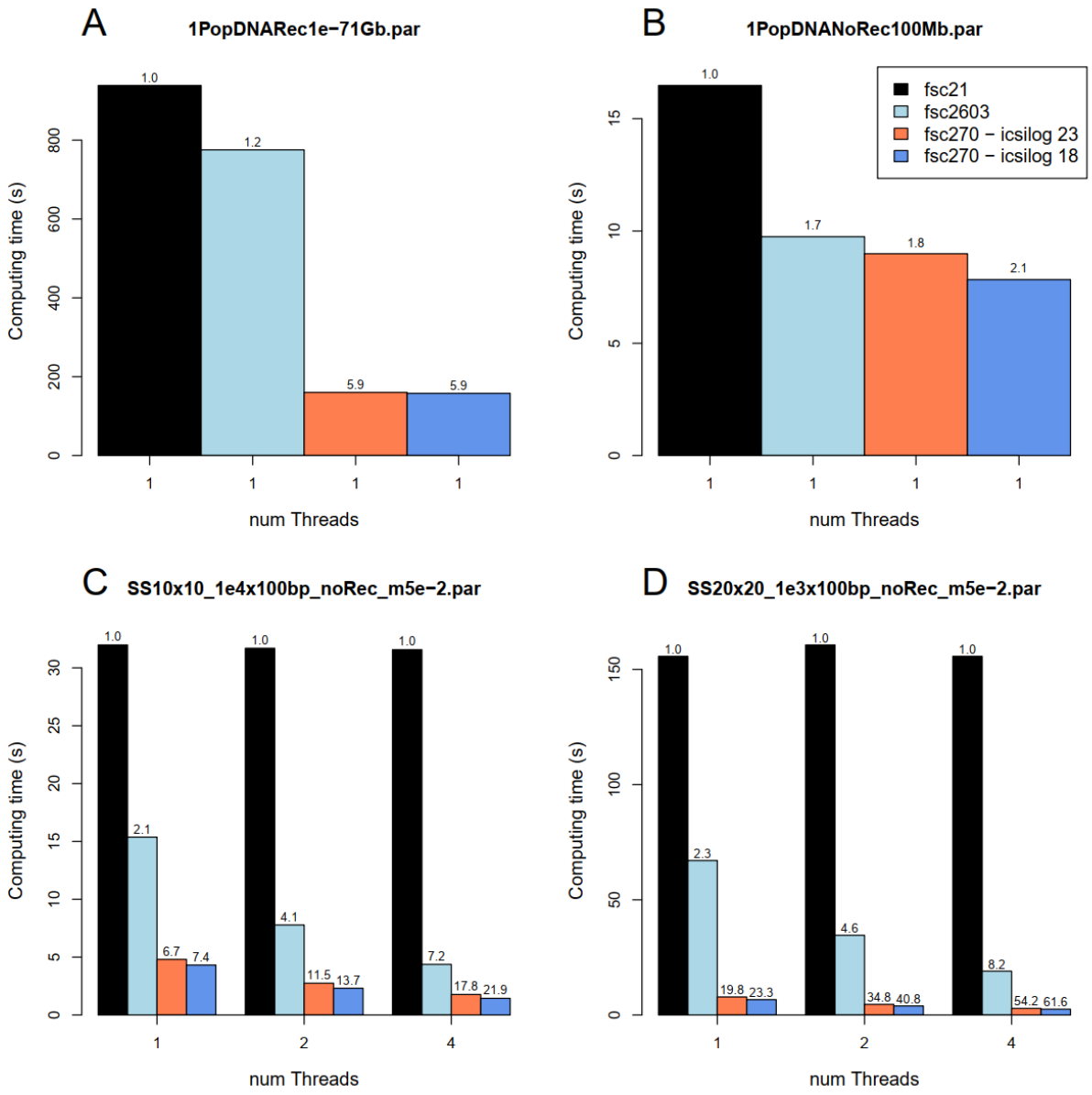
For fsc27

```
./ fsc -t 3PopExpBot20Mb.tpl -e 3PopExpBot20Mb.est -d -M -n100000 -L5 --seed 1234 --
          logprecision xx --multiSFS -q -cyy -B8

./ fsc -t 3PopBot20Mb.tpl -e 3PopBot20Mb.est -d -M  -n100000 -L5 --seed 1234 --
          logprecision xx --multiSFS -q -cyy -B8
```

Where *fsc* is one of the three *fsc* programs, *xx* is the log precision for *fsc27*, and *yy* is the number of threads.

