fastsimcoal ver 2.7
fsc27

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

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2. INTRODUCTION

This manual describes the use of *fastsimcoal27,* shortened to *fsc27,* a program to generate the neutral genomic molecular diversity in current or ancient samples drawn from a population with a complex demographic history. *fsc27* is the fourth 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 *fsc27* is based on a much faster continuous time approximation. Despite a completely new coalescent engine, *fsc27* uses exactly the same input files as *simcoal2*, and it produces very similar output files.

fsc27 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).

fsc27 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).

fsc27 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 *fsc27* for Windows, Linux or MacOs X are available on http://cmpg.unibe.ch/software/fastsimcoal27

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 *fsc27*:

- 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. (submitted) *fastsimcoal2*: demographic inference under complex evolutionary scenarios-

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

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 (FSC27) 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
     o *-N* option has been suppressed, and maximum no. of iteration is now equal to that set by the *-n* option.
     o The number of cycles to performed is now fixed and only specified with option *-L.*
     o 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).
     o 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.

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

```
Command Prompt

Microsoft Windows [Version 6.1.7600]
Copyright (c) 2009 Microsoft Corporation.  All rights reserved.

C:\Users\Laurent\Documents\My Dropbox\fastsimcoal\manual\examples files>fastsimcoal.exe -i 2popSTRDiv.par -n1

Random generator initialized with : 825704

Deme sizes
Deme 0  1000
Deme 1  1000


Sample sizes
Deme 0   5
Deme 1   5

Sample ages
Deme 0   0
Deme 1   0

Growth rates
Deme 0   0
Deme 1   0


Migration matrix
 0.0000000  0.0005000
 0.0001000  0.0000000


Migration matrix
 0.0000000  0.0000000
 0.0000000  0.0000000


Historical events
Event 0

#Time            : 1000
#Source          : 0
#Sink            : 0
#Migrants        : 1.0000000
#New size        : 1.0000000
#New growth rate : 0.0000000
#New migr. matrix : 1

Event 1

#Time            : 10000
#Source          : 1
#Sink            : 0
#Migrants        : 1.0000000
#New size        : 10.0000000
#New growth rate : 0.0000000
#New migr. matrix : 1


Number of independent loci to simulate : 1
with the same chromosomal structure

Number of linkage blocks to simulate in structure 1: 1

    10 partially linked MICROSAT:
       recombination, mutation rates, geometric parameter, and range constraint : 0.0000000000   0.0005000000   0.00000
    0

Fastsimcoal is building 1 genealogies ...
MRCA time for non-recombining chromosome structure 0: 15083
       Genealogy # 1/1

Iteration 1/1 done in 0.000sec


C:\Users\Laurent\Documents\My Dropbox\fastsimcoal\manual\examples files>_
```

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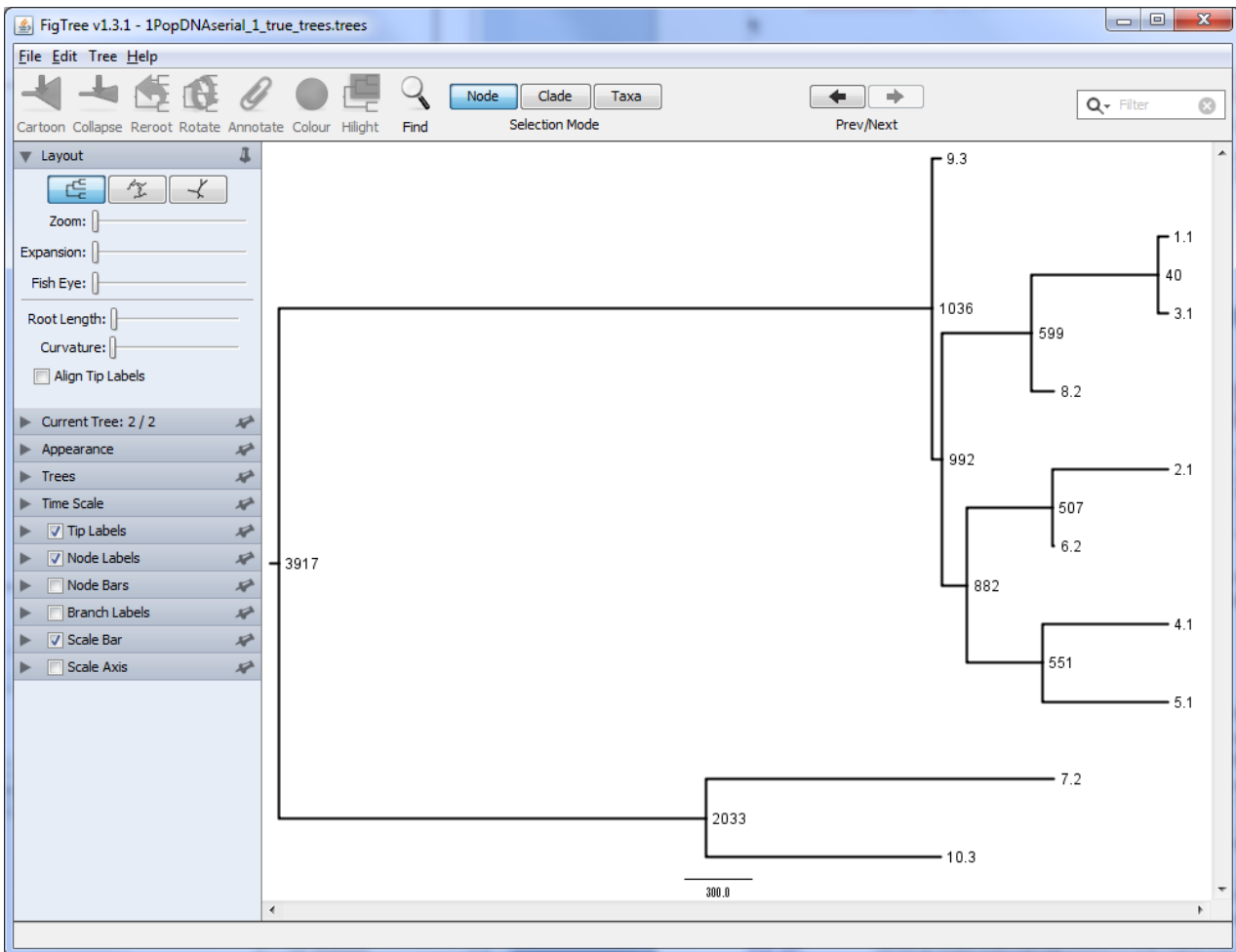
We indeed see that the first 5 sequences from deme 0 were sampled at time 0, that the 3 sequences from deme 1 were sampled at time 500, and that the 2 sequences of deme 2 were sampled at time 1000.

