Swiss Institute of Bioinformatics

UNIVERSITÄT BERN

ARLEQUIN

Ver 3.5

An Integrated Software Package for Population Genetics Data Analysis

http://cmpg.unibe.ch/software/arlequin3

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1 ARLEQUIN VER 3.5.1.3 USER MANUAL

Arlequin ver 3.5

An Integrated Software Package for

Population Genetics Data Analysis

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2 INTRODUCTION

2.1 Why Arlequin?

Arlequin is the French translation of "Arlecchino", a famous character of the Italian "Commedia dell'Arte". As a character he has many aspects, but he has the ability to switch among them very easily according to its needs and to necessities. This polymorphic ability is symbolized by his colorful costume, from which the Arlequin icon was designed.

2.2 Arlequin philosophy

The goal of Arlequin is to provide the average user in population genetics with quite a large set of basic methods and statistical tests, in order to extract information on genetic and demographic features of a collection of population samples.

The graphical interface is designed to allow users to rapidly select the different analyses they want to perform on their data. We felt important to be able to explore the data, to analyze several times the same data set from different perspectives, with different selected options.

The statistical tests implemented in Arlequin have been chosen such as to minimize hidden assumptions and to be as powerful as possible. Thus, they often take the form of either permutation tests or exact tests, with some exceptions.

Finally, we wanted Arlequin to be able to handle genetic data under many different forms, and to try to carry out the same types of analyses irrespective of the format of the data.

Because Arlequin has a rich set of features and many options, it means that the user has to spend some time in learning them. However, we hope that the learning curve will not be that steep.

Arlequin is made available free of charge, as long as we have enough local resources to support the development of the program.

2.3 About this manual

The main purpose of this manual is to allow you to use Arlequin on your own, **in order to limit as far as possible e-mail exchange with us**.

In this manual, we have tried to provide a description of

1) The data types handled by Arlequin
2) The way these data should be formatted before the analyses
3) The graphical interface

4) Output files
5) The impact of different options on the computations
6) Methodological outlines describing which computations are actually performed by Arlequin.

Even though this manual contains the description of some theoretical aspects, it should not be considered as a textbook in basic population genetics. **We strongly recommend you to consult the original references provided with the description of a given method if you are in doubt with any aspect of the analysis**.

2.4 Data types handled by Arlequin

Arlequin can handle several types of data either in *haplotypic* or *genotypic* form. The basic data types are:

- DNA sequences
- RFLP data
- Microsatellite data
- Standard data
- Allele frequency data

By *haplotypic form* we mean that genetic data can be presented under the form of haplotypes (i.e. a combination of alleles at one or more loci). This haplotypic form can result from the analyses of haploid genomes (mtDNA, Y chromosome, prokaryotes), or from diploid genomes where the gametic phase could be inferred by one way or another. Note that allelic data are treated here as a single locus haplotype.

```
Ex 1: Haplotypic RFLP data          : 1001101001010010 10
Ex 2: Haplotypic standard HLA data  : DRB1*0101 DQB1*0102 DPB1*0201
```

By *genotypic form*, we mean that genetic data is presented under the form of diploid genotypes (i.e. a combination of pairs of alleles at one or more loci). Each genotype is entered on two separate lines, with the two alleles of each locus being on a different line.

Ex1: Genotypic DNA sequence data:

```
ACGGCATTTAAGCATGACATACGGATTGACA
ACGGGATTTTAGCATGACATTCGGATAGACA
```

Ex 2: Genotypic Microsatellite data:

```
63    24    32
62    24    30
```

The gametic phase of a multi-locus genotype may be either known or unknown. If the gametic phase is known, the genotype can be considered as made up of two well-defined haplotypes. For genotypic data with unknown gametic phase, you can consider the two alleles present at each locus as codominant, or you can allow for the presence of a recessive allele. This gives finally four possible forms of genetic data:

- Haplotypic data,
- Genotypic data with known gametic phase,
- Genotypic data with unknown gametic phase (no recessive alleles)
- Genotypic data with unknown gametic phase (recessive alleles).

2.4.1 DNA sequences

Arlequin can accommodate DNA sequences of arbitrary length. Each nucleotide is considered as a distinct locus. The four nucleotides "C", "T", "A", "G" are considered as unambiguous alleles for each locus, and the "-" is used to indicate a deleted nucleotide. Usually the question mark "?" codes for an unknown nucleotide. The following notation for ambiguous nucleotides are also recognized:

R: A/G (purine)

Y: C/T (pyrimidine)

M: A/C

W: A/T

S: C/G

K: G/T

B: C/G/T

D: A/G/T

H: A/C/T

V: A/C/G

N: A/C/G/T

2.4.2 RFLP Data

Arlequin can handle RFLP haplotypes of arbitrary length. Each restriction site is considered as a distinct locus. The presence of a restriction site should be coded as a "1", and its absence as a "0". The "-" character should be used to denote the deletion of a site, not its absence due to a point mutation.

2.4.3 Microsatellite data

The raw data consist here of the allelic state of one or an arbitrary number of microsatellite loci. For each locus, one should **provide the number of repeats of the microsatellite motif** as the allelic definition, if one wants his data to be analyzed according to the step-wise mutation model (for the analysis of genetic structure). It may occur that the absolute number of repeats is unknown. If the difference in length

between amplified products is the direct consequence of changes in repeat numbers, then the minimum length of the amplified product could serve as a reference, allowing to code the other alleles in terms of additional repeats as compared to this reference. If this strategy is impossible, then any other number could be used as an allelic code, but the stepwise mutation model could not be assumed for these data.

2.4.4 Standard data

Data for which the molecular basis of the polymorphism is not particularly defined, or when different alleles are considered as mutationally equidistant from each other. Standard data haplotypes are thus compared for their content at each locus, without taking special care about the nature of the alleles, which can be either similar or different. For instance, HLA data (human MHC) enters the category of standard data.

2.4.5 Allele frequency data

The raw data consist of only allele frequencies (**single-locus treatment only**), so that no haplotypic information is needed for such data. Population samples are then only compared for their allelic frequencies.

2.5 Methods implemented in Arlequin

The analyses Arlequin can perform on the data fall into two main categories: intra-population and inter-population methods. In the first category statistical information is extracted independently from each population, whereas in the second category, samples are compared to each other.

Intra-population methods:	***Short description:***
Standard indices	Some diversity measures like the number of polymorphic sites, gene diversity.
Molecular diversity	Calculates several diversity indices like nucleotide diversity, different estimators of the population parameter θ.
Mismatch distribution	The distribution of the number of pairwise differences between haplotypes, from which parameters of a demographic (*NEW*) or spatial population expansion can be estimated
Haplotype frequency estimation	Estimates the frequency of haplotypes present in the population by maximum likelihood methods.
Gametic phase estimation (*NEW*)	Estimates the most like gametic phase of multi-locus genotypes using a pseudo-Bayesian approach (ELB algorithm).
Linkage disequilibrium	Test of non-random association of alleles at different loci.
Hardy-Weinberg equilibrium	Test of non-random association of alleles within diploid individuals.
Tajima's neutrality test (infinite site model)	Test of the selective neutrality of a random sample of DNA sequences or RFLP haplotypes under the infinite site model.
Fu's F_S neutrality test (infinite site model)	Test of the selective neutrality of a random sample of DNA sequences or RFLP haplotypes under the infinite site model.
Ewens-Watterson neutrality test (infinite allele model)	Tests of selective neutrality based on Ewens sampling theory under the infinite alleles model.
Chakraborty's amalgamation test (infinite allele model)	A test of selective neutrality and population homogeneity. This test can be used when sample heterogeneity is suspected.
Minimum Spanning Network (MSN)	Computes a Minimum Spanning Tree (MST) and Network (MSN) among haplotypes. This tree can also be computed for all the haplotypes found in different populations if activated under the AMOVA section.

Inter-population methods:	***Short description:***
Search for shared haplotypes between populations	Comparison of population samples for their haplotypic content. All the results are then summarized in a table.
AMOVA	Different hierarchical **A**nalyses of **Mo**lecular **Va**riance to evaluate the amount of population genetic structure.
Pairwise genetic distances	F_{ST} based genetic distances for short divergence time.
Exact test of population differentiation	Test of non-random distribution of haplotypes into population samples under the hypothesis of panmixia.
Assignment test of genotypes	Assignment of individual genotypes to particular populations according to estimated allele frequencies.
Detection of loci under selection from F-statistics	Detection of loci under selection by the examination of the joint distribution of FST and heterozygosity under a hierarchical island model.
Mantel test:	***Short description:***
Correlations or partial correlations between a set of 2 or 3 matrices	Can be used to test for the presence of **isolation-by-distance**

2.6 System requirements

- Windows XP/Vista/7.
- A minimum of 256 MB RAM, and more to avoid swapping.
- At least 50Mb free hard disk space.

2.7 Installing and uninstalling Arlequin

2.7.1 Installation

2.7.1.1 Arlequin 3.5 installation

1) Download Arlequin35.zip to any temporary directory.
2) Extract all files contained in Arlequin35.zip in the directory of your choice.
3) Start Arlequin by double-clicking on the file WinArl35.exe, which is the main executable file.

2.7.1.2 Arlequin 3.5 uninstallation

Simply delete the directory where you installed Arlequin. The registries were not modified by the installation of Arlequin.

2.8 List of files included in the Arlequin package

Files	Description	Required by Arlequin to run properly
Arlequin files		
WinArl35.exe	Arlequin main application file including graphical interface and computational routines.	✓
Arlequin.ini	A file containing the description of the last custom settings defined by the user. (NOT TO BE MODIFIED BY HAND)	✓
Arl_run.ars	A file containing all the computation settings selected by the user to perform some calculation with Arlequin. (NOT TO BE MODIFIED BY HAND)	✓
Arl_run.txt	A file containing information about Arlequin working directory and path to working project file. (NOT TO BE MODIFIED BY HAND)	✓
recent_pro.txt	A file containing the list of up to the last ten projects loaded into Arlequin. (NOT TO BE MODIFIED BY HAND)	✓
ua js. And ftiens4.js	ua.js and ftiens4.js contain the Java scripts that allows the browsing of the result HTML files. This script needs gif files.	✓
14 gif files	These gif files are used by the java scripts for graphical display in the main result html file.	✓
14 gif files	These gif files are used by the java scripts for graphical display in the main result html file.	✓
Qtinf.dll	A dynamic link library necessary for the display of graphical components of the application	✓
ArlequinStyleSheet.xsl	Extensible Stylesheet for the formatting of Arlequin xml result file.	
Arlequin35.pdf	Arlequin 3.5 user manual in pfd format	

Various Arlequin example files are found in subdirectory **Example Files**

R scripts to produce graphics in output files are found in subdirectory **Rfunctions**

2.9 Arlequin computing limitations

The amount of data that Arlequin can handle mostly depends on the memory available on your computer. However, a few parameters are limited to values within the range shown below.

Portions of Arlequin concerned by the limitations	Limited parameter	Maximum value
Ewens-Watterson and Chakraborty's neutrality tests	Sample size	2,000
Ewens-Watterson and Chakraborty's neutrality tests	Number of haplotypes	1,000
DNA sequence	Maximum length	249,000

Other limitations:

- Line length in input file is limited to 250,000 characters
- Interleaved format is not supported in Arlequin. This concerns haplotype definition, multilocus genotypes, and distance matrices.

2.10 How to cite Arlequin

A manuscript describing the new functionalities of Arlequin ver 3.5 is in preparation. Until it is out, please cite:

Excoffier, L. G. Laval, and S. Schneider (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online **1**:47-50.

2.11 Acknowledgements

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Stefan Schneider, David Roessli, and Jean-Marc Kuffer have been involved the development of versions 1 and 2, contributing very significantly to several of its components.

The following people of the CMPG lab have also detected many bugs in development and released versions: Nicolas Ray, Samuel Neuenschwander, Daniel Wegmann, Carlo Largiadèr, Pierre Berthier, Mathias Currat, Guillaume Laval, Isabelle Dupanloup, Tamara Hofer, Martin Fisher, Gerald Heckel, and Benjamin Peter.

Friends and colleagues have also provided useful comments and suggestions. We would like to thank Yannis Michalakis, Montgomery Slatkin, David Balding, Peter Smouse, Oscar Gaggiotti, Giorgio Bertorelle, Guido Barbujani, Michele Belledi, Evelyne Heyer Philippe Jarne, Manuel Ruedi, Peter de Knijff, Peter Beerli, Matthew Hurles, Mark Stoneking,

Rosalind Harding, Steve Carr, John Novembre, Nelson Fagundes, Eric Minch, Pierre Darlu, Jérôme Goudet, François Balloux, Eric Petit, Ettore Randi, and Sergey Gavrilets.
Finally, we would like to thank all the other beta-testers and users of Arlequin that have send us their comments and detected sometimes serious bugs.

2.12 How to get the last version of the Arlequin software?

Arlequin will be updated regularly and can be freely retrieved on

http://cmpg.unibe.ch/software/arlequin3

2.13 What's new in version 3.5

Compared to version 3.11, Arlequin 3.5 includes several bug corrections, addition of new computations, and several significant improvements. The main improvement is its interfacing with the R statistical package, allowing one to produce high qualitiy graphs of many results found in the result files. We also introduce new console versions of Arlequin for both Windows and Linux.

Additions:

- New procedure to detect loci under selection from hierarchical F-statistics, as implemented in Excoffier et al. (2009)
- Computation of allele frequencies at all loci for all populations, which are output in locus-specific files.
- Computation of the genetic distance $(\delta\mu)^2$ for microsatellite data.
- Possibility to output results as an XML file with a dedicated style sheet.
- R-lequin:
  - Developments of R functions to parse the XML output file and produce publication quality graphics
  - Graphics can be directly embedded into the XML result file below result tables.
  - R functions can be modified by the user to customize graphics.
- Console version of Arlequin, arlecore, for Windows and Linux, allowing the analysis of a large number of files with bash scripts.
- Modified console version of Arlequin, called arlsumstat, for Windows and Linux, to compute specific summary statistics for each project

Modifications:

- All computations can now be performed at the group level, by automatically pooling all population samples from a given group defined in the [STRUCTURE] section into a single artificial population.
- Maximum number of characters in input line is now 250,000, which limits the maximum sizes of, say, DNA sequences that can be read.
- Removed the computation of population specific FSTs
- Changed the order of the presentation of the results. Now it begins with the intra-population computations and then output inter-population computations.
- Individuals with partially missing data at a given locus are now excluded in the locus by locus AMOVA analysis when taking individual level into account.

Bug corrections:

- In the summary statistics, the reported mean number of alleles was zero when there was a single monomorphic locus.
- LocusSeparator = None was not recognized (NONE was needed)
- Chakraborty's neutrality test: there was an overflow when the number of allele was larger than 265. Larger numbers of alleles are now possible.
- In the molecular diversity summary table, the number of sites with transversion was incorrectly reported as the number of sites with transitions
- The total number of polymorphic sites reported in summary stat table was not really the total number of polymorphic sites that would be computed on the pooled populations. It was rather the total number of sites that were found polymorphic within populations.
- Errors when computing average summary statistics within-samples, if some loci were monomorphic in some populations.
- Wrong computations of standard deviations of some summary statistics (Garza-Williamson, modified Garza-Williamson, total range) and Theta(H) for microsatellite data.
- Option to use associated settings did not work anymore
- Error when computing statistics within groups and within samples when DNA sequences contained white spaces and LocusSeparator was set to Whitespace.
- No message was issued when a population contained only missing data at a given locus and one was attempting to perform a locus-by-locus analysis. The locus was just not listed in the locus-by-locus AMOVA.Now, a warning message is issued.
- Bad handling of diploid individuals having partially missing data (on one chromosome only) when one attempts to compute locus by locus AMOVA with individual level (FIS and FIT).
- Setting file (arl_run.ars) was always saved in the arlequin directory instead of the directory chosen in the dialog box.
- It was impossible to compute the expected mismatch distribution under the demographic and the range expansions models at the same time
- Mantel test was not performed when a custom Ymatrix was provided.
- When there is a single polymorphic microsat locus, the reported average Garza-Williamson statistics was the number of loci.
- Since version 3.0, the name of external files containing information on Distance matrix, Haplotype List, or Sample Data, could not contain an absolute path. This is now possible again.

2.13.1 Changes introduced in previous releases

2.13.1.1 Version 3.11 compared to version 3.1

Compared to version 3.1, Arlequin 3.11 is mainly an update of ver 3.1, and there was no new manual.

Bug corrections:

1. Significance level of FSC and Var(b). The p-value associated to the variance component due to differences between populations within groups was erroneously computed when the number of samples in the genetic structure to test was identical to the total number of samples defined in the Samples section but the order of the samples in the Genetic Structure section was different from that in the Samples section.This bug has been around since the first release of Arlequin 2.0... Thanks to Romina Piccinali for finding it.
2. The expected homozygosity reported in the Ewens-Watterson test in the samples summary section was that of the last simulated sample. Correct value was reported only if no permutations were done.
3. Total number of alleles reported in the statistics summary section also included the missing data allele.
4. The population labels were incorrectly reported when computing population-specific FIS statistics. The reported order corresponded to that of the last

permutation. The population labels were only correct when the significance of the globalFIS statistic was not tested. Thanks to Jeff Lozier for finding this bad bug.

Modifications:

- Mean expected heterozygosity and mean allele number are reported over polymorphic sites in the Sample section, while they are reported over all loci in the statistics summaries at the end of the result file.

Additions:

- Sample allele frequencies can now be output in locus-specific files, if this option is selected in the Molecular Diversity tab. Locus-specific files are output in the Arlequin project result directory.

2.13.1.2 Version 3.1 compared to version 3.01

Arlequin 3.1 includes some bug corrections, some improvements and additional features:

Improvements

- Locus-by-locus AMOVA can now be performed independently from conventional AMOVA. This can lead to faster computations for large sample sizes and large number of population samples.
- Faster routines to handle long DNA sequences or large number of microsatellites.
- Faster reading of input file
- Faster computation of demographic parameters from mismatch distribution. Improved convergence of least-square fitting algorithm.

Additions:

- Computations of population specific inbreeding coefficients and computations of their significance level.
- Computation of the number of alleles as well as observed and expected heterozygosity per locus
- Computation of the Garza-Williamson statistic for MICROSAT data.
- In batch mode, the summary file (*.sum) now report the name of the analyzed file as well as the name of the analyzed population sample.
- When saving current settings, user are now asked to choose a file name. Default is "project file name".ars.
- New sections are provided at the end of the result file, in order to report summary statistics computed over all populations:
    - Basic properties of the samples (size, no. of loci, etc...)
    - Heterozygosity per locus
    - Number of alleles + total no. of alleles over all pops
    - Allelic range + total allelic range over all pops (for microsatellite data)
    - Garza-Williamson index (for microsatellite data)
    - Number of segregating sites, + total over all pops
    - Molecular diversity indices (theta values)
    - Neutrality tests summary statistics and p-values
    - Demographic parameters estimated from the mismatch distribution and p-values.
- New shortcuts are provided in the left pane of the html result file for F-statistics bootstrap confidence intervals, population specific FIS, and summary of intra-population statistics.

2.13.1.3 Version 3.01 compared to version 3.0

Arlequin 3.01 include some bug corrections and some additional features:

Additions:

- New **editor of genetic structure** allowing one to modify the current Genetic Structure directly in the graphical interface (see section Defining the Genetic Structure to be tested 3.2.1)

- Computation of **population-specific F_{ST} indices**, when a single group is defined in the Genetic Structure. This may be useful to recognize population contributing particularly to the global F_{ST} measure. This is also available in the locus-by-locus AMOVA section (discontinued in ver 3.5).

2.13.1.4 Version 3.0 compared to version 2

Arlequin version 3 now integrates the core computational routines and the interface in a single program written in C++. Therefore Arlequin does not rely on Java anymore. This has two consequences: the new graphical interface is nicer and faster, but it is less portable than before. At the moment we release a Windows version (2000, XP, and above) and we shall probably release later a Linux. Support for the Mac has been discontinued.

Other main changes include:

1. Correction of many small bugs
2. Incorporation of two new methods to estimate gametic phase and haplotype frequencies
    a. EM zipper algorithm: An extension of the EM algorithm allowing one to handle a larger number of polymorphic sites than the plain EM algorithm.
    b. ELB algorithm: a pseudo-Bayesian approach to specifically estimate gametic phase in recombining sequences.
3. Incorporation of a least-square approach to estimate the parameters of an instantaneous spatial expansion from DNA sequence diversity within samples, and computations of bootstrap confidence intervals using coalescent simulations.
4. Estimation of confidence intervals for *F*-statistics, using a bootstrap approach when genetic data on more than 8 loci are available.
5. Update of the java-script routines in the output html files, making them fully compatible with Firefox 1.X.
6. A completely rewritten and more robust input file parsing procedure, giving more precise information on the location of potential syntax and format mistakes.
7. Use of the ELB algorithm described above to generate samples of phased multi-locus genotypes, which allows one to analyse unphased multi-locus genotype data as if the phase was known. The phased data sets are output in Arlequin projects that can be analysed in a batch mode to obtain the distribution of statistics taking phase uncertainty into account.
8. No need to define a web browser for consulting the results. Arlequin will automatically present the results in your default web browser (we recommend the use of Firefox freely available on http://www.mozilla.org/products/firefox/central.html.

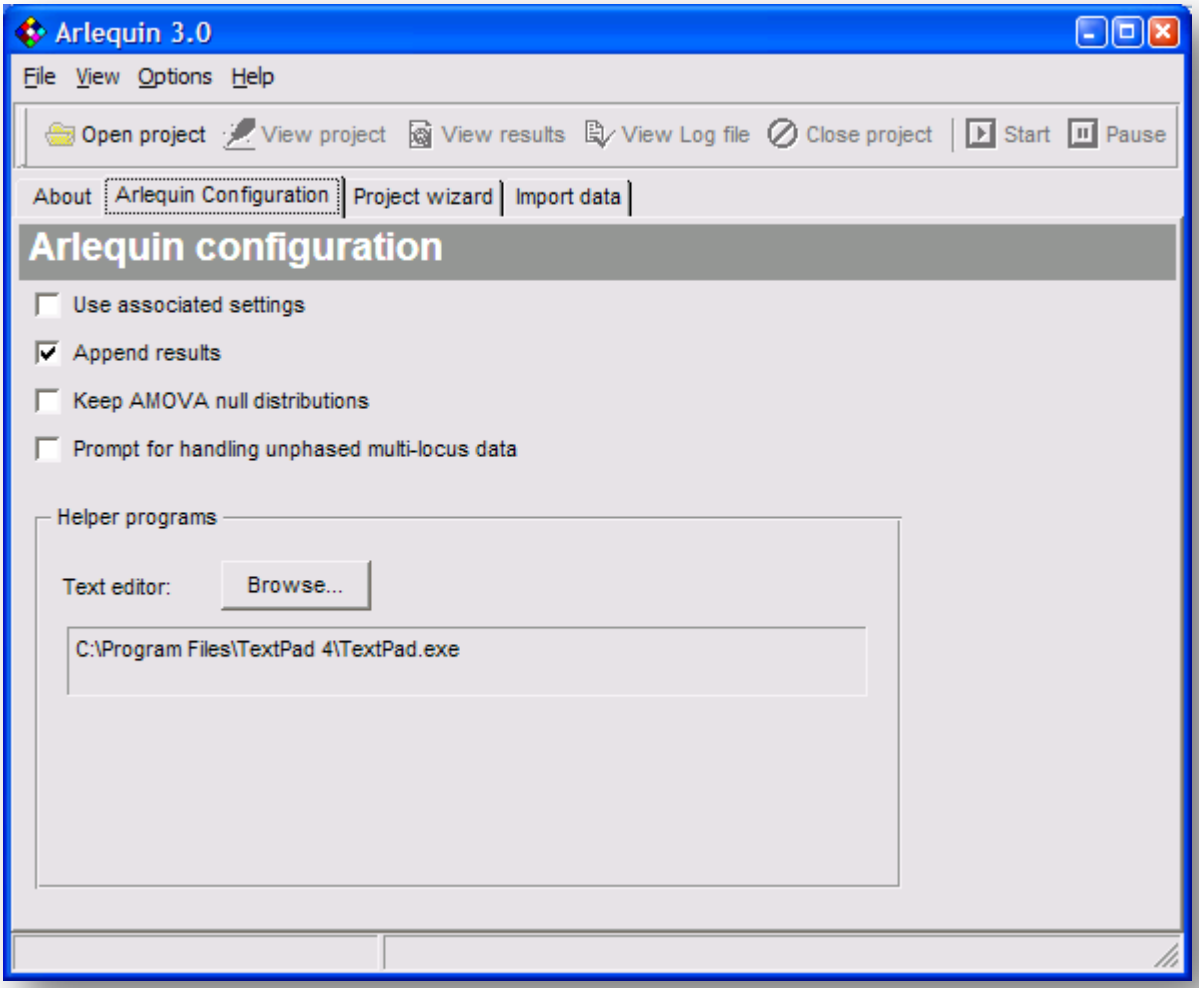
2.14 Reporting bugs and comments

Problems about Arlequin computations and interface can be reported to laurent.excoffier@iee.unibe.ch. Problems concerning graphical outputs (R-lequin) can be reported to heidi.lischer@iee.unibe.ch

3 GETTING STARTED

The first thing to do before running Arlequin for the first time is certainly to **read the present manual** . It will provide you with most of the information you are looking for. So, **take some time to read it before you seriously start analyzing your data**.

3.1 Arlequin configuration



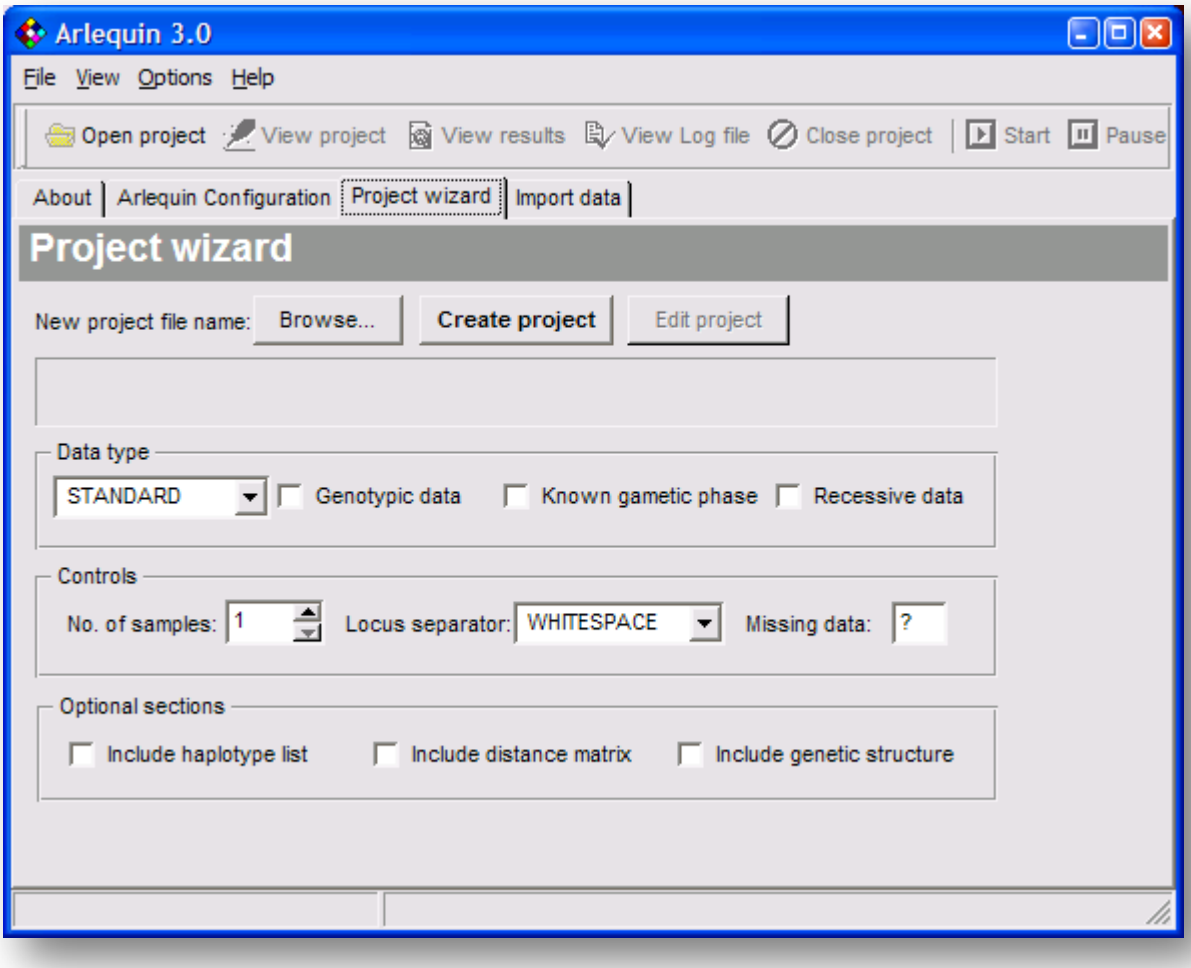
Before a first use of Arlequin, you need to specify which text editor will be used by Arlequin to edit project files or view the log file. We recommend the use of a powerful text editor like TextPad, freely available on http://www.textpad.com.

3.2 Preparing input files

The first step for the analysis of your data is to prepare an input data file for Arlequin. This input file is called here a *project file*. As Arlequin is quite a versatile program able to analyze several data types, you have to include some information about the properties of your data in the project file together with the raw data.

There are two ways to create Arlequin projects:

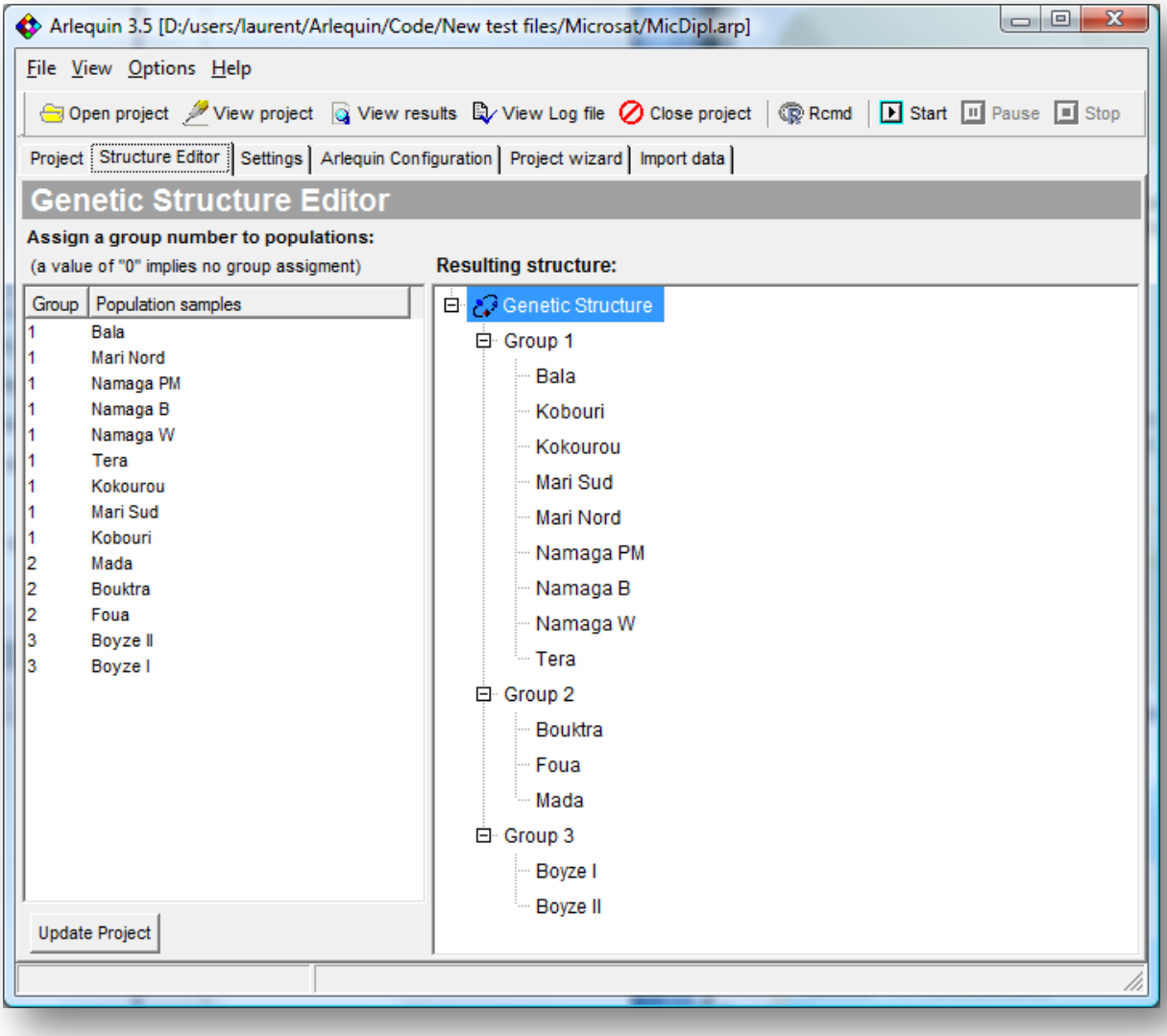
1) You can start from scratch and use a text editor to define your data using reserved keywords.
2) You can let Arlequin's create the outline of a project by selecting the tab panel *Project Wizard* (see section Project Wizard 6.3.4).



The controls on this tab panel allow you to specify the type of project outline that should be build. Use the *Browse* button to choose a name and a hard disk location for the project. Once all the settings have been chosen, the project outline is created by pressing the "*Create Project*" button. Note that it is not automatically loaded into Arlequin. The name of the data file should have a "**.arp*" extension (for ARlequin Project). You can then edit the project by pressing the *Edit Project* button.

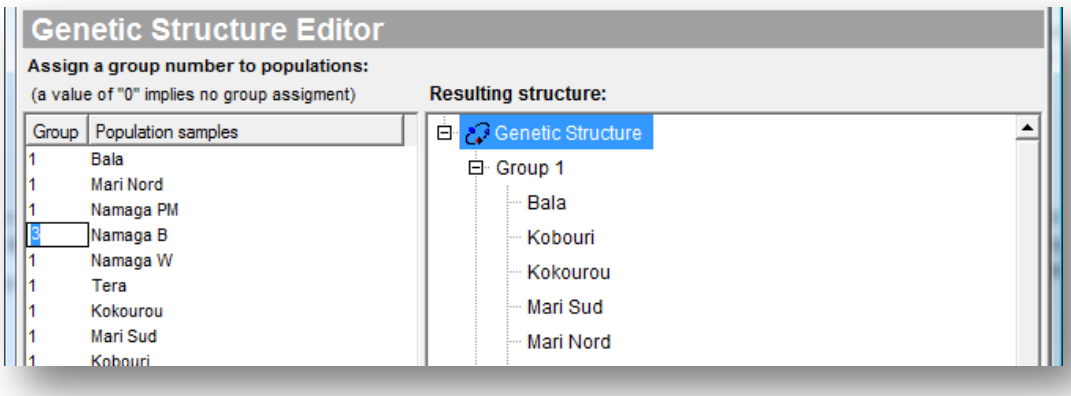
Note that this wizard only creates an outline and that you manually need to fill in the data, and specify your genetic structure.

3.2.1 Defining the Genetic Structure to be tested



A new **Genetic Structure Editor** has been implemented in version 3.01. In the left pane, all population samples found in the opened project are listed in the right column, with a corresponding group identifier in the left column. If no Genetic Structure is defined, the "0" identifier will be listed. In the right pane, the resulting structure is shown.