COMPARATIVE SPEED TESTS: FSC2.1.1 VS. FSC2.6.0.3 VS. FSC2.7.0

fsc27 has been optimized for the handling of large recombining DNA segments and for the simulation of samples in large, subdivided populations (e.g. 2D stepping stone models). We report below some tests done in such cases, showing the net gain speed of *fsc27* compared to previous versions.



The figure above reports the speed comparison between different version of *fsc2*. *fsc21*: released in 2013, single-threaded. *fsc2603*: released in 2017, multi-threading but no log acceleration. *fsc270*:

current release, log acceleration, optimized for large chromosomes and highly subdivided populations. A: Simulation of 100 1Gb chromosomes, *r*=1e-7. B: Simulation of 100 haploid genomes consisting of 1 million unlinked segments of 100 bp, u=1.4e-8. C: Simulation of 2 haploid genomes of 10,000 unlinked segments of 100 bp in a 2D stepping-stone of 10x10 demes. D: Simulation of 2 haploid genomes of 1,000 unlinked segments of 100 bp in a 2D stepping-stone of 20x20 demes. In the two top cases, the mutation rate *u*=1.4e-8 per bp, and the haploid population size is 20,000. In the two bottom cases, u=1.25e-8, *m*=0.05 to each of the 4 adjacent demes and the haploid population size of each deme is 200.

The following command lines have been used to launch these computations:

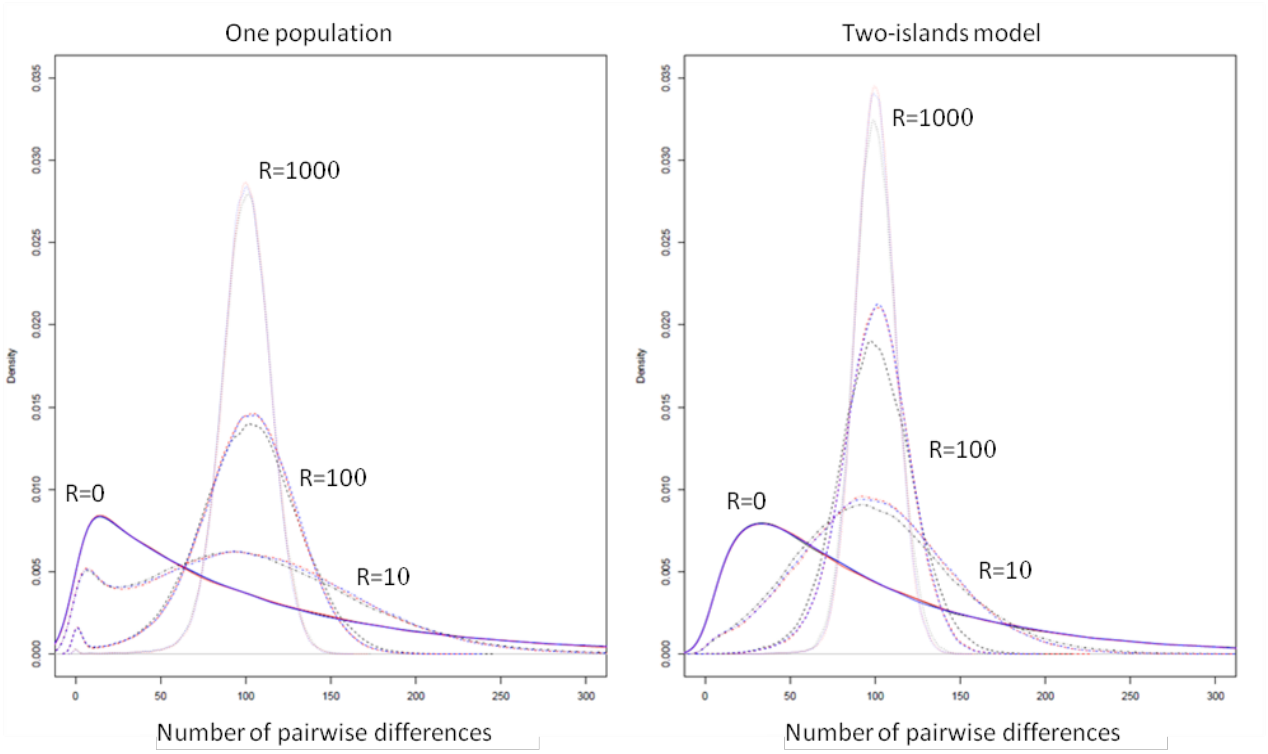
```
./ fsc -i 1PopDNARec1e-71Gb.par -n1 -x --seed 1234 -q -c1 -B4
./ fsc -i 1PopDNARec100Mb.par -n1 -x --seed 1234 -q -c1 -B4
./ fsc -i SS10x10_1e4x100bp_noRec_m5e-2.par -n1 -x --seed 1234 -q -c1 -B4
./ fsc -i SS20x20_1e3x100bp_noRec_m5e-2.par -n1 -x --seed 1234 -q -c1 -B4
```