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.
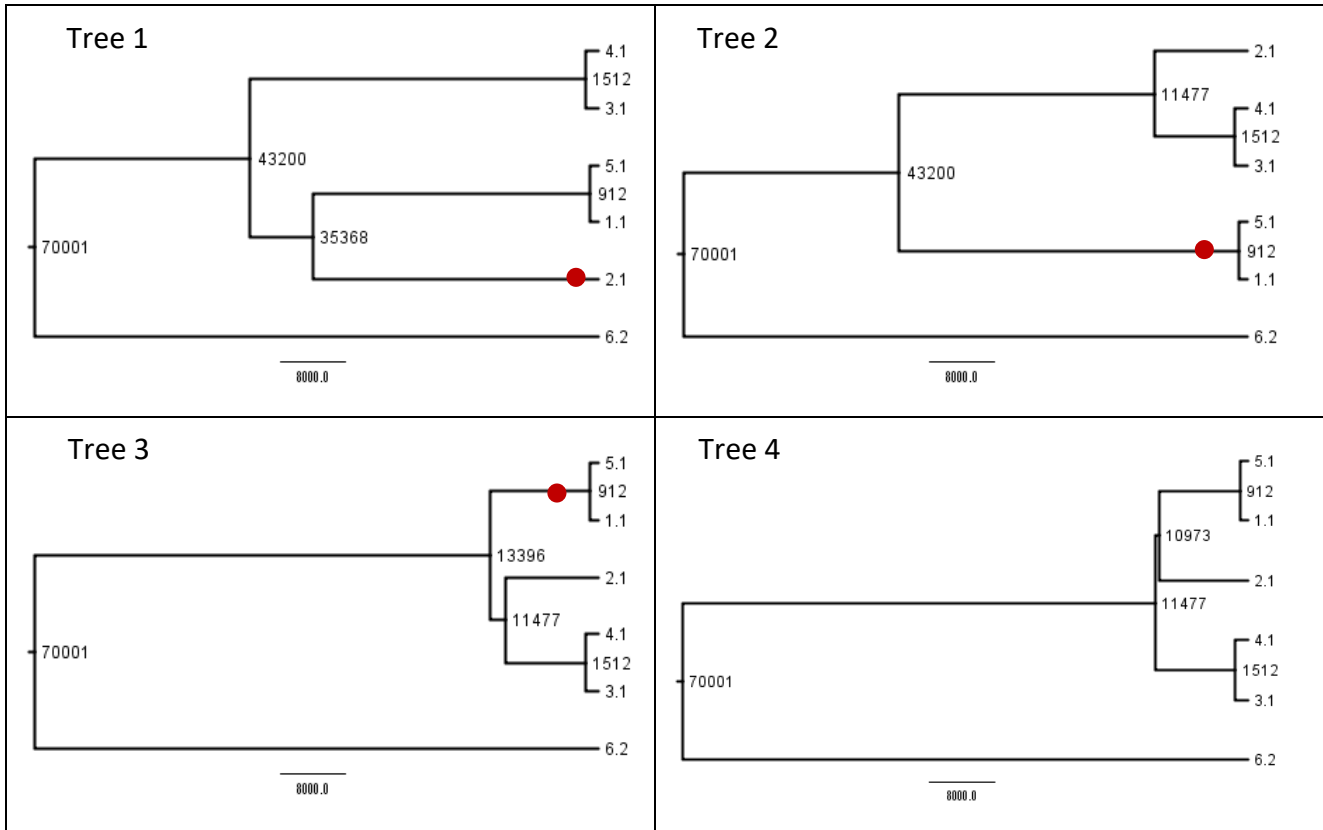
INPUT FILE SYNTAX

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

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.