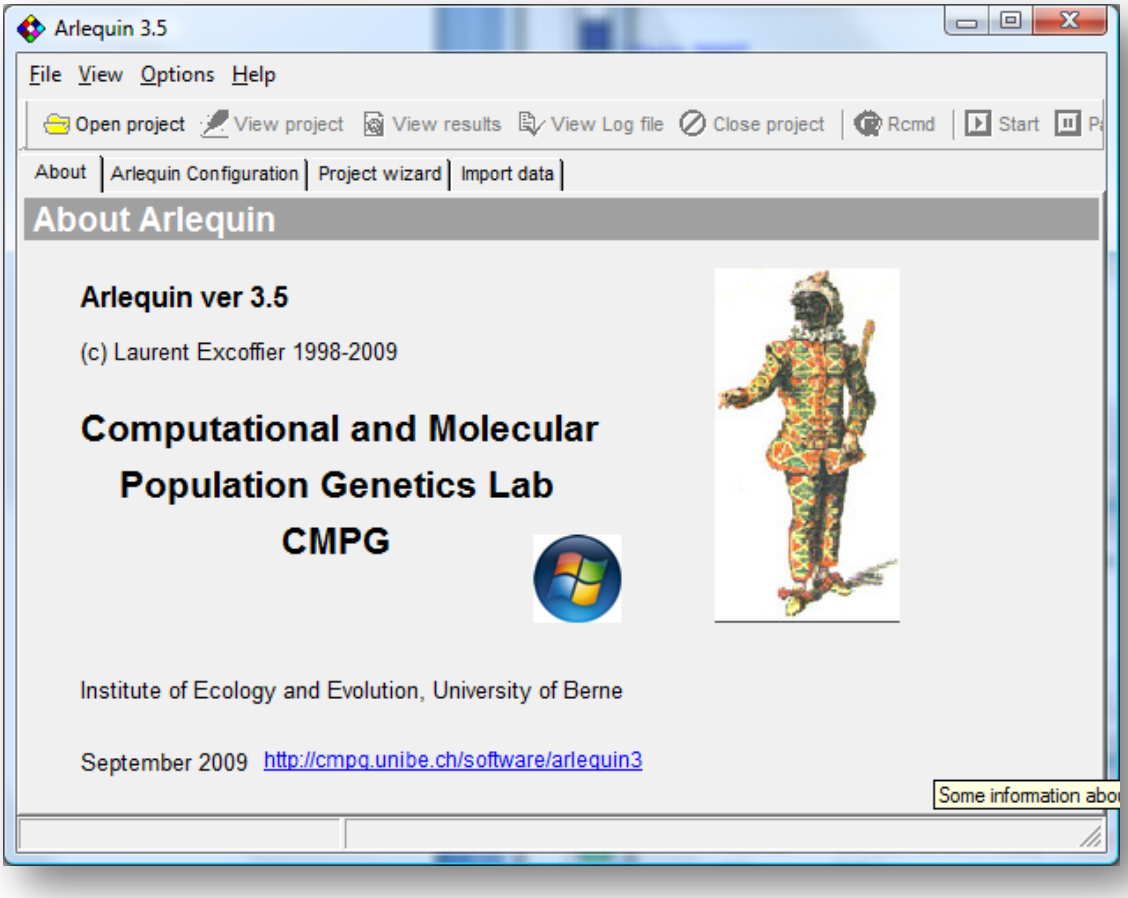
Population samples can be assigned to different groups by giving them a new group identifier, like:



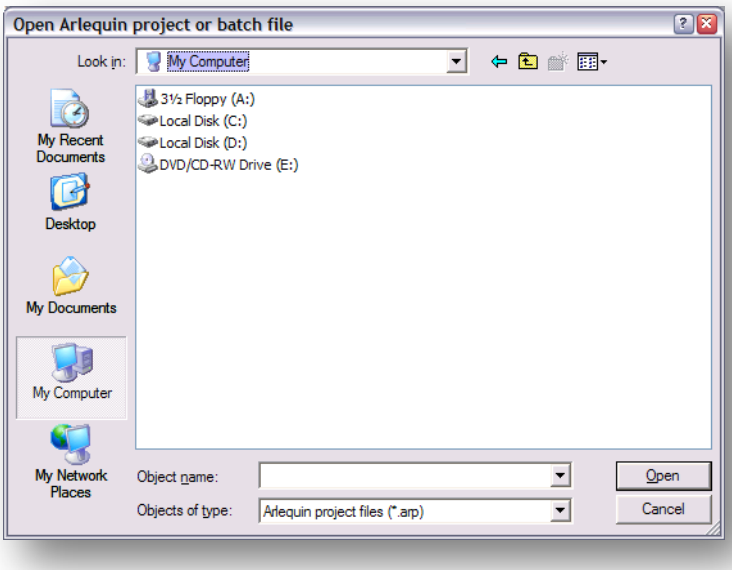
By pressing on the button "Update Project", this new Structure will be added in the project file, a backup-copy of the old project will be created (with the extension *.arp.bak), and the new revised project will be reloaded into Arlequin.

3.3 Loading project files into Arlequin

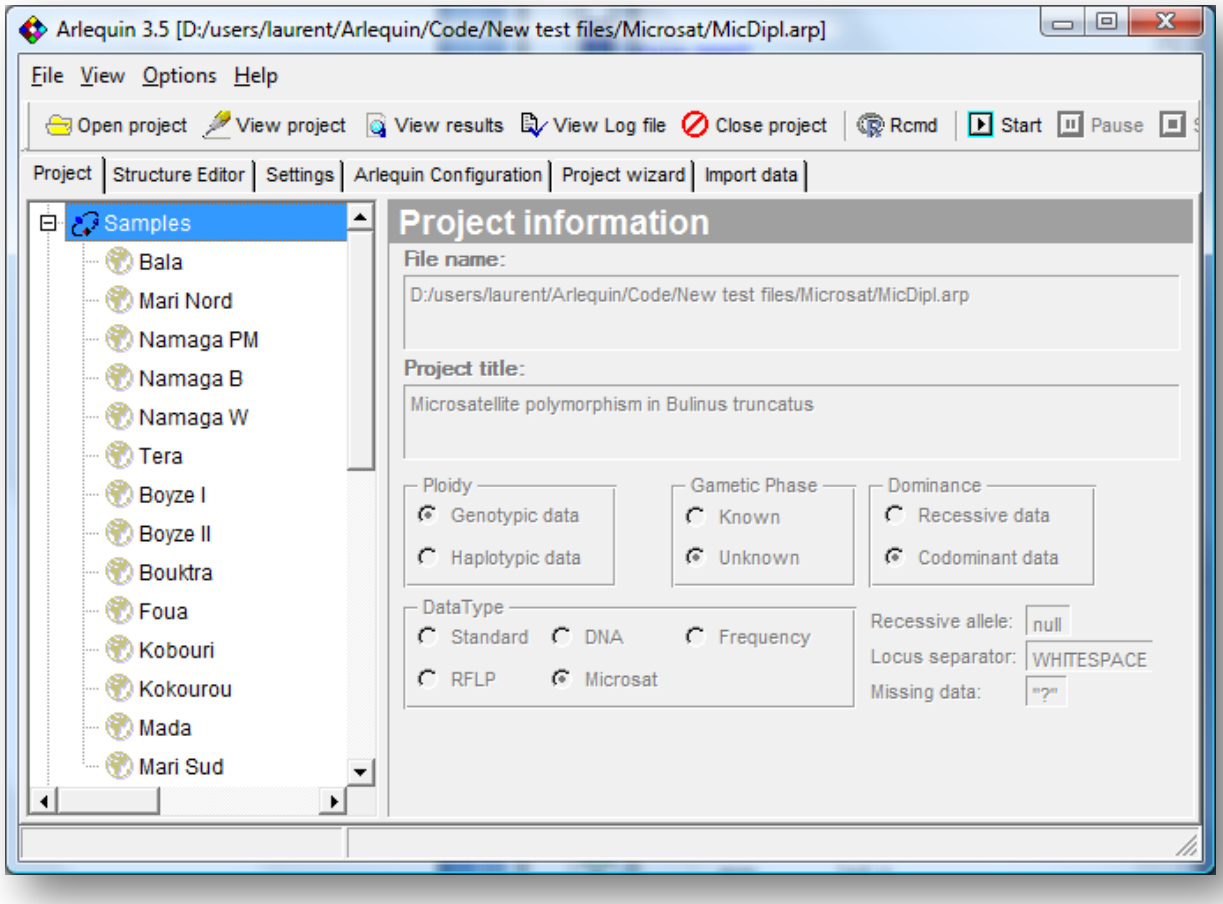
Once the project file is built, you must load it into Arlequin. You can do this either by activating the menu *File* | *Open project*, by clicking on the *Open project* button on the toolbar, or by activating the *File* | *Recent projects…* menu.



A dialog box should open to allow the selection of an existing project you want to work on, like

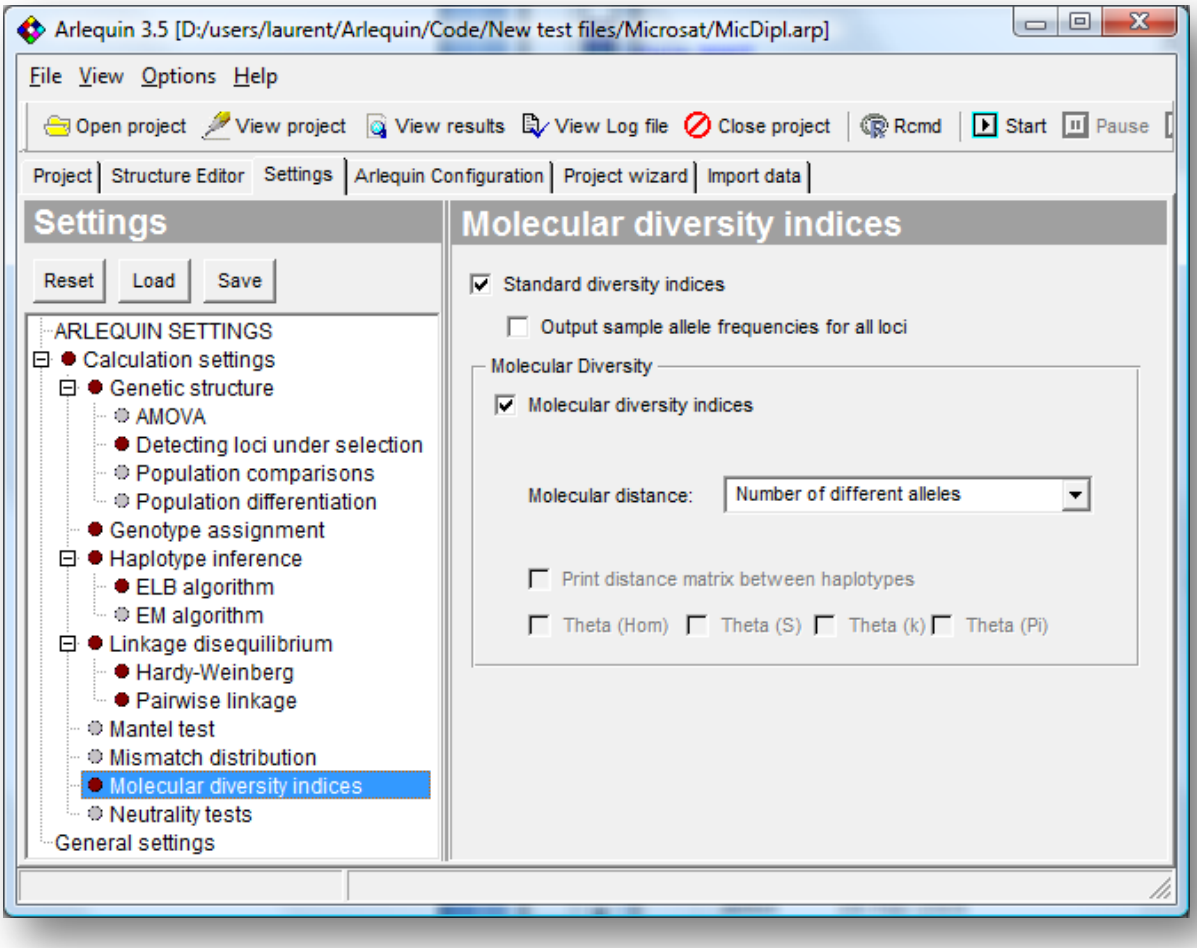


The Arlequin project files must have the **.arp* extension. If your project file is valid, its main properties will be visible in the *Project* tab, as shown below:



3.4 Selecting analyses to be performed on your data

Different analyses can be selected and their parameters tuned in the *Settings* tab.



You can navigate in the tree on the left side to select different types of computations you whish the set up. Depending on your selection, the right part of the tab dialog is will show you different parameters to set up.

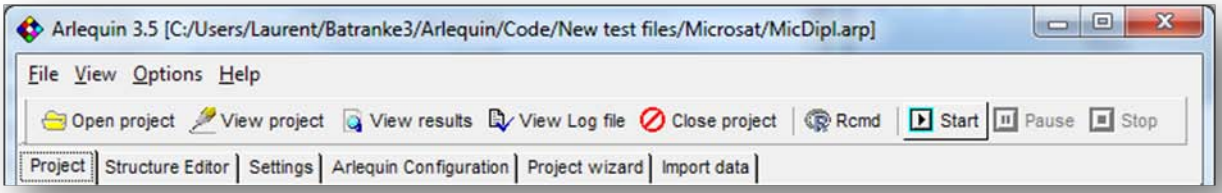
3.5 Creating and using Setting Files

By *settings* we mean any alternative choice of analyses and their parameters that can be set up in Arlequin. As you can choose different types of analyses, as well as different options for each of these analyses, all these choices can be saved into *setting files*. These files generally take the same name as the project files, but with the extension **.ars*. Setting files can be created at any time of your work by clicking on the *Save* button on top of the settings tree. Alternatively, if you activate the *Use associated settings* in the *Arlequin configuration* pane (see Arlequin configuration – section 3.1), the last used settings used on this project will be automatically saved when you close the project and reloaded when you open it later again. The setting are stored in a file having same name as the project file, and the *.ars* extension. These setting files are convenient when you want to repeat some analyses done previously, or when you want to make different types

of computations on several projects, as it is possible using batch files (see Batch files in section 4.6) giving you considerable flexibility on the analyses you can perform, and avoiding tedious and repetitive mouse-clicks.

3.6 Performing the analyses

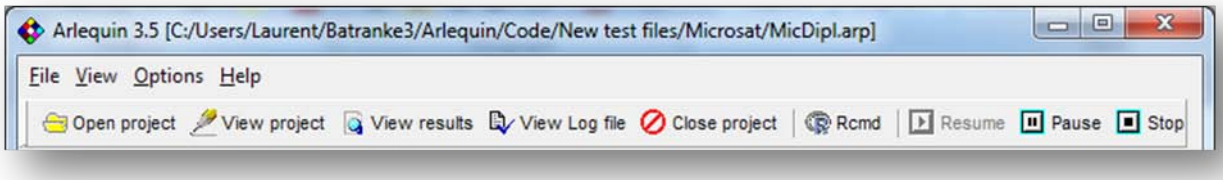
The selected analyses can be performed either by clicking on the *Start* button.



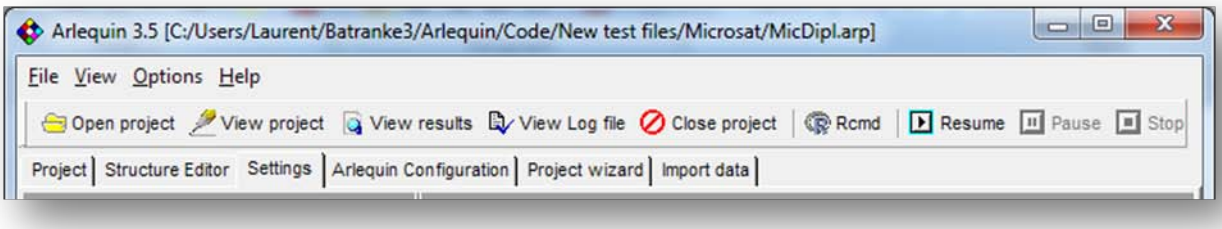
If an error occurs during the execution, Arlequin will write diagnostic information in a log file. If the error is not too severe, Arlequin will open the web browser where you can consult the log file. If there is a memory error, Arlequin will shut down itself. In the latter case, you should consult the Arlequin log file ***before*** launching a new analysis in order to get some information on where or at which stage of the execution the problem occurred. To do that, just reopen your last project, and press on the *View Log File button* on the ToolBar above. In any case, the file *Arlequin_log.txt* is located in the project results directory.

3.7 Interrupting the computations

The computations can be stopped at any time by pressing either the *Pause* or the *Stop* buttons on the toolbar.



After pressing on the Pause button, computations can be resumed by pressing on the Resume button.



Note that by pressing the *Stop* button you have no guarantee that the current computations give correct results. For very large project files, you may have to wait for a few seconds before the calculations are stopped.

3.8 Checking the results

When the calculations are over, Arlequin will create a result directory, which has the same name as the project file, but with the **.res* extension. This directory contains all the result files, particularly the main result file with the same name as the project file, but with the **.htm* or **.xml* extension depending on the option defined in the *Option* menu. After the computations, the result file *[project name]_main.html* is automatically loaded in the default html browser. You can also view your results at anytime by clicking on the *View results* button.

4 INPUT FILES

4.1 Format of Arlequin input files

Arlequin input files are also called project files. The project files contain the description of the properties of the data, as well as the raw data themselves. The project file may also refer to one or more external data files.

Note that comments beginning by a "#" character can be put anywhere in the Arlequin project files. Everything that follows the "#"character on a line will be ignored by Arlequin.

Also note that Arlequin does not support interleaved data, implying that haplotypes, multi-locus genotypes, as well as entire rows of distance matrices must be entered on a single line. A maximum of 100,000 characters can be entered on each line.

4.2 Project file structure

Input files are structured into two main sections with additional subsections that must appear in the following order:

1) Profile section (mandatory)
2) Data section (mandatory)
   - 2a) Haplotype list (optional)
   - 2b) Distance matrices (optional)
   - 2c) Samples (mandatory)
   - 2d) Genetic structure (optional)
   - 2e) Mantel tests (optional)

We now describe the content of each (sub-) section in more detail.

4.2.1 Profile section

The properties of the data must be described in this section. The beginning of the profile section is indicated by the keyword [Profile] (within brackets).

One must also specify

- *The title of the current project* (used to describe the current analysis)

  Notation: **Title**=

  Possible value: Any string of characters within double quotes

  Example: `Title="An analysis of haplotype frequencies in 2 populations"`

- *The number of samples or populations present in the current project*

  Notation: **NbSamples** =

Possible values: Any integer number between 1 and 1000.

Example: `NbSamples =3`

- *The type of data to be analyzed*. Only one type of data is allowed per project

  Notation: **DataType** =

  Possible values: DNA, RFLP, MICROSAT, STANDARD and FREQUENCY

  Example: `DataType = DNA`

- *If the current project deals with haplotypic or genotypic data*

  Notation: **GenotypicData** =

  Possible values: 0 (haplotypic data), 1 (genotypic data)

  Example: `GenotypicData = 0`

One can also optionally specify

- *The character used to separate the alleles at different loci* (the locus separator)

  Notation: **LocusSeparator** =

  Possible values: WHITESPACE, TAB, NONE, or any character other than "#", or the character specifying missing data.

  Example: `LocusSeparator = TAB`

  Default value: WHITESPACE

- *If the gametic phase of genotypes is known*

  Notation: **GameticPhase** =

  Possible values: 0 (gametic phase not known), 1 (known gametic phase)

  Example: `GameticPhase = 1`

  Default value: 1

- *If the genotypic data present a recessive allele*

  Notation: **RecessiveData** =

  Possible values: 0 (co-dominant data), 1 (recessive data)

  Example: `RecessiveData =1`

  Default value: 0

- *The code for the recessive allele*

  Notation: **RecessiveAllele** =

  Possible values: Any string of characters within double quotes. This string can be explicitly used in the input file to indicate the occurrence of a recessive homozygote at one or several loci.

  Example: `RecessiveAllele ="xxx"`

  Default value: "null"

- *The character used to code for missing data*

Notation: **MissingData** =

Possible values: A character used to specify the code for missing data, **entered between single or double quotes**.

Example: `MissingData ='$'`

Default value: `'?'`

- *If haplotype or phenotype frequencies are entered as absolute or relative values*

  Notation: **Frequency** =

  Possible values: ABS (absolute values), REL (relative values: absolute values will be found by multiplying the relative frequencies by the sample sizes)

  Example: `Frequency = ABS`

  Default value: ABS

- *The number of significant digits for haplotype frequency outputs*

  Notation: **FrequencyThreshold** =

  Possible values: A real number between 1e-2 and 1e-7

  Example: `FrequencyThreshold = 0.00001`

  Default value: 1e-5

- *The convergence criterion for the EM algorithm used to estimate haplotype frequencies and linkage disequilibrium from genotypic data*

  Notation: **EpsilonValue** =

  Possible values: A real number between 1e-7 and 1e-12.

  Example: `EpsilonValue = 1e-10`

  Default value: 1e-7

4.2.2 Data section

This section contains the raw data to be analyzed. The beginning of the profile section is indicated by the keyword [Data] (within brackets).

It contains several sub-sections:

4.2.2.1 Haplotype list (optional)

In this sub-section, one can define a list of the haplotypes that are used for all samples. This section is most useful in order to avoid repeating the allelic content of the haplotypes present in the samples. For instance, it can be tedious to write a full sequence of several hundreds of nucleotides next to each haplotype in each sample. It is much easier to assign an identifier to a given DNA sequence in the haplotype list, and then use this identifier in the sample data section. This way Arlequin will know exactly the DNA sequences associated to each haplotype.

However, this section is optional. The haplotypes can be fully defined in the sample data section.

An identifier and a combination of alleles at different loci (one or more) describe a given haplotype. The locus separator defined in the profile section must separate each adjacent allele from each other.

It is also possible to have the definition of the haplotypes in an external file. Use the keyword *EXTERN* followed by the name of the file containing the definition of the haplotypes. Read *Example 2* to see how to proceed. If the file "*hapl_file.hap*" contains exactly what is between the braces of Example 1, the two haplotype lists are equivalent.

Example 1:

```
[[HaplotypeDefinition]]  #start the section of Haplotype definition
  HaplListName="list1"  #give any name you whish to this list
  HaplList={
      h1  A T     #on each line, the name of the haplotype is
      h2  G C     # followed by its definition.
      h3  A G
      h4  A A
      h5  G G
  }
```

Example 2:

```
[[HaplotypeDefinition]] #start the section of Haplotype definition
  HaplListName="list1"  #give any name you whish to this list
  HaplList = EXTERN "hapl_file.hap"
```

4.2.2.2 Distance matrix (optional)

Here, a matrix of genetic distances between haplotypes can be specified. This section is here to provide some compatibility with earlier WINAMOVA files. The distance matrix must be a lower diagonal with zeroes on the diagonal. This distance matrix will be used to compute the genetic structure specified in the genetic structure section. As specified in AMOVA, the elements of the matrix should be squared Euclidean distances. In practice, they are an evaluation of the number of mutational steps between pairs of haplotypes.

One also has to provide the labels of the haplotypes for which the distances are computed. The order of these labels must correspond to the order of rows and columns of the distance matrix. If a haplotype list is also provided in the project, the labels and their order should be the same as those given for the haplotype list.

Usually, it will be much more convenient to let Arlequin compute the distance matrix by itself.

It is also possible to have the definition of the distance matrix given in an external file. Use the keyword *EXTERN* followed by the name of the file containing the definition of the matrix. Read Example 2 to see how to proceed.

Example 1:

```
[[DistanceMatrix]]    #start the distance matrix definition section
  MatrixName= "none"  # name of the distance matrix
  MatrixSize= 4       # size = number of lines of the distance matrix
  MatrixData={
       h1 h2 h3 h4  # labels of the distance matrix (identifier of the
         0.00000    # haplotypes)
         2.00000   0.00000
         1.00000   2.00000   0.00000
         1.00000   2.00000   1.00000   0.00000
  }
```

Example2:

```
[[DistanceMatrix]]     #start the distance matrix definition section
  MatrixName= "none"  # name of the distance matrix
  MatrixSize= 4       # size = number of lines of the distance matrix
  MatrixData= EXTERN "mat_file.dis"
```

4.2.2.3 Samples

In this obligatory sub-section, one defines the haplotypic or genotypic content of the different samples to be analyzed.

Each sample definition begins by the keyword *SampleName* and ends after a *SampleData* has been defined.

One must specify:

- *A name for each sample*

  Notation: **SampleName** =

  Possible values: Any string of characters within quotes.

  Example: `SampleName= "A first example of a sample name"`

  Note: This name will be used in the Structure sub-section to identify the different samples, which are part of a given genetic structure to test.

- *The size of the sample*

  Notation: **SampleSize** =

  Possible values: Any integer value.

  Example: `SampleSize=732`

  Note: For haplotypic data, the sample size is equal to the haploid sample size. For genotypic data, the sample size should be equal to the number of diploid individuals present in the sample. When absolute frequencies are entered, the size of each sample will be checked against the sum of all haplotypic frequencies will check. If a discrepancy is found, a *Warning message* is issued in the log file, and the sample size is set to the sum of haplotype frequencies. When relative frequencies are specified, no such check is

possible, and the sample size is used to convert relative frequencies to absolute frequencies.

- *The data itself*

  Notation: **SampleData** =

  Possible values: A list of haplotypes or genotypes and their frequencies as found in the sample, entered within braces

  Example:

```
SampleData={
 id1 1  ACGGTGTCGA
 id2 2  ACGGTGTCAG
 id3 8  ACGGTGCCAA
 id4 10 ACAGTGTCAA
 id5 1  GCGGTGTCAA
}
```

  Note: The last closing brace marks the end of the sample definition. A new sample definition begins with another keyword *SampleName*.

  FREQUENCY data type:

  If the data type is set to FREQUENCY, one must only specify for each haplotype its identifier (a string of characters without blanks) and its sample frequency (either relative or absolute). In this case the haplotype should not be defined.

  Example:

```
SampleData={
   id1      1
   id2      2
   id3      8
   id4      10
   id5      1
}
```

Haplotypic data

For all data types except *FREQUENCY*, one must specify for each haplotype its identifier and its sample frequency. If no haplotype list has been defined earlier, one must also define here the allelic content of the haplotype. The haplotype identifier is used to establish a link between the haplotype and its allelic content maintained in a local database.

Once a haplotype has been defined, it needs not be defined again. However the allelic content of the same haplotype can also be defined several times. The different definitions of haplotypes with same identifier are checked for equality. If they are found identical, a warning is issued is the log file. If they are found to be different at some loci, an error is issued and the program stops, asking you to correct the error.

For complex haplotypes like very long DNA sequences, one can perfectly assign different identifiers to all sequences (each having thus an absolute frequency of 1), even if some sequences turn out to be similar to each other. If the option *Infer Haplotypes from Distance Matrix* is checked in the General Settings dialog box, Arlequin will check whether haplotypes are effectively different or not. This is a good precaution when one tests the selective neutrality of the sample using Ewens-Watterson or Chakraborty's tests, because these tests are based on the observed number of effectively different haplotypes.

Genotypic data

For each genotype, one must specify its identifier, its sample frequency, and its allelic content. Genotypic data can be entered either as a list of individuals, all having an absolute frequency of 1, or as a list of genotypes with different sample frequencies. During the computations, Arlequin will compare all genotypes to all others and recompute the genotype frequencies.

The allelic content of a genotype is entered on two separate lines in the form of two pseudo-haplotypes.

Examples:

1):

```
Id1 2  ACTCGGGTTCGCGCGC    # the first pseudo-haplotype
       ACTCGGGCTCACGCGC    # the second pseudo-haplotype
```

2)

```
my_id  4       0 0 1 1 0 1
               0 1 0 0 1 1
```

If the gametic phase is supposed to be known, the pseudo-haplotypes are treated as truly defined haplotypes.

If the gametic phase is not supposed to be known, only the allelic content of each locus is supposed to be known. In this case an equivalent definition of the upper phenotype would have been:

```
my_id  4       0 1 1 0 0 1
               0 0 0 1 1 1
```

4.2.2.4 Genetic structure

The hierarchical genetic structure of the samples is specified in this optional sub-section. It is possible to define groups of populations. This subsection starts with the keyword *[[Structure]]*. The definition of a genetic structure is only required for AMOVA analyses.

One must specify:

- *A name for the genetic structure*

Notation: **StructureName** =

Possible values: Any string of characters within quotes.

Example: `StructureName= "A first example of a genetic structure"`

Note: This name will be used to refer to the tested structure in the output files.

- *The number of groups defined in the structure*

  Notation: **NbGroups** =

  Possible values: Any integer value.

  Example: `NbGroups = 5`

  Note: If this value does not correspond to the number of defined groups, then calculations will not be possible, and an error message will be displayed.

- *The group definitions*

  Notation: **Group** =

  Possible values: A list containing the names of the samples belonging to the group, entered within braces. Repeat this for as many groups you have in your structure. It is of course not allowed to put the same population in different groups. Also note that a comment sign (#) is not allowed after the opening brace and would lead to an error message. Comments about the group should therefore be done *before* the definition of the group.

```
Example ( NbGroups=2 ) :
 Group ={
       population1
       population2
       population3
 }
 Group ={
       population4
       population5
 }
```

A new genetic Structure Editor is now available to help you with the process of defining the genetic Structure to be tested (see section Defining the Genetic Structure to be tested 3.2.1).

4.2.2.5 Mantel test settings

This subsection allows to specify some distance matrices (*Ymatrix*, *X1* and *X2*). The goal is to compute a correlation between the *Ymatrix* and *X1* or a partial correlation between the *Ymatrix*, *X1* and *X2*. The *Ymatrix* can be either a pairwise population F_{ST} *matrix* or a custom matrix entered into the project by the user. X1 (and X2) have to be defined in the project.

This subsection starts with the keyword `[[Mantel]]`. The matrices, which are used to test correlation between genetic distances and one or two other distance matrices, are defined in this section.

One must specify:

- *The size of the matrices used for the Mantel test.*

  Notation: **MatrixSize**=

  Possible values: Any positive integer value.

  Example: `MatrixSize= 5`

- *The number of matrices among which we compute the correlations. If this number is 2 the correlation coefficient between the* ***YMatrix*** *(see next keyword) and the matrix defined after the* ***DistMatMantel*** *keyword. If this number is 3 the partial correlation between the* ***YMatrix*** *(see next keyword) and the two other matrices are computed. In this case the Mantel section should contain two* ***DistMatMantel*** *keywords followed by the definition of a distance matrix.*

  Notation: **MatrixNumber**=

  Example: `MatrixNumber= 2`

- *The matrix that is used as genetic distance. If the value is* "fst*" then the correlation between the population pairwise* F_{ST} *matrix other another matrix is computed. . If the value is* "custom*" then the correlation between a project defined matrix and other matrix is computed*

  Notation: **YMatrix**=

Possible values:	*Corresponding YMatrix*
"fst"	Y=Fst
"log_fst"	Y=log(Fst)
"slatkinlinearfst"	Y=Fst/(1-Fst)
"log_slatkinlinearfst"	Y=log(Fst/(1-Fst))
"nm"	Y=(1-Fst)/(2 Fst)
"custom"	Y= user-specified in the project

  Example: `YMatrix = "fst"`

- *Labels that identify the columns of the* ***YMatrix***. *In case of YMatrix* = "fst" *the labels should be the names of population from witch we use the pairwise* F_{ST} *distances. In case of YMatrix* = "custom" *the labels can be chosen by the user. These labels will be used to select the sub-matrices on which correlation (or partial correlation) is computed.*

  Notation: **YMatrixLabels** =

Possible values: A list containing the names of the label name belonging to the group, entered within braces.

Example:
```
YMatrixLabels =  {
                 "Population1 " "Population4" "Population2"
                 "Population8" "Population5"
        }
```

- *A keyword that allows to define a matrix with witch the correlation with the* ***YMatrix*** *is computed.*

  Notation: **DistMatMantel** =

  Example:
```
DistMatMantel={
            0.00
            3.20 0.00
            0.47 0.76 0.00
            0.00 1.23 0.37 0.00
            0.22 0.37 0.21 0.38 0.00
        }
```

- *Labels defining the sub-matrix on witch the correlation is computed.*

  Notation: **UsedYMatrixLabels**=

  Possible values: A list containing the names of the label name belonging to the group, entered within braces.

  Example:
```
UsedYMatrixLabels={
            "Population1 "
           "Population5"
            "Population8"
        }
```

Note: If you want to compute the correlation between entirely user-specified matrices, you need to list a dummy population sample in the *[[Sample]]* section, in order to allow for a proper reading of the Arlequin project. We hope to remove this weird limitation, but it is the way it works for now !

Two complete examples:

Example 1: We compute the partial correlation between the YMatrix and two other matrices X1 and X2. The YMatrix will be the pairwise F_{ST} matrix between the population listed after *YMatrixLabels* . The partial correlations will be based on the 3 by 3 matrix whose labels are listed after *UsedYMatrixLabels*.

```
[[Mantel]]
     #size of the distance matrix:
     MatrixSize= 5
     #number of declared matrixes:
     MatrixNumber=3
     #what to be taken as the YMatrix
     YMatrix="Fst"
     #Labels to identify matrix entry and Population
     YMatrixLabels ={
           "pop 1"
           "pop 2"
           "pop 3"
           "pop 4"
           "pop 5"
     }
     # distance matrix: X1
     DistMatMantel={
       0.00
       1.20 0.00
       0.17 0.84 0.00
       0.00 1.23 0.23 0.00
       0.12 0.44 0.21 0.12 0.00
     }

     # distance matrix: X2
     DistMatMantel={
       0.00
       3.20 0.00
       0.47 0.76 0.00
       0.00 1.23 0.37 0.00
       0.22 0.37 0.21 0.38 0.00
     }

     UsedYMatrixLabels ={
           "pop 1"
           "pop 3"
           "pop 4"
     }
```

Example 2: we compute the correlation between the YMatrix and another matrix X1. The YMatrix will be defined after the keyword **YMatrix**. The correlation will be based on the 3 by 3 matrix whose labels are listed after *UsedYMatrixLabels*.

```
[[Mantel]]
     #size of the distance matrix:
     MatrixSize= 5
     #number of declared matrixes: 1 or 2
     MatrixNumber=2
     #what to be taken as YMatrix
     YMatrix="Custom"
     #Labels to identify matrix entry and Population
     YMatrixLabels ={
```

```
            "1" "2" "3"
            "4" "5"
          }
          #This will be the Ymatrix
          DistMatMantel={
            0.00
            1.20 0.00
            1.17 0.84 0.00
            1.00 1.23 0.23 0.00
            2.12 0.44 0.21 0.12 0.00
          }
          #This will be X1
          DistMatMantel={
            0.00
            3.20 0.00
            2.23 1.73 0.00
            2.55 2.23 0.35 0.00
            2.23 1.62 1.54 2.32 0.00
          }
          UsedYMatrixLabels ={
           "1" "2"
           "3"
           "4" "5"
          }
```

4.3 Example of an input file

The following small example is a project file containing four populations. The data type is *STANDARD* genotypic data with unknown gametic phase.

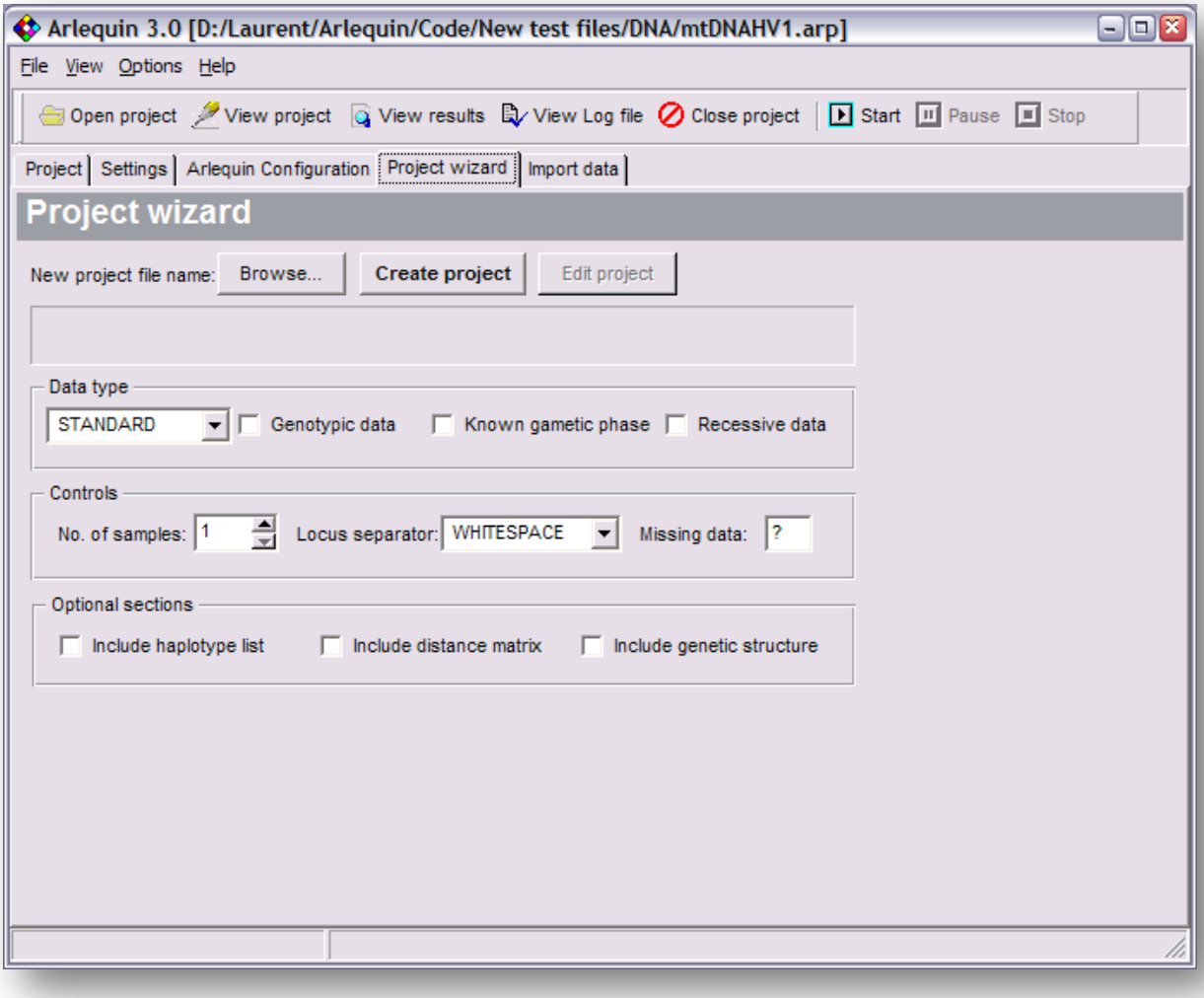
```
[Profile]
    Title="Fake HLA data"
    NbSamples=4
    GenotypicData=1
    GameticPhase=0
    DataType=STANDARD
    LocusSeparator=WHITESPACE
    MissingData='?'

[Data]

[[Samples]]
    SampleName="A sample of 6 Algerians"
    SampleSize=6
    SampleData={
           1  1 1104 0200
                0700 0301
           3  3 0302 0200
                1310 0402
           4  2 0402 0602
                1502 0602
    }
    SampleName="A sample of 11 Bulgarians"
    SampleSize=11
    SampleData={
```

```
             1  1   1103    0301
                    0301    0200
             2  4   1101    0301
                    0700    0200
             3  1   1500    0502
                    0301    0200
             4  1   1103    0301
                    1202    0301
             5  1   0301    0200
                    1500    0601
             6  3   1600    0502
                    1301    0603
    }
    SampleName="A sample of 12 Egyptians"
    SampleSize=12
    SampleData={
          1    2      1104   0301
                      1600   0502
          3    1      1303   0301
                      1101   0502
          4    3      1502   0601
                      1500   0602
          6    1      1101   0301
                      1101   0301
          8    4      1302   0502
                      1101   0609
          9    1      1500   0302
                      0402   0602
    }
    SampleName="A sample of 8 French"
    SampleSize=8
    SampleData={
          219    1    0301   0200
                      0101   0501
          239    2    0301   0200
                      0301   0200
          249    1    1302   0604
                      1500   0602
          250    3    1401   0503
                      1301   0603
          254    1    1302   0604
    }

[[Structure]]

    StructureName="My population structure"
    NbGroups=2
    Group={
          "A sample of 6 Algerians"
          "A sample of 12 Egyptians"
    }
    Group={
          "A sample of 11 Bulgarians"
          "A sample of 8 French"
    }
```

4.4 Automatically creating the outline of a project file

In order to help you setting up quickly a project file, Arlequin can create the outline of a project file for you.