where *fsc* stands for either *fsc2.1, fsc2.6, or fsc2.7*, and options –logprecision 18 or –logprecision 23 were additionally used for *fsc* ver *2.7.*

COMPARATIVE PATTERNS OF SIMULATED MOLECULAR DIVERSITY

NUMBER OF PAIRWISE DIFFERENCES

We report below a comparison of the patterns of diversity within and between populations simulated by *ms*, *MaCs* and *fastsimcoal*.



ms results are shown with a black line, *MaCs* with a red line, and *fastsimoal*, with a blue line. In all cases, the empirical distributions of the number of pairwise differences were computed from 100,000 simulations of the coalescent of 2 genes. The 2 genes were drawn from a single population for the one-population case, and were drawn each from a different population in the two-island model case. We used the following population parameters: $\theta = 4N\mu = 100$ for the entire sequence

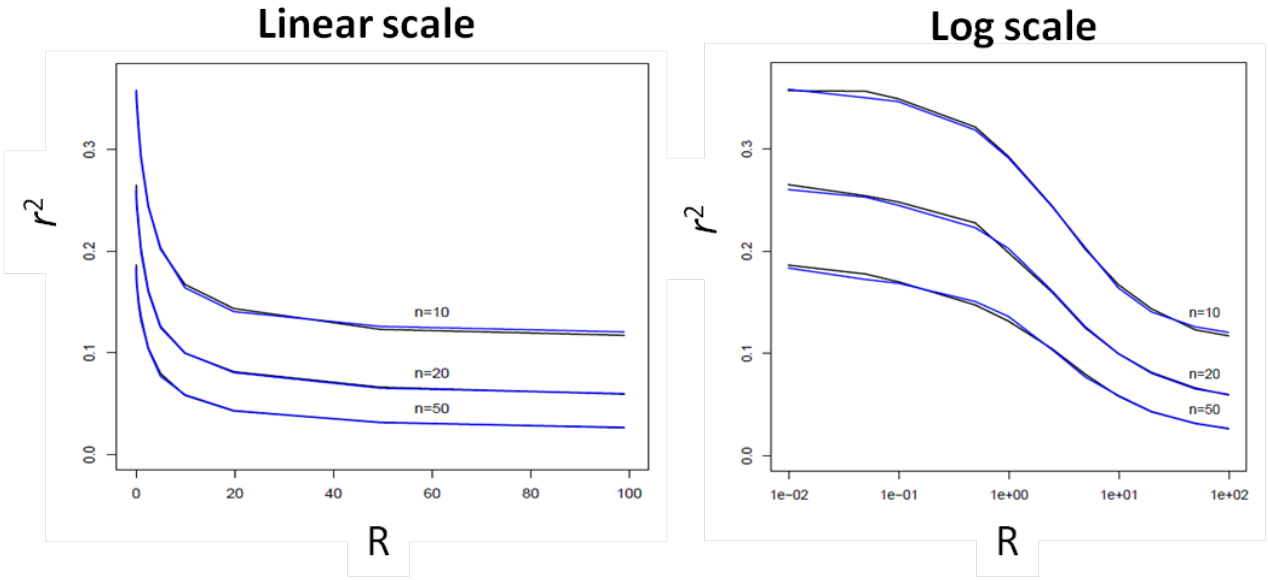
in the one population case; $\theta = 4N_0\mu = 4N_1\mu = 25$ and $M = 4Nm = 0.5$ for the two-island case; and $R = 4Nr$ (where *r* is the total recombination rate between the two ends of the sequence to be simulated) was varied between 0 and 1000, as shown above.