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
```

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.

Data types[1]	Extra parameters		
	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).
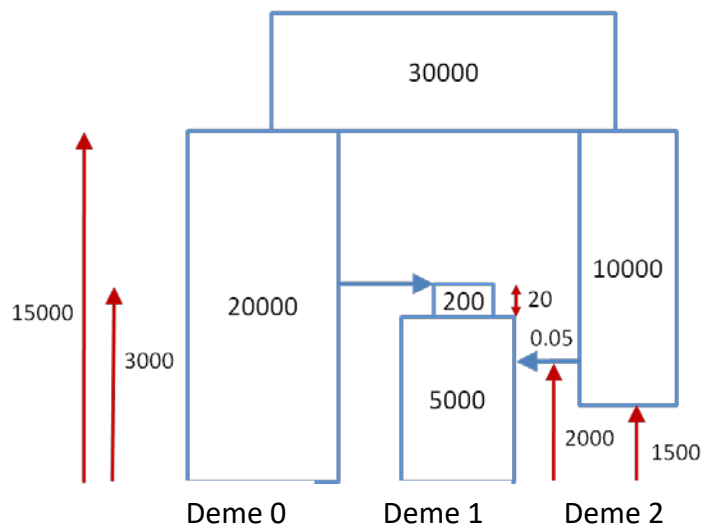
This scenario assumes that genes from deme 2 were sampled 1500 generations ago, and that 5% of the genes present in deme 1 2000 generations ago actually came from deme 2 (which implies an introgression or admixture event). It also assumes that deme 1 originated from deme 0 3000 generations ago and went 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:
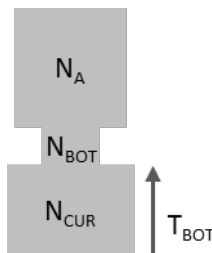
Note that more complex examples are provided in the Appendix section "Example files for the estimation of demography from the (joint) SFS".

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 **–I** 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 **-I** 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.

- **-I** 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 **–I** 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**.

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.

Short cut (-)	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
`-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*.
	`--logprecision 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
	`--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.
	`--initValues my.pv`	Specifies a file (*.pv*) containing initial parameter values for parameter estimation. This is especially useful to reduce the number of runs necessary to estimate parameters when estimating confidence intervals by bootstrap.
`-G`	`--indgenot`	Generates an individual genotype table.

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 -I -x --seed 1234 - 10 simulations of 100,000 segments of 100 bp
Additional options: -c1 to -c12 and -B4 to -B12

Computing time as a function of threads used
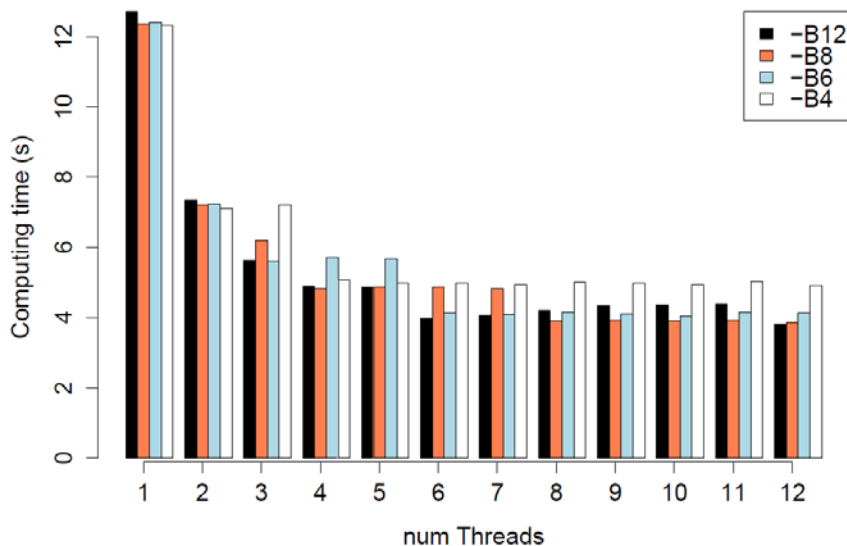
./$fsc25 -t 1PopExpInst20Mb.tpl -e 1PopExpInst20Mb.est -d -M0.001 -n200000 -N200000 -I -l5 -L5 --seed 1234 -q

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

Computing time as a function of threads used