In order to do this, use the ***Project wizard*** tab.



See section Project Wizard (6.3.4) for more information on how to setup up the different parameters.

4.5 Conversion of data files

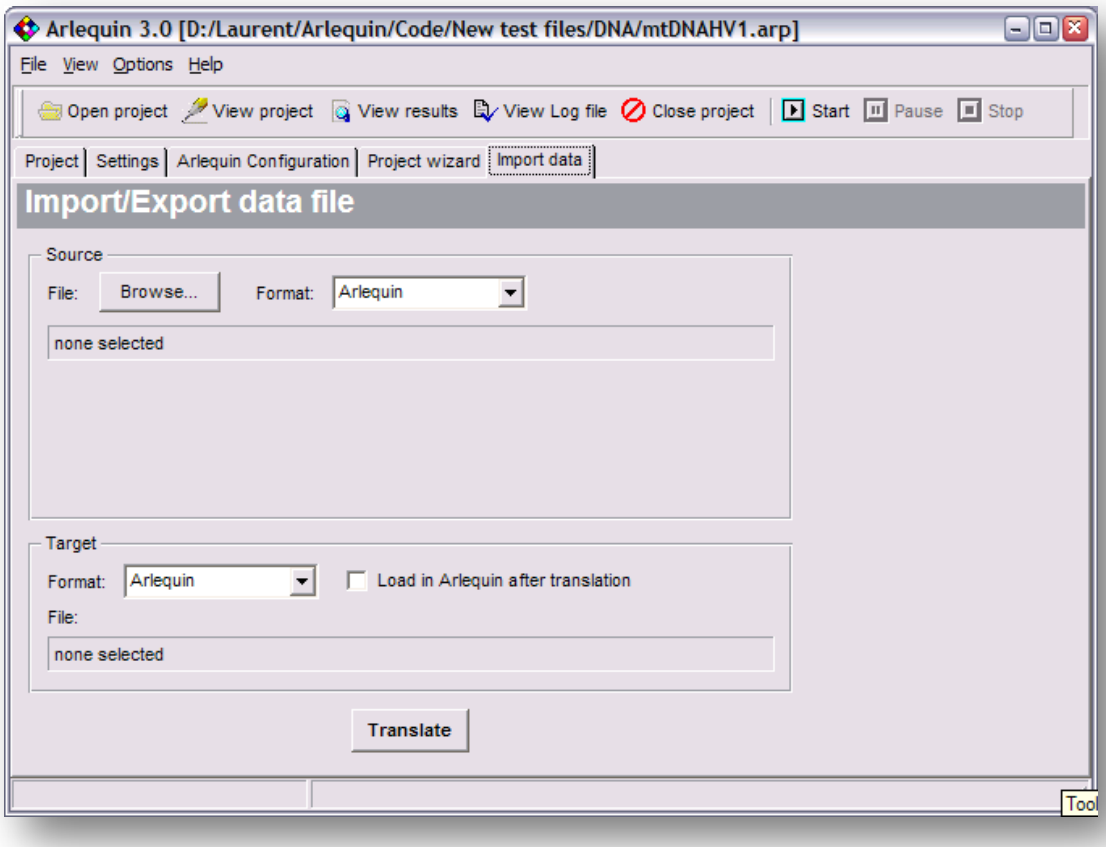
Selecting the *Import Data* tab opens a tab for the conversion of data files from one format to an other.

This might be useful for users already having data files set up for other data software packages. It is also possible to convert Arlequin data files into other formats.

The currently recognized data formats are:

- Arlequin

- GenePop ver. 3.0,
- Biosys ver.1.0,
- Phylip ver. 3.5
- Mega ver. 1.0
- Win Amova ver. 1.55.

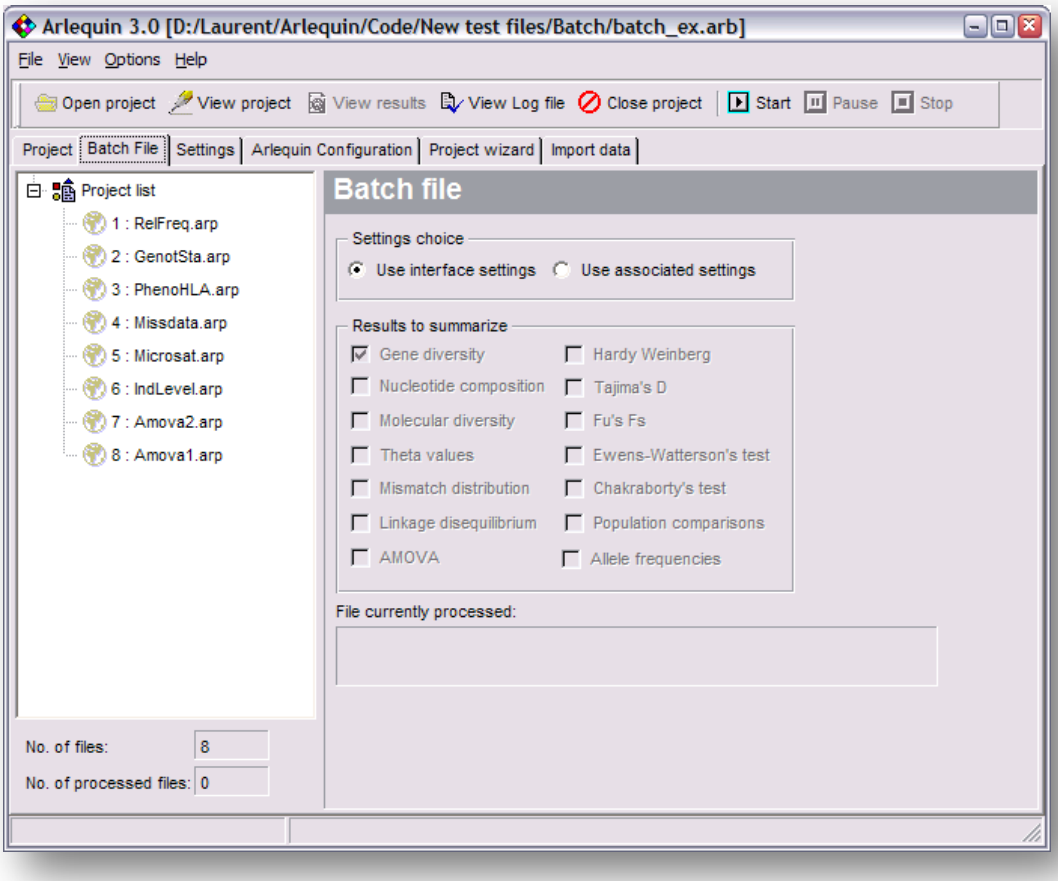


The translation procedure is more fully described in the Project Wizard section 6.3.5. These conversion routines were done on the basis of the description of the input file format found in the user manuals of each of aforementioned programs. The tests done with the example files given with these programs worked fine. However, the original reading procedures of the other software packages may be more tolerant than our own, and some data may be impossible to convert. Thus, some small corrections will need to be done by hand, and we apologize for that.

4.6 Arlequin batch files

A batch file (with the *.arb* extension) is simply a text file having on each line the name of the project files that should be analyzed by Arlequin. The number of data files to be analyzed can be arbitrary large.

If the project type you open is of *Batch file* type, the *Batch file* tab panel opens up automatically and allows you to tune the settings of your batch run.



On the left tree pane you can see project files listed in the batch file.

Settings choice:

You can either use the same options for all project files by selecting ***Use interface settings***, or use the setting file associated with each project file by selecting ***Use associated settings***. In the first case, the same analyses will be performed on all project files listed in the batch file. In the second case, you can perform different computations on each project file listed in the batch file, giving you much more flexibility on what should be done. However, it implies that setting files have been prepared previously, recording the analyses needing to be performed on the data, as well as the options of these analyses.

Results to summarize:

Some results can be collected from the analysis of each batch file, and put into summary files. See section Batch files 6.3.7 for additional information.

If the associated project file does not exist, the current settings are used.

Note that the batch file, the project files, and the setting files should all be in the same folder

5 EXAMPLES OF INPUT FILES

5.1 Example of allele frequency data

The following example is a file containing *FREQUENCY* data. The allelic composition of the individuals is not specified. The only information we have are the frequencies of the alleles.

```
[Profile]
     Title="Frequency data"
     NbSamples=2
     GenotypicData=0
     DataType=FREQUENCY
[Data]
 [[Samples]]
     SampleName="Population 1"
     SampleSize=16
     SampleData= {
          000 1
          001 3
          002 1
          003 7
          004 4
     }
     SampleName="Population 2"
     SampleSize=23
     SampleData= {
          000 3
          001 6
          002 2
          003 8
          004 4
    }
```

5.2 Example of standard data (Genotypic data, unknown gametic phase, recessive alleles)

In this example, the individual genotypes for 5 HLA loci are output on two separate lines. We specify that the gametic phase between loci is unknown, and that the data has a recessive allele. We explicitly define it to be "xxx". Note that with recessive data, all single locus homozygotes are also considered as potential heterozygotes with a null allele. We also provide Arlequin with the minimum frequency for the estimated haplotypes to be listed (0.00001), and we define the minimum epsilon value (sum of haplotype frequency differences between two steps of the EM algorithm) to be reached for the EM algorithm to stop when estimating haplotype frequencies.

```
[Profile]
     Title="Genotypic Data, Phase Unknown, 5 HLA loci"
     NbSamples=1
     GenotypicData=1
```

```
      DataType=STANDARD
      LocusSeparator=WHITESPACE
      MissingData='?'
      GameticPhase=0
      RecessiveData=1
      RecessiveAllele="xxx"
  [Data]
   [[Samples]]
      SampleName="Population 1"
      SampleSize=63
      SampleData={
          MAN0102   12  A33   Cw10   B70    DR1304  DQ0301
                        A33   Cw10   B7801  DR1304  DQ0302
          MAN0103   22  A33   Cw10   B70    DR1301  DQ0301
                        A33   Cw10   B7801  DR1302  DQ0501
          MAN0108   23  A23   Cw6    B35    DR1102  DQ0301
                        A29   Cw7    B57    DR1104  DQ0602
          MAN0109   6   A30   Cw4    B35    DR0801  xxx
                        A68   Cw4    B35    DR0801  xxx
      }
```

5.3 Example of DNA sequence data (Haplotypic)

Here, we define 3 population samples of haplotypic DNA sequences. A simple genetic structure is defined that just incorporates the three population samples into a single group of populations.

```
  [Profile]
      Title="An example of DNA sequence data"
      NbSamples=3
      GenotypicData=0
      DataType=DNA
      LocusSeparator=NONE
  [Data]
   [[Samples]]
      SampleName="Population 1"
      SampleSize=6
      SampleData= {
          000    3   GACTCTCTACGTAGCATCCGATGACGATA
          001    1   GACTGTCTGCGTAGCATACGACGACGATA
          002    2   GCCTGTCTGCGTAGCATAGGATGACGATA
      }
      SampleName="Population 2"
      SampleSize=8
      SampleData= {
          000    1   GACTCTCTACGTAGCATCCGATGACGATA
          001    1   GACTGTCTGCGTAGCATACGACGACGATA
          002    1   GCCTGTCTGCGTAGCATAGGATGACGATA
          003    1   GCCTGTCTGCCTAGCATACGATCACGATA
          004    1   GCCTGTCTGCGTACCATACGATGACGATA
          005    1   GCCTGTCCGCGTAGCGTACGATGACGATA
          006    1   GCCCGTGTGCGTAGCATACGATGGCGATA
          007    1   GCCTGTCTGCGTAGCATGCGACGACGATA
      }
      SampleName="Population 3"
      SampleSize=6
      SampleData= {
          023    1   GCCTGTCTGCGTAGCATACGATGACGGTA
```

```
          024    1   GCCTGTCTGCGTAGCGTACGATGACGATA
          025    1   GCCTGTCTGCGTAGCATACGATGACGATA
          026    1   GCCTGTCCGCGTAGCATACGGTGACGGTA
          027    1   GCCTGTCTGCGTGGCATACGATGACGATG
          028    1   GCCTGTCTGCGTAGCATACGATGACGATA
     }
[[Structure]]
     StructureName="A group of 3 populations analyzed for DNA"
     NbGroups=1
     Group= {
          "Population 1"
          "Population 2"
          "Population 3"
     }
```

5.4 Example of microsatellite data (Genotypic)

In this example, we show how to prepare a project file consisting in microsatellite data. Four population samples are defined. Three microsatellite loci only have been analyzed in diploid individuals. The different genotypes are output on two separate lines. The frequencies of the different genotypes are listed in the second column of the first line of each genotype. Alternatively, one could just output the genotype of each individual, and simply set its frequency to 1. One should however be careful to use different identifiers for each individual. It does not matter if different genotype labels refer to the same genotype content. Here, only a few different genotypes have been found in each of the populations (which should not correspond to most real situations, but we wanted to save space). The genotypes consist in the number of repeats found at each locus. The genetic structure to be analyzed consists in 2 groups, each made up of 2 populations.
To make things clear, the genotype "Genot1" in the first population, has been observed 27 times. For the first locus, 12 and 13 repeats were observed, 22 and 23 repeats were observed for the second locus, and finally 16 and 17 repeats were found at the third locus.

```
[Profile]
     Title="A small example of microsatellite data"
     NbSamples=4
     GenotypicData=1
     #Unknown gametic phase between the 2 loci
     GameticPhase=0
     DataType=MICROSAT
     LocusSeparator=WHITESPACE
[Data]
  [[Samples]]
     SampleName="MICR1"
     SampleSize=28
     SampleData=      {
          Genot1      27    12 23 17
                            13 22 16
          Genot2      1     15 22 16
                            13 22 16
```

```
      }
      SampleName="MICR2"
      SampleSize=59
      SampleData=      {
           Genot3      37    12 24 18
                             12 22 16
           Genot4      1     15 20 18
                             13 22 18
           Genot5      21    14 22 16
                             14 23 16
      }
      SampleName="MICR3"
      SampleSize=30
      SampleData=      {
           Genot6      17    12 21 16
                             13 22 15
           Genot7      1     12 20 16
                             13 23 16
           Genot8      12    10 22 15
                             12 22 15
      }
      SampleName="MICR4"
      SampleSize=16
      SampleData=      {
           Genot9      15    13 24 16
                             13 23 17
           Genot10     1     12 24 16
                             13 23 16
      }
[[Structure]]
      StructureName="Test microsat structure"
      NbGroups=2
      #The first group is made up of the first 2 samples
      Group={
                 "MICR1"
                 "MICR2"
       }
      #The last 2 samples will be put into the second group
      Group={
                 "MICR3"
                 "MICR4"
       }
```

5.5 Example of RFLP data(Haplotypic)

In this example, we show how to use a definition list of RFLP haplotypes. Different RFLP haplotypes are first defined in the *[[HaplotypeDefinition]]* section. The allelic content of each haplotype is then defined after a given identifier. The identifier is then used at the population samples level. Note that the list of haplotypes can include haplotypes that are not listed in the population samples. The genetic diversity of the samples is then simply described as a list of haplotypes found in each population as well as their sample frequencies.

```
[Profile]
      Title="A small example of RFLP data: 3 populations"
      NbSamples=3
```

```
      GenotypicData=0
      DataType=RFLP
      LocusSeparator=WHITESPACE
      #We tell Arlequin to compute Euclidian square distances between
      #the haplotypes listed below
      MissingData='?'
  [Data]
   [[HaplotypeDefinition]]
      HaplListName="A fictive list of RFLP haplotypes"
      HaplList= {
        1     00001110011101001101100100101100110111010010110 1100
        2     10001110011101001101100100101100110111010010110 0100
        6     00001110011101001001100100101100110111010010110 1100
        7     10001110011101001101100100101100110111010010110 1100
        8     00001110011101001101100100100100110111010010110 1100
        11    00000110011101101101100100101100110111010010111 1100
        12    00001110011101001101100110101100110111010010110 1100
        17    00001110011101001101100100101100110011010010110 1100
        22    00001110011101101101100100101100110111010010110 0100
        36    00001110011101001101100100101000110011010010110 1100
        37    00001110011101101101100100111100110111010010110 0100
        38    00011110011101001101100100101100110111010010110 1100
        40    00001110011100001101100100101100110111010010110 1100
        47    00001110011101001101100100101100110111010010110 0100
        139   00001110011101001101100100101100111111010010100 1110
        140   00001110011101001101100100101100110111010010110 0101
        141   00001110011101001001100100001100110111010010110 0100
      }
   [[Samples]]
      #1
      SampleName="pop 1"
      SampleSize=28
      SampleData=      {
           1     27
           40    1
      }
      #2
      SampleName="pop 2"
      SampleSize=75
      SampleData=      {
           1     37
           17    1
           6     21
           7     1
           2     1
           22    5
           11    2
           36    1
           139   1
           47    1
           140   1
           141   1
           37    1
           38    1
      }
      #3
      SampleName="pop 3"
      SampleSize=48
      SampleData=      {
           1     46
           8     1
```

```
          12    1
     }
[[Structure]]
     StructureName="A single group of 3 samples"
     NbGroups=1
     Group={
          "pop 1"
          "pop 2"
          "pop 3"
     }
```

5.6 Example of standard data (Genotypic data, known gametic phase)

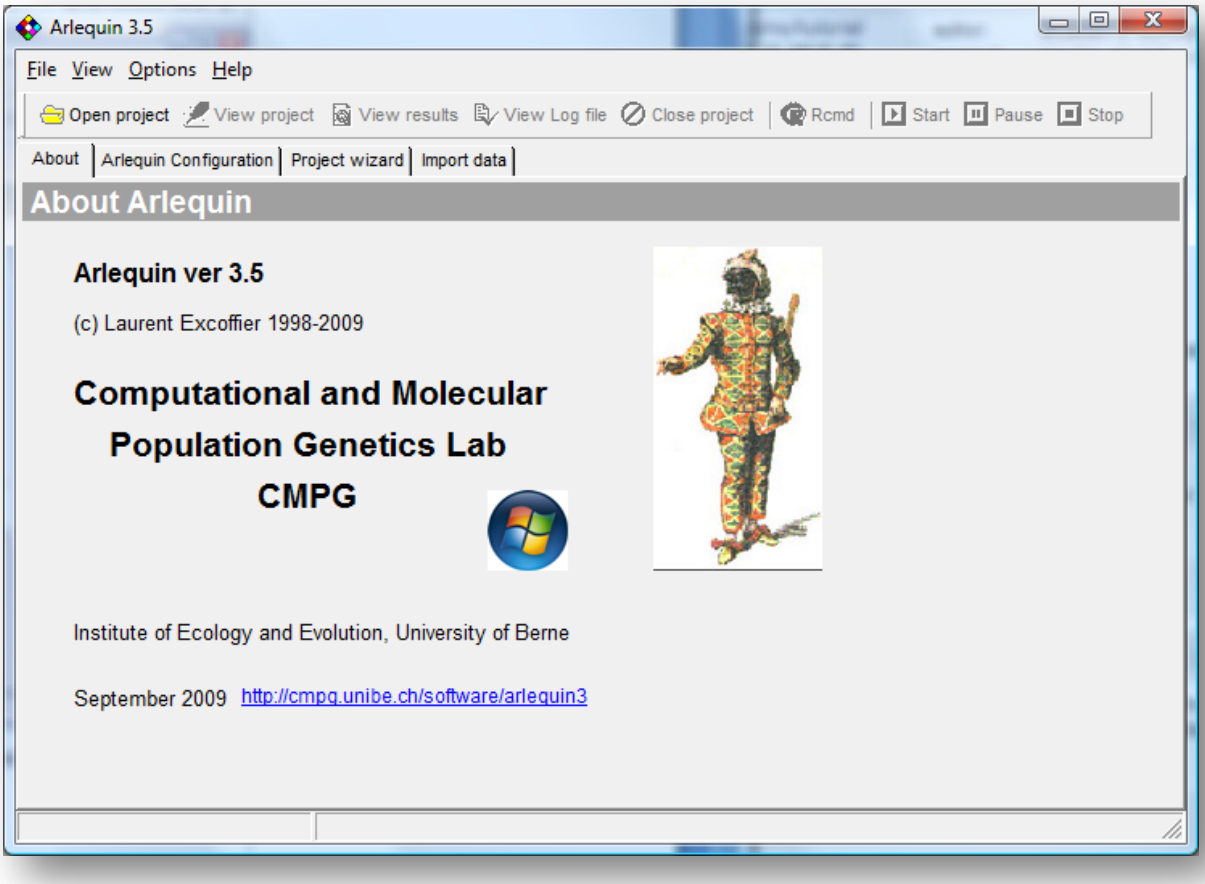
In this example, we have defined 3 samples consisting of standard multi-locus data with known gametic phase. It means that the alleles listed on the same line constitute a haplotype on a given chromosome. For instance, the genotype G1 is made up of the two following haplotypes: AD on one chromosome and BC on the second, A and b being two alleles at the first locus, and C and D being two alleles at the second locus. Note that the same allele identifier can be used in different loci. This is obviously true for Dna sequences, but it also holds for all other data types.

```
[Profile]
     Title="An example of genotypic data with known gametic phase"
     NbSamples=3
     GenotypicData=1
     GameticPhase=1
     #There is no recessive allele
     RecessiveData=0
     DataType=STANDARD
     LocusSeparator=WHITESPACE
[Data]
  [[Samples]]
     SampleName="standard_pop1"
     SampleSize=20
     SampleData=      {
          G1   4    A  D
                    B  C
          G2   5    A  B
                    A  A
          G3   3    B  B
                    B  A
          G4   8    D  C
                    D  C
     }
     SampleName="standard_pop2"
     SampleSize=10
     SampleData=      {
          G5   5    A  C
                    C  B
          G6   5    B  C
                    D  B
     }
     SampleName="standard_pop3"
     SampleSize=15
```

```
      SampleData=      {
           G7   3    A  D
                     C  A
           G8   12   A  C
                     B  B
      }

  [[Structure]]
      StructureName="Two groups"
      NbGroups=2
      Group={
           "standard_pop1"
      }
      Group={
           "standard_pop2"
           "standard_pop3"
```

6 ARLEQUIN INTERFACE

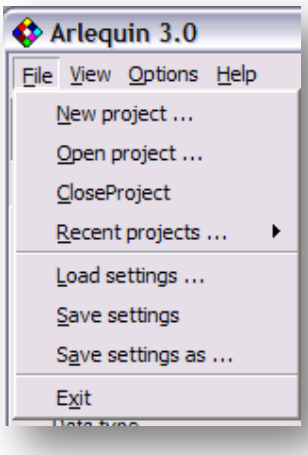
The interface of Arlequin (since ver. 3.0) is written in C++ and looks like:



The graphical interface is made up of a series of tabbed dialog boxes, whose content vary dynamically depending on the type of data currently analyzed.

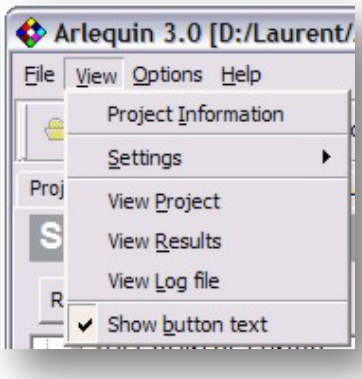
6.1 Menus

6.1.1 File Menu



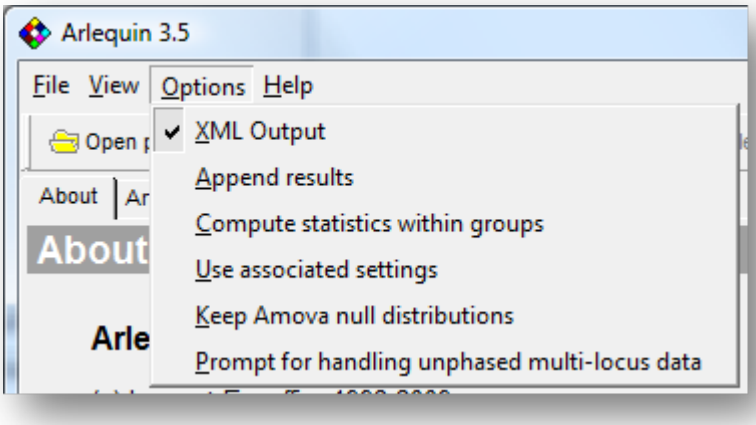
New project...	Prompts the Project Wizard dialog box
Open project...	Opens a dialog box to locate an existing project
Close project	Closes the current project.
Recent projects...	Open a submenu with the last 10 more recently opened projects
Load settings...	Load previously saved computation settings
Save settings	Save current computation settings
Save settings as ...	Save current computation settings under a specific name
Exit	Exit Arlequin and close current project

6.1.2 View Menu



Project information	Open tab dialog with information on current project
Settings	Open specific tab dialogs to active some computations and choose their associated settings
View Project	View current project in text editor
View Results	View computation result in default web browser
View Log file	View log file in text editor
Show button text	Toggle presence/absence of text associated to toolbar buttons

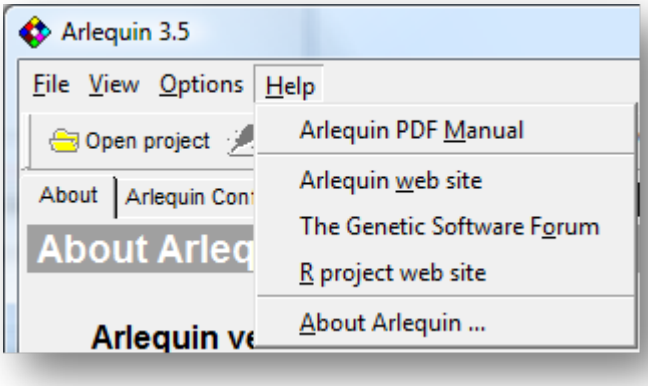
6.1.3 Options Menu



XML Output	Check this menu item if you want Arlequin to generate output files in xml format, allowing for more flexibility in the formatting of the output, and the inclusion of graphics generated by an R script (see section 7.6). If this menu is unchecked, conventional html files are generated, and graphs cannot be incorporated into output files.
Append results	If checked, Add results of a new analysis at the end of the current result file. Otherwise, previous results are

	deleted before adding the new results.
Use associated settings	Check this box if you want Arlequin to automatically load the settings associated to each project. If this box is unchecked, the same settings will be used for different projects (see section 6.3.2).
Keep Amova null distributions	If checked, the nulle distribution of variance compoents are written in specific files (see section 6.3.2).
Prompt for handling unphased multi-locus data	If checked, you will have the option of estimating the gametic phase of unphased genotype data with the ELB algorithm (see section 6.3.8.4.2.1).

6.1.4 Help Menu



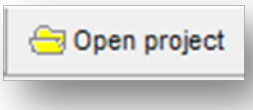
The menu to get access to the Help File System

Arlequin PDF Help file	Open Arlequin help file. Actually it tries to open the file "arlequin.pdf". You thus need to have installed the Adobe Acrobat extensions in your web browser.
Arlequin web site	Link to Arlequin web site http://cmpg.unibe.ch/software/arlequin3"
R project	Link to the R project web page http://www.r-project.org/ R is a language and environment for statistical computing and graphics, that needs to be installed on your computer to include graphs in output files
About Arlequin…	Some information about Arlequin, its authors, contact address and the Swiss NSF grants that supported its development.

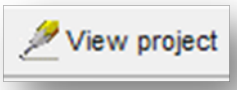
6.2 Toolbar

Arlequin's toolbar contains icons that are shortcuts to some commonly used menu items as shown below. Clicking on one of these icons is equivalent to activating the corresponding menu item.

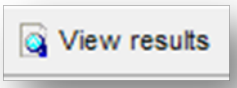
The individual buttons perform the following actions:



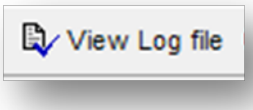
Opens a dialog box to choose an Arlequin project to open (see section 6.3.1)



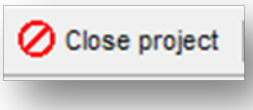
Calls the text editorspecified in the *Arlequin Configuration* tab (see section6.3.3) and loads the current Arlequin project, allowing you to edit it.



View results in your web browser

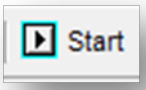


View Arlequin log file in the selected text editor



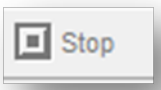
Closes the current project

Calls R routines to integrate graphics of results into the xml output file



Start currently selected computations

Pauses current computations



Stops current computations

6.3 Tab dialogs

Most of the methods implemented in Arlequin can be computed irrespective of the data type. Nevertheless, the testing procedure used for a given task (e.g. linkage disequilibrium test) may depend on the data type. The aim of this section is to give an overview of the numerous options which can be set up for the different ananlyses.

The items that appear «grayed» in Arlequin's dialog boxes indicate that a given task is impossible in the current situation. For example, if you open a project containing haplotypic data, it is not possible to test for Hardy-Weinberg equilibrium or for *STANDARD* data it is not possible to set up the transversion or transition weights, which can only be set up for *DNA* data.

Arlequin's interface usually prevents the user from selecting tasks impossible to perform, or from setting up parameters that are not taken into account in the analyses.

When describing the different dialog boxes accessible in Arlequin, we have sometimes used the following symbols to specify which types of user input were expected:

[f] : parameter to be set in the dialog box as a floating number.

[i] : parameter to be set in the dialog box as an integer.

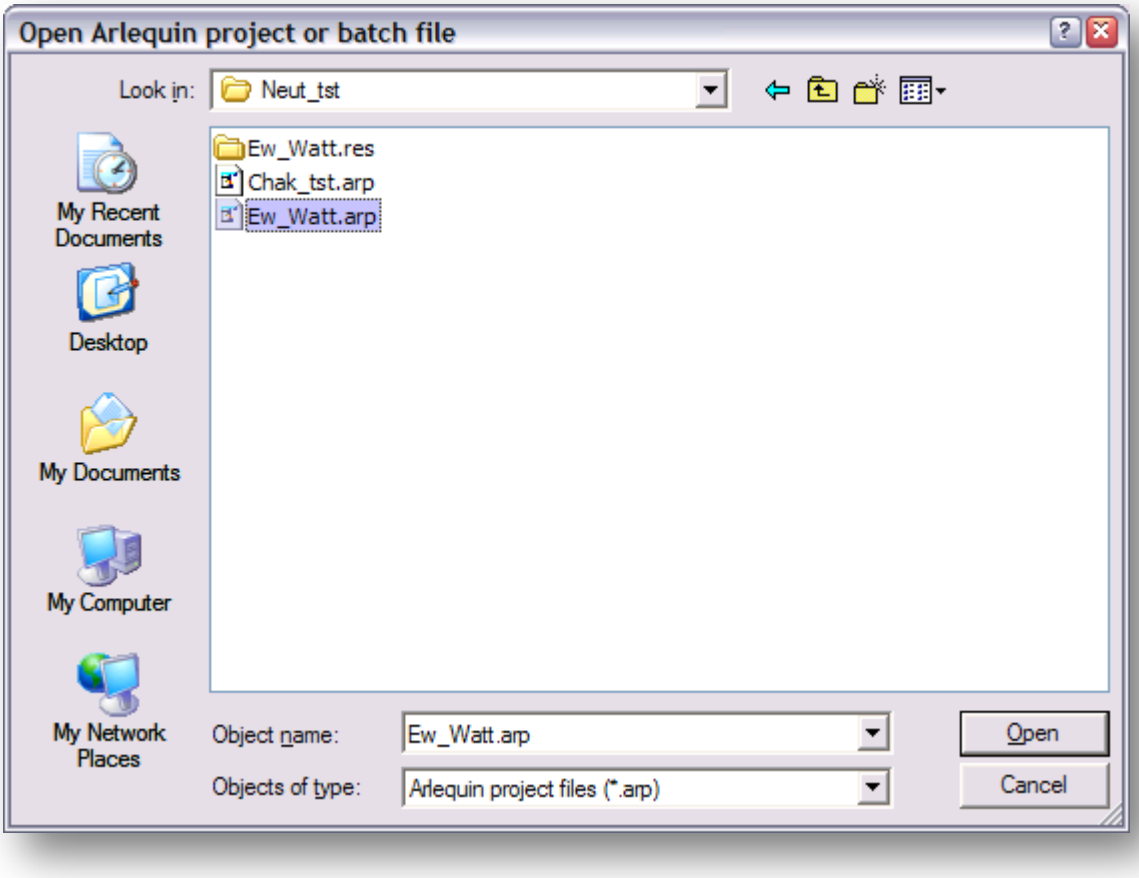
[b] : check box (two states: checked or unchecked).

[m] : multiple selection radio buttons.

[l] : List box, allowing the selection of an item in a downward scrolling list.

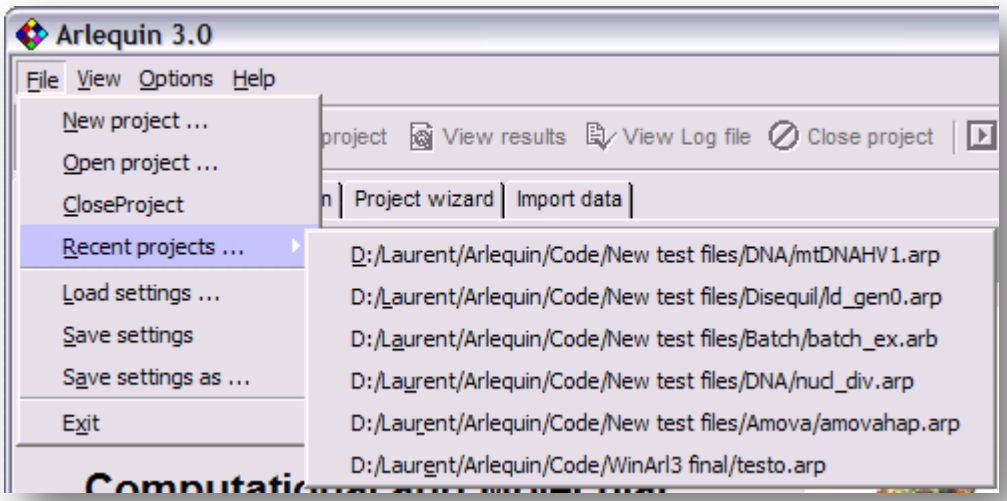
[r] : read only setting, cannot be changed by the user.