In all cases, *MaCS* and *fastsimcoal* lead to identical distributions, which is expected as they are both based on the same SMC' approximation. In absence of recombination *MaCS* and *fastsimcoal* give also exactly the same distributions as *ms*, but are just running 7-10 times slower, as was seen in the previous section. With very high recombination rates, the SMC-based approximation of *MaCS* and *fastsimcoal* is extremely close to the ancestral recombination graph (ARG) implemented in *ms*, in keeping with previous results (McVean and Cardin 2005). For "intermediate" recombination rates (*R*=10, *R*=100), some slight differences do emerge between ARG- and SMC-based programs, and these differences are slightly more pronounced in the 2-island model. However, it seems that these differences are much less than differences due to the choice of different demographic, mutation, or recombination parameters.

LINKAGE DISEQUILIBRIUM

Previous studies have shown that patterns of LD were virtually undistinguishable between *ms* and SMC'-based programs (Marjoram and Wall 2006, Chen et al. 2009) along DNA sequences.

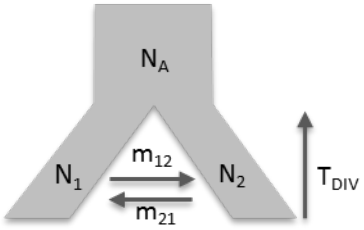
Here, we report a comparison of the average LD (as measured by r^2) between *simcoal2* and *fastsimcoal* between two SNP markers located at a given recombination distance expressed in *R* units.



The results are based on 20,000 simulations and confirm that average r^2 values are virtually undistinguishable between the two approaches.

EXAMPLE FILES FOR THE ESTIMATION OF DEMOGRAPHY FROM THE (JOINT) SFS

ISOLATION WITH MIGRATION (IM) SCENARIO



IM20Mb.tpl

```
//Parameters for the coalescence simulation program : simcoal.exe
2 samples to simulate :
//Population effective sizes (number of genes)
NPOP1
NPOP2
//Samples sizes and samples age
20
30
//Growth rates: negative growth implies population expansion
0
0
//Number of migration matrices : 0 implies no migration between demes
2
//Migration matrix 0
0 MIG21
MIG12 0
//Migration matrix 1
0 0
0 0
//historical event: time, source, sink, migrants, new deme size, growth rate, migr mat index
1 historical event
TDIV 0 1 1 RESIZE 0 1
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of contiguous linkage Block: a block is a set of contiguous loci
1
//per Block:data type, number of loci, per gen recomb and mut rates
FREQ 1 0 2.5e-8e-8
```

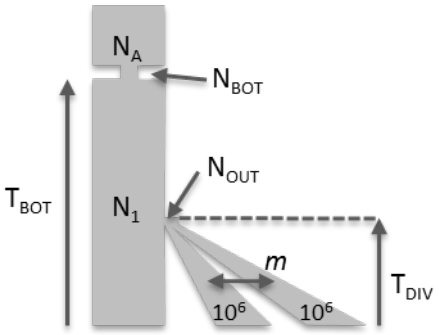
IM20Mb.est

```
// Priors and rules file
// *********************

[PARAMETERS]
//#isInt? #name   #dist.#min  #max
//all N are in number of haploid individuals
1  ANCSIZE     unif     100  100000   output
1  NPOP1       unif     100  100000   output
1  NPOP2       unif     100  100000   output
0  N1M21       logunif  1e-2 20       hide
0  N2M12       logunif  1e-2 20       hide
1  TDIV        unif     100   20000   output

[COMPLEX PARAMETERS]

0  RESIZE = ANCSIZE/NPOP2     hide
0  MIG21  = N1M21/NPOP1       output
0  MIG12  = N2M12/NPOP2       output
```

COMMAND LINE FOR PARAMETER ESTIMATION

```
./ fsc27 -t IM20Mb.tpl -n100000 -d -e IM20Mb.est -M  -L 40 -c6 -q
```