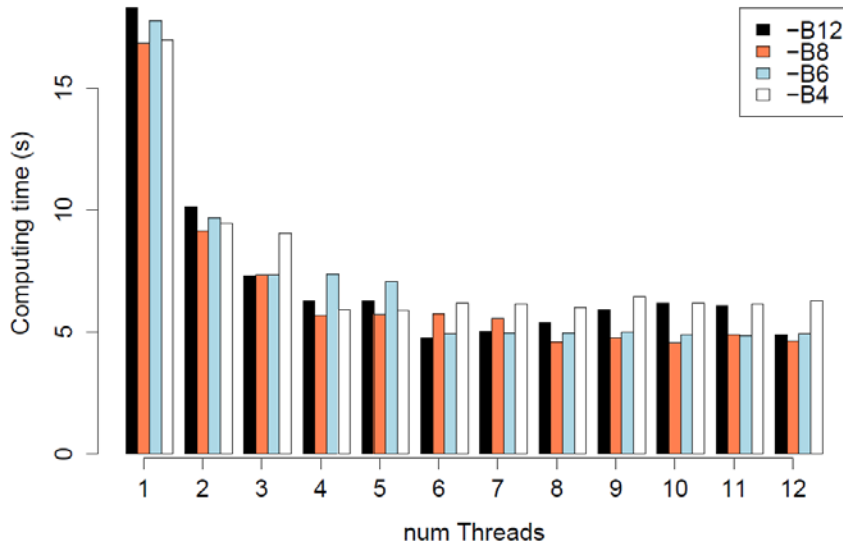
SEQUENTIAL MARKOV COALESCENT APPROXIMATION

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 |
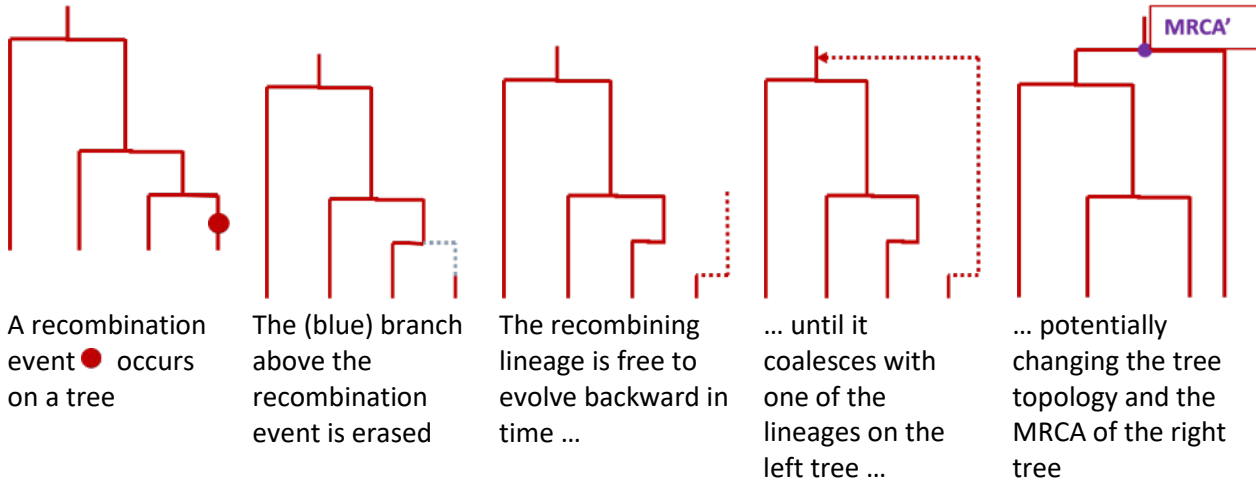
Marjoram and Wall (2006) proposed a slight modification of the SMC algorithm consisting in removing step 5 of the SMC algorithm above, and thus keeping the blue branch, and thus leaving the possibility to the recombining lineage to coalesce with it. The blue branches are only erased at the end of the 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. |
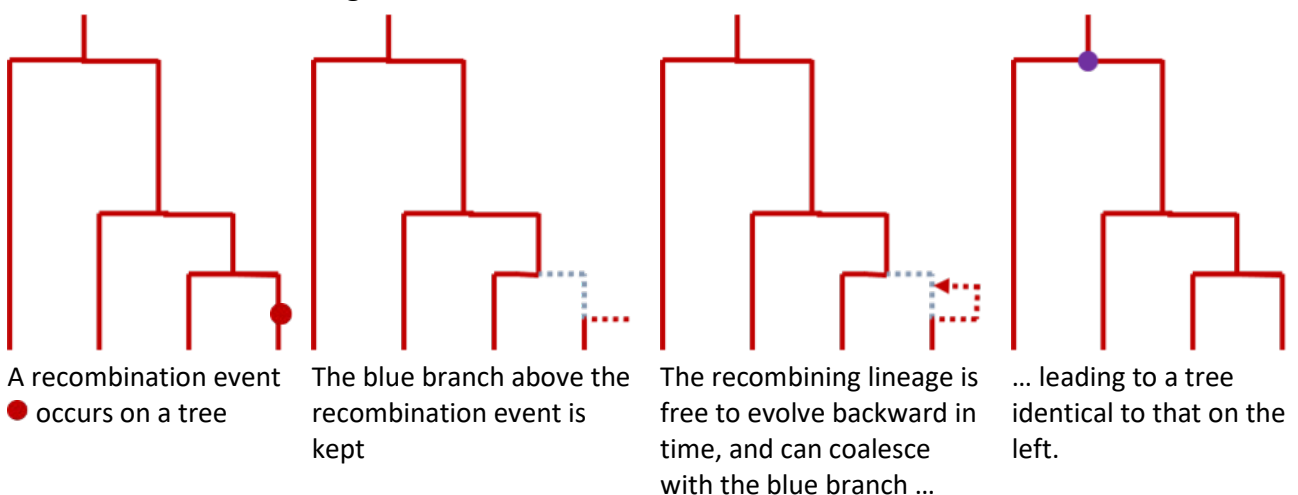
SITE FREQUENCY SPECTRUM

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   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 SITE FREQUENCY SPECTRUM

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	3PopDNASFSsmall_jointMAFpop2_0.obs	3PopDNASFSsmall_jointMAFpop2_1.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
```

```
 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
```

```
 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	3PopDNASFSsmall_jointMAFpop2_0.obs	3PopDNASFSsmall_jointMAFpop2_1.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
```

```
 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
```

```
 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				3PopDNASFSsmall_jointDAFpop2_0.obs				3PopDNASFSsmall_jointDAFpop2_1.obs			
1 observations				1 observations				1 observations			
	d0_0	d0_1	d0_2		d0_0	d0_1	d0_2		d1_0	d1_1	d1_2	d1_3
d1_0	9976936	6160	2036	d2_0	9969356	7535	4048	d2_0	9970975	2918	1926	5120
d1_1	2866	644	519	d2_1	4210	218	299	d2_1	4196	85	55	391
d1_2	1435	650	618	d2_2	2435	157	307	d2_2	2369	124	76	330
d1_3	2409	2469	3258	d2_3	1735	247	325	d2_3	1742	124	64	377
				d2_4	1162	149	354	d2_4	1155	93	89	328
				d2_5	1106	226	465	d2_5	1157	103	40	497
				d2_6	3642	1391	633	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:
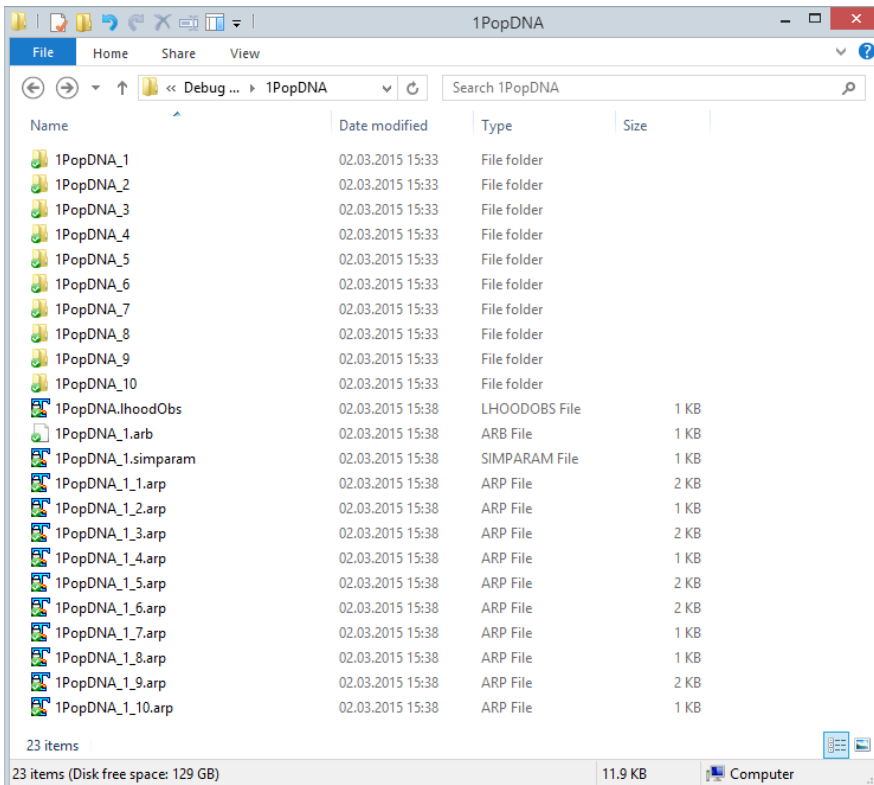
and each subdiretory will contain a separate SFS file, like:
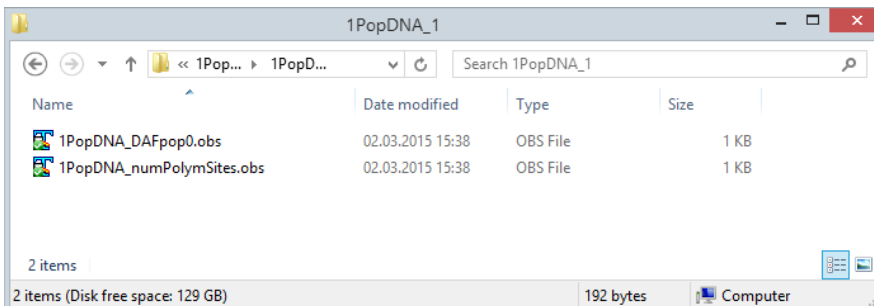
GENERATING NON-PARAMETRIC BOOTSTRAPED SFS

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.
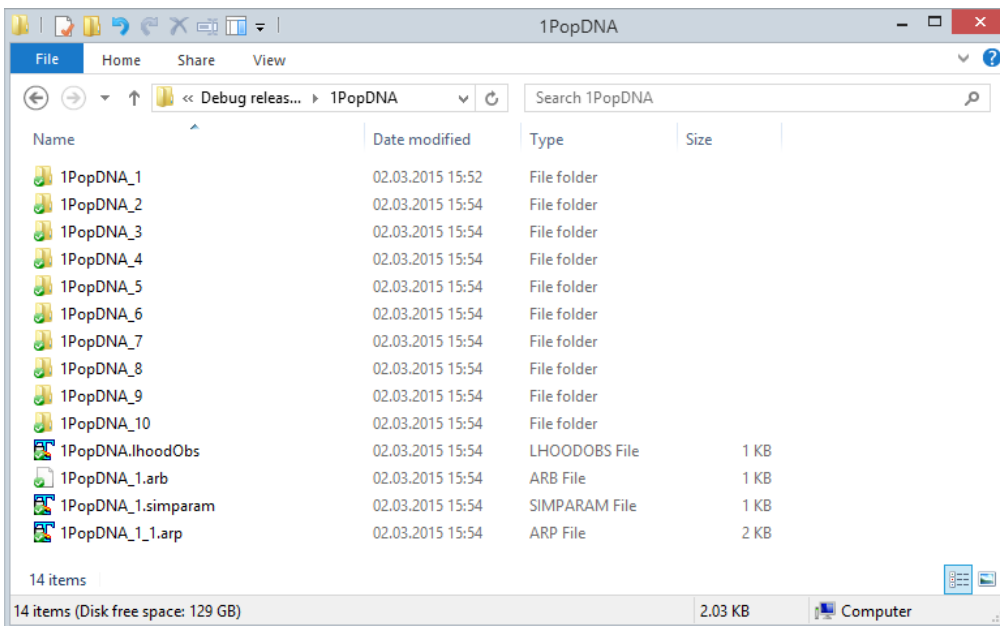
CAUTION AND USE OF BLOCK-BOOTSTRAP WITH REAL DATA