6.3.1 Open project

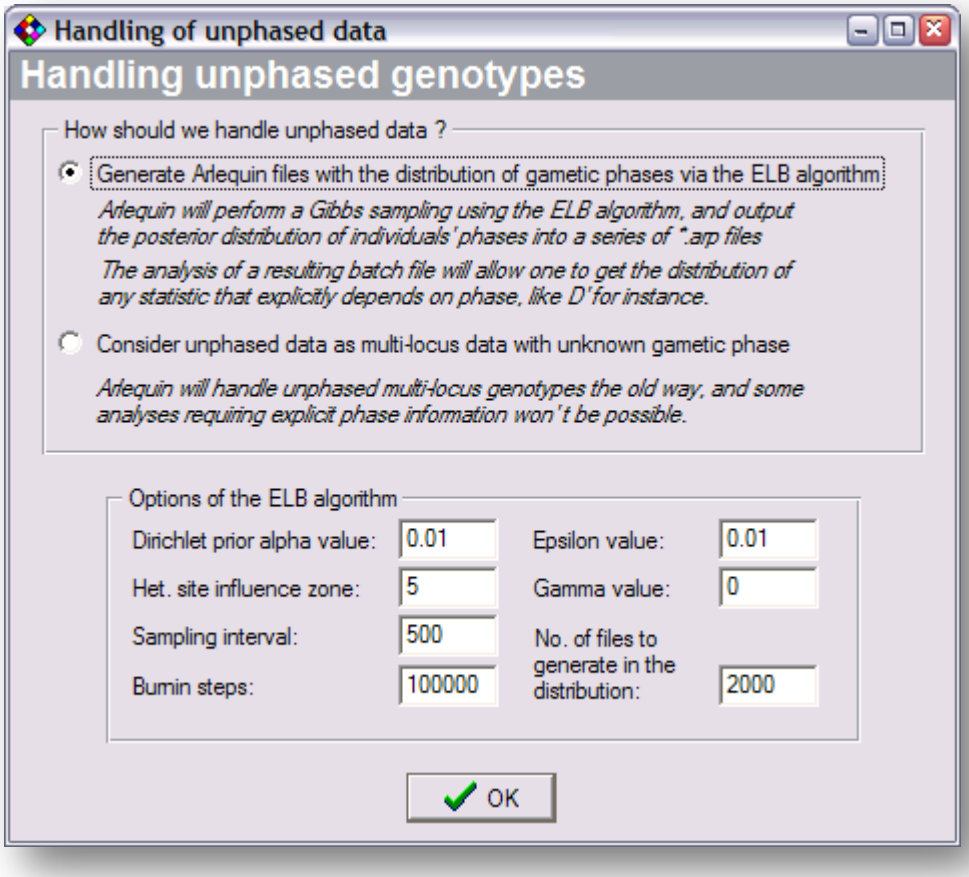


In this dialog box, you can locate an existing Arlequin project on your hard disk. Alternatively you can use the *File* | *Recent Projects* menu to reload one the last 10

projects on which you worked on.



6.3.2 Handling of unphased genotypic data



If the menu "*Prompt for handling unphased multi-locus data*" is checked in the *Option* menu (see section 6.1.3), this dialog box will appear when projects containing genotypic data with unknown phase are loaded. The two options appearing in the dialog box are

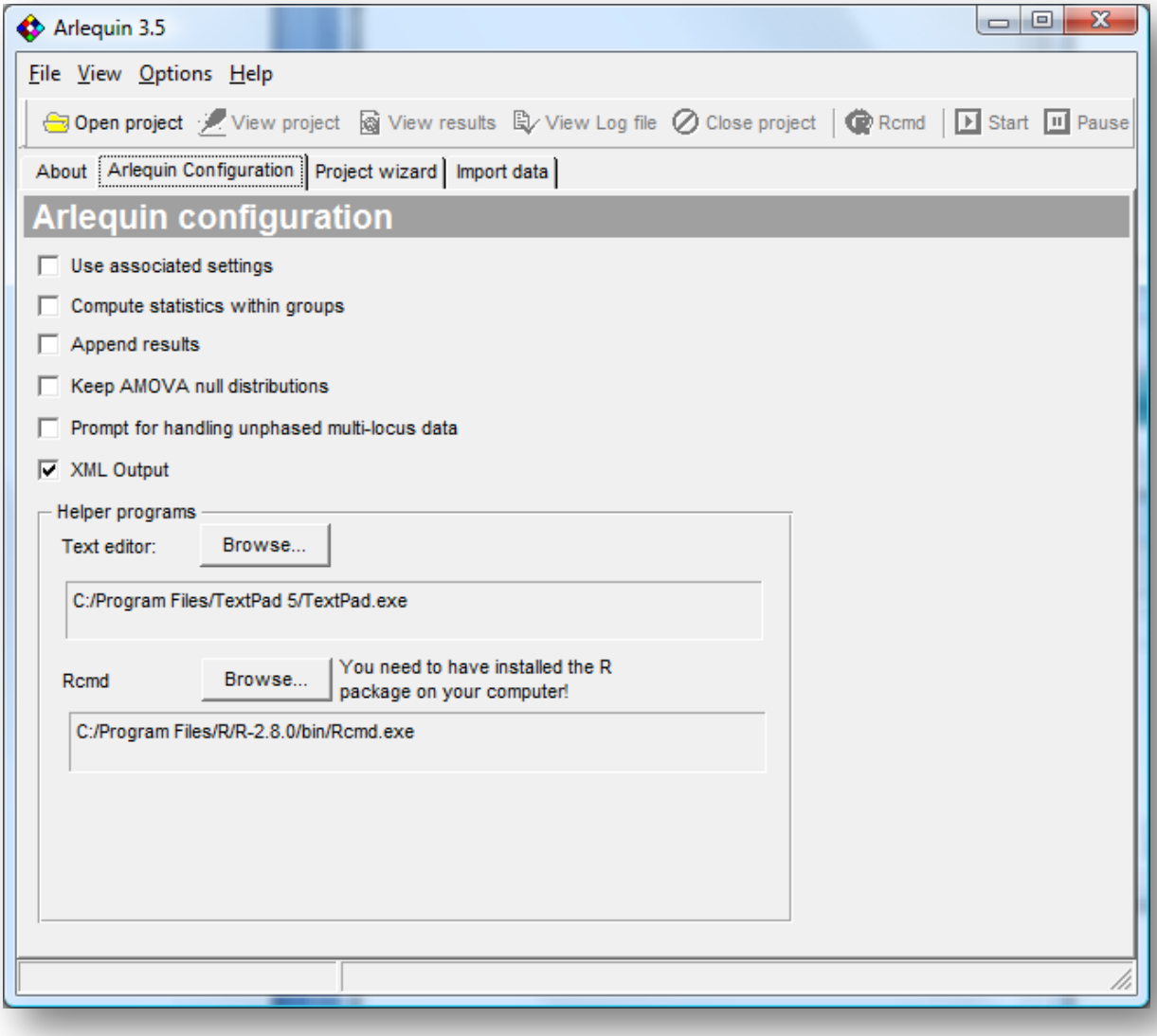
self-explanatory, and the settings for the ELB algorithm are described in the Settings for the ELB algorithm and ELB algorithm sections (6.3.8.4.2.1 and 8.1.3.2.3).

If you choose to estimate the gametic phase with the ELB algorithm, then Arlequin project files (as many as the variable *No. of files to generate in the distribution* defined above) are written in a subdirectory of the result directory called *PhaseDistribution*. They have the name ELB_EstimatedPhase#<Sample number>.arp. Arlequin also outputs a file called *ELB_Best_Phases.arp* containing for each individual the gametic phases estimated with the ELB algorithm, as well as batch file *ELB_PhaseDistribution.arb* listing all aforementioned project files.

The file *ELB_Best_Phases.arp* can then be analyzed as if gametic phases were known for the different samples.

Keep however in mind that the gametic phases are not necessarily correct, and that analyses assuming that the gametic phase is unknown will not take into account possible gametic phase estimation errors.

6.3.3 Arlequin Configuration



Different options can be specified in this tab dialog.

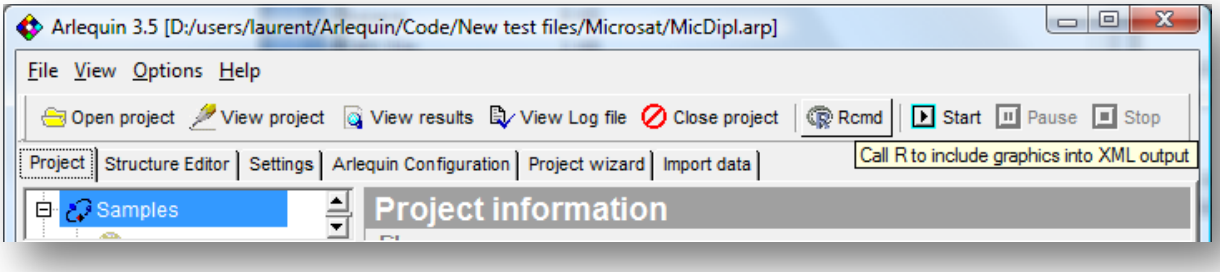
- **Use associated settings**: By checking the *Use associated settings* checkbox, the settings and options last specified for your project will be used when opening a project file. When closing a project file, Arlequin automatically saves the current calculation settings for that particular project. Check this box if you want Arlequin to automatically load the settings associated to each project. If this box is unchecked, the same settings will be used for different projects.
- **Append results**: If the option *Append Results* is checked, the results of the current computations are appended to those of previous analyses. Otherwise, only the results of the last analysis are written in the result file, and previous results are erased.
- **Keep AMOVA null distributions**: If this option is checked, the null distributions of σ_a^2, σ_b^2, σ_c^2, and σ_d^2 generated by an AMOVA analysis are written in files having

the same name as the project file, but with the extensions *.va*, *.vb*, *.vc*, and *.vd,* respectively.

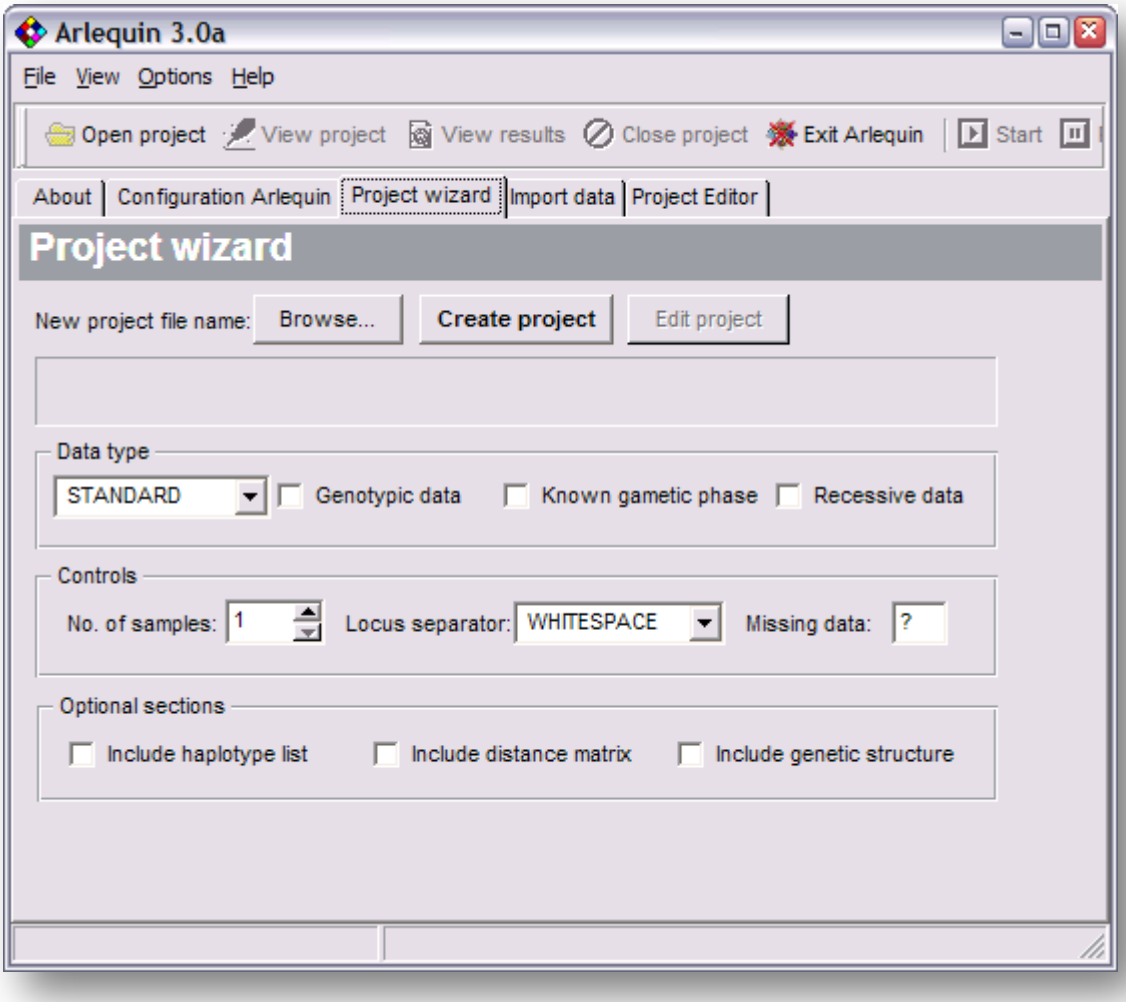
- **XML Output**: This option has the same effect as activating the menu XML output in the *Options* menu (see section 6.1.3). Check this box if you want Arlequin to generate output files in xml format, allowing for more flexibility in the formatting of the output, and the inclusion of graphics generated by an R script (see section 7.6. If this menu is unchecked, conventional html files are generated, and graphs cannot be incorporated into output files.

Helper programs:

- **Text editor**: press on the Browse button to locate the text editor you want to use to edit or view your project file and to view the Arlequin Log File.
- **Rcmd**: RCmd is a console version of the R statistical package, which needs to be installed on your computer. The R environment for statistical computing and graphics is a GNU project, which can be downloaded from the R project page http://www.r-project.org/.
  After installation of the R package on your computer, you need to tell Arlequin where the Rcmd executable file is located (usually in {R version directory}/bin). This program is called when you press on the *Rcmd* button located on Arlequin toolbar.



6.3.4 Project Wizard



In order to help you setting up quickly a project file, Arlequin can create the outline of a project file for you. This tab dialog should allow you to quickly define which type of data you have and some of its properties.

- **Browse** button

  It allows you to specify the name and the directory location of the new project file. Pressing on that buttons opens a File dialog box. The project file should have the extension ".arp".

- **Create project** button

  Press on that button once you have specified all other properties of the project.

- **Edit project** button

  This button become active once you have created an outline and allows you to begin editing the outline and fill in some data.

- **Data type**

Specify which **type of data** you want to analyze (DNA, RFLP, Microsat, Standard, or Frequency).

Specify if the data is under **genotypic** or **haplotypic** form.

Specify if the **gametic phase** is known (for genotypic data only).

Specify if there are **recessive alleles** (for genotypic data only)

- **Controls**

  Specify the **number of population samples** defined in the project

  Choose a **locus separator**

  Specify the character coding for **missing data**

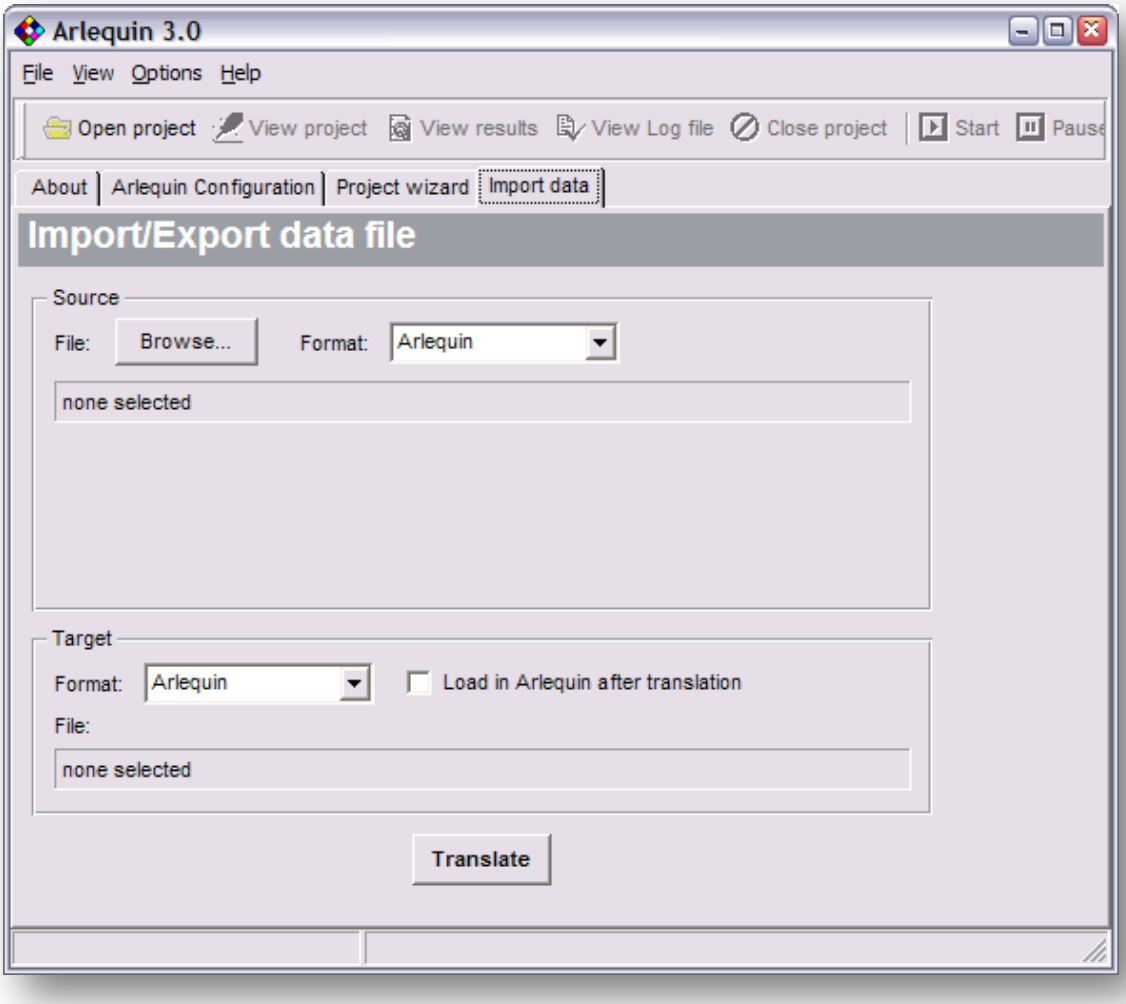
- **Optional sections**

  Specify if you want to include a global **list of haplotypes**

  Specify if you want to include a predefined **distance matrix**

  Specify if you want to include a **group structure**

6.3.5 Import data



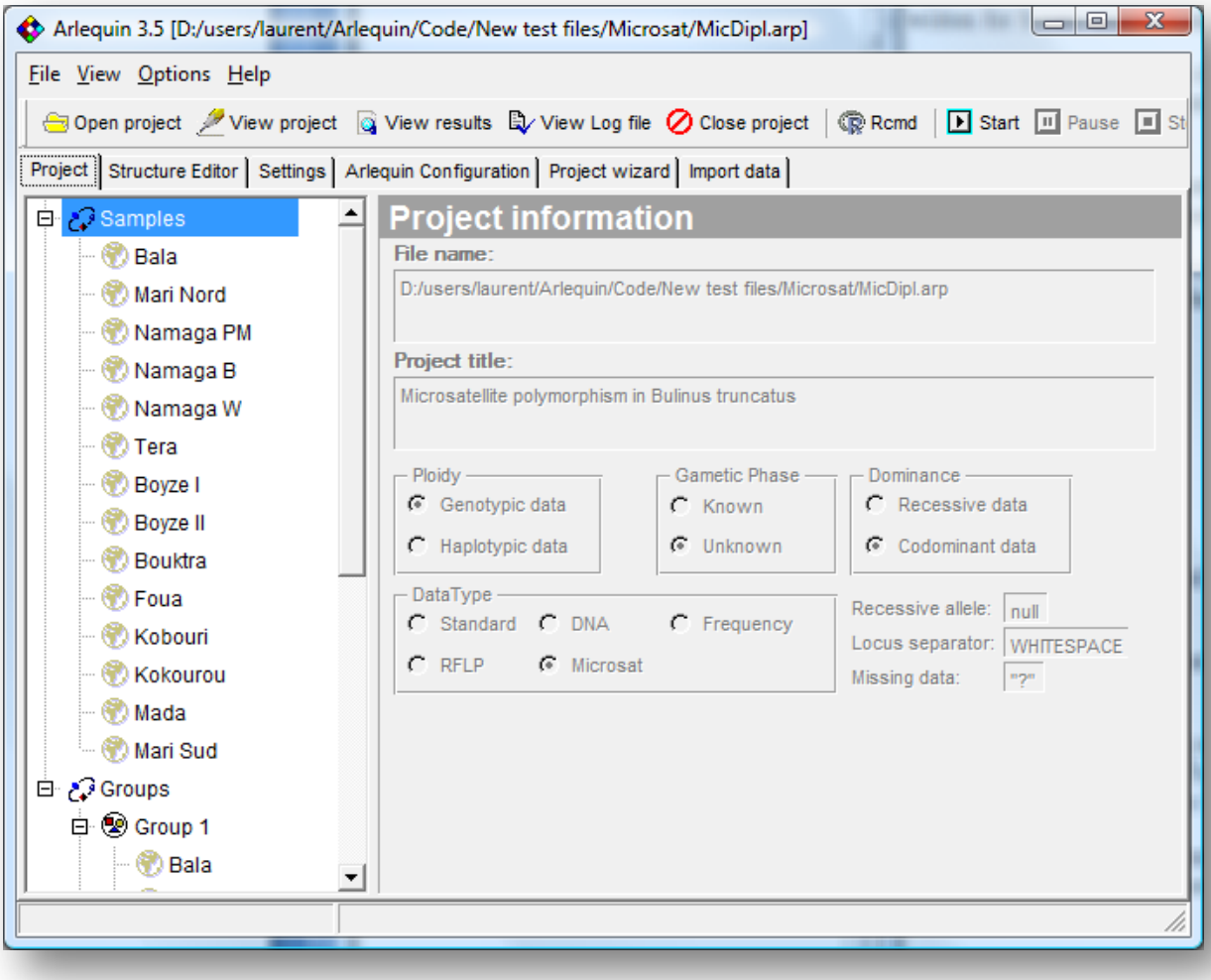
With this dialog box you can quickly translate data into several other file formats often us in population genetics analyses. The currently supported formats are:

Arlequin	GenePop ver. 1.0	Phylip ver. 3.5
Mega ver. 1.0	Biosys ver.1.0	Win Amova ver. 1.55

The translation procedure is as follows:

1) Select the source file with the upper left *Browse* button.
2) Select the format of the source data file, as well as that of the target file.
3) A default extension depending on the data format is automatically given to the target file.
4) The file conversion is launched by pressing on *Translate* button.
5) In some cases, you might be asked for some additional information, for instance if input data is split into several input files (like in WinAmova).
6) If you have selected the translation of a data file into the Arlequin file format, you'll have the option to load the newly created project file into the Arlequin Java Interface.

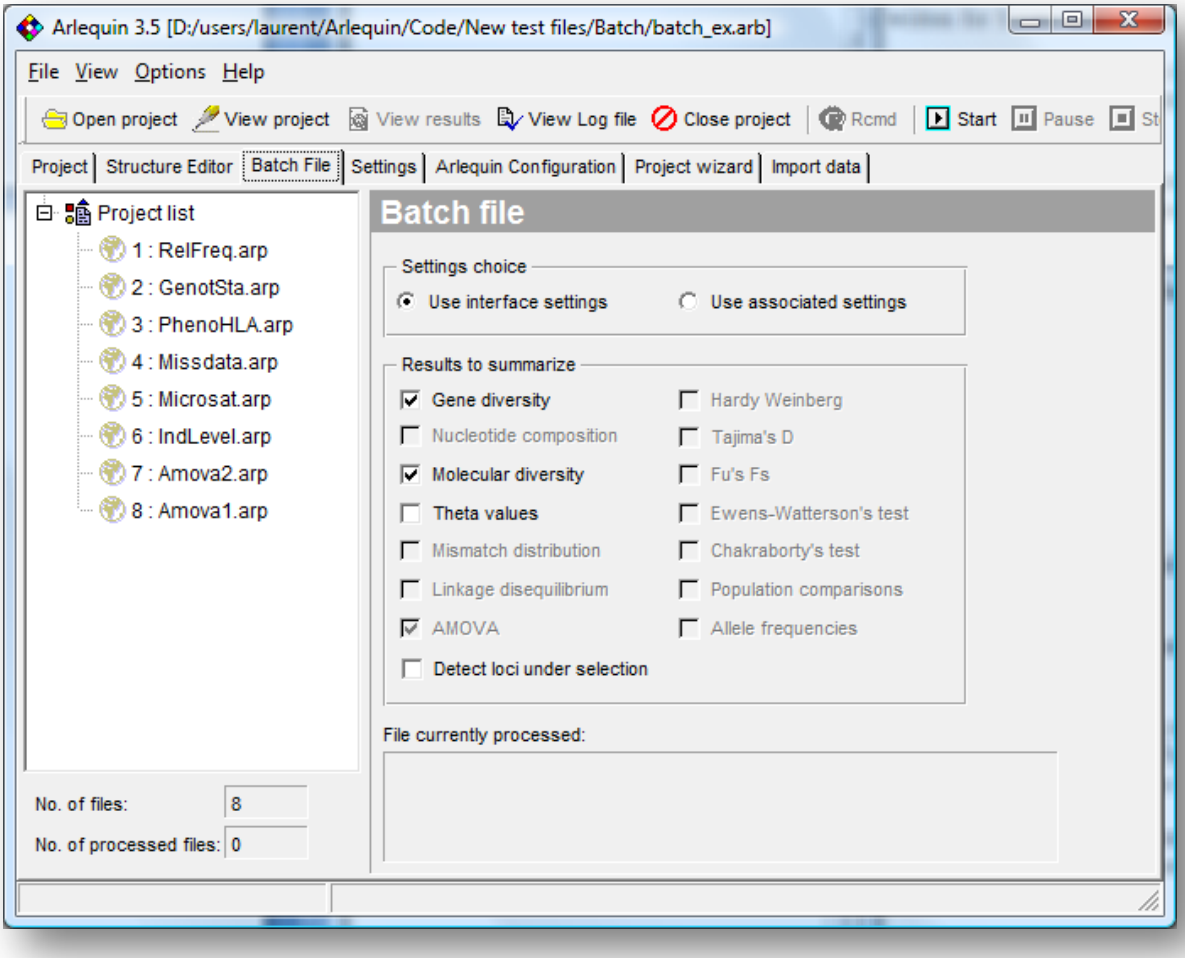
6.3.6 Loaded Project



Once a project has been loaded, the *Project* tab dialog becomes active. It shows a brief outline of the project in an explorable tree pane, and a few information on the data type. The project can be edited by pressing the *View Project* button on the Toolbar, which will launch the text editor currently specified in the *Arlequin Configuration* tab. All the information shown under the project profile section is read only. In order to modify them, you need to edit the project file with your text editor and reload the project with the *File* | *Recent projects* menu.

- **File name** [r]: The location and the name of the current project.
- **Project title**[r]: The title of the project as entered in the input file.
- **Ploidy** [r]: Specifies whether input data consist of diploid genotypic data or haplotypic data. For genotypic data, the diploid information of each genotype is entered on separate lines in the input file.
- **Gametic phase** [r]: Specifies whether the gametic phase is known or unknown when the input file is made up of genotypic data. If the gametic phase is known, then the treatment of the data will be essentially similar to that of haplotypic data.
- **Data type** [r]: Data type specified in the input file.
- **Dominance** [r]: Specifies if the data consists of only co-dominant data or if some recessive alleles can occur.
- **Recessive allele** [r]: Specifies the identifier of the recessive allele.
- **Locus separator**[r]: The character used to separate allelic information at adjacent loci.
- **Missing data**[r]: The character used to represent missing data at any locus. By default, a question mark (?) is used for unknown alleles.

6.3.7 Batch files



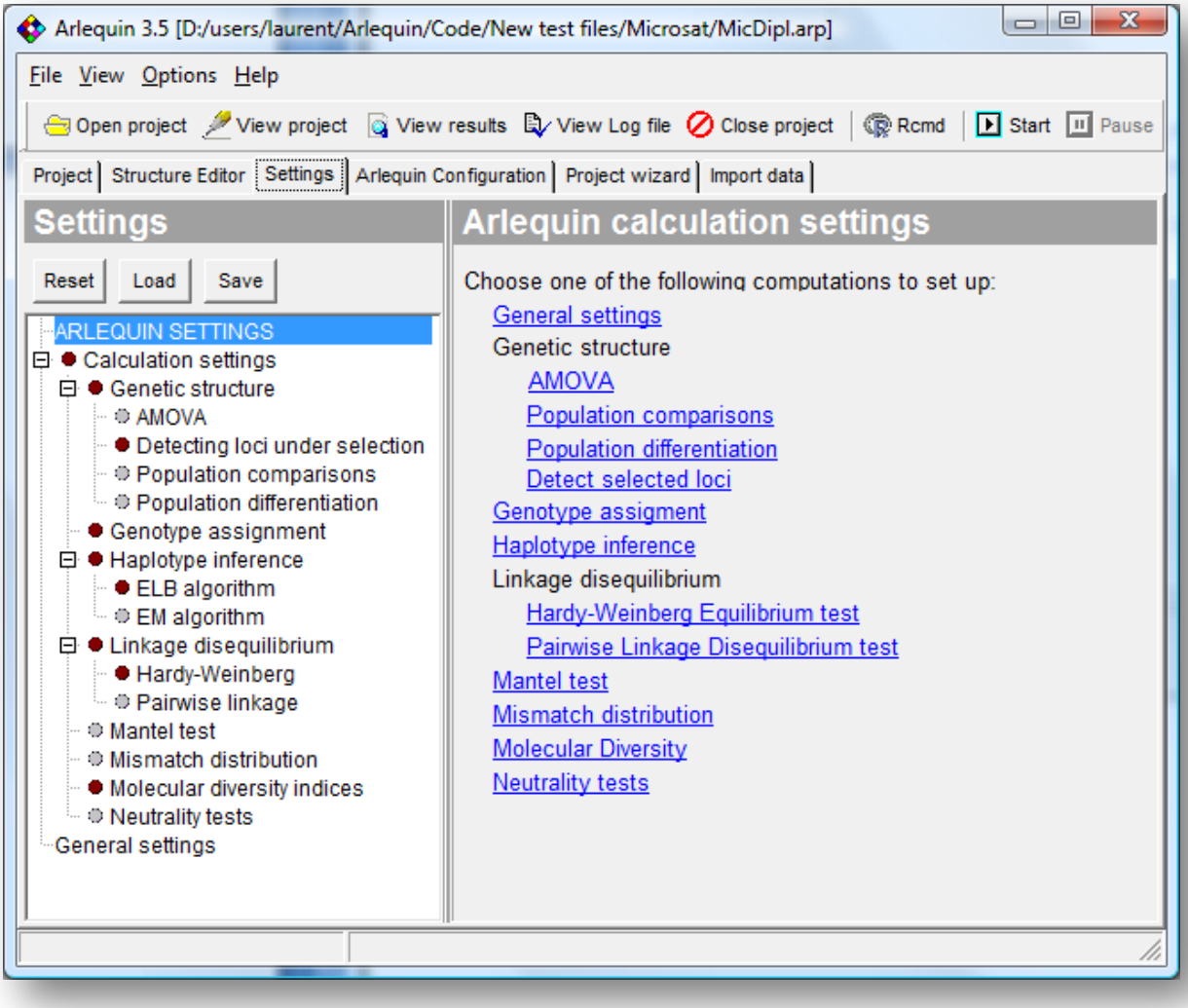
The project files found in the selected batch file appear listed in the left pane window.

- **Use associated settings** [b].: Use this button if you have prepared settings files associated to each project.
- **Use interface settings** [b] : Use this button if you want to use the same predefined calculation settings for all project files.
- **Results to summarize**: This option allows you to collect a summary of the results for each file found in the batch list. These results are written in different files, having the extension **.sum*. These *summary files* will be placed into the same directory as the batch file.

List of summary files created by activating different checkboxes

Checkbox	***Summary file***	***Description***
Gene diversity	gen_div.sum	Gene diversity of each sample
Nucleotide composition	nucl_comp.sum	Nucleotide composition of each sample
Molecular diversity	mold_div.sum	Molecular diversity indexes of each sample
Mismatch distribution	mismatch.sum	Mismatch distribution for each sample
Theta values	theta.sum	Different theta values for each sample
Linkage disequilibrium	l_d_pro.sum	Significance level of linkage disequilibrium for each pair of loci
	link_dis.sum	Number of significantly linked loci per locus
Hardy Weinberg	hw.sum	Test of departure from Hardy-Weinberg equilibrium
Tajima's test	tajima.sum	Tajima's test of selective neutrality
Fu's Fs test	fu_fs.sum	Fu's F_S test of selective neutrality
Ewens Watterson	ewens.sum	Ewens-Watterson tests of selective neutrality
Chakraborty's test	chakra.sum	Chakraborty's test of population amalgamation
Population comparisons	coanst_c.sum	Matrix of Reynolds genetic distances (in linear form)
	NM_value.sum	Matrix of Nm values between pairs of populations (in linear form)
	slatkin.sum	Matrix of Slatkin's genetic distance (in linear form)
	tau_uneq.sum	Matrix of divergence times between populations, taking into account unequal population sizes (in linear form)
	pairdiff.sum	Matrix of mean number of pairwise differences between pairs of samples (in linear form)
	pairdist.sum	Different genetic distances for each pair of population (only clearly readable if 2 samples in the project)
Allele frequencies	allele_freqs.sum	List allele frequencies for all populations in turn. It becomes difficult to read when more than a single population is present in the project file.
Detect loci under selection	selectionDetection.sum	Reports just the fraction of loci that have significant FSTs or FCTs for different significance levels. Locus specific F-statistics, heterozygosities, and p-values can be found in the file "fdist2_ObsOut.txt" located in the result directory of each arp file.

6.3.8 Calculation Settings



The *Settings* tab is divided into two zones:

On the left, a **tree structure** allows the user to quickly select which task to perform. The options for those tasks (**settings**) will appear on the right pane of the tab dialog.

If you select the first *Arlequin settings* node on the tree, a list of the different tasks that can be set up appears on the right pane. Clicking on these underlined blue links will lead you to the appropriate settings panes.

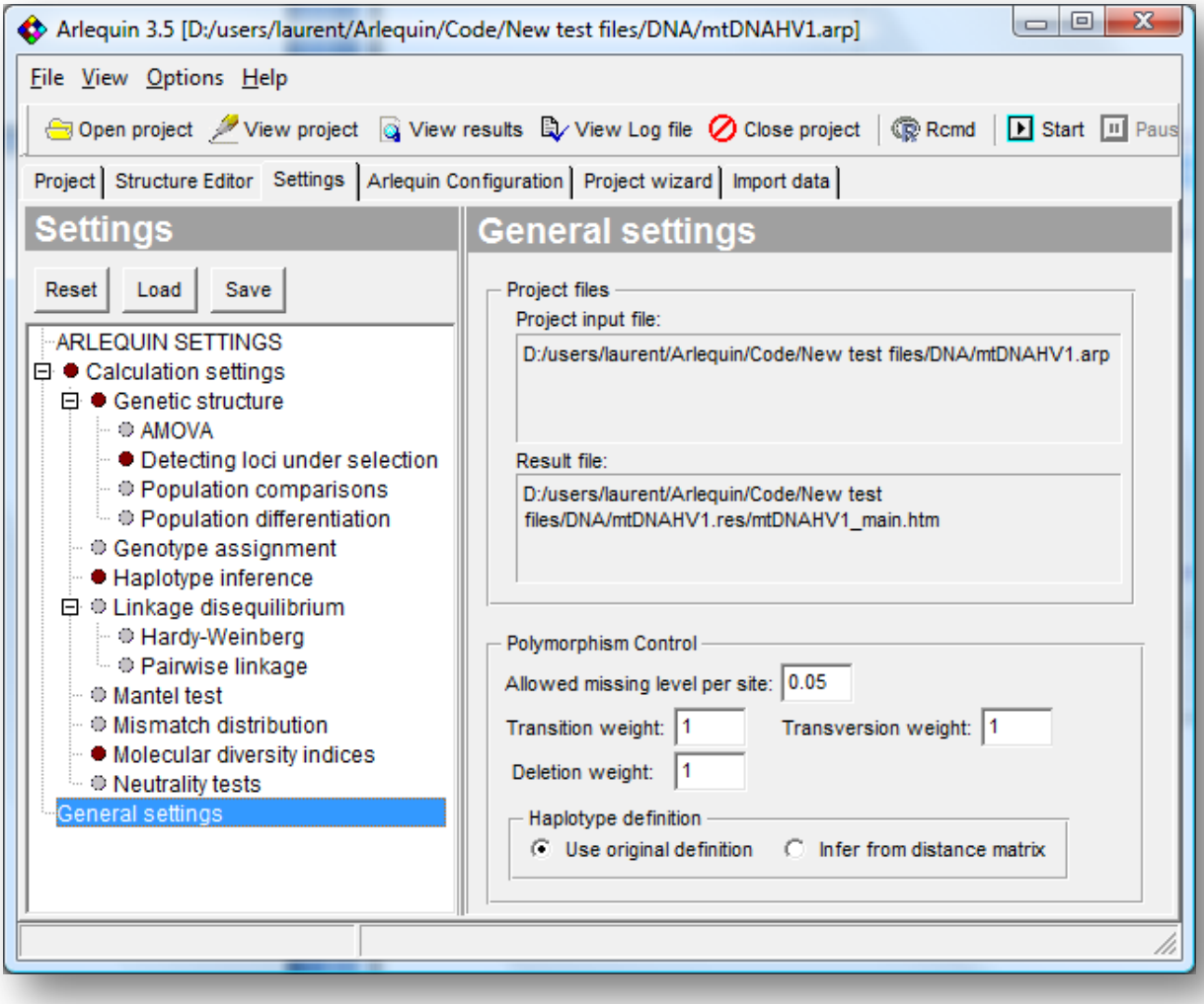
If a particular task has been selected, it will be reflected by a red dot on the left side of the task in the tree structure.