DIVERGENCE OF THREE POPULATIONS



3PopExpBot20Mb.tpl

```
//Parameters for the coalescence simulation program : fastsimcoal.exe
3 samples to simulate :
//Population effective sizes (number of genes)
NPOPAF
2000000
2000000
//Samples sizes and samples age
20
20
20
//Growth rates : negative growth implies population expansion
0
R1
R1
//Number of migration matrices : 0 implies no migration between demes
2
//Migration matrix 0
0.0000 0.0000 0.0000
0.0000 0.0000 MIG
0.0000 MIG 0.0000
//Migration matrix 1
0 0 0
0 0 0
0 0 0
//historical event: time, source, sink, migrants, new deme size, growth rate, migr mat index
4 historical event
TDIV 2 0 1 1 0 1
TDIV 1 0 1 1 0 1
TBOT 0 0 0 RES1 0 1
TENDBOT 0 0 0 RES2 0 1
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of contiguous linkage Block: a block is a set of contiguous loci
1
//per Block:data type, number of loci, per gen recomb and mut rates
FREQ 1 0 2.5e-8
```

3PopExpBot20Mb.est

```
// Priors and rules file
// *********************

[PARAMETERS]
//#isInt? #name   #dist.#min  #max
//all Ns are in number of haploid individuals
1  ANCSIZE     unif     1000  100000  output
1  NBOT        unif     10    2000    output
1  NPOPAF      unif     1000 100000   output
1  NPOPOOA     unif     10    10000   output
1  TDIV        unif     10    10000   output
1  TPLUSDIV    unif     10    10000   hide
0  MIG         logunif  1e-5 1e-2     output

[COMPLEX PARAMETERS]
1  TBOT = TDIV+TPLUSDIV           output
```

```
0  RATIO_OOA_EA = NPOPOOA/2000000 hide
0  RTEA = log(RATIO OOA EA)       hide
0  R1 = RTEA/TDIV                 hide
1  TENDBOT = TBOT+500             hide
0  RES1 = NBOT/NPOPAF             hide
0  RES2 = ANCSIZE/NBOT            hide
```

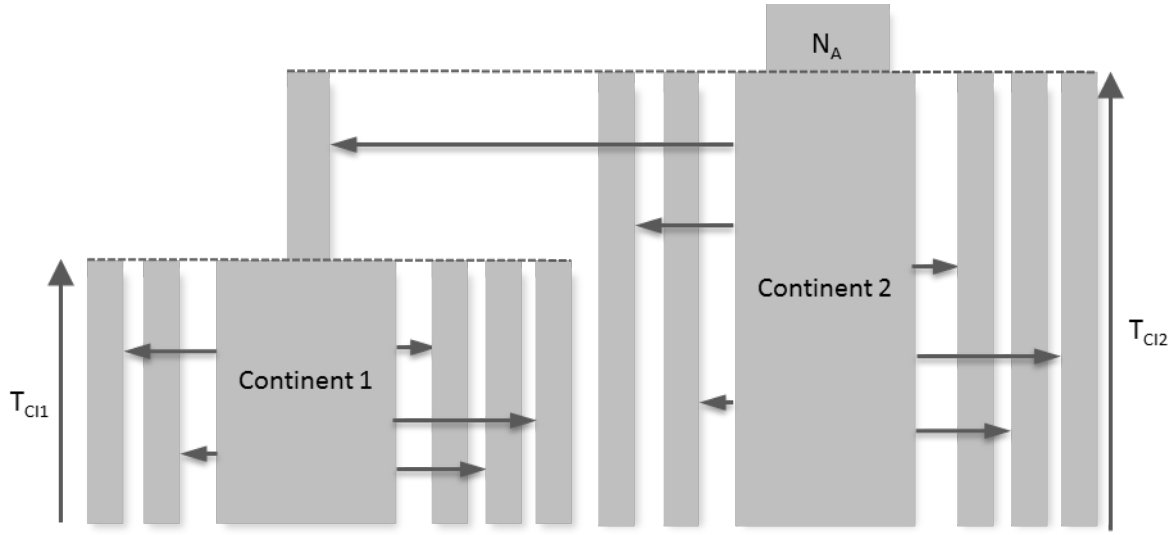
COMMAND LINE FOR PARAMETER ESTIMATION

```
./ fsc27 -t 3PopExpBot20Mb.tpl -n100000 -d -e 3PopExpBot20Mb.est -M -L40 -q --multiSFS -C10 -c8
```

Comments:

- Here we use the multidimensional SFS (*--multiSFS*)
- We also impose a minimum observed SFS entry of 10 (*-C10*) to appear in the likelihood, entries of the observed SFS with lower observations wil be collapsed into a single entry
- We use 8 threads to speed up computations (*-c8*)