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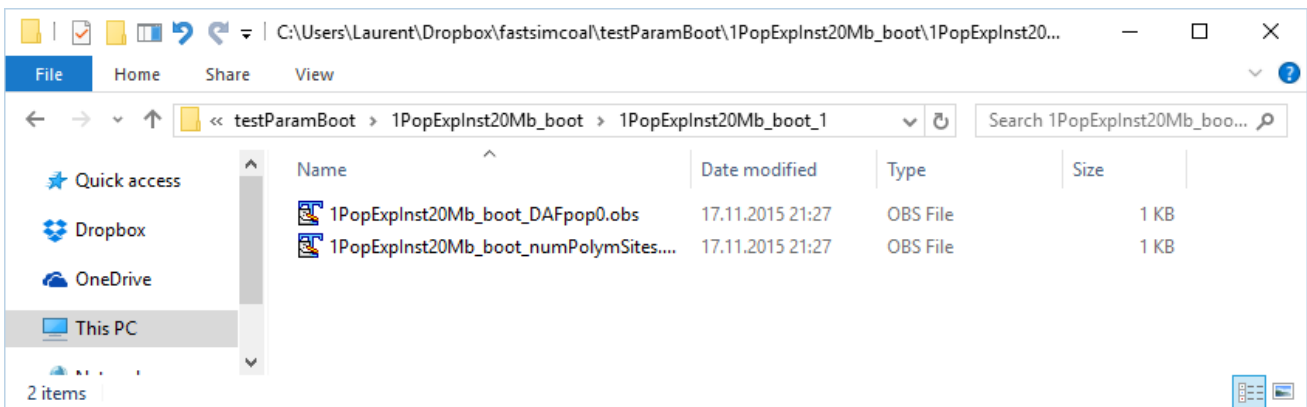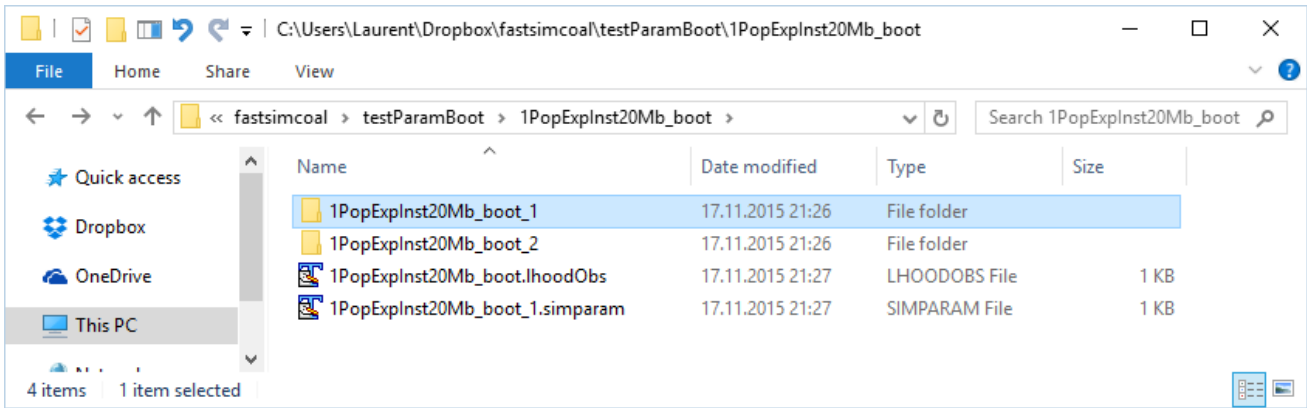
With this procedure one can easily generate more than 2 SFS for these sets of parameters (say 100) and then estimate parameters from these pseudo-observed data sets using the same *.tpl* and *.est* files as those used to get the **_maxL.par* file.

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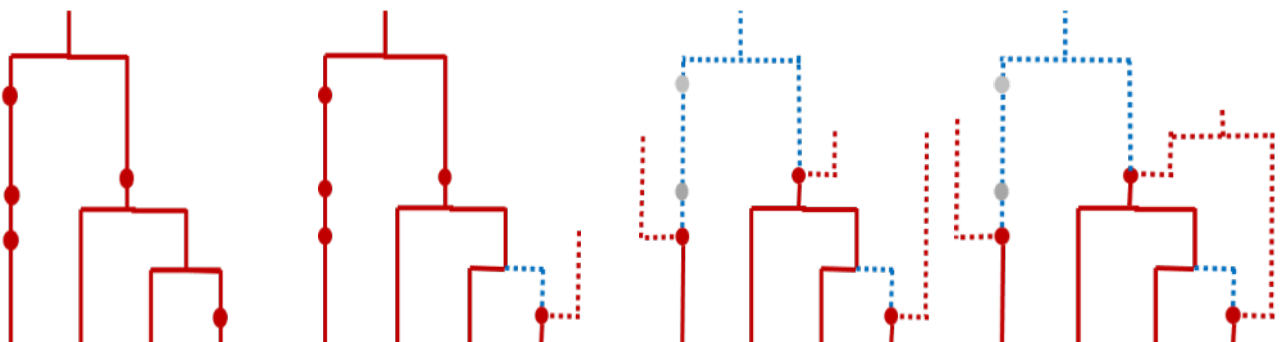
The *z* recombination events are randomly positioned on the tree

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

The recombination event on blue branches is deactivated (grayed)

Two recombinant lineages can coalesce
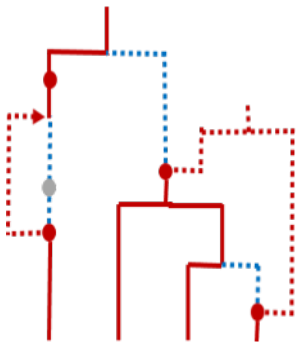
A recombinant lineage coalesces with a blue branch, which reactivates the recombination events above it.
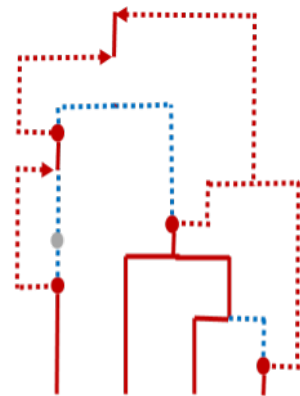
The final coalescent event is reached.
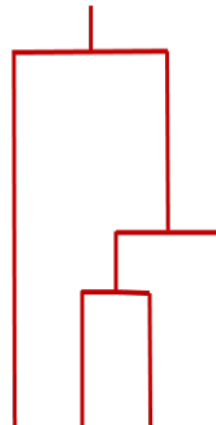
All blue branches are erased to produce the final tree for the right marker.
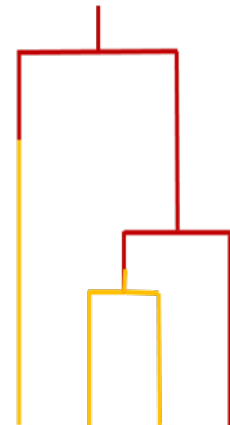
The shared lineages between the two trees are shown in yellow.

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):

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

$$\hat{p}_i = \sum_k^Z \sum_{j \in B_i} b_{ijk} \Big/ \sum_k^Z T_k .$$
(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
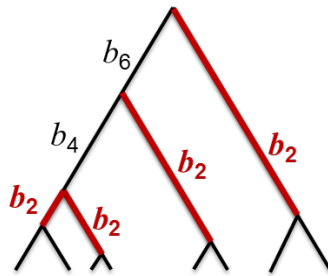
COMPOSITE LIKELIHOODS

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)

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

where $X = \{m_1, ..., m_{n-1}\}$ 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

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

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/.

Ascertained alleles
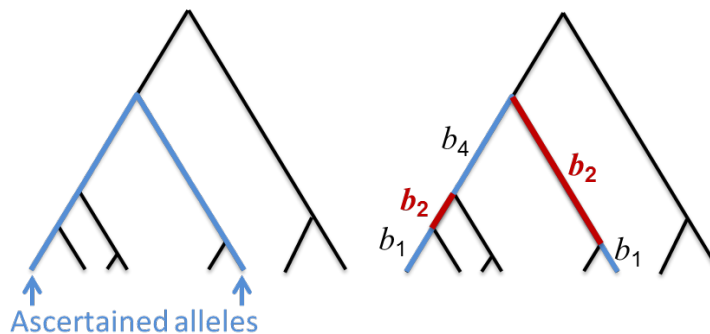
The ascertainment scheme of this array is simple and homogeneous over a given panel. However, the SFS inferred from this array is biased as only mutations that occur in the ancestry of the two compared chromosomes will be considered (see Figure above). Nielsen et al. (2004) have shown how to correct 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`