Settings management

Three buttons are also shown on the upper left of the tab dialog:

- **Reset**: Reset all settings to default values and uncheck all tasks.
- **Load**: Load a particular set of settings previously saved into a settings file (extension ".*ars*").
- **Save**: Saves the current settings into a given setting file (extension ".*ars*").

6.3.8.1 General Settings

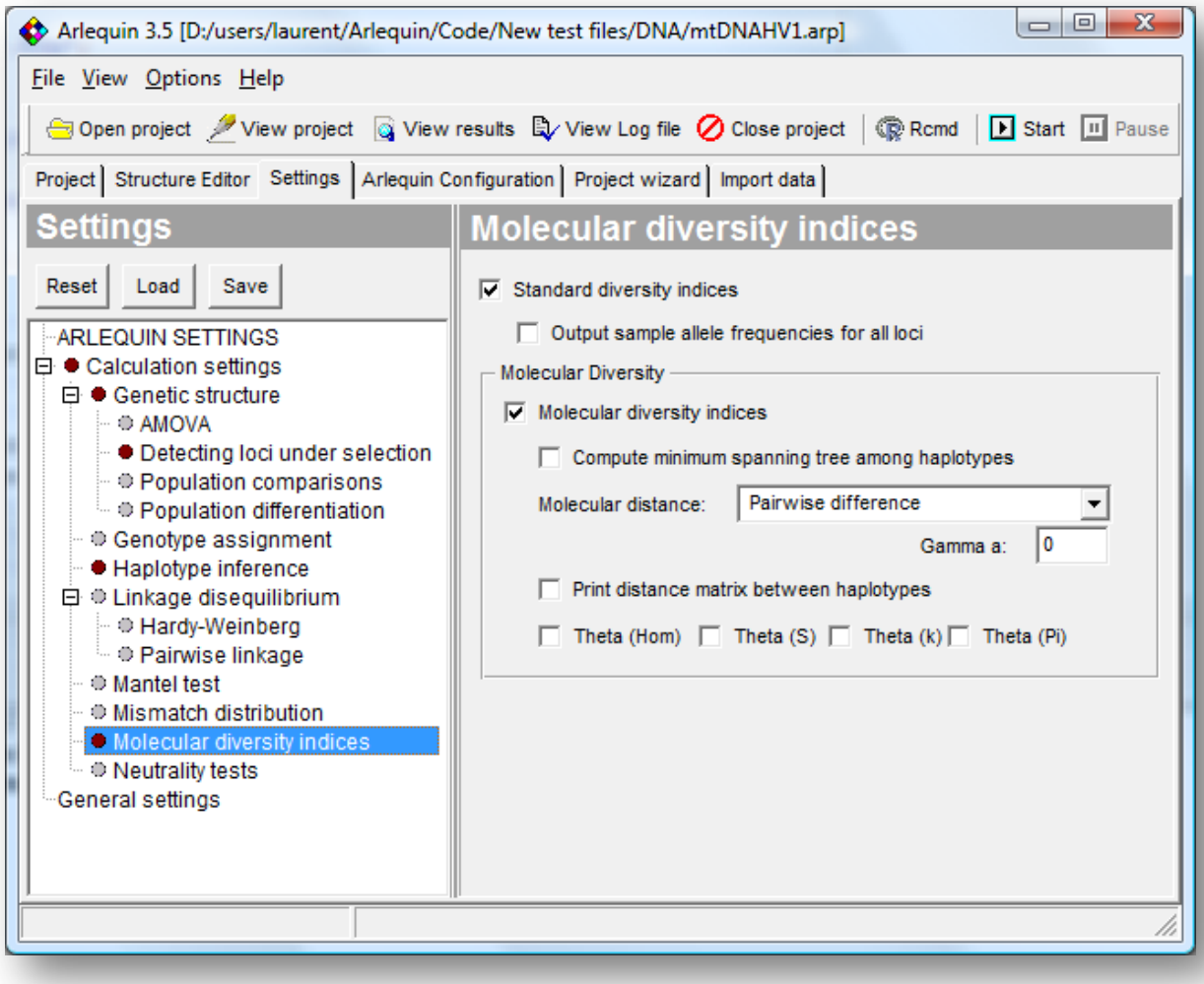


- **Project file** [r]: The name of the project file containing the data to be analyzed (it usually has the "*.arp*" extension).
- **Result files**: The html file containing the results of the analyses generated by Arlequin (it has the same name as the project file, but the "*.htm"* extension).
- **Polymorphism control:**
  - **Allowed missing level per site** [f]: Specify the fraction of missing data allowed for any locus to be taken into account in the analyses. For instance, a level of 0.05 means that a locus with more than 5% of missing data will not be considered in any analysis. This option is especially useful when dealing with DNA data where different individuals have been sequenced for slightly different fragments. Setting a level of zero will force the analysis to consider only those sites that have been sequenced in all individuals. Alternatively, choosing a level of one means that all sites will be considered in the analyses,

even if they have not been sequenced in any individual (not a very smart choice, however).

- **Transversion weight** [f]: The weight given to transversions when comparing DNA sequences.
- **Transition weight** [f]: The weight given to transitions when comparing DNA sequences.
- **Deletion weight** [f]: The weight given to deletions when comparing DNA or RFLP sequences.
- **Haplotype definition**
  - **Use original definition** [m]: Haplotypes are identified according to their original identifier, without considering the fact that their molecular definition could be identical.
  - **Infer from distance matrix** [m]
    Similar haplotypes will be identified by computing a distance matrix based on the settings chosen above. **When this option is activated, a search for shared haplotypes is automatically performed at the beginning of each run, and new haplotypes definitions and frequencies are computed for each population.**

6.3.8.2 Diversity indices



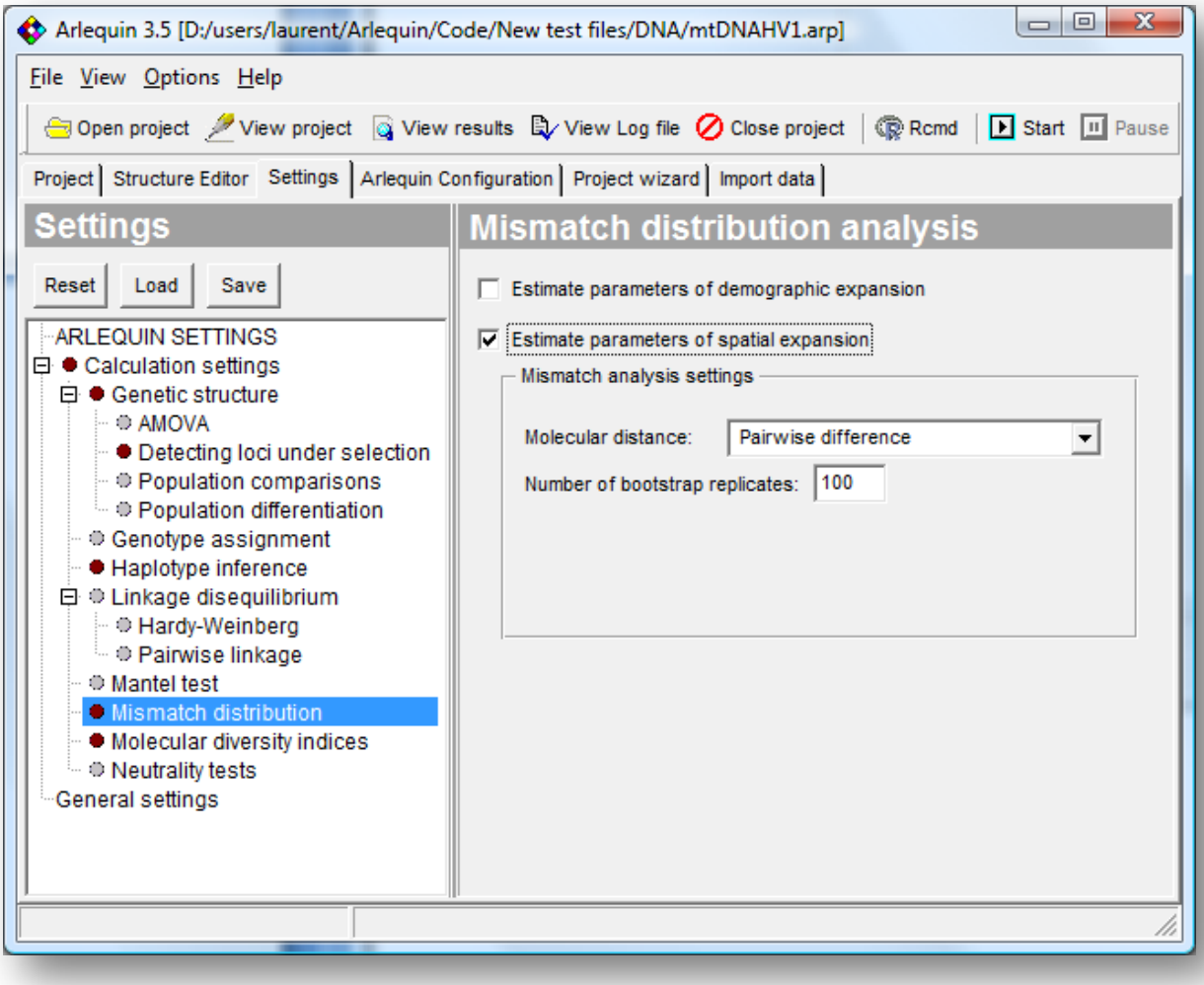
- **Standard diversity indices** [b]: Compute several common indices of diversity, like the number of alleles, the number of segregating loci, the heterozygosity level, etc. (see section 8.1.1).
  - **Output sample allele frequencies for all loci** [b]: Output allele frequencies at all loci for all populations. Creates a separate file for each locus in the result directory. File names are "AllFreqLocus_XXX.txt", where XXX is the locus number. On each row, the frequencies of an allele are listed for all sampled populations. The names of the populations are listed in a separate file, called "PopNames.txt".
- **Molecular diversity indices** [b]: Check box for computing several indices of diversity at the molecular level.
  - **Compute minimum spanning tree among haplotypes** [b]: Computes a minimum spanning tree and a minimum spanning network among the

haplotypes found in each population sample (see section 8.1.2.9). This option is only valid for haplotypic data.

- **Molecular distance** [l]: Choose the type of distance used when comparing haplotypes (see section 8.1.2 and below).
  - **Gamma a value** [f]: Set the value for the shape parameter of the gamma function, when selecting a distance allowing for unequal mutation rates among sites. This option is only valid for some distances computed between DNA sequences. Note that a value of zero deactivates here the Gamma correction of these distances, whereas in reality, a value of infinity would deactivate the Gamma correction procedure. This option is only valid for DNA data.
- **Print distance matrix between haplotypes** [b]: If checked, the inter-haplotypic distance matrix used to evaluate the molecular diversity is printed in the result file.
- **Theta(Hom)** [b]: An estimation of θ obtained from the observed homozygosity H (see section 8.1.2.3.1).
- **Theta(S)** [b]: An estimation of θ obtained from the observed number of segregating site S (see section 8.1.2.3.2).
- **Theta(k)** [b]: An estimation of θ obtained from the observed number of alleles k (see section 8.1.2.3.3).
- **Theta(π)** [b]: An estimation of θ obtained from the mean number of pairwise differences $\hat{\pi}$ (see section 8.1.2.3.4).

6.3.8.3 Mismatch distribution

Compute the distribution of the observed number of differences between pairs of haplotypes in the sample (see section 8.1.2.4). It also estimates parameters of a sudden demographic (or spatial) expansion using a generalized least-square approach, as described in Schneider and Excoffier (1999) (see section 8.1.2.4).



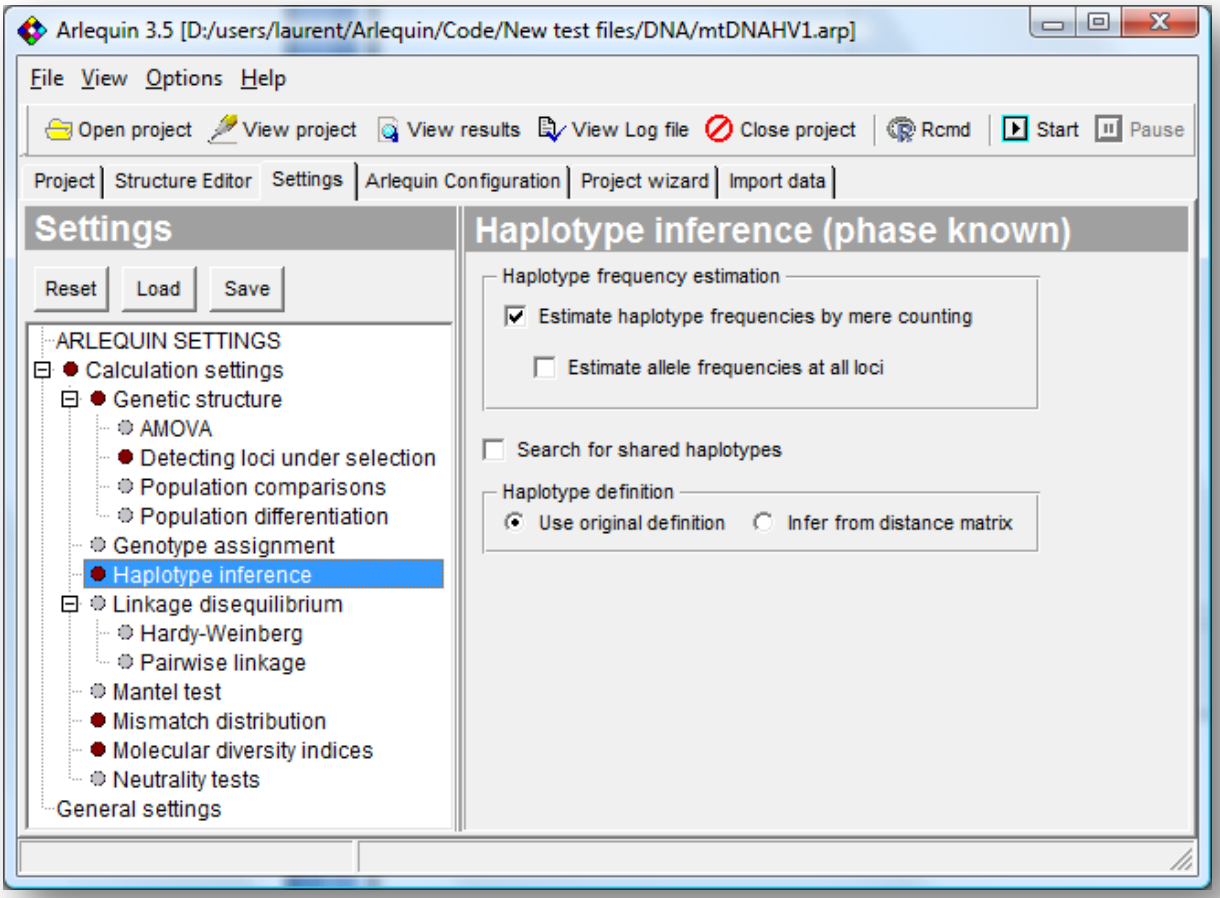
- **Estimate parameters of demographic expansion** [b]: The parameters of an instantaneous demographic expansion are estimated from the mismatch distribution (see section 8.1.2.4) using a generalized least-square approach, as described in Schneider and Excoffier (1999) (see section 8.1.2.4.1).
- **Estimate parameters of spatial expansion** [b]: Estimate the specific parameters of spatial expansion, following Excoffier (2004). (see section 8.1.2.4.2).
- **Molecular distance** [l]: Here we only allow one genetic distance: the mere number of observed differences between haplotypes.
- **Number of bootstrap replicates** [l]: The number of coalescent simulations performed using the estimated parameters of the demographic or spatial expansion. These parameters will be re-estimated for each simulation in order to obtain their empirical confidence intervals, and the empirical distribution of the output statistics such as the sum of squared deviations between the observed and the expected mismatch, the raggedness index, or percentile values for each

point of the expected mismatch (see section 8.1.2.4). Hundreds to thousands of simulations are necessary to obtain meaningful estimates.

6.3.8.4 Haplotype inference

Depending on the data type, different methods are used to estimate the haplote frequencies.

6.3.8.4.1 Haplotypic data, or genotypic (diploid) data with known gametic phase



- **Search for shared haplotypes** [b]: Look for haplotypes that are effectively similar after computing pairwise genetic distances according to the distance calculation settings in the General Settings section. For each pair of populations, the shared haplotypes will be printed out. Then will follow a table that contains, for every group of identified haplotypes, its absolute and relative frequency in each population. This task is only possible for haplotypic data or genotypic data with known gametic phase.

Haplotype definition:

- **Use original definition** [m]: Haplotypes are identified according to their original identifier, without considering the fact that their molecular definition could be identical.

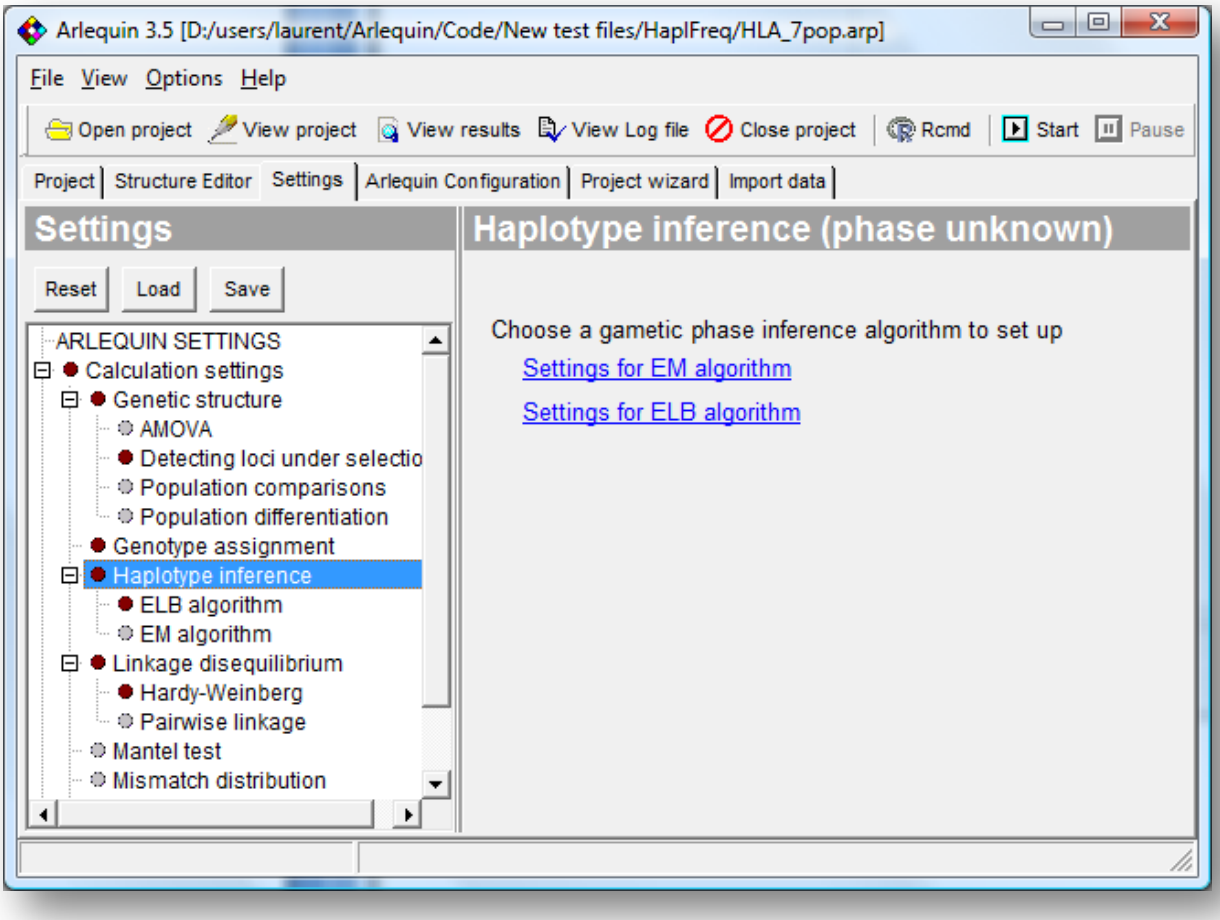
- **Infer from distance matrix** [m]: Similar haplotypes will be identified by computing a molecular distance matrix between haplotypes.

Haplotype frequency estimation:

- **Estimate haplotype frequencies by mere counting** [b]: Estimate the maximum-likelihood haplotype frequencies from the observed data using a mere gene counting procedure.
- **Estimate allele frequencies at all loci**: Estimate allele frequencies at all loci separately.

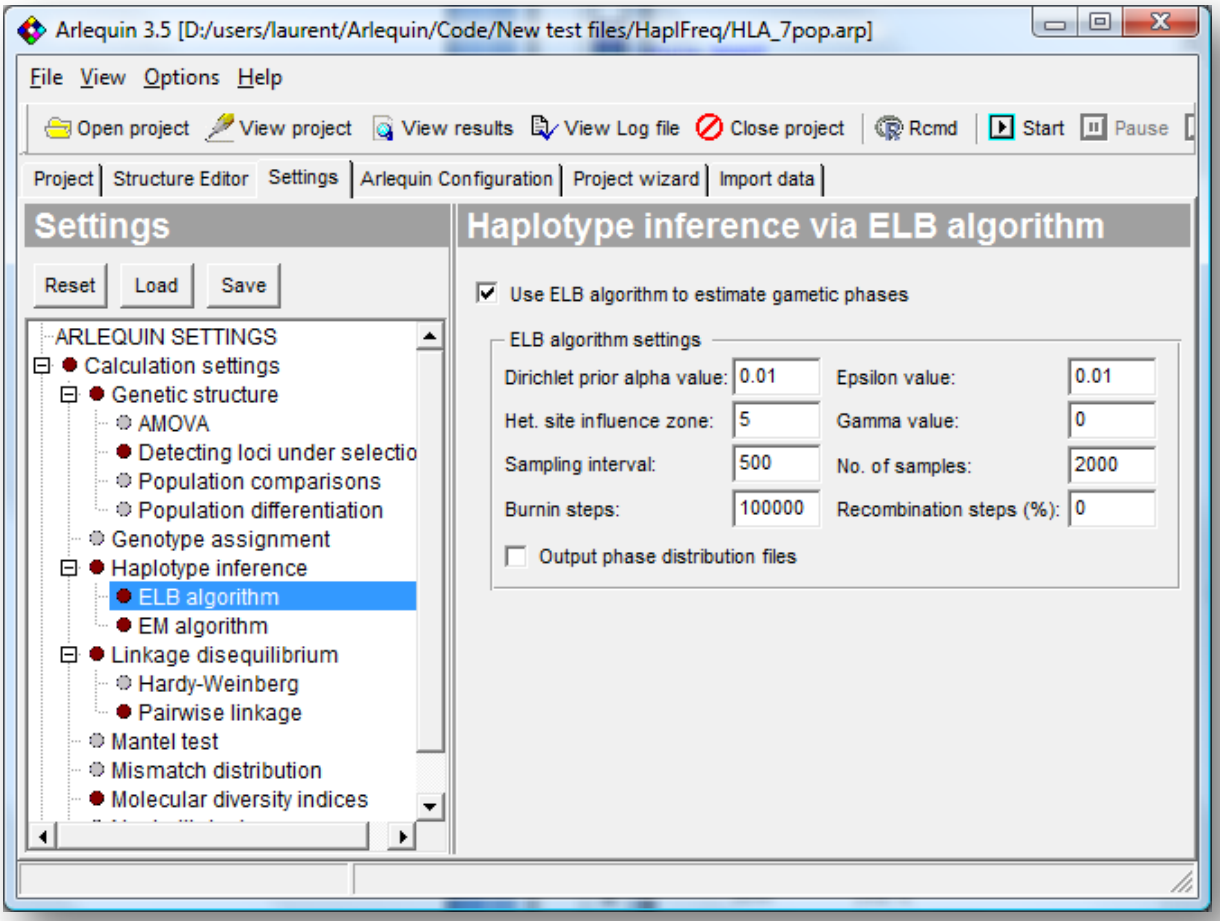
6.3.8.4.2 Genotypic data with unknown gametic phase

When gametic phase is unknown, two methods can be used to infer haplotypes: The (maximum-likelihood) EM algorithm or or the (Bayesian) ELB algorithm.



6.3.8.4.2.1 Settings for the ELB algorithm

The ELB algorithm has been described recently in Excoffier et.al (2003).

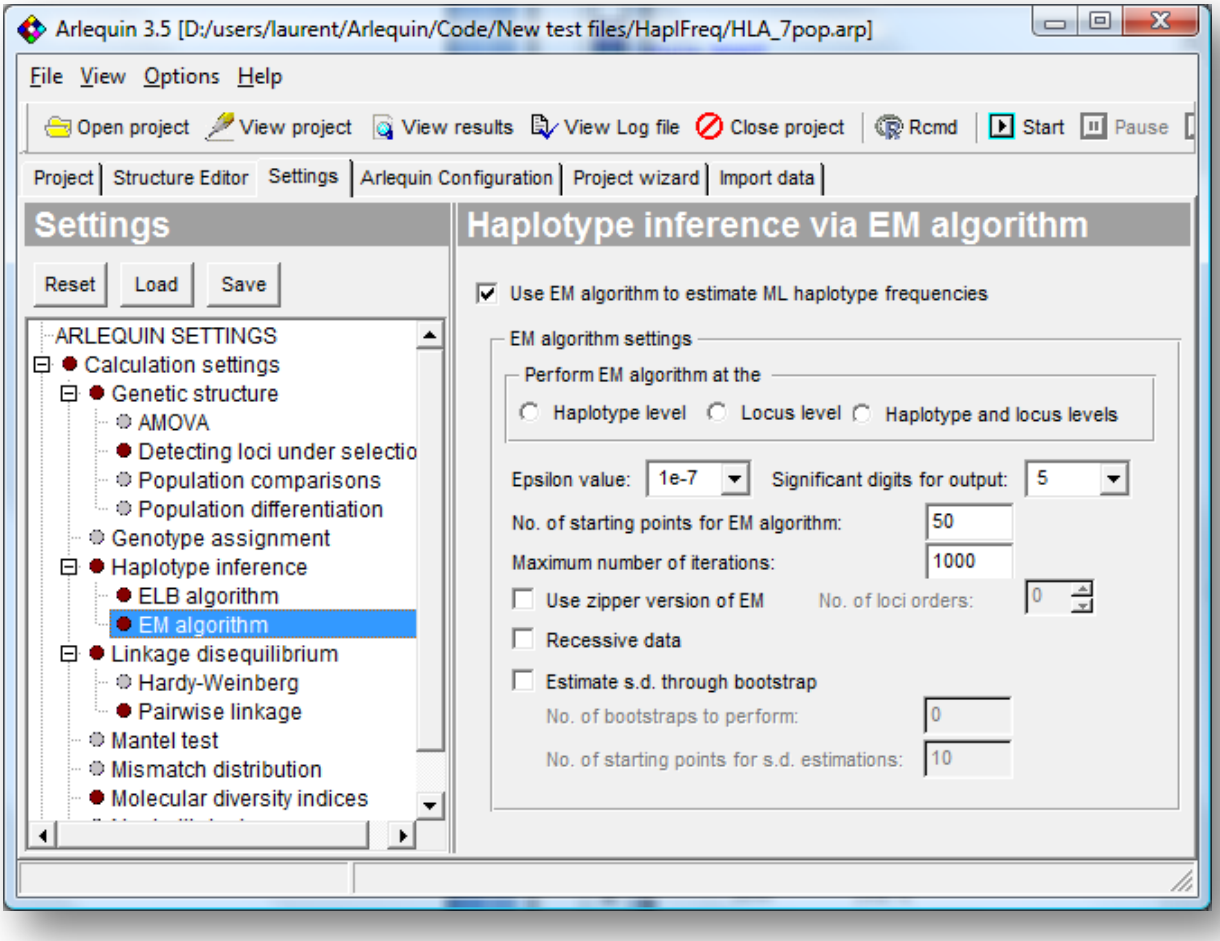


- **Use ELB algorithm to estimate gametic phase [b]**: Check this box if you want to estimate the gametic phase of multi-locu genotypes with the ELB algorithm. See methodological section on ELB algorithm (8.1.3.2.3) for a description of the algorithm.
  - **Dirichlet prior alpha value** [f]: Value of the alpha parameter of the prior dirichlet distribution of haplotype frequencies. Recommended value: a small value like 0.01 for all data types has been found to work well (Excoffier et al. 2003). (see section 8.1.3.2.3 details)
  - **Epsilon value** [f]: Value of the parameter controlling how much haplotypes differing by a single mutation from potentially present haplotypes are weighted. Recommended values: 0.1 for microsatellite data, and 0.01 for other data types. (see section 8.1.3.2.3 details)
  - **Heterozygote site influence zone** [i]: Defines the number of sites adjacent to heterozygote sites that need to be taken into account when computing haplotype frequencies in the Gibbs chain. A value of zero implies that gametic phase will be

estimated only on the basis of heterozygote sites. A negative value will indicate that all sites (homozygotes and heterozygotes will be used). This parameter is mostly useful for inferring gametic phase of DNA sequences where there is only a few heterozygote sites among long stretches of homozygous sites. (see section 8.1.3.2.3 details)

- **Gamma value** [f]: This parameter prevents adaptive windows where gametic phase is estimated to grow too much. It can be set to zero for microsatellite data, and to a small value for other data sets, like 0.01. (see section 8.1.3.2.3 details)
- **Sampling interval** [i]: It is the number of steps in the Gibbs chain between two consecutive samples of gametic phases.
- **Number of samples** [i]: It represents the number of samples of gametic phases one wants to draw in the Gibbs chain to get the posterior distribution of gametic phases (and haplotype frequencies) for each individual. (see section 8.1.3.2.3 details)
- **Burnin steps** [i]: It is the number of steps to perform in the Gibbs chain before sampling gametic phases. The total number of steps in the chain will thus be: Burnin steps + (Number of samples H Sampling interval). (see section 8.1.3.2.3 details)
- **Recombination steps** [i]: It is the proportion of steps in the Gibbs chain consisting in implementing a pseudo-recombination phase update instead of a simple phase switch (corresponding to a double recombination around a heterozygous site) (see section 8.1.3.2.3 details).
- **Output phase distribution files** [b]: Controls if one wants to output Arlequin files with the gametic phase of each sample in the Gibbs chain. The arlequin files (as many as the variable *Number of samples* defined above) are written in a subdirectory of the result directory called *PhaseDistribution*. They have the name ELB_EstimatedPhase#<Sample number>.arp. Arlequin also outputs a file called *ELB_Best_Phases.arp* containing for each individual the gametic phases estimated with the ELB algorithm, as well as batch file *ELB_PhaseDistribution.arb* listing all aforementioned project files.

6.3.8.4.2.2 Settings for the EM algorithm



- ***Use EM algorithm to estimate ML haplotype frequencies*** [b]: We estimate the maximum-likelihood (ML) haplotype frequencies from the observed data using an Expectation-Maximization (EM) algorithm for multi-locus genotypic data when the gametic phase is not known, or when recessive alleles are present (see section 8.1.3.2).

  Perform EM algorithm at the:

  - **Haplotype level** [m]: Estimate haplotype frequencies for haplotypes defined by alleles at all loci.
  - **Locus level** [m]: Estimate allele frequencies for each locus.
  - **Haplotype and locus levels** [m]: The two previous options are performed one after the other.

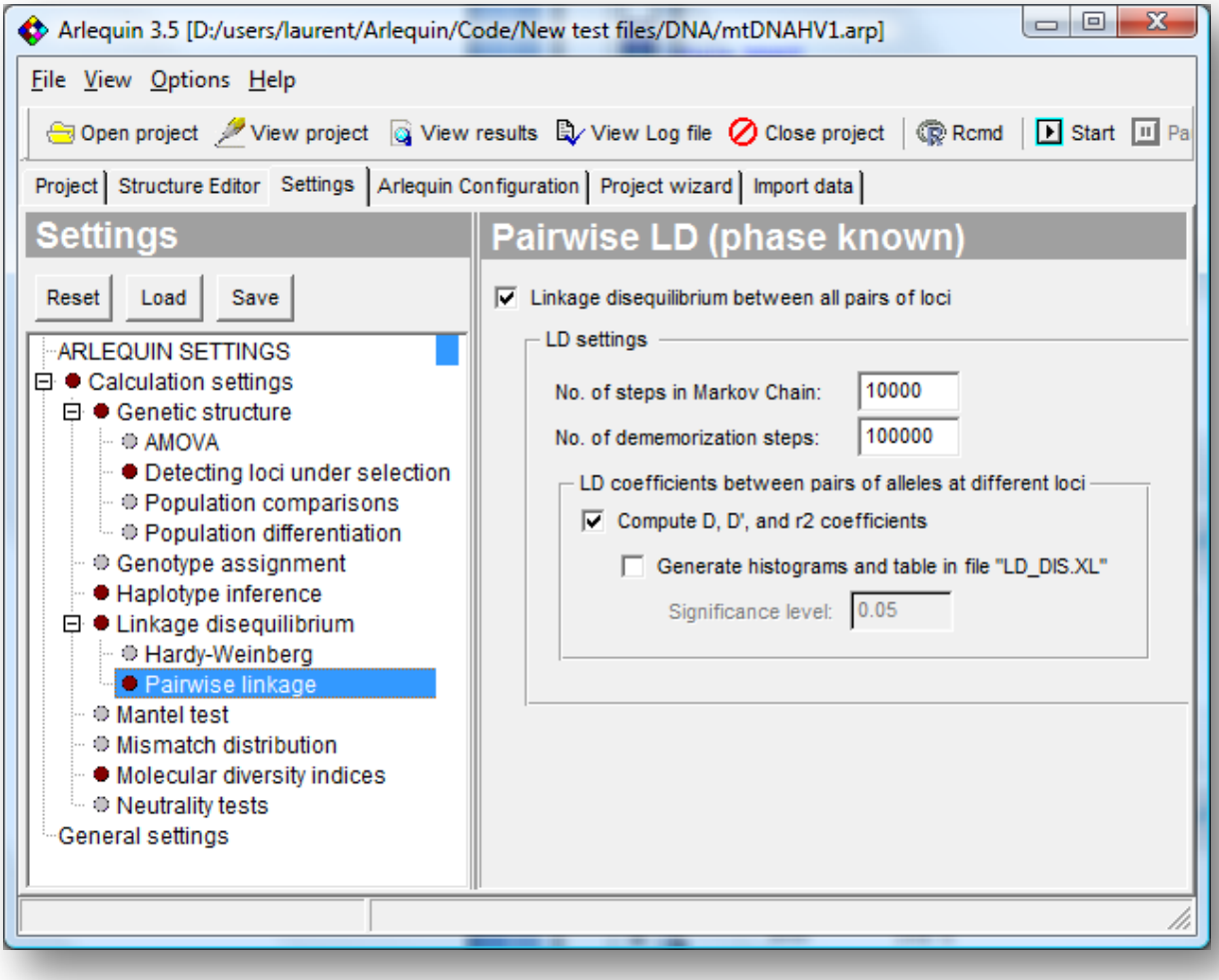
- **Epsilon value** [l]: Threshold for stopping the EM algorithm. After each iteration, Arlequin checks if the current haplotype frequencies are different from those at the previous iteration. If the sum of difference is smaller than epsilon, the algorithm stops.