HIERARCHICAL ISLAND MODEL



10Pop2ContiIsl.tpl

```
//Parameters for the coalescence simulation program : fastsimcoal.exe
12 samples to simulate :
//Population effective sizes (number of genes)
1000
1000
1000
1000
1000
1000
1000
1000
1000
1000
20000000
20000000
//Samples sizes and samples age
20
20
20
20
20
20
20
20
20
20
0
0
//Growth rates      : negative growth implies population expansion
0
0
0
0
0
0
0
0
```

```
0
0
0
0
//Number of migration matrices : 0 implies no migration between demes
3
//Migration matrix 0
0         0         0         0         0         0         0         0         0         0         M010      0
0         0         0         0         0         0         0         0         0         0         M110      0
0         0         0         0         0         0         0         0         0         0         M210      0
0         0         0         0         0         0         0         0         0         0         M310      0
0         0         0         0         0         0         0         0         0         0         M410      0
0         0         0         0         0         0         0         0         0         0         0         M511
0         0         0         0         0         0         0         0         0         0         0         M611
0         0         0         0         0         0         0         0         0         0         0         M711
0         0         0         0         0         0         0         0         0         0         0         M811
0         0         0         0         0         0         0         0         0         0         0         M911
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
//Migration matrix 1
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         M511
0         0         0         0         0         0         0         0         0         0         0         M611
0         0         0         0         0         0         0         0         0         0         0         M711
0         0         0         0         0         0         0         0         0         0         0         M811
0         0         0         0         0         0         0         0         0         0         0         M911
0         0         0         0         0         0         0         0         0         0         0         M1211
0         0         0         0         0         0         0         0         0         0         0         0
//Migration matrix 2
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
0         0         0         0         0         0         0         0         0         0         0         0
//historical event: time, source, sink, migrants, new deme size, new growth rate, migration matrix index
13 historical event
TISLAND1 0 10 1 1 0 1
TISLAND1 1 10 1 1 0 1
TISLAND1 2 10 1 1 0 1
TISLAND1 3 10 1 1 0 1
TISLAND1 4 10 1 1 0 1
TISLAND1 10 10 0 0.0001 0 1
TISLAND2 5 11 1 1 0 2
TISLAND2 6 11 1 1 0 2
TISLAND2 7 11 1 1 0 2
TISLAND2 8 11 1 1 0 2
TISLAND2 9 11 1 1 0 2
TISLAND2 10 11 1 1 0 2
TISLAND2 11 11 0 RESIZE 0 2//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of contiguous linkage Block: a block is a set of contiguous loci
1
//per Block:data type, number of loci, per gen recomb and mut rates
FREQ 1 0 2.5e-8
```

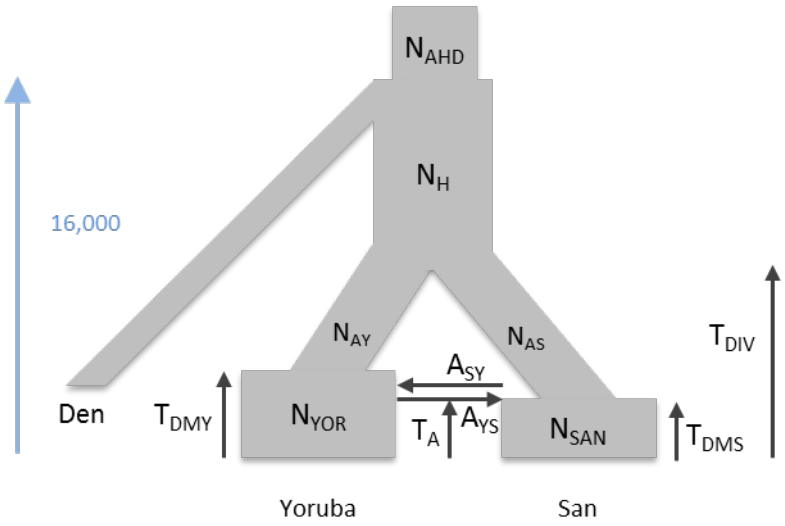
10Pop2ContiIsl.est

```
// Priors and rules file
// *********************

[PARAMETERS]
//#isInt? #name   #dist.#min  #max
//all Ns are in number of haploid individuals
1  ANCSIZE     unif     10  100000    output
0  NM0         logunif  0.01  100     output
0  NM1         logunif  0.01  100     output
0  NM2         logunif  0.01  100     output
0  NM3         logunif  0.01  100     output
0  NM4         logunif  0.01  100     output
0  NM5         logunif  0.01  100     output
0  NM6         logunif  0.01  100     output
0  NM7         logunif  0.01  100     output
0  NM8         logunif  0.01  100     output
0  NM9         logunif  0.01  100     output
0  NM_12        logunif  0.01  100    output
1  TISLAND1    unif     10    20000   output
1  TPLUS       unif     10    20000   hide

[COMPLEX PARAMETERS]

//Assume an island haploid population size of 1000 for all islands
1  TISLAND2 = TISLAND1+TPLUS output
0  M010 =  NM0/1000          hide
0  M110 =  NM1/1000          hide
0  M210 =  NM2/1000          hide
0  M310 =  NM3/1000          hide
0  M410 =  NM4/1000          hide
0  M511 =  NM5/1000          hide
0  M611 =  NM6/1000          hide
0  M711 =  NM7/1000          hide
0  M811 =  NM8/1000          hide
0  M911 =  NM9/1000          hide
0  M1211 = NM_12/2000        hide
0  RESIZE = ANCSIZE/20000000 hide
```