```bash
#!/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
```

```bash
#!/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} × bp^{-1})	Recombination rate (gen^{-1} × 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*
	1000	1 Mb	3.7	28.1	25.2
1popLargeSample	100	10 Mb	x	327.5	235.7
	100	100 Mb	x	3700.8	2635.4
	1000	1 Mb	3.9	33.3	25.9
2popLargeSample	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
10Pop2ContiIsl 1 -n1000 -L5	191.11 s	129.35 s	1.48	32.54 s	5.87
10Pop2ContiIsl 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.
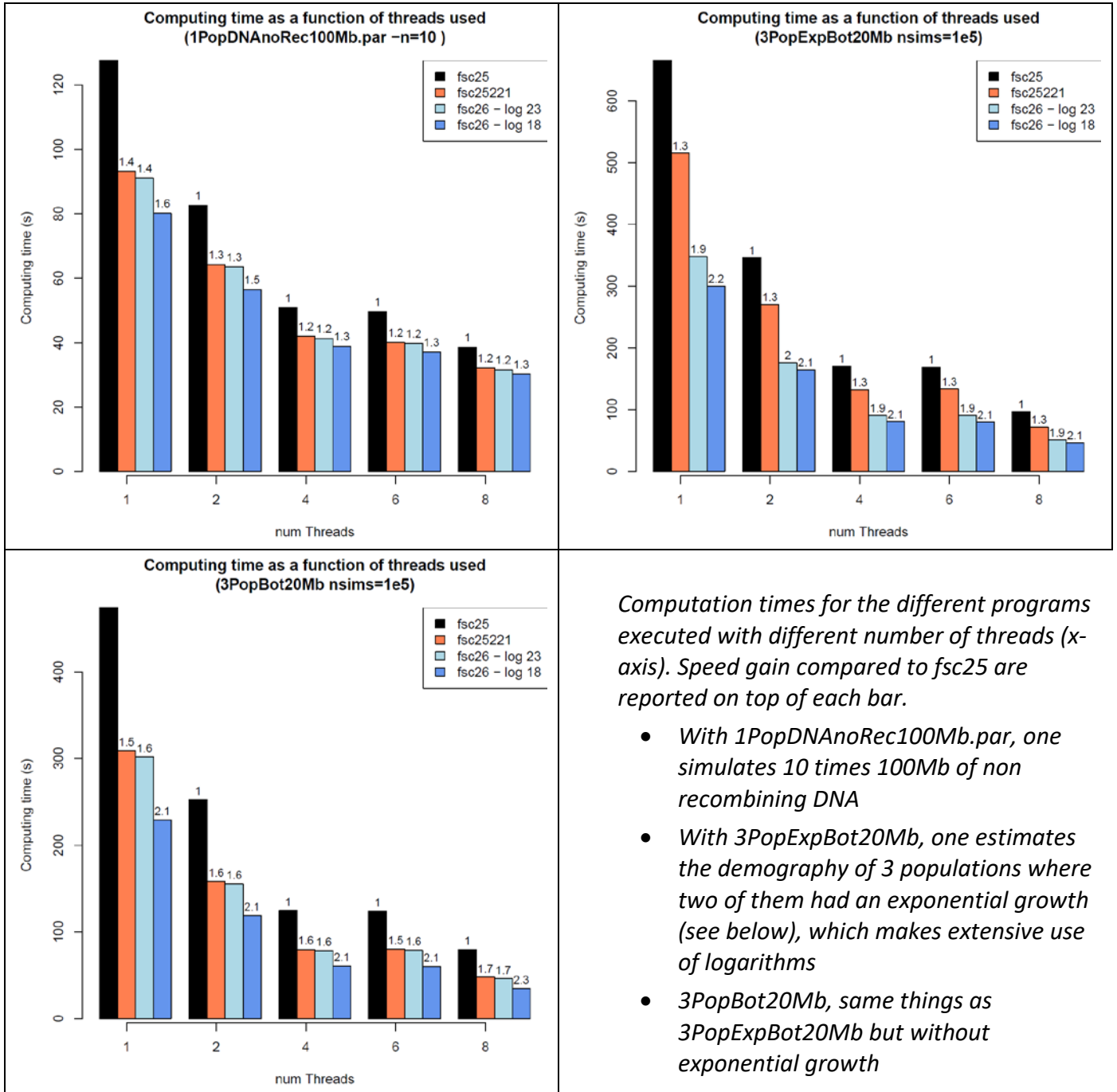
Computation times for the different programs executed with different number of threads (x-axis). Speed gain compared to fsc25 are reported on top of each bar.