- **Significant digits for output** [l]: Precision required for output of haplotype frequencies. Haplotypes having a zero frequency given the required precisin are not output in the result file.
- **Number of starting points for EM algorithm**:[i]: Set the number of random initial conditions from which the EM algorithm is started to repeatedly estimate haplotype frequencies. The haplotype frequencies globally maximizing the likelihood of the sample will be kept eventually. Figures of 50 or more are usually in order.
- **Maximum no. of iterations** [i]: Set the maximum number of iterations allowed in the EM algorithm. The iterative process will have at most this number of iterations, but may stop before if convergence has been reached. Here, convergence is reached when the sum of the differences between haplotypes frequencies between two successive iterations is smaller than the epsilon value defined above.
- **Use Zipper version of EM** [b]: Use the zipper version of the EM algorithm consisting in building haplotypes progressively by adding one locus at a time (see section 8.1.3.2.2).
  - **No. of loci orders** [l]: Defines how many random loci orders should be used in the zipper version of the EM algorithm. Results about haplotype frequencies obtained for the locus order leading to the best likelihood is shown in the result file.
- **Recessive data** [b]: Specify whether a recessive allele is present. This option applies to all loci. The code for the recessive allele can be specified in the project file (see section 4.2.1).
- **Estimate standard deviation through bootstrap [b]**: Uses a bootstrap approach to estimate the standard deviation of haplotype frequencies.
  - **No. of bootstrap to perform** [i]: Set the number of parametric bootstrap replicates of the EM estimation process on random samples generated from a fictive population having haplotype frequencies equal to previously estimated ML frequencies. This procedure is used to generate the standard deviation of haplotype frequencies. When set to zero, the standard deviations are not estimated.
  - **No. of starting points for s.d. estimation** [i]: Set the number of initial conditions for the bootstrap procedure. It may be smaller than the number of initial conditions set when estimating the haplotype frequencies, because the bootstrap replicates are quite time-consuming. Setting this number to small values is conservative, in the sense that it usually inflates the standard deviations.

6.3.8.5 Linkage disequilibrium

6.3.8.5.1 Linkage disequilibrium between pairs of loci

6.3.8.5.1.1 Gametic phase known



- **Linkage disequilibrium between all pairs of loci**[b]: Test for the presence of significant association between pairs of loci, based on an exact test of linkage disequilibrium. This test can be done with all data types except FREQUENCY data type. The number of loci can be arbitrary, but if there are less than two polymorphic loci, there is no point performing this test. The test procedure is analogous to Fisher's exact test on a two-by-two contingency table but extended to a contingency table of arbitrary size (see section 8.1.4.1).
  - **No. of steps in Markov chain** [i]: The maximum number of alternative tables to explore. Figures of 100,000 or more are in order. Larger values will lead to a better precision of the *P*-value as well as its estimated standard deviation.
  - **No. of dememorization steps** [i]: The number of steps to perform before beginning to compare the alternative table probabilities to that of the observed table. It corresponds to a burnin. A few thousands steps are necessary to reach a

random starting point corresponding to a table independent from the observed table.

LD coefficients between pairs of alleles at different loci

- **Compute *D*, *D'* and r^2 coefficients** [b] (between all pairs of alleles at different loci):

  See section 8.1.4.3

  1) *D*: The classical linkage disequilibrium coefficient measuring deviation from random association between alleles at different loci (Lewontin and Kojima, 1960) expressed as $D = p_{ij} - p_i p_j$.

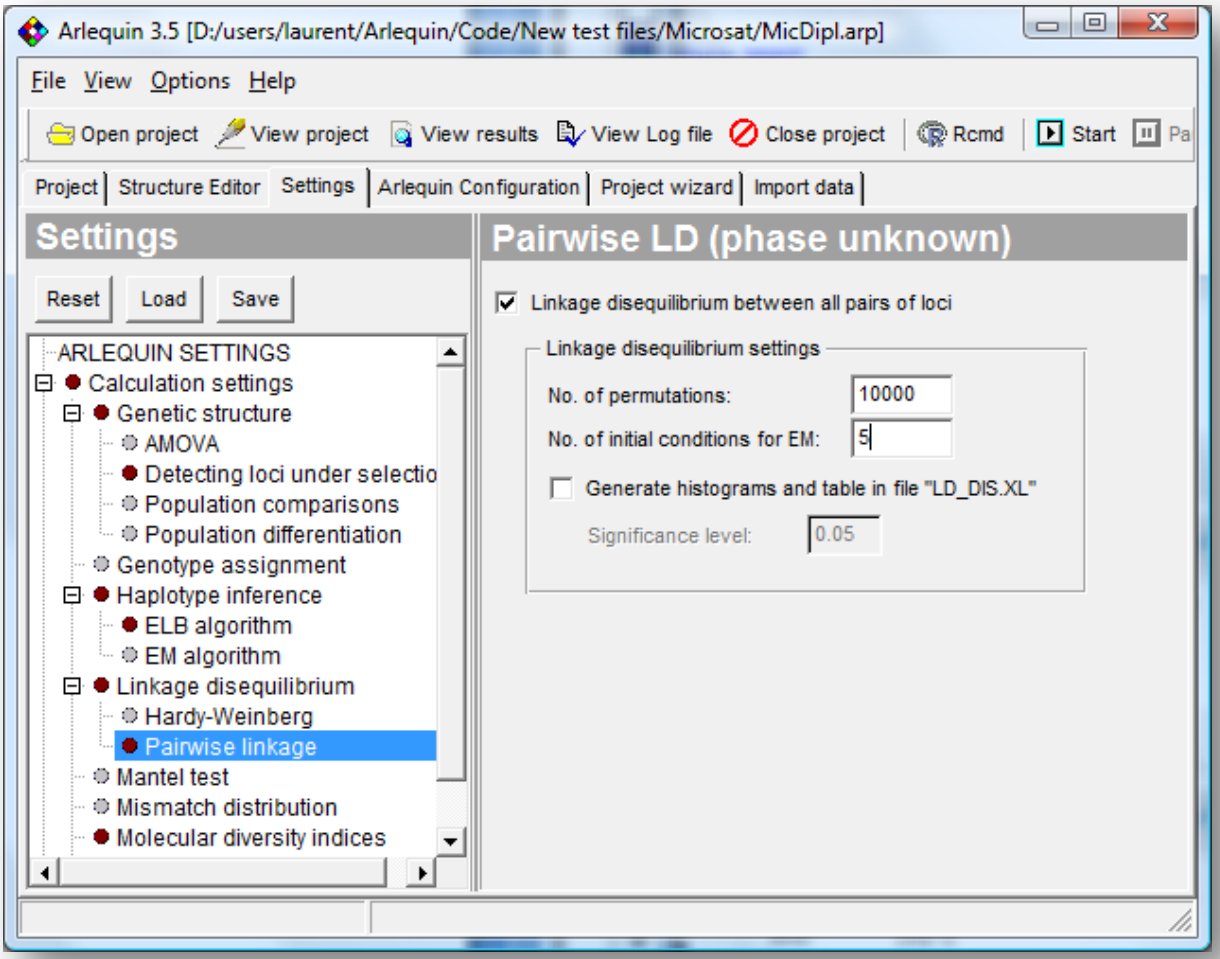
  2) *D'*: The linkage disequilibrium coefficient *D* standardized by the maximum value it can take ($D_{\max}$), given the allele frequencies (Lewontin 1964).

  3) r^2: It is another way to standardise the simple measure of linkage disequilibrium *D* as $r^2 = \frac{D^2}{p_i(1-p_i)p_j(1-p_j)}$.

  - **Generate histogram and table** [b]: Generates a histogram of the number of loci with which each locus is in disequilibrium, and an *S* by *S* table (*S* being the number of polymorphic loci) summarizing the significant associations between pairs of loci. This table is generated for different levels of polymorphism, controlled by the value *y*: a locus is declared polymorphic if there are at least 2 alleles with *y* copies in the sample (Slatkin, 1994a). This is done because the exact test is more powerful at detecting departure from equilibrium for higher values of *y* (Slatkin 1994a). The results are output in a file called "*ld_dis.xl*".
    - **Significance level** [f]: The level at which the test of linkage disequilibrium is considered significant for the output table

6.3.8.5.1.2 Gametic phase unknown

When the gametic phase is not known, we use a different procedure for testing the significance of the association between pairs of loci (see section 8.1.4.2). It is based on a likelihood ratio test, where the likelihood of the sample evaluated under the hypothesis of no association between loci (linkage equilibrium) is compared to the likelihood of the sample when association is allowed (see Slatkin and Excoffier, 1996). The significance of the observed likelihood ratio is found by computing the null distribution of this ratio under the hypothesis of linkage equilibrium, using a permutation procedure.

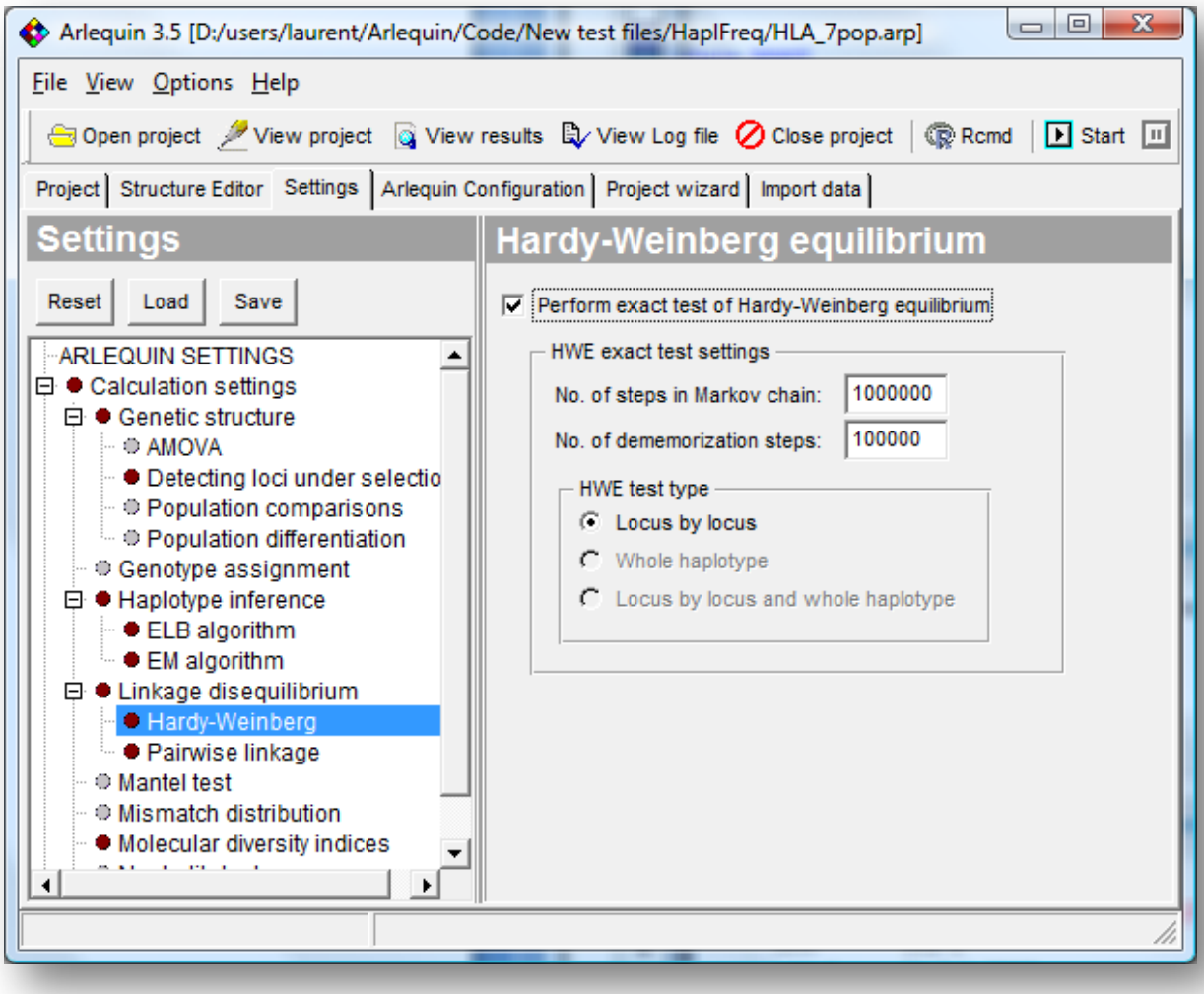


- **Linkage disequilibrium between all pairs of loci**[b]: perform the likelihood-ratio test (see section 8.1.4.2).
  - **No. of permutations** [i]: Number of random permuted samples to generate. Figures of several thousands are in order, and 16,000 permutations guarantee to have less than 1% difference with the exact probability in 99% of the cases (Guo and Thomson, 1992). A standard error for the estimated *P*-value is estimated using a system of batches (Guo and Thomson, 1992).
  - **No. of initial conditions for EM** [i]: Sets the number of random initial conditions from which the EM is started to repeatedly estimate the sample likelihood. The haplotype frequencies globally maximizing the sample likelihood will be eventually kept. Figures of 3 or more are in order.
  - **Generate histogram and table** [b]: Generates an histogram of the number of loci with which each locus is in disequilibrium, and an *S* by *S* table (*S* being the number of polymorphic loci) summarizing the significant associations between pairs of loci. This table is generated for different levels of polymorphism, controlled by the value *y*: a locus is declared polymorphic if there are at least 2 alleles with *y*

copies in the sample (Slatkin, 1994a). This is done because the exact test is more powerful at detecting departure from equilibrium for higher values of *y* (see Slatkin 1994a). The results are output in a file called "*ld_dis.xl*".

- **Significance level** [f]: The level at which the test of linkage disequilibrium is considered significant for the output table.

6.3.8.5.2 Hardy-Weinberg equilibrium

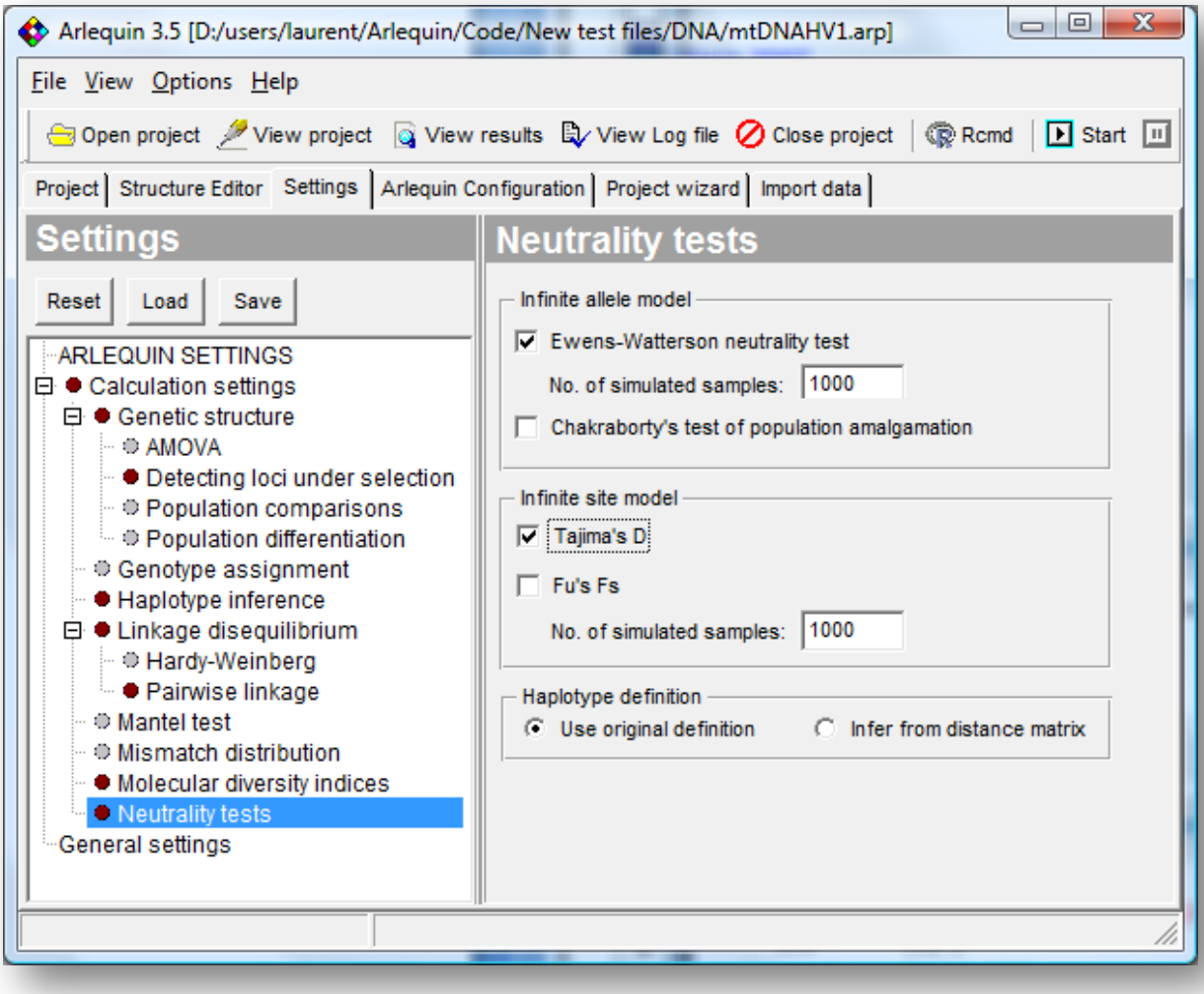


- **Perform exact test of Hardy-Weinberg equilibrium** [b]: Test of the hypothesis that the observed diploid genotypes are the product of a random union of gametes. This test is only possible for genotypic data. Separate tests are carried out at each locus.

  This test is analogous to Fisher's exact test on a two-by-two contingency table but extended to a contingency table of arbitrary size (see section 8.1.5). If the gametic phase is unknown the test is only possible locus by locus. For data with known gametic phase, it is also possible to test the association at the haplotypic level within individuals.

- **No. of steps in Markov chain** [i]: The maximum number of alternative tables to explore. Figures of 100,000 or more are in order.
- **No. of dememorisation steps** [i]: The number of steps to perform before beginning to compare the alternative table probabilities to that of the observed table. A few thousands steps are necessary to reach a random starting point corresponding to a table independent from the observed table.
- HWE test type
  - **Locus by locus** [m]: Perform separate HWE test for each locus.
  - **Whole haplotype** [m]: Perform a HWE test at the haplotype level (if gametic phase is available).
  - **Locus by locus and whole haplotype** [m]: Perform both kinds of tests (if gametic phase is available).

6.3.8.6 Neutrality tests



Tests of selective neutrality, based either on the infinite-allele model or on the infinite-site model (see section 8.1.6).

Infinite allele model

- **Ewens-Watterson neutrality test** [b]: Performs tests of selective neutrality based on Ewens sampling theory in a population at equilibrium (Ewens 1972). These tests are currently limited to sample sizes of 2000 genes or less and 1000 different alleles (haplotypes) or less.
  - Ewens-Watterson homozygosity test: This test, devised by Watterson (1978, 1986), is based on Ewens' sampling theory, but uses as a statistic the quantity F equal to the sum of squared allele frequencies, equivalent to the sample homozygosity in diploids (see section 8.1.6.1).
  - Exact test based on Ewens' sampling theory: In this test, devised by Slatkin (1994b, 1996), the probability of the observed sample is compared to that of a random neutral sample with same number of alleles and identical size. The probability of the sample selective neutrality is obtained as the proportion of random samples, which are less or equally probable than the observed sample.
  - **No. of simulated samples** [i]: Number of random samples to be generated for the two neutrality tests mentioned above. Values of several thousands are in order, and 16,000 permutations guarantee to have less than 1% difference with the exact probability in 99% of the cases (see Guo and Thomson 1992).
- **Chakraborty's test of population amalgamation** [b]: A test of selective neutrality and population homogeneity and equilibrium (Chakraborty, 1990). This test can be used when sample heterogeneity is suspected. It uses the observed homozygosity to estimate the population mutation parameter θ_{Hom}. The estimated value of this parameter is then used to compute the probability of observing k alleles or more in a neutral sample drawn from a stationary population. This test is based on Chakraborty's observation that the observed homozygosity is not very sensitive to population amalgamation or sample heterogeneity, whereas the number of observed (low frequency) alleles is more affected by this phenomenon.

Infinite site model

- **Tajima's D** [b]: This test described by Tajima (1989a, 1989b, 1993) compares two estimators of the population parameter θ, one being based on the number of segregating sites in the sample, and the other being based on the mean number of pairwise differences between haplotypes. Under the infinite-site model, both estimators should estimate the same quantity, but differences can arise under selection, population non-stationarity, or heterogeneity of mutation rates among sites (see section 8.1.6.4).

- **Fu's F_S** [b]: This test described by Fu (1997) is based on the probability of observing *k* or more alleles in a sample of a given size, conditioned on the observed average number of pairwise differences. The distribution of the statistic is obtained by simulating samples according to a given θ value taken as the average number of pairwise differences. This test has been shown to be especially sensitive to departure from population equilibrium as in case of a population expansion (see section 8.1.6.4).

- **Haplotype definition**

  The way haplotypes are defined is important here since some tests are based on the number of alleles in the samples, and therefore it is better to re-evaluate this quantity before doing these tests (Chakraborty's test, Ewens-Watterson, and Fu's Fs).

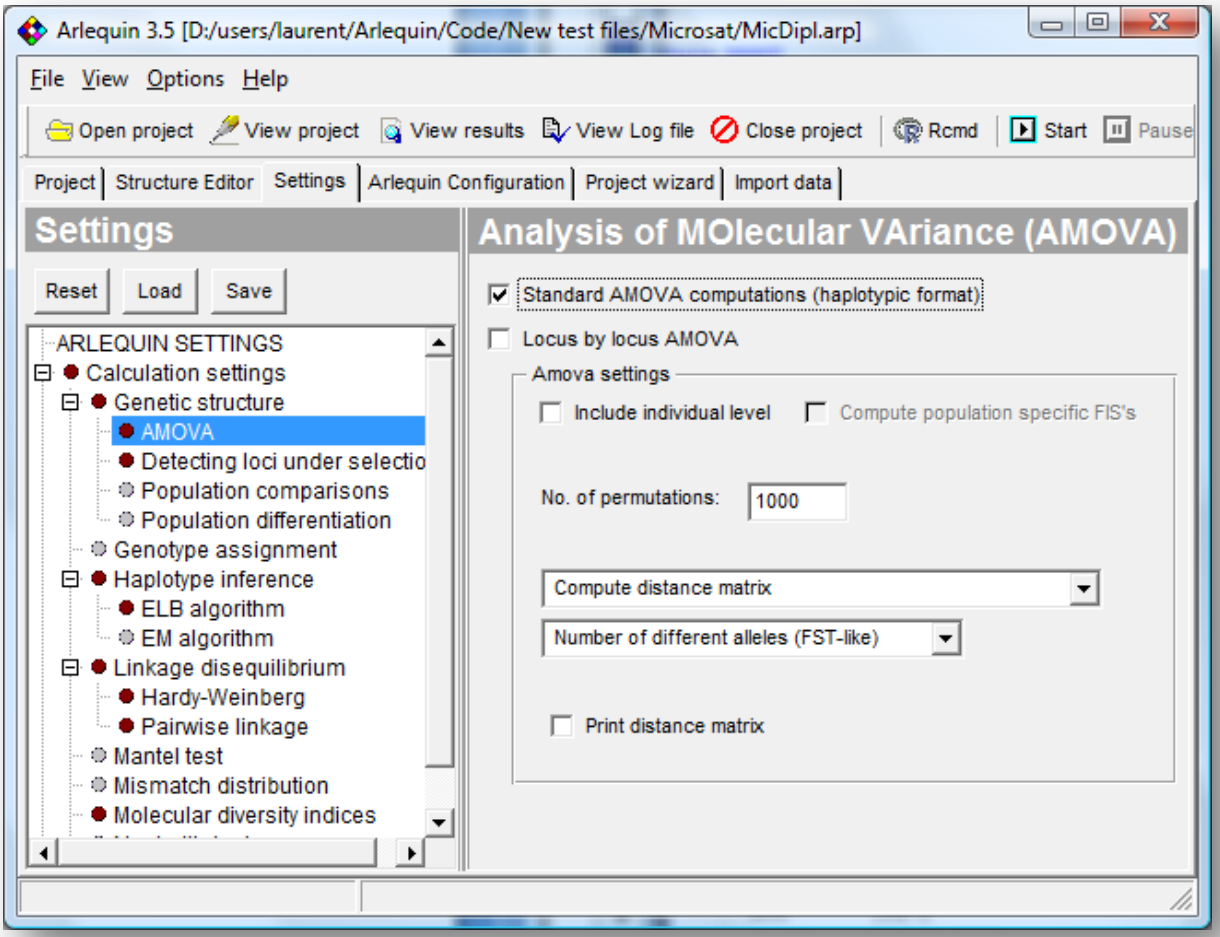
  - **Use original definition** [m]: Haplotypes are identified according to their original identifier, without considering the fact that their molecular definition could be identical.
  - **Infer from distance matrix** [m]

    Similar haplotypes will be identified by computing a distance matrix based on the settings chosen above. **When this option is activated, a search for shared haplotypes is automatically performed at the beginning of each run, and new haplotypes definitions and frequencies are computed for each population.**

6.3.8.7 Genetic structure

6.3.8.7.1 AMOVA

6.3.8.7.1.1 AMOVA with haplotypic data



- **Standard AMOVA** [b]: **A**nalysis of **MO**lecular **VA**riance framework and computation of a Minimum Spanning Network among haplotypes. Estimate genetic structure indices using information on the allelic content of haplotypes, as well as their frequencies (Excoffier et al. 1992). The information on the differences in allelic content between haplotypes is entered as a matrix of Euclidean squared distances. The significance of the covariance components associated with the different possible levels of genetic structure (within individuals, within populations, within groups of populations, among groups) is tested using non-parametric permutation procedures (Excoffier et al. 1992). The type of permutations is different for each covariance component (see section 8.2).
  The minimum spanning tree and network is computed among all haplotypes defined in the samples included in the genetic structure to test (see section 8.2.2).
  The number of hierarchical levels of the variance analysis and the kind of permutations that are done depend on the kind of data, the genetic structure that

is tested, and the options the user might choose. All details will be given in section 8.2.

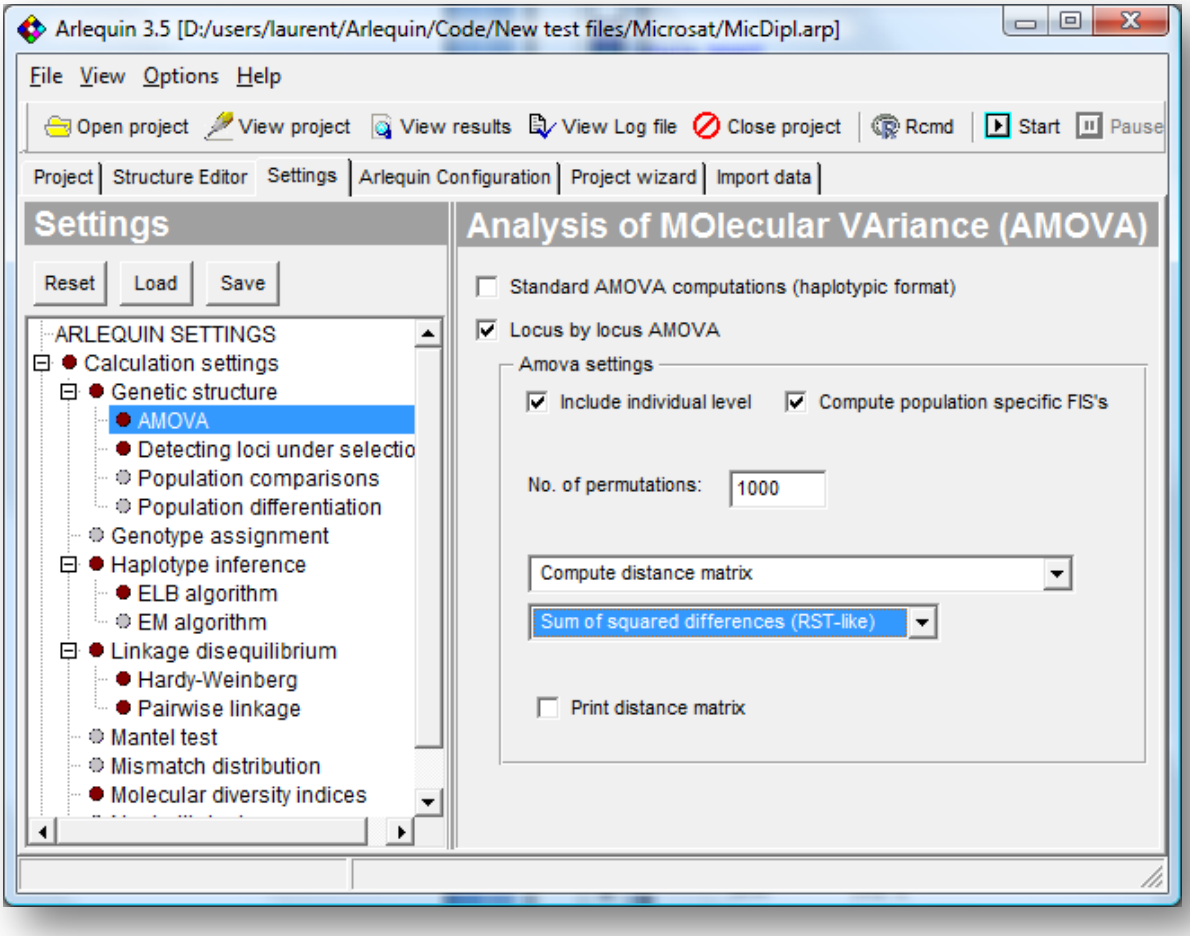
- **Locus by locus AMOVA** [b]: A separate AMOVA can be performed for each locus separately. For this purpose, we use the same number of permutations as in the global Amova. **This procedure should be favored when there is some missing data.** Note that diploid individuals that are found with missing data for one of their two alleles at a given locus are removed from the analysis for that locus.
  - **Compute Population Specific FST's** [b]: Population specific F_{ST} indices will be computed (as defined in section **Error! Reference source not found.**) for ll loci and for each locus separately if the Locus by locus AMOVA option is checked. Note that this option is only available if a single group is defined in the [[Structure]] section. No test of these coefficients is performed as they are only provided for exploratory purposes.
  - **No. of permutations** [i]: Enter the number of permutations used to test the significance of covariance components and fixation indices. A value of zero will not lead to any testing procedure. Values of several thousands are in order for a proper testing scheme, and 16 000 permutations guarantee to have less than 1% difference with the exact probability in 99% of the cases (Guo and Thomson 1992).
    The number of permutations used by the program might be slightly larger. This is the consequence of subdivision of the total number of permutation in batches for estimating the standard error of the *P*-value.
    Note that if several covariance components need to be tested, the probability of each covariance component will be estimated with this number of permutation. The distribution of the covariance components is output into a tabulated text file called *amo_hist.xl*, which can be directly read into MS-EXCEL .
  - **Compute Minimum Spanning Network (MSN) among haplotypes**. A Minimum Spanning Tree and a Minimum Spanning Network are computed from the distance matrix used to perform the AMOVA calculations.
  - **Choice of Euclidian square distances** [m]:
    - **Use project distance matrix** [m]**:** Use the distance matrix defined in the project file (if available)
    - **Compute distance matrix** [m]**:** Compute a given distance matrix based on a method defined below. With this setting selected, the distance matrix potentially defined in the project file will be ignored. This matrix can be

generated either for haplotypic data or genotypic data (Michalakis and Excoffier, 1996)

- **Use conventional F-statistics** [m]**:** With this setting activated, we will use a lower diagonal distance matrix, with zeroes on the diagonal and ones as off-diagonal elements. It means that all distances between non-identical haplotypes will be considered as identical, implying that one will bas the analysis of genetic structure only on allele frequencies.

- **Distance between haplotypes** [m]**:** Select a distance method to compute the distances between haplotypes. Different square Euclidean distances can be used depending on the type of data analyzed.
  - **Gamma *a* value** [f]: Set the value for the shape parameter α of the gamma function, when selecting a distance allowing for unequal mutation rates among sites. See the Molecular diversity section 6.3.8.2.
- **Print distance matrix** [b]**:** If checked, the inter-haplotypic distance matrix used to evaluate the molecular diversity is printed in the result file.

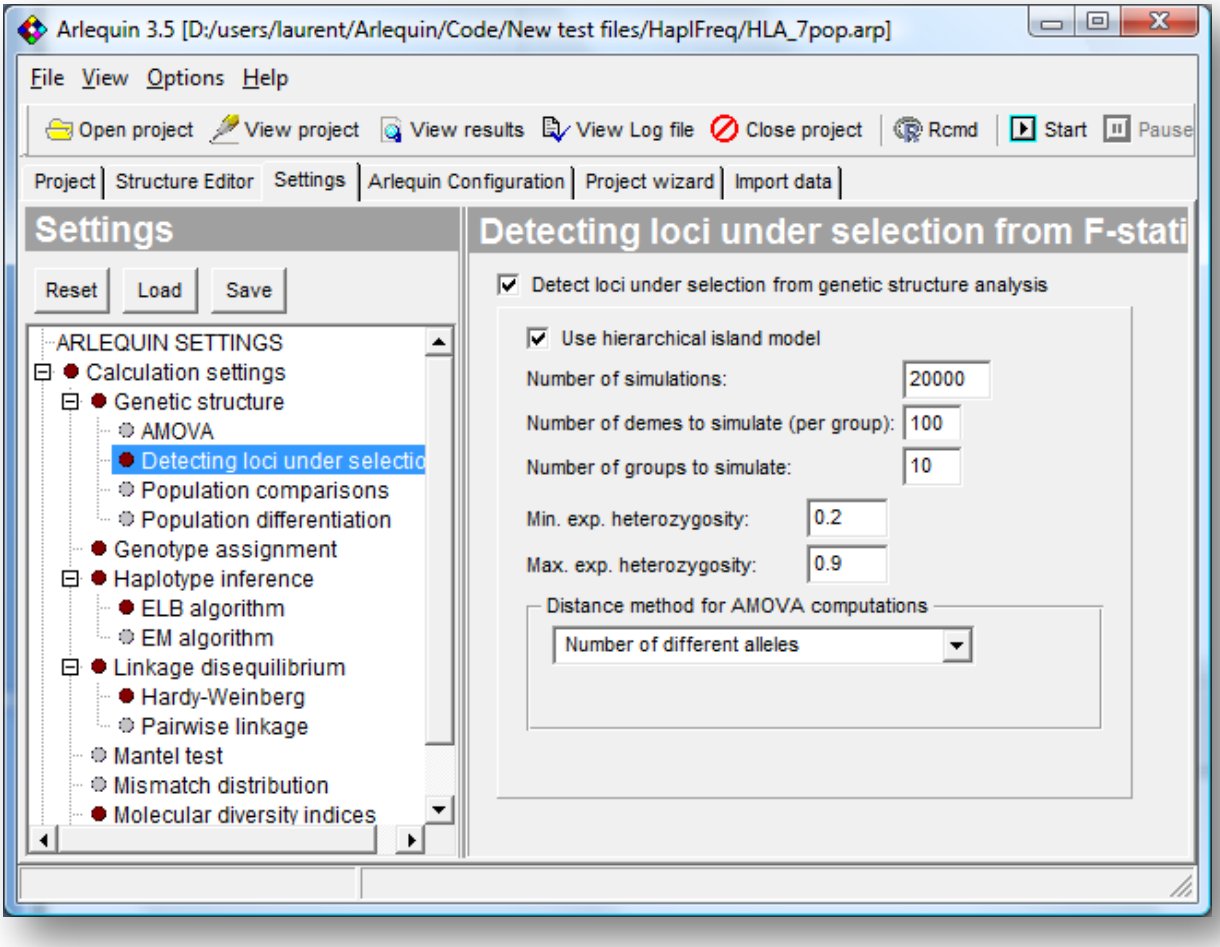
6.3.8.7.1.2 AMOVA with genotypic data



Compared to haplotypic data, it becomes possible to compute the average inbreeding coefficient F_{IS} with diploid genotypic data.

- **Include individual level for genotype data** [b]: Include the intra-individual covariance component of genetic diversity, and its associated inbreeding coefficients (F_{IS} and F_{IT}). It thus takes into account the differences between genes found within individuals. This is another way to test for global departure from Hardy-Weinberg equilibrium.
- **Compute population specific FIS's** [b]: Compute inbreeding coefficients (F_{IS}) separately for each population and test it by permutation of gene copies between individuals within population. The checkbox *Include individual level* must be checked to enable this option.