COMMAND LINE FOR PARAMETER ESTIMATION

```
./ fsc27 -t 10Pop2ContiIsl.tpl -n50000 -d -e 10Pop2ContiIsl.est -L30 -M -q
```

HUMAN AFRICAN DEMOGRAPHY WITH SNP ASCERTAINMENT



p4NocpgSanYor4.tpl

```
//Parameters for the coalescence simulation program : simcoal.exe
3 samples to simulate : Exponential growth : 1000 to 100,000,000 started 3000 generations ago
//Population effective sizes (number of genes)
NSan
NYor
1000
//Samples sizes
12
44
1 1600
//Growth rates : negative growth implies population expansion
0
0
0
//Number of migration matrices : 0 implies no migration between demes
0
//historical event: time, source, sink, migrants, new deme size, new growth rate, migration matrix
index
6  historical event
TDMS 0 0 0 RES_SAN 0 0
TDMY 1 1 0 RES_YOR 0 0
TAdm 0 1 AYS 1 0 0
TAdm 1 0 ASY 1 0 0
TDIVSanYor 1 0 1 RES_AF 0 0
16000 2 0 1 RES_ANC 0 0
//Number of independent loci [chromosome]
1 0
//Per chromosome: Number of contiguous linkage Block: a block is a set of contiguous loci
1
//per Block:data type, number of loci, per gen recomb and mut rates
FREQ 1 0 1e-7
```

Note that the divergence time with Denisovans is fixed, and this time is used to calibrate the other parameters

p4NocpgSanYor4.tpl

```
// Priors and rules file
// *********************

[PARAMETERS]
//#isInt? #name   #dist.#min  #max
//all Ns are in number of haploid individuals
1  NSan         unif     1000   2e6  output
1  NYor         unif     1000   2e6  output
1  NASan        unif     1000   1e5  output
1  NAYor        unif     1000   1e5  output
1  HSIZE        unif     1000   1e5  output
1  ANCSIZE      unif     1000   1e5  output
1  TDMS         unif     10     500  output
1  TDMY         unif     10     500  output
1  TAdm         unif     10     500  output
1  TPlusAdm     unif     1     5000  hide
0  AYS          unif     0      0.2  output
0  ASY          unif     0      0.2  output

[COMPLEX PARAMETERS]
1  TDIVSanYor = TAdm+TPlusAdm   output
0  RES_SAN  = NASan/NSan        hide
0  RES_YOR  = NAYor/NYor        hide
0  RES_AF   = HSIZE/NASan       hide
0  RES_ANC  = ANCSIZE/HSIZE     hide
```

COMMAND LINE FOR PARAMETER ESTIMATION

```
./ fsc27 -t p4NocpgSanYor4.tpl -n100000 -d -e p4NocpgSanYor4.est -M -L 20 -a0 -A2 -q -0 -C2 -multiSFS
```

Comments:

- ***-a0*** indicates that the ascertainment occurred in population 0 (San)
- ***-A2*** indicates that 2 chromosomes were used to infer the polymorphism status for each SNP
- ***-0*** indicates that one does not use information on the number of monomorphic sites
- ***--multiSFS*** indicates that the 3D SFS is used

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