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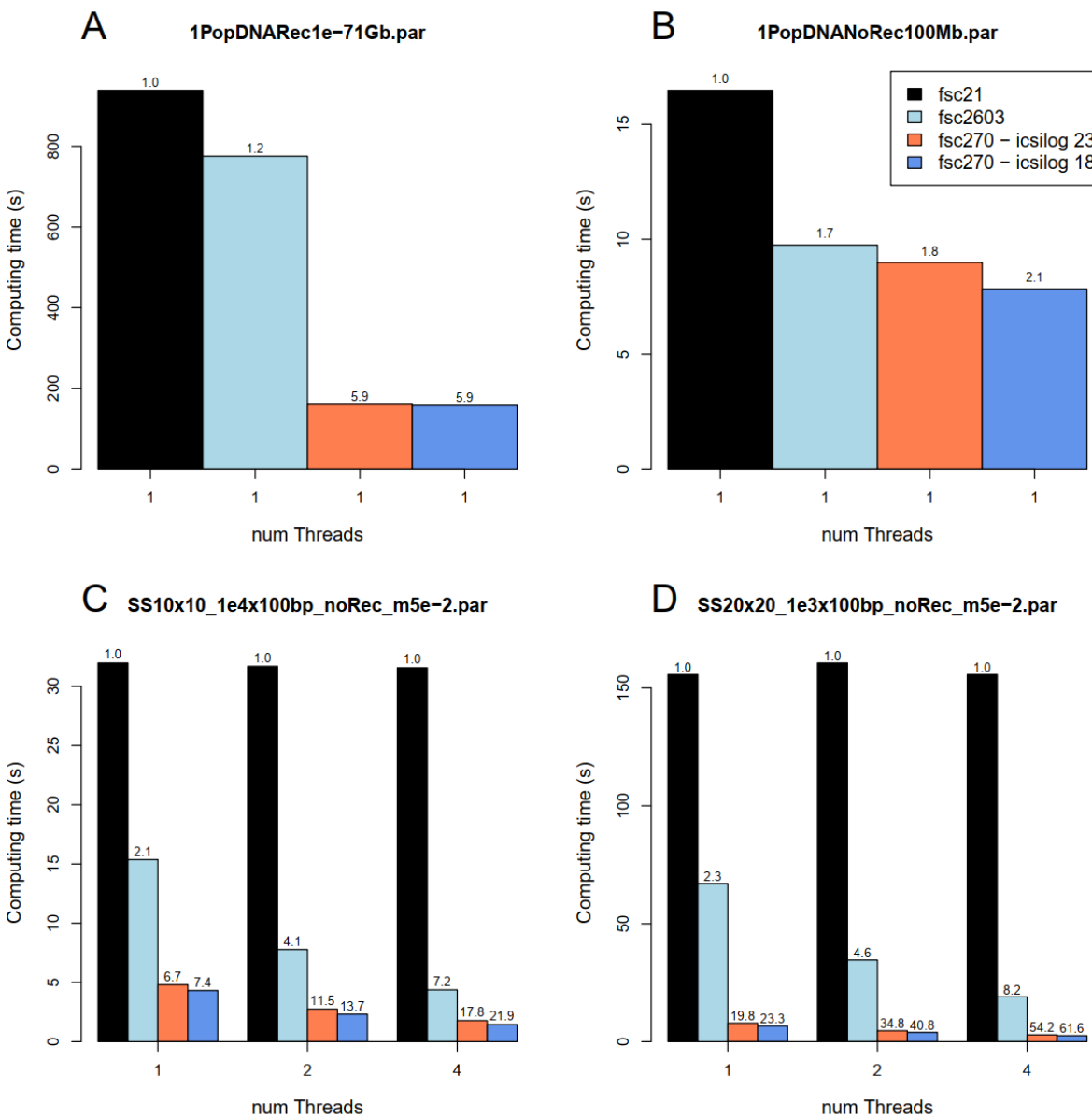
The figure above reports the speed comparison between different version of *fsc2*. *fsc21*: released in 2013, single-threaded. *fsc2603*: released in 2017, multi-threading but no log acceleration. *fsc270*:

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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*.
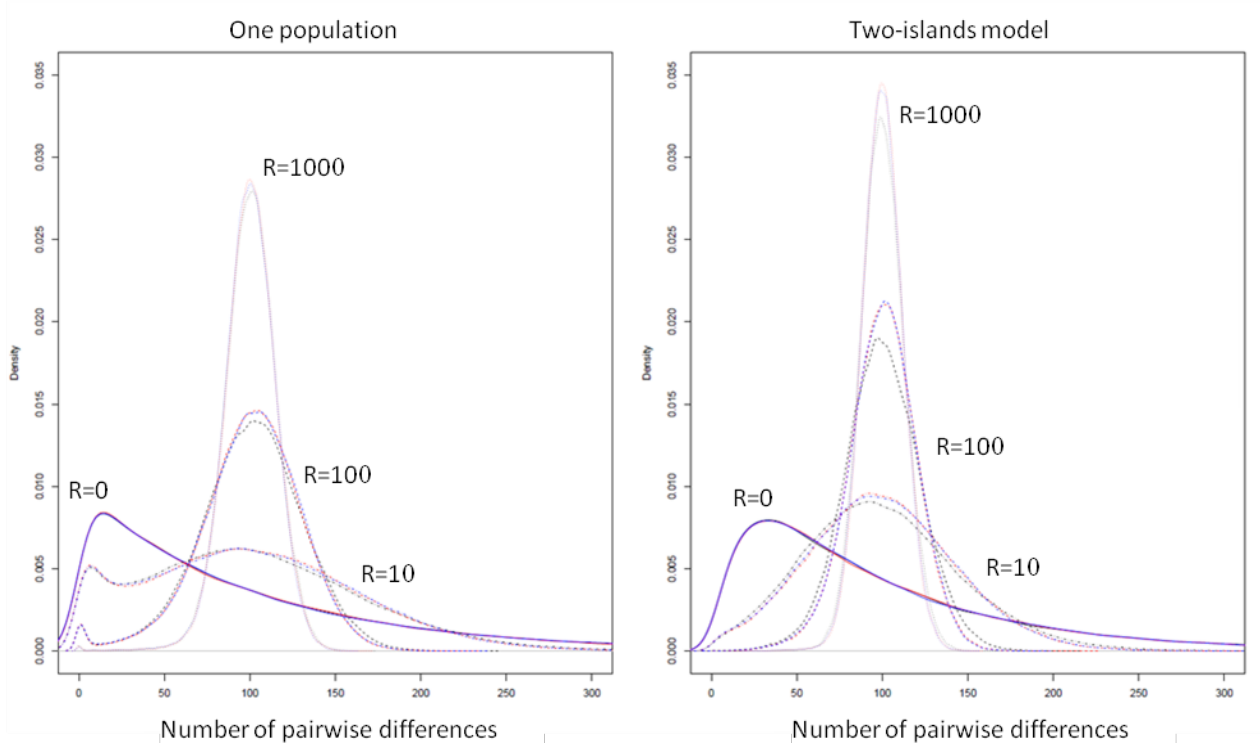
ms results are shown with a black line, *MaCs* with a red line, and *fastsimoal*, with a blue line. In all cases, the empirical distributions of the number of pairwise differences were computed from 100,000 simulations of the coalescent of 2 genes. The 2 genes were drawn from a single population for 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.
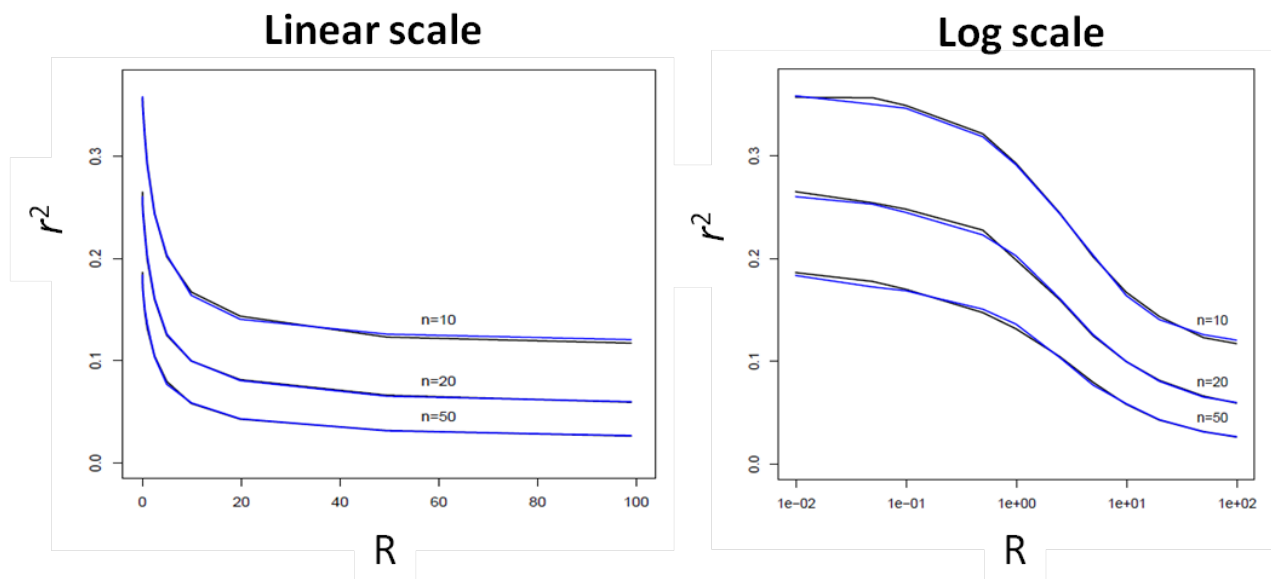
The results are based on 20,000 simulations and confirm that average r^2 values are virtually undistinguishable between the two approaches.