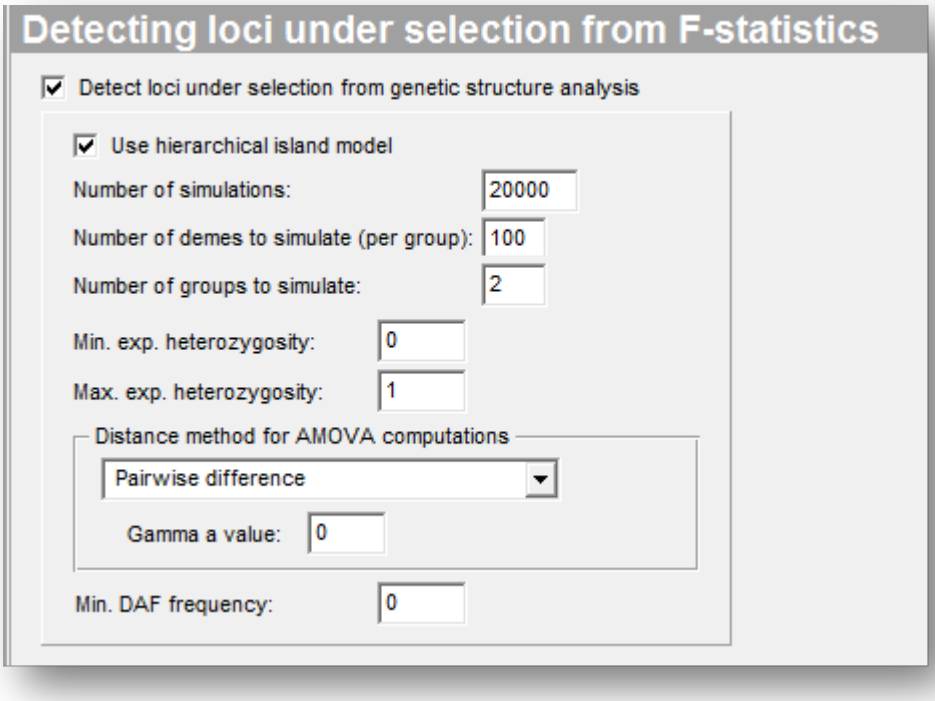
6.3.8.7.2 Detection of loci under selection



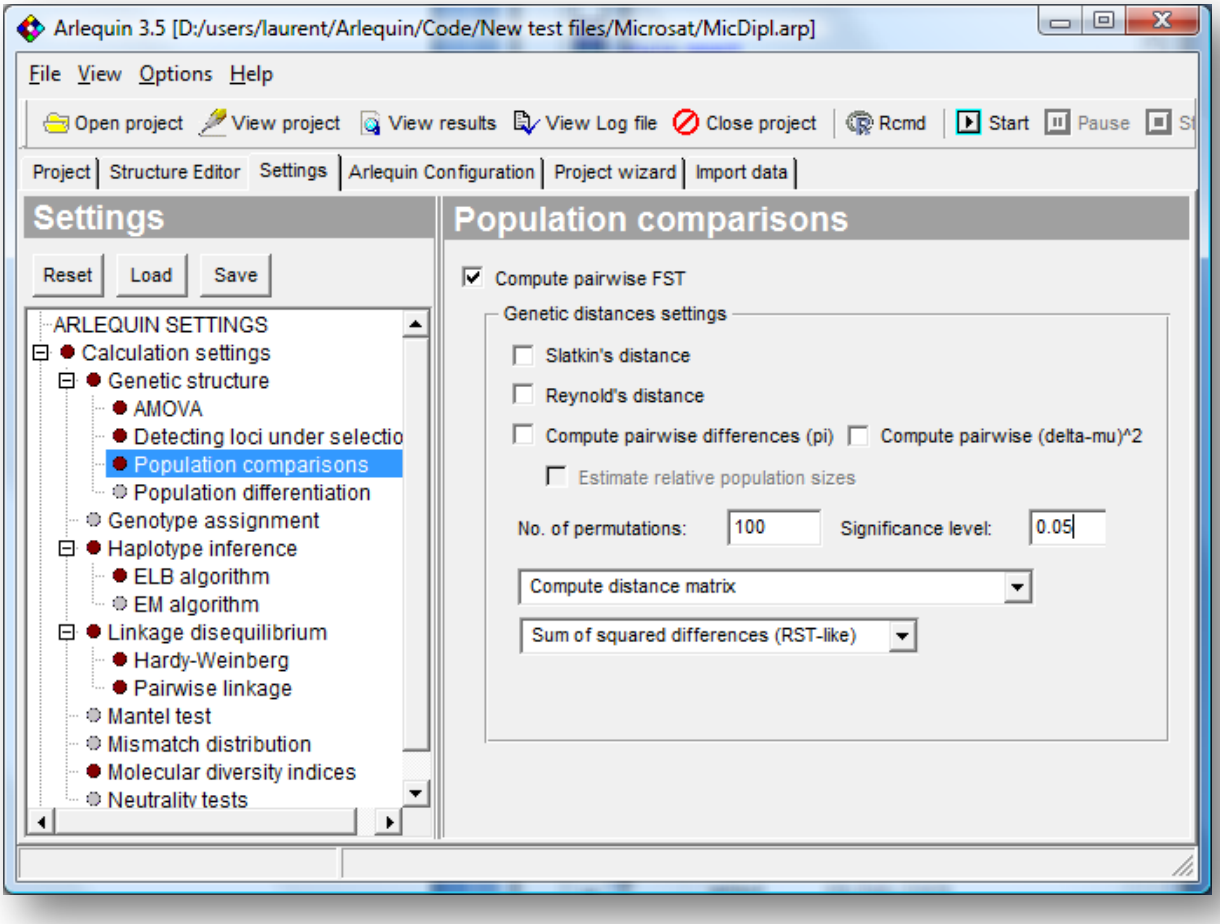
- **Detecting loci under selection from genetic structure analysis** [b]: Uses coalescent simulations to get the p-values of locus-specific *F*-statistics conditioned on observed levels of heterozygosities. See Excoffier et al. (2009) and section 8.2.8 for methodological details about these computations.

- **Use hierarchical island model** [b]: If checked, Arlequin uses a hierarchical island model (Slatkin and Voelm, 1991) to perform coalescent simulations leading to the joint null distributions of hierarchical F-statistics (F_{SC}, F_{CT}, and F_{ST}) and heterozygosities, from which locus-specific p-values are estimated. The used hierarchical population structure is that defined in the [Structure] section of your Arlequin input file. Note that a hierarchical structure canonly be used if more than one group is defined in Arlequin genetic structure. If unchecked, a non-hierarchical finite island model is used instead. In that case, all populations found in different groups of the [Structure] section are pooled into a single group for this analysis. Populations present in the Arlequin input file that are not listed as belonging to a group in the [Structure] section are discarded from the analysis.
    - **Number of simulations:** [i] Number of coalescent simulations to perform to get the null distribution of F-statistics. Some large number is expected (10,000-50,000) to get correct estimates.
    - **Number of demes to simulate:** [i] Number of simulated demes per group. If no hierarchical structure is assumed, this is the total number of simulated demes. A value of 100 is adequate in most situations.
    - **Number of groups to simulate:** [i] Number of simulated groups in the hierarchical genetic structure. This number should be equal or larger to the number of groups defined in the Arlequin genetic structure. If this number is smaller than the number of defined groups, then simulations are done with the number of groups in the Arlequin structure. A value larger than the number of groups in the structure is in order, as this does not have too much influence on the resulting p-values (see Excoffier et al. 2009).
    - **Min. exp. Heterozygosity**: [f] Minimum value of simulated target heterozygosity. This setting combined with the next one can be useful to make simulations around the heterozygosities of the tested loci. This is because the locus specific p-values are computed by using simulations that have matching heterozygosities. It is therefore useless to simulate very low heterozygosities if all tested loci have high heterozygosities, because these simulations won't be used to compute p-values.
    - **Max. exp. Heterozygosity**: [f] maximum value of simulated target heterozygosity. See above for an explanation of the usefulness of thiss setting.

- **Distance method for AMOVA computations**: [m] Select a distance method to compute the distances between haplotypes. Different squared Euclidean distances can be used depending on the type of data analyzed. See sections 0 for DNA, 8.1.2.6 for RFLP, 8.1.2.7 for microsatellite, and 8.1.2.8 for standard data.
- **Min DAF frequency**: [f] For DNA sequence only. One can specify a minimum value for the simulated Derived Allele Frequencies (DAF) of individual SNPs that are simulated. It can be useful if all observed SNPs have some minimum frequency, e.g. due to some ascertainment bias procedure.



6.3.8.7.3 Population comparison



- **Compute pairwise F_{ST}** [b]: Computes pairwise F_{ST} 's for all pairs of populations, as well as different indexes of dissimilarities (genetic distances) between pairs of populations, like transformed pairwise F_{ST} 's that can be used as short term genetic distances between populations (Reynolds et al. 1983; Slatkin, 1995), but also Nei's mean number of pairwise differences within and between pairs of populations.

  The significance of the genetic distances is tested by permuting the haplotypes or individuals between the populations. See section **8.2.3** for more details on the output results (genetic distances and migration rates estimates between populations).

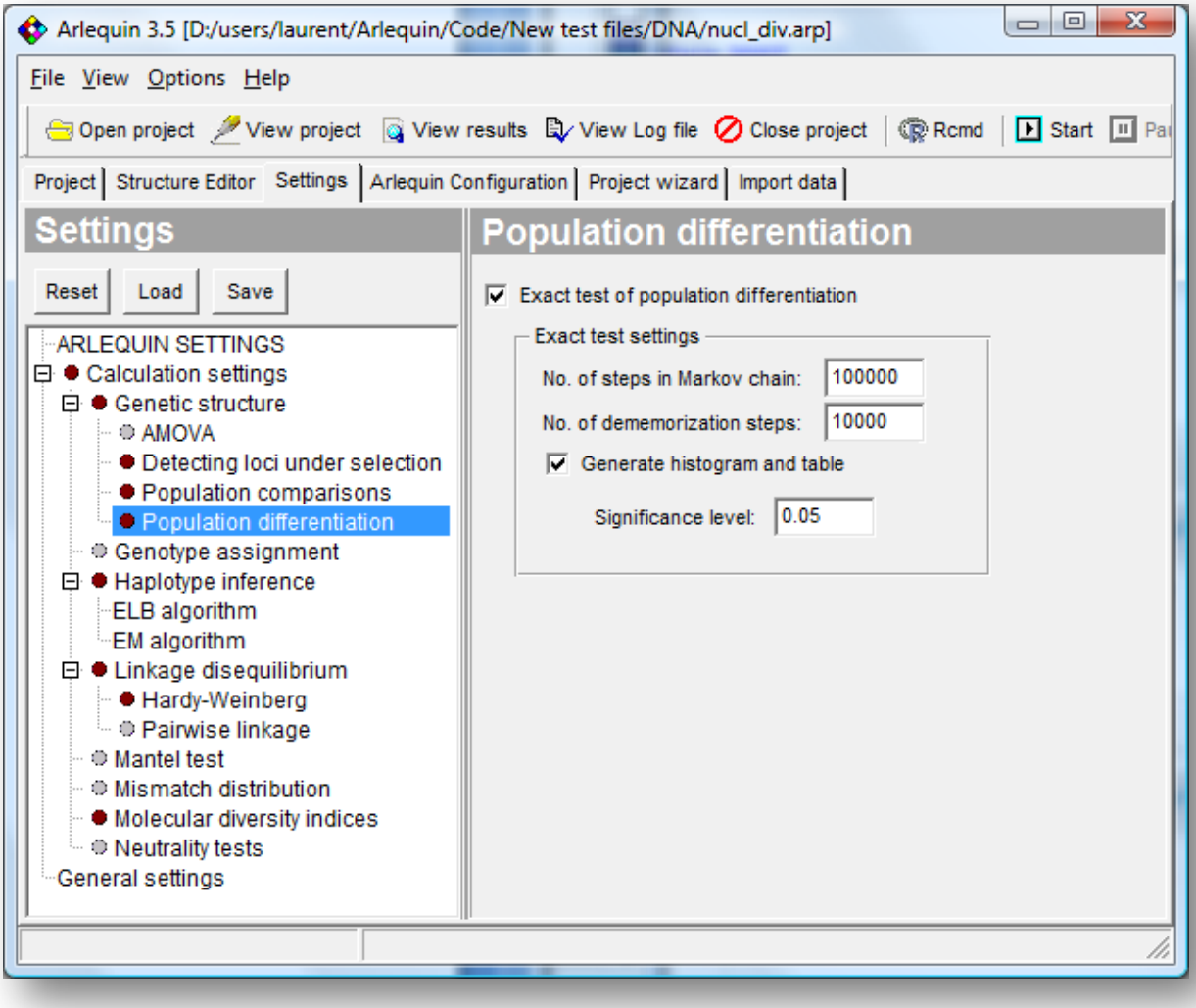
  - **Slatkin's distance** [b]: Computes Slatkin's (1995) genetic distance derived from pairwise F_{ST} (see section 8.2.4.2).
  - **Reynolds's distance** [b]: Computes Reynolds' et al. (1983) linearized F_{ST} for short divergence time (see section 8.2.4.1).

- **Compute pairwise differences** [b]: Computes Nei's average number of pairwise differences within and between populations (Nei and Li, 1979) (see section 8.2.4.4)
    - **Estimate relative population sizes** [b]: Computes relative population sizes for al pairs of populations, as well as divergence times between populations taking into account these potential differences between population sizes (Gaggiotti and Excoffier 2000) (see section 8.2.4.5).
- **Compute pairwise** $\delta\mu^2$: [b] For microsatellite data only. Computes a genetic distance between all pairs of populations that should be linearly related to divergence time (see section 8.2.4.5)
- **No. of permutations** [i]: Enter the required number of permutations to test the significance of the derived genetic distances.. If this number is set to zero, no testing procedure will be performed. Note that this procedure is quite time consuming when the number of populations is large.
- **Significance level** [f]: The level at which the test of differentiation is considered significant for the output table. If the *P*-value is smaller than the *Significance level*, then the two populations are considered as significantly different.

Choice of Euclidian distance [m]: Select a distance method to compute the distances between haplotypes. Different square Euclidean distances can be used depending on the type of data analyzed.

- **Use project distance matrix** [m]**:** Use the distance matrix defined in the project file (if available)
- **Compute distance matrix** [m]**:** Compute a given distance matrix based on a method defined below. With this setting selected, the distance matrix potentially defined in the project file will be ignored. This matrix can be generated either for haplotypic data or genotypic data (Michalakis and Excoffier, 1996).
    - **Gamma *a* value** [f]: Set the value for the shape parameter a of the gamma function, when selecting a distance allowing for unequal mutation rates among sites. See the Molecular diversity section 0. This parameter only applies to DNA data.
- **Use conventional F-statistics** [m]**:** With this setting activated, we will use a lower diagonal distance matrix, with zeroes on the diagonal and ones as off-diagonal elements. It means that all distances between non-identical haplotypes will be considered as identical, implying that one will bas the analysis of genetic structure only on allele frequencies.

6.3.8.7.4 Population differentiation

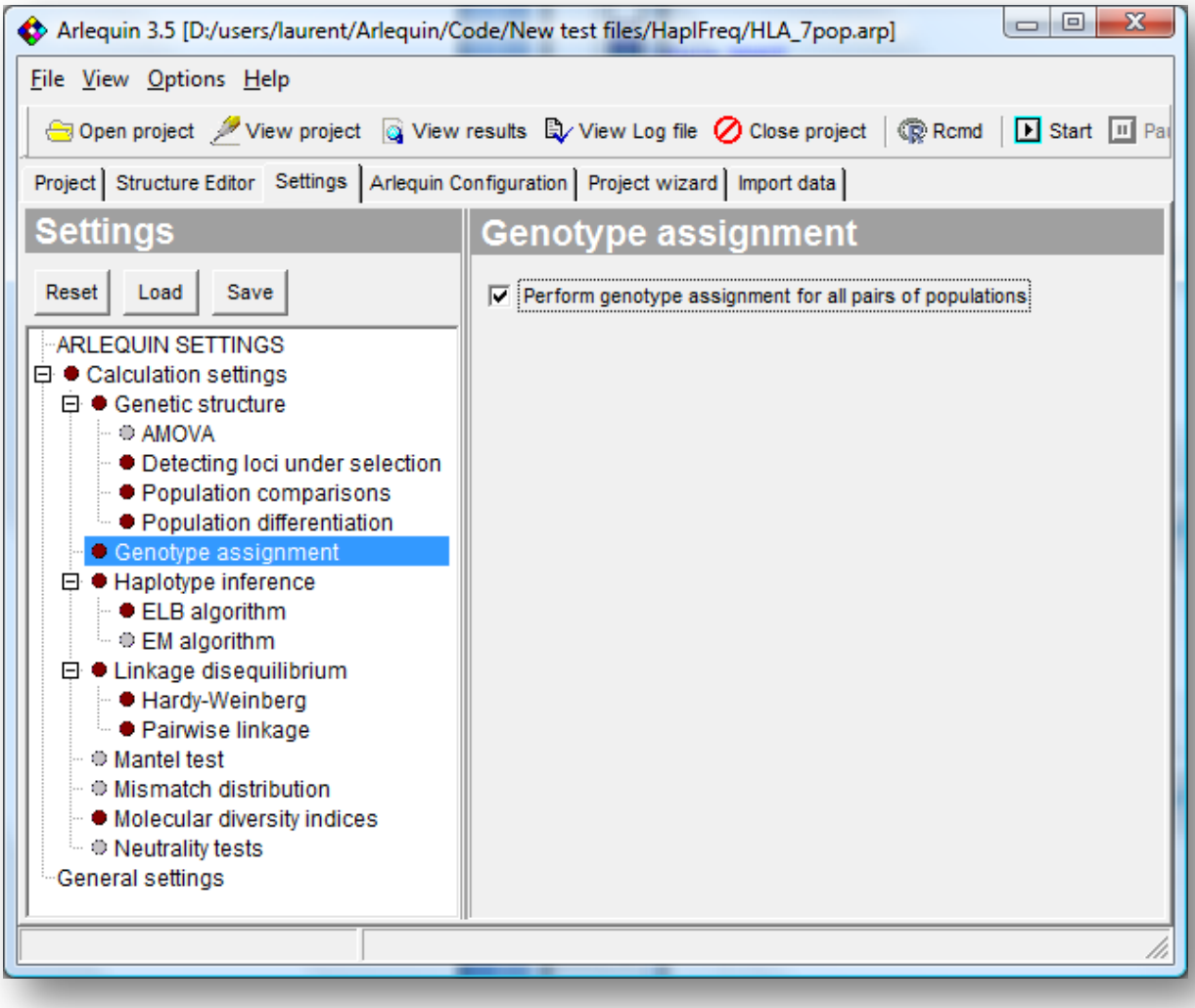


- **Exact test of population differentiation** [b]: We test the hypothesis of random distribution of the individuals between pairs of populations as described in Raymond and Rousset (1995) and Goudet et al. (1996). This test is analogous to Fisher's exact test on a two-by-two contingency table, but extended to a contingency table of size two by (no. of haplotypes). We do also an exact differentiation test for all populations defined in the project by constructing a table of size (no. of populations) by (no. of haplotypes). (Raymond and Rousset, 1995).
  - **No. of steps in Markov chain** [i]: The maximum number of alternative tables to explore. Figures of 100,000 or more are in order. Larger values of the step number increases the precision of the *P*-value as well as its estimated standard deviation.
  - **No. of dememorisation steps** [i]: The number of steps to perform before beginning to compare the alternative table probabilities to that of the observed

table. Corresponds to a burnin. A few thousands steps are necessary to reach a random starting point corresponding to a table independent from the observed table.

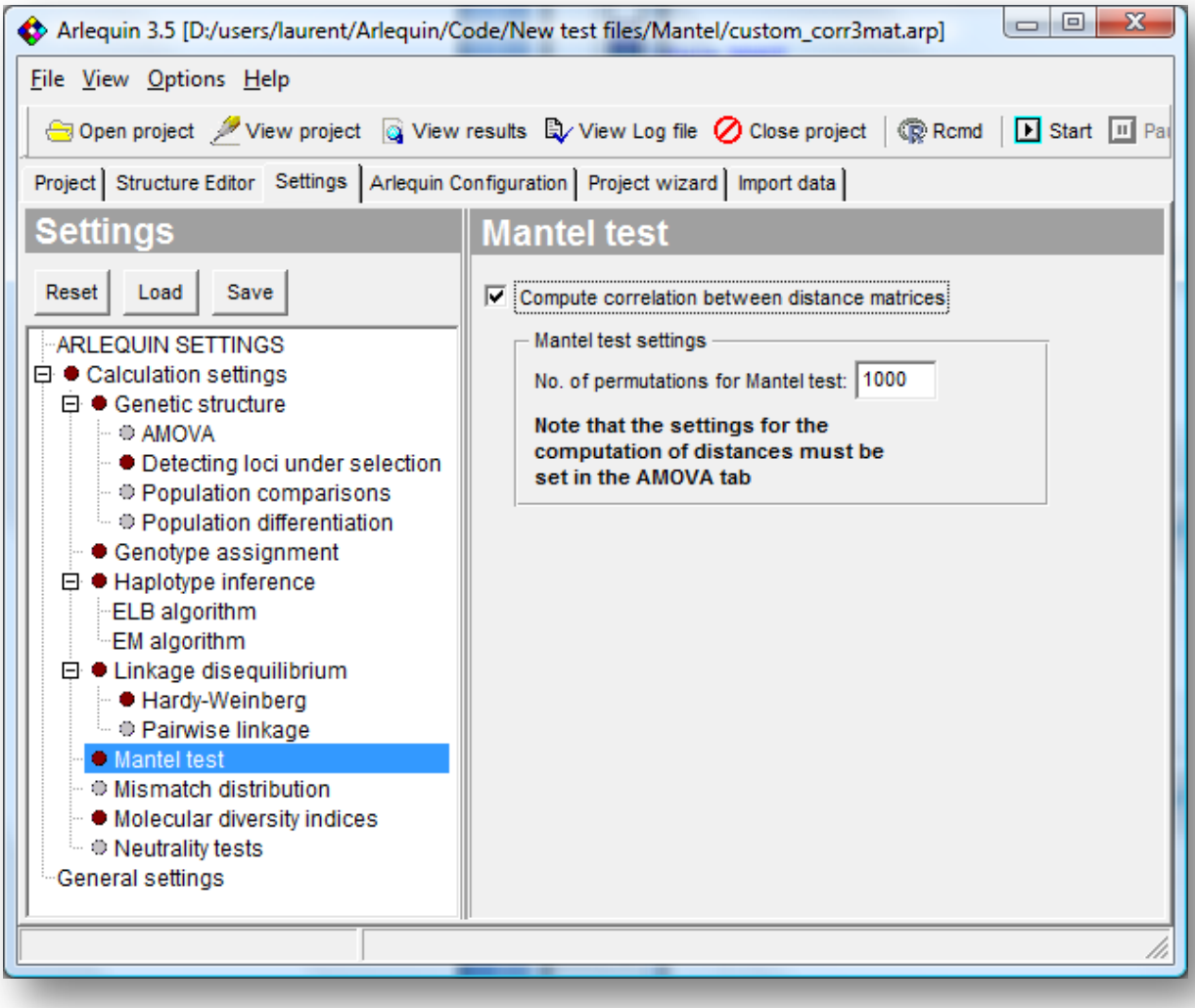
- **Generate histogram and table** [b]: Generates a histogram of the number of populations which are significantly different from a given population, and a $P \times P$ table (P being the number of populations) summarizing the significant associations between pairs of populations. An association between two populations is considered as significant or not depending on the significance level specified below.
- **Significance level** [f]: The level at which the test of differentiation is considered significant for the output table. If the P-value is smaller than the *Significance level*, then the two populations are considered as significantly different.

6.3.8.8 Genotype assignment



- **Perform genotype assignment for all pairs of populations**: Computes the log likelihood of the genotype of each individual in every sample, as if it was drawn from a population sample having allele frequencies equal to those estimated for each sample (Paetkau et al. 1997; Waser and Strobeck, 1998). Multi-locus genotype likelihoods are computed as the product of each locus likelihood, thus assuming that the loci are independent. The output result file lists, for each population, a table of the log-likelihood of each individual genotype in all populations (see section 8.2.6).

6.3.8.9 Mantel test



- **Compute correlation between distance matrices**: Test the correlation or the partial correlations between 2 or 3 matrices by a permutation procedure (Mantel, 1967; Smouse et al. 1986).
  - **Number of permutations**: Sets the number of permutations for the Mantel test

7 OUTPUT FILES

The result files are all output in a special sub-directory, having the same name as your project, but with the "*.res*" extension. This has been done to structure your result files according to different projects. For instance, if your project file is called *my_file.arp*, then the result files will be in a sub-directory called [*my_file.res*]

7.1 Result file

The file containing all the results of the analyses just performed. You can choose to have results shown in an html file, as with previous versions (before 3.5), or by outputting results into an xml file (see options in section 6.1.3). By default, it has these result files have the same name than the Arlequin input file, with the extension *.htm* or *.xml*. The result file is opened in the right frame of the html browser at the end of each run.
If the option *Append Results* of the *Configuration Arlequin* tab is checked, the results of the current computations are appended to those of previous calculations, otherwise the results of previous analyses are erased, and only the last results are output in the result file.

7.2 Arlequin log file

A file where run-time *WARNINGS* and *ERRORS* encountered during any phases of the current Arlequin session are issued. The file has the name *Arlequin_log.txt* and **is located in the result directory of the opened project.** You should consult this file if you observe any warning or error message in your result file. If Arlequin has crashed then consult *Arlequin_log.txt* **before** running Arlequin again. It will probably help you in finding where the problem was located. A reference to the log file is provided in the left pane of the html result file and can be activated in your web browser. The log file of the current project can also be viewed by pressing on the *View Log File* button on the Toolbar

7.3 Linkage disequilibrium result file

This file contains the results of pairwise linkage disequilibrium tests between all pairs of loci. By default, it has the name *LD_DIS.XL*. As suggested by its extension, this file can be read with MS-Excel without modification. The format of the file is tab separated.

7.4 Allele frequencies

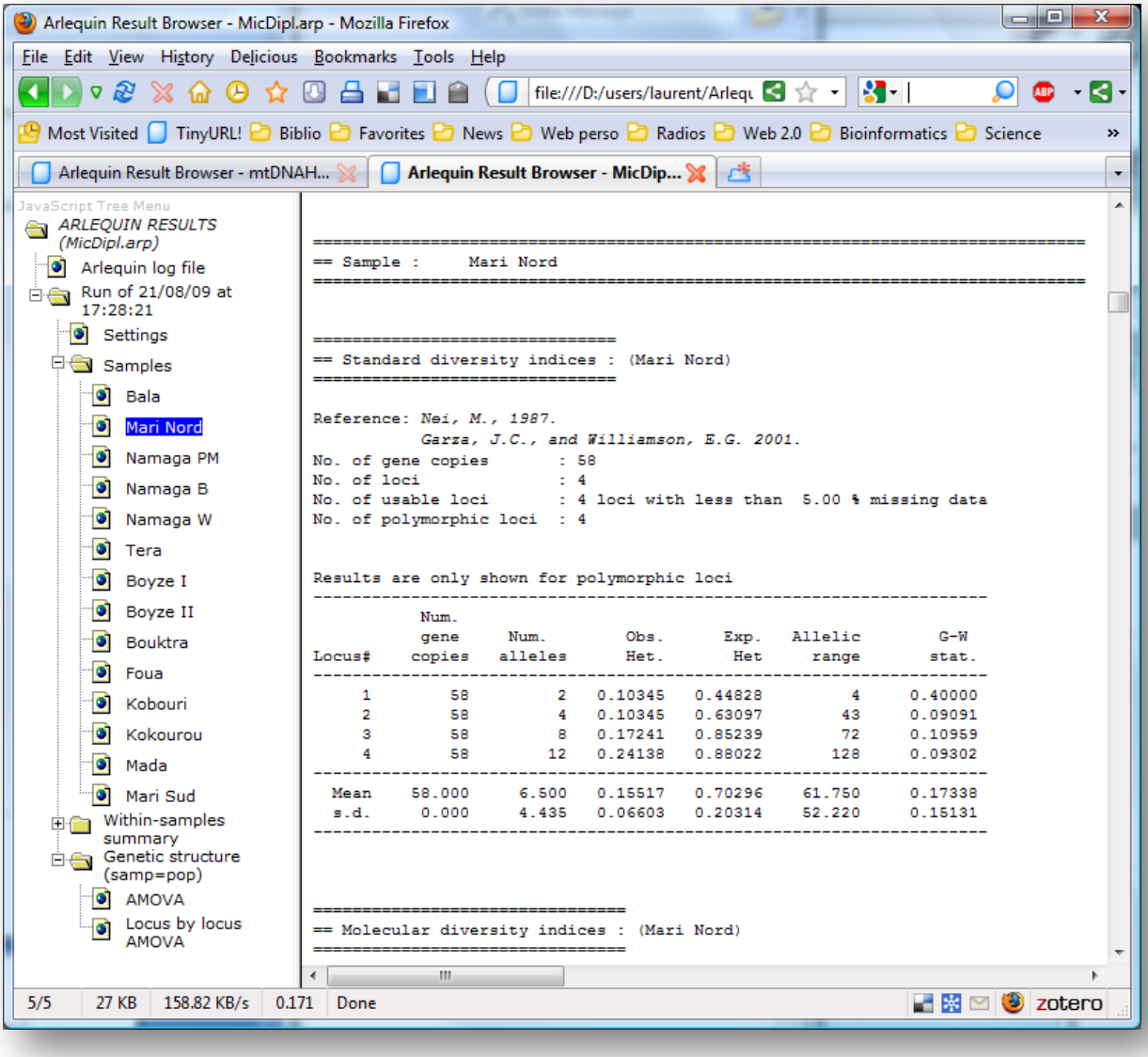
By checking the option "Output sample allele frequencies for all loci" in the *Molecular diversity indices* tab, it is possible to output allele frequencies at all loci for all populations in a series of files called "AllFreqLocus_XXX.txt", where XXX is the locus number. On each

row, the frequencies of an allele are listed for all sampled populations. The names of the populations are listed in a separate file, called "PopNames.txt".

7.5 View results in your HTML browser

For very large result files or result files containing the product of several analyses, it may be of practical interest to view the results in an HTML browser. This can be simply done by activating the button *Browse results* of the project tab panel, which will then load the result files into your default web browser.

If the XML output is inactivated, Arlequin will produce conventional html file, looking like this in your web browser:



1) The **left pane** contains a tree where each first level branch corresponds to a run. For each run we have several entries corresponding to the settings used for the calculation, the inter-population analyses (Genetic structure, shared haplotypes, etc…) and finally all intra-population analyses with one entry per population sample.

The description of this tree is stored in *[project name]_tree.html*. At this point it is important to notice that this tree uses the java script files *ftiens4.js* and *ua.js* located in Arlequin's installation directory. If you move Arlequin to another location, or uninstall Arlequin, the left pane will not work anymore.

2) The **right pane** shows the results concerning the selected item in the left pane. The HTML code of this pane is in the main result file. This file is located in result sub-directory of your project and is named *[project name].htm or [project name].xml*, depending on your choice of output in the *Option* menu (see section 6.1.3).

7.6 XML output file

In Arlequin ver 3.5, the user has the choice to produce result files in conventional html file or in the Extensible Markup Language (http://www.w3.org/XML/), by checking the *XML Output* menu in the Options menu. Output files include additional formatting options in the xml versions, as well as the possibility to extract data from tables and use it to produce graphics that are integrated directly into the xml file.

7.6.1 Potential XML formatting problem with Firefox ver 3.x

In Firefox ver 3.x, your xml may appear as unformatted, which is because the XSLT style sheet is outside the current XML file's path.

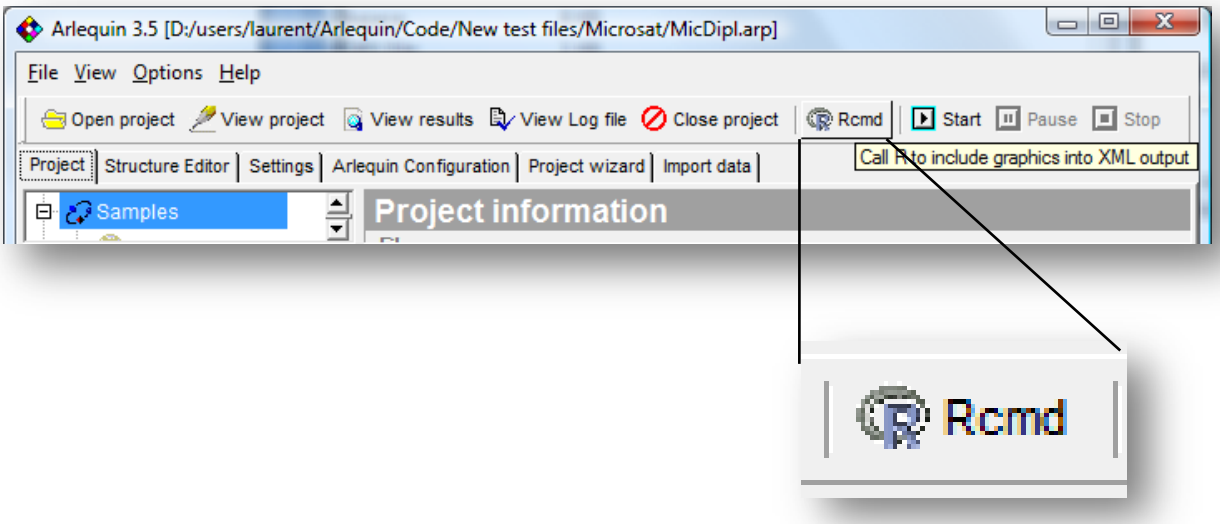
A workaround is to edit Firefox settings as follows:

1) Type **about:config** in Firefox address bar

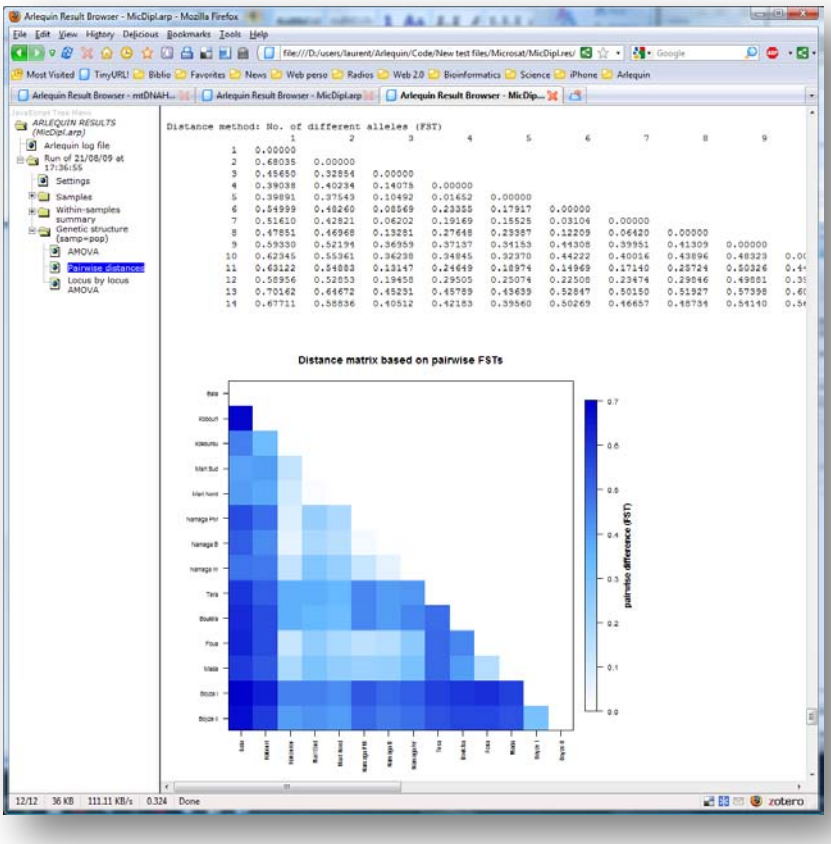
2) Change **security.fileuri.strict_origin_policy** to **false**

7.6.2 Include graphics into the xml output file

If results have been generated in an XML result file, it is then possible to create graphs from specific tables found in the xml output file. Graphics are generated automatically by a series of R scripts triggered by the **Rcmd** button on the toolbar (see also section 6.2).



The **Rcmd** command is active if the path to the Rcmd program has been specified in the *Arlequin configuration* tab (see section 6.3.3), implying that the R package has been installed on your computer. This package can be freely downloaded from http://www.r-project.org/. Note again that html outputs cannot be used to produce graphs.
For instance, a graph conveniently representing **pairwise FSTs between populations** has been added below the FST distance matrix in the xml result file.



7.6.3 Why use R to make graphs?

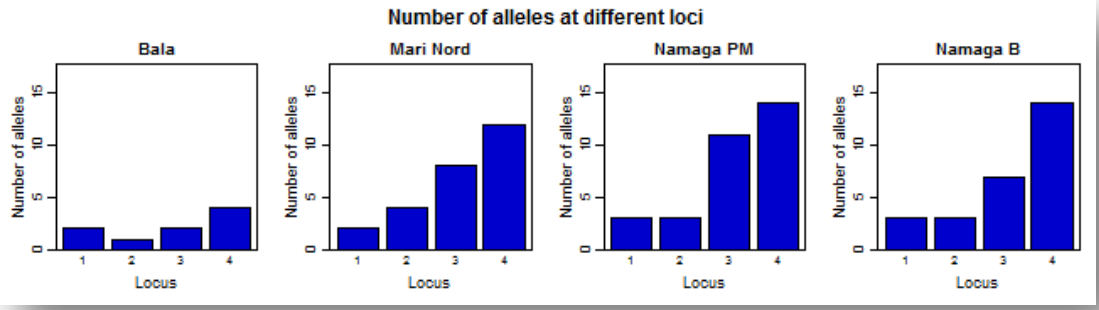
R is a language and environment for statistical computing and graphics production. It provides a wide variety of statistical and graphical techniques and is highly extensible. R is available as a free package and it compiles and runs on Linux, Windows and MacOS X. Therefore R functions are portable under many computer systems. R also provides very powerful graphic facilities for the production of many different diagrams and plots.
R also includes an XML packages, which contains tools for parsing and generating XML within R. These tools allow one to get an R structure representing the XML file, to access tags of interest with R and to get their attribute values and their containing data. It also allows one to manipulate the XML structure, e.g. to add additional tags or attributes. After this manipulation it is possible to save the R structure back into the xml file.
The possibility to parse XML files, the ability to produce complex graphics and the portability of R make it an ideal tool to parse the XML output of ARLEQUIN and to generate graphics based on the extracted data.