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

ISOLATION WITH MIGRATION (IM) SCENARIO
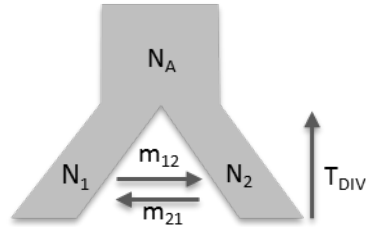
IM20Mb.tpl

```
//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
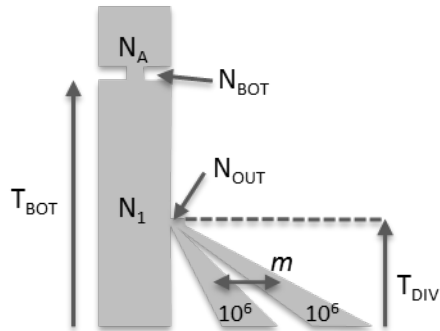
3PopExpBot20Mb.tpl

```
//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
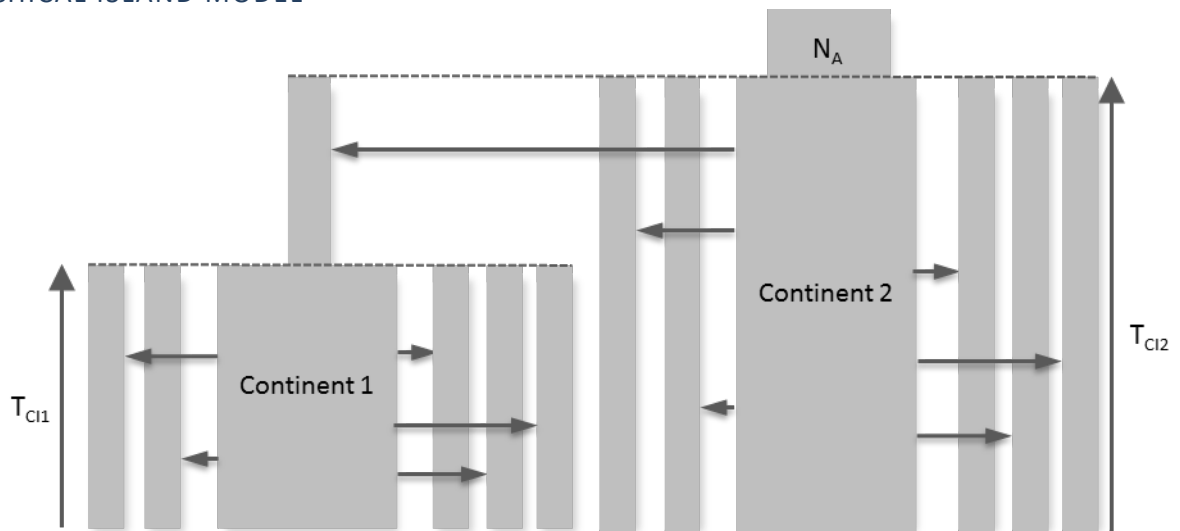
10Pop2ContiIsl.tpl

```
//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
```
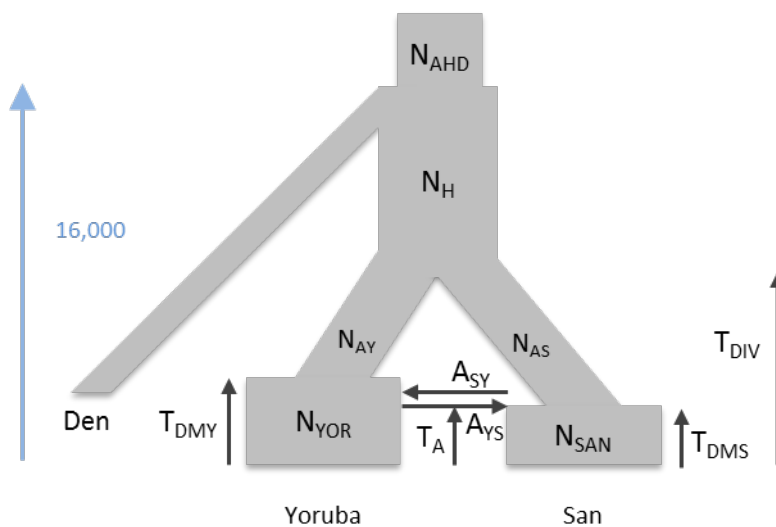
COMMAND LINE FOR PARAMETER ESTIMATION

```
./ 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

COMMAND LINE FOR PARAMETER ESTIMATION

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

Comments:

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