7.6.4 Example of R-lequin graphical outputs

We report below some examples of the graphs produced by R-lequin for different summary statistics, and the name of the R function used to produce it. These R functions can be found in the *Rfunctions* directory located at the root of the Arlequin directory. Users can modify them to customize their graphs at will. We also report for each graph, the XML tag surrounding the data used to generate these graphs, as well as which Arlequin computations produce the results used to generate these graphs.

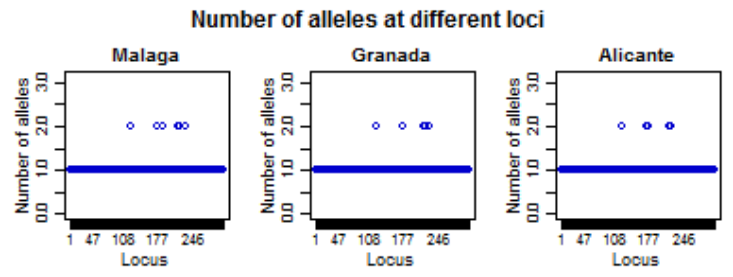
7.6.4.1 Genetic diversity

7.6.4.1.1 Number of alleles per locus

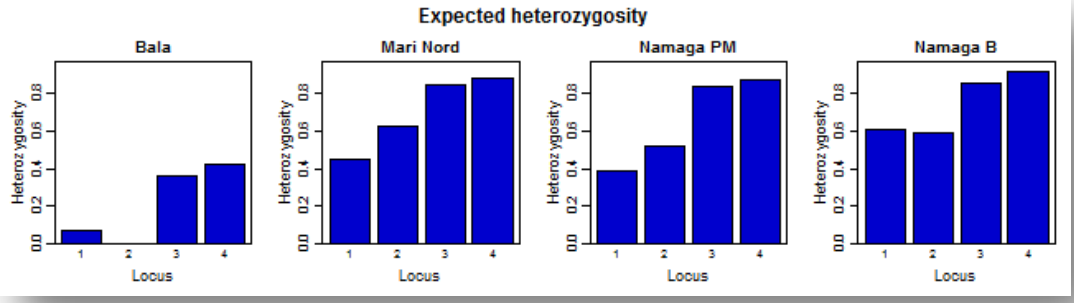


XML tag in output file: sumNumAlleles
R-function: sumNumAllelesFunction.r
Arlequin computation: Standard diversity indices (see section 6.3.8.2).

When the number of loci is large, we use another representation, like below for DNA sequences:



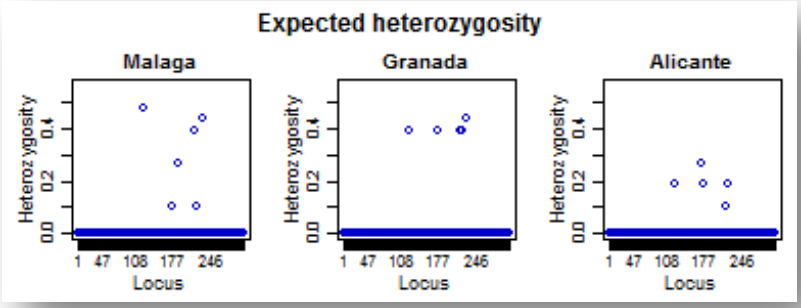
7.6.4.1.2 Expected heterozygosity



XML tag in output file: sumExpHeterozygosity

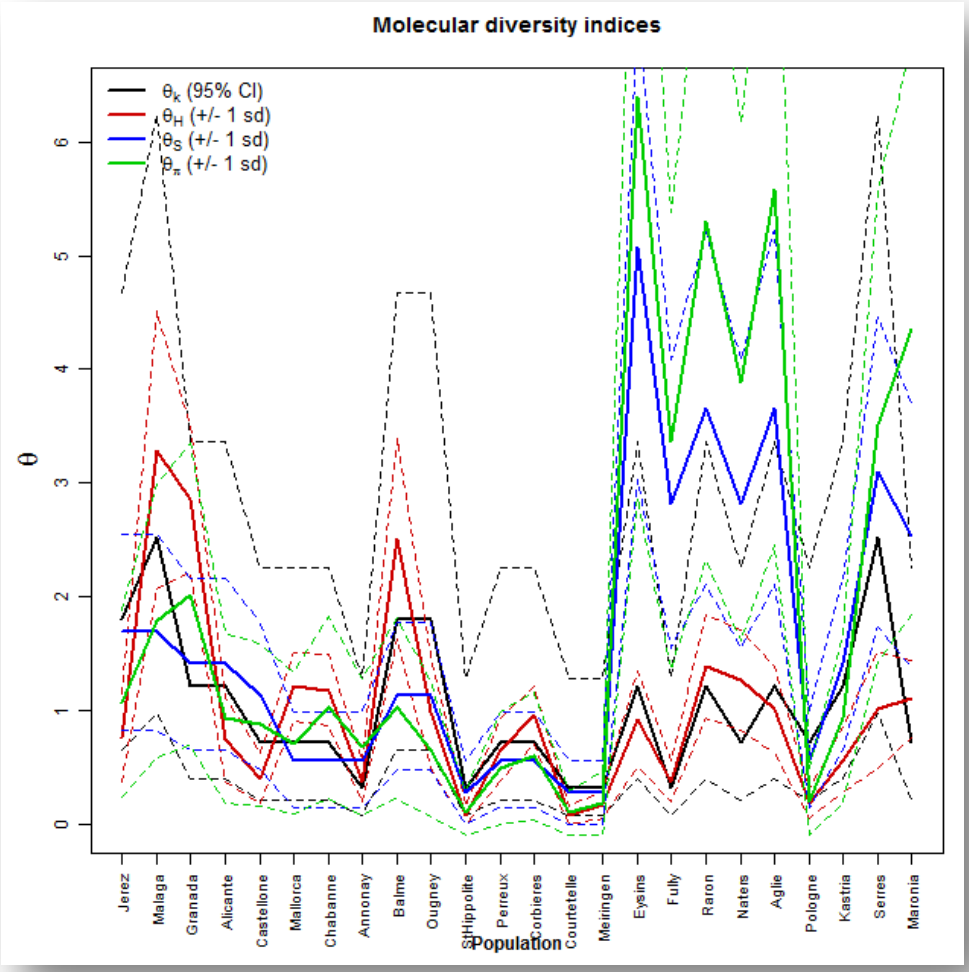
R-function: sumExpectedHeterozygosity.r
Arlequin computation: Standard diversity indices (see section 6.3.8.2).

Again, when the number of loci is large, we use another representation, like below for DNA sequences:



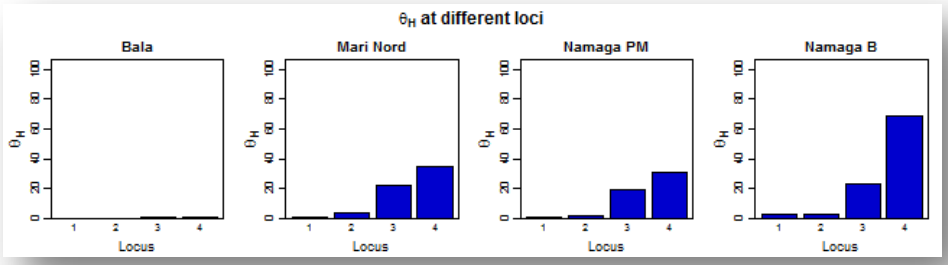
7.6.4.1.3 Theta values

We plot theta values estimated from different aspects of the data for all populations. Note that the comparison of different theta values is the basis of several neutrality tests, like that based on Tajima's D (see section 8.1.6.4).



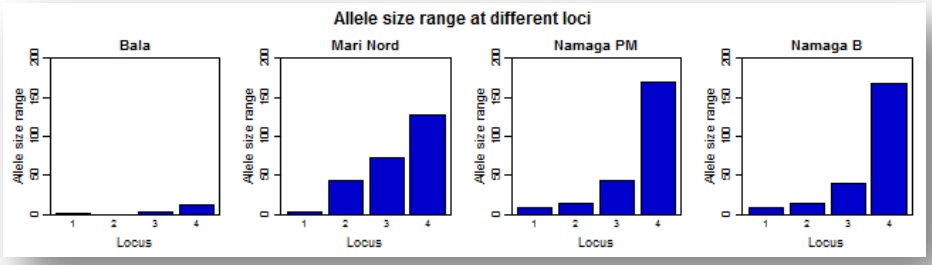
XML tag in output file: sumMolecDivIndexes
R-function: sumMolecularDivIndexes.r
Arlequin computation: Molecular distance (see section 6.3.8.2).

7.6.4.1.4 Theta (H) for microsatellite data



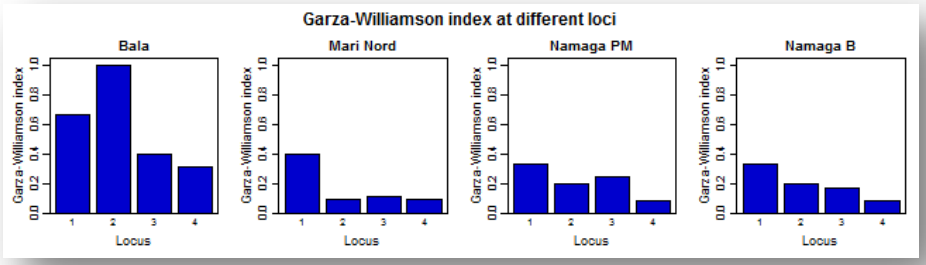
XML tag in output file: sumThetaH
R-function: sumThetaHFunction.r
Arlequin computation: Molecular distance (see section 6.3.8.2).

7.6.4.1.5 Allele size range at different loci (microsatellite data)



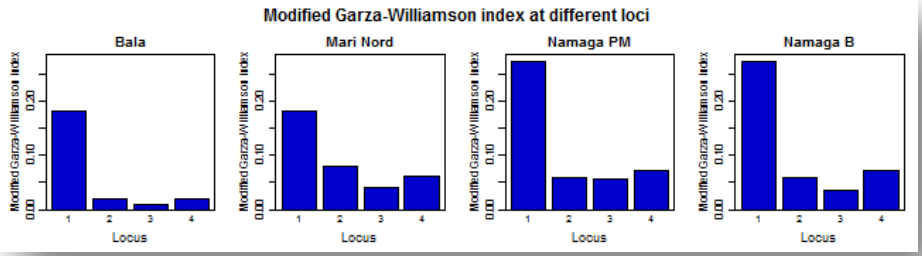
XML tag in output file: sumAllelicSizeRange
R-function: sumAllelicSizeRangeFunction.r
Arlequin computation: Molecular distance (see section 6.3.8.2).

7.6.4.1.6 Garza-Williamson index (microsatellite data)



XML tag in output file: sumGWIndex
R-function: sumGWIndexFunction.r
Arlequin computation: Molecular distance (see section 6.3.8.2).

7.6.4.1.7 Modified Garza-Williamson index (microsatellite data)

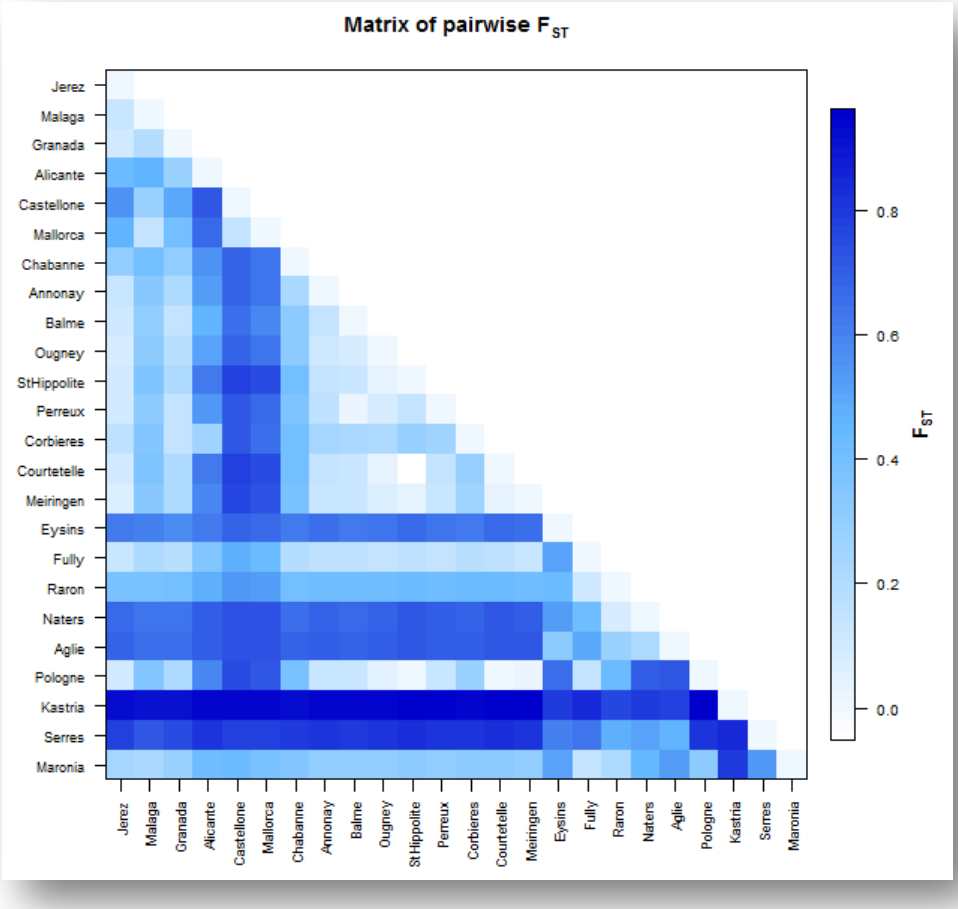


XML tag in output file: sumModGWIndex
R-function: sumModGWIndexFunction.r
Arlequin computation: Molecular distance (see section 6.3.8.2).

7.6.4.2 Genetic distances between populations

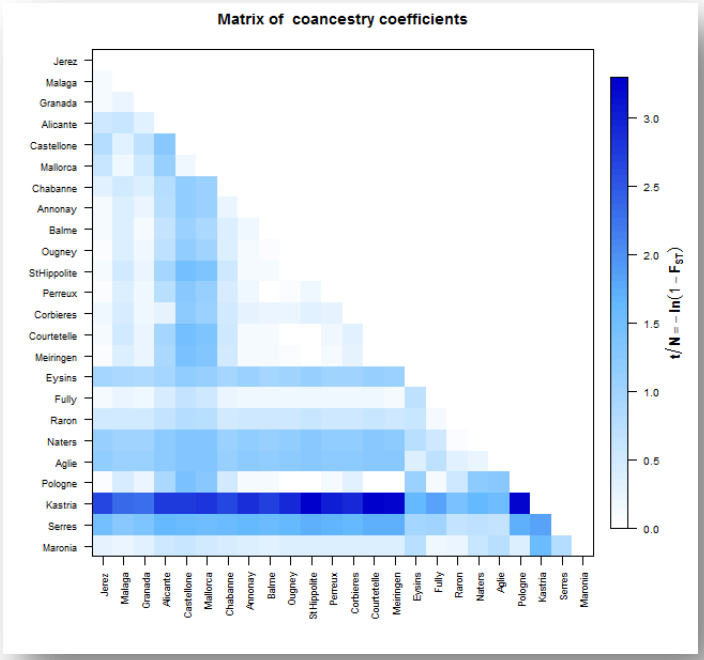
7.6.4.2.1 Matrix of pairwise F_{ST}'s

We make a simple graphic representation of the population relationships as described by F_{ST} computed between pairs of populations. As an example, we show pairwise distances between *Myotis myotis* bat populations from Europe as inferred from mtDNA control region. This representation allows one to quickly perceive genetic affinities between populations.



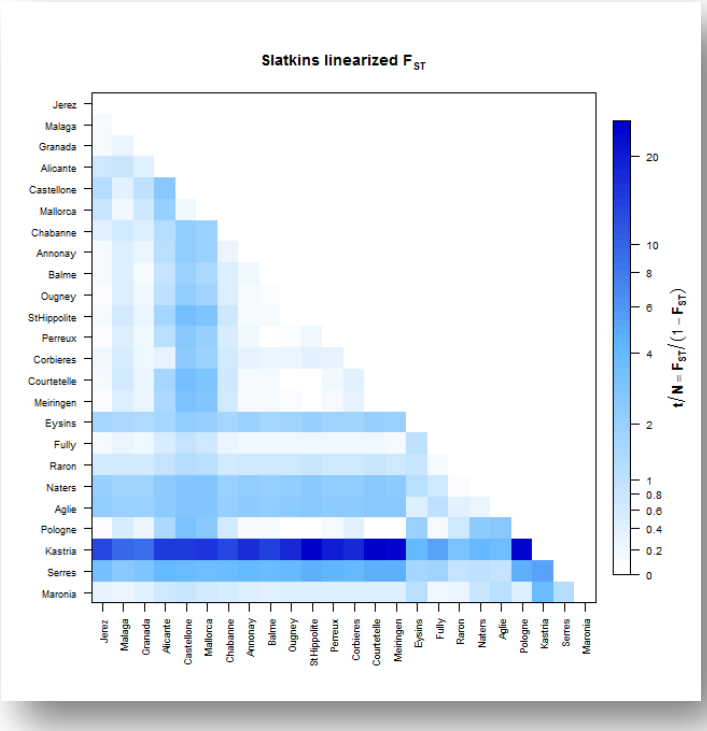
XML tag in output file: pairwiseDifferenceMatrix
R-function: pairFstMatrix.r
Arlequin computation: Compute pairwise F_{ST} (see section 6.3.8.7.3)

7.6.4.2.2 Matrix of Reynold's coancestry coefficient



XML tag in output file: coancestryCoefficients
R-function: coancestryCoeff.r
Arlequin computation: Compute pairwise F_{ST} and Reynolds's distance (see section 6.3.8.7.3)

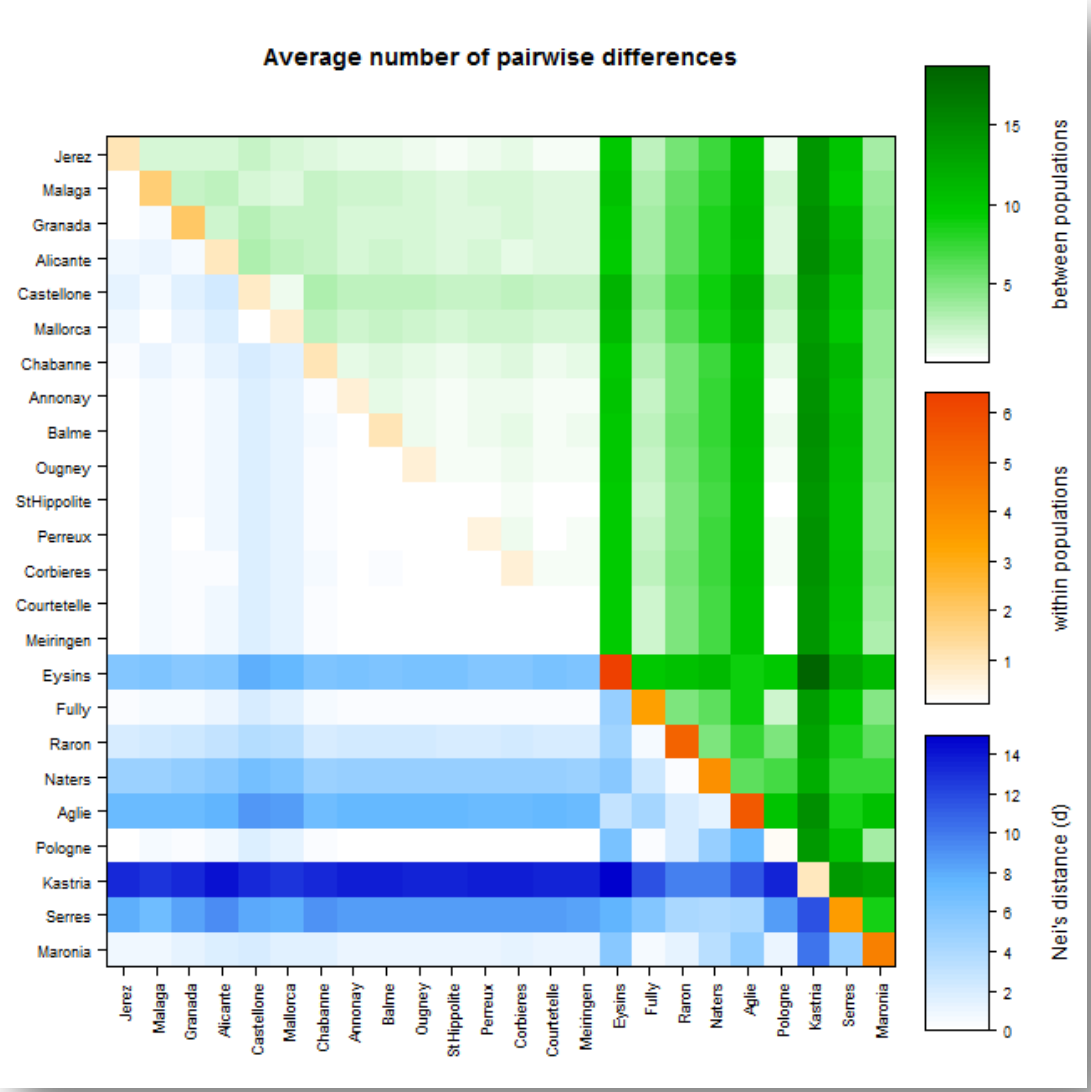
7.6.4.2.3 Slatkin's linearized F_{ST}'s



XML tag in output file: slatkinFst
R-function: slatkinFstFunction.r
Arlequin computation: Compute pairwise F_{ST} and Slatkin's distances (see section 6.3.8.7.3)

7.6.4.2.4 Average number of pairwise differences within and between populations

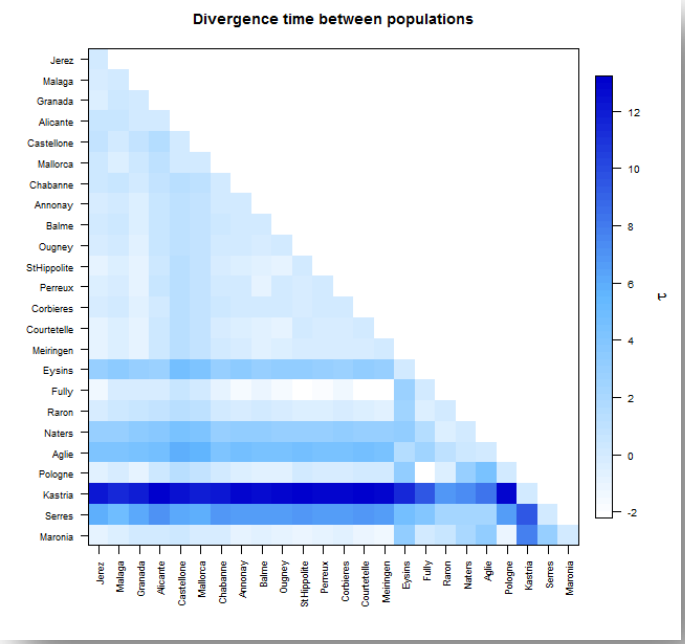
In this graph we represent on three different colour scales the average number of pairwise differences (π) between sampled populations. Orange on diagonal: π within populations; Green above diagonal: π_{xy} between pairs of populations Blue below diagonal: net number of nucleotide differences between populations (D_A, see section 8.2.4.4).



XML tag in output file: pairwiseDiffMatrix
R-function: pairwiseDiffMatrix.r
Arlequin computation: Compute pairwise differences (see section 6.3.8.7.3)

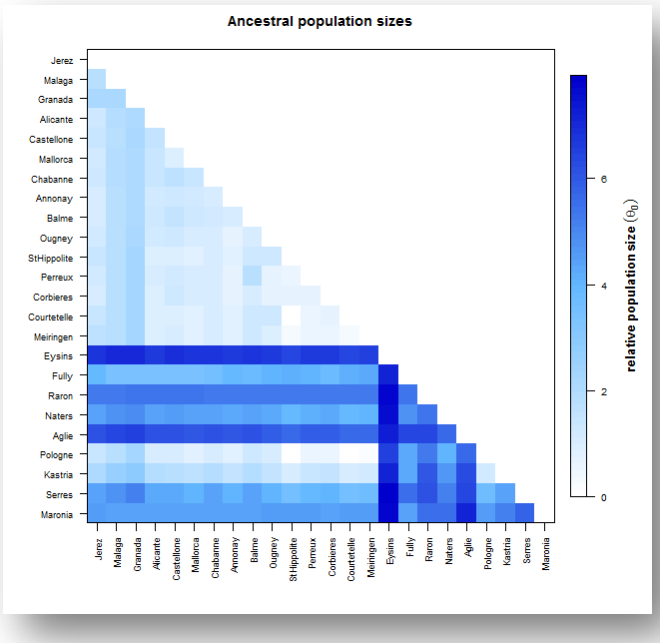
7.6.4.2.5 Model of population divergence allowing for unequal derived population size

7.6.4.2.5.1 Divergence time between populations assuming different derived population sizes



XML tag in output file: tauMatrix
R-function: tauMatrixFunction.r
Arlequin computation: Compute pairwise differences and Estimate relative population sizes (see section 6.3.8.7.3)

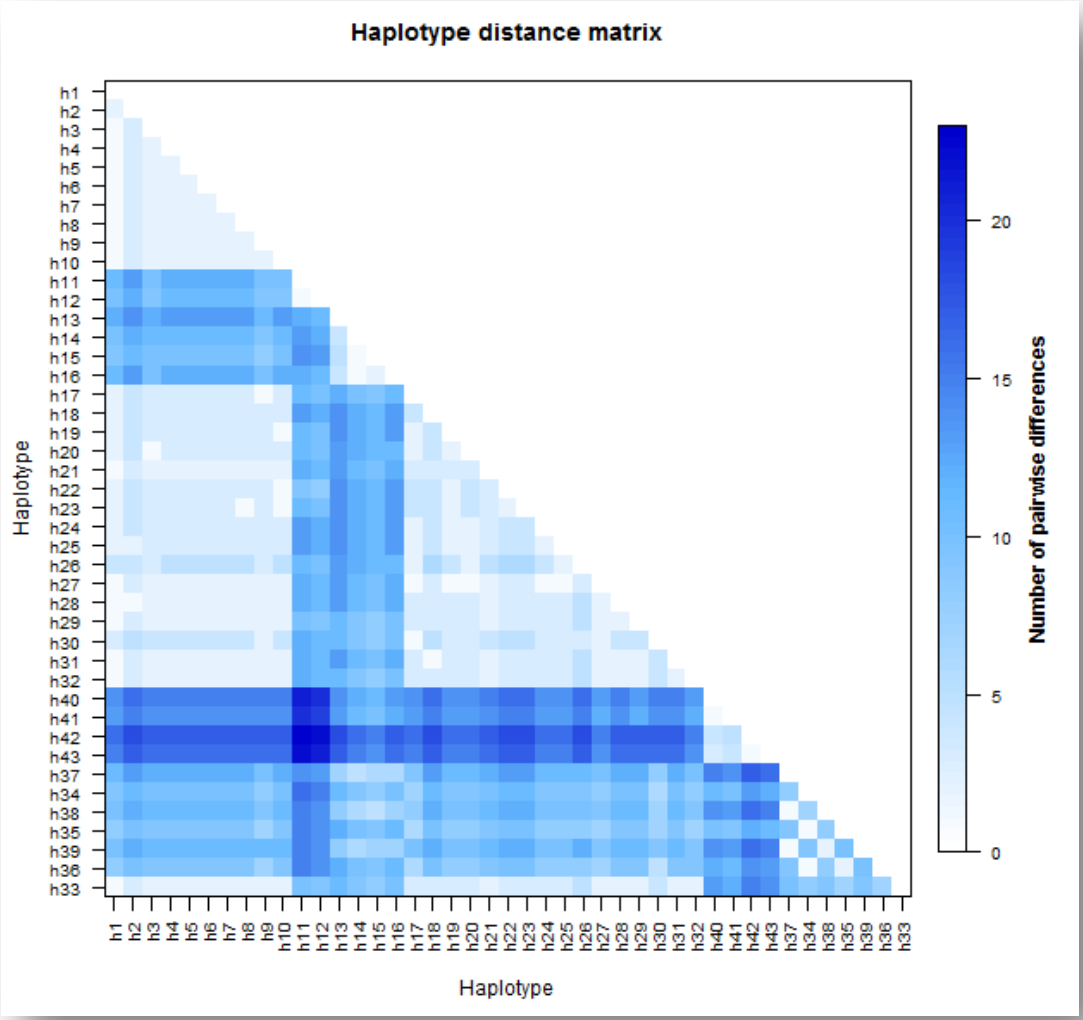
7.6.4.2.5.2 Ancestral population sizes



XML tag in output file: ancestralPopSize
R-function: ancestralPopulationSize.r
Arlequin computation: Compute pairwise differences and Estimate relative population sizes (see section 6.3.8.7.3)

7.6.4.3 Matrix of molecular distance between haplotypes

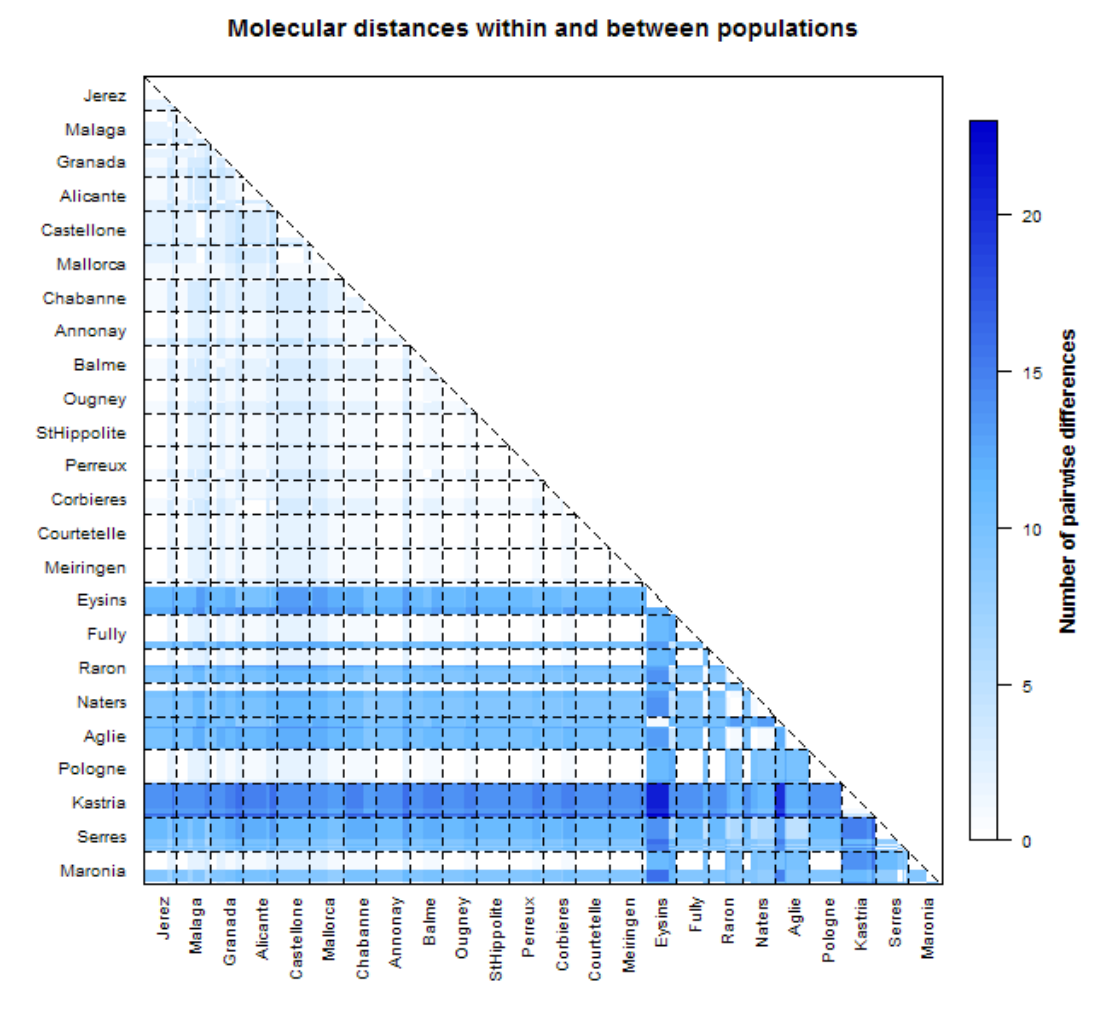
This graph represents the number of molecular differences between all different haplotypes found in the project. It is output when one selects the option “Print distance matrix” in the AMOVA tab (see section 6.3.8.7.1).



XML tag in output file: hapDistMatrix
R-function: haplotypeDistMatrix.r
Arlequin computation: Print distance (see AMOVA section 6.3.8.7.1.1)

7.6.4.4 Matrix of molecular distances between gene copies within and between populations (phase known only)

This graphic is produced by combining information from the table of haplotype frequencies and the inter-haplotype distance matrix. It implies that both tables must be computed in the same run (see below on how to activate these computations). The graphic is displayed after the haplotype distance matrix graphic in the XML output file.

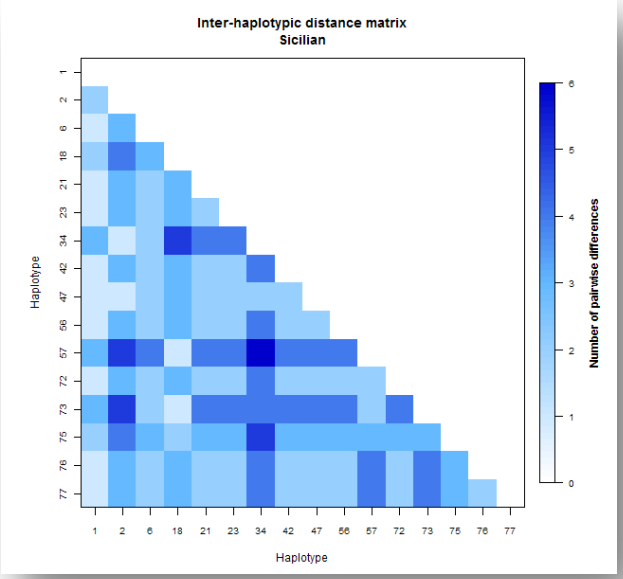


The dashed lines separate population samples. Like the graph of the Matrix of pairwise F_{ST}'s (see section 7.6.4.2.1), this graph allows one to quickly visualize population genetic affinities, but provides a more detailed view, at the individual haplotype (sequence) level.

XML tag in output file:	hapDistMatrix
R-function:	hapDistMatrix_withinBetweenComplete.r
Arlequin computations:	Search for shared haplotypes and Print distance (see sections 6.3.8.4.1 and 6.3.8.7.1.1)

7.6.4.5 Matrix of molecular distances between haplotypes within populations

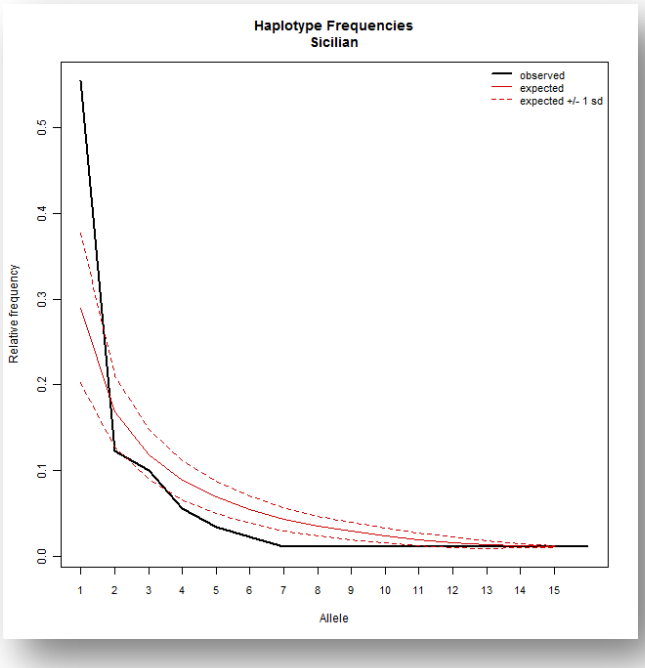
This graph represents the matrix of the number of pairwise distances between all haplotypes found in a given population. Note that it does not use information on haplotype frequencies.



XML tag in output file: interHapDistMatrix
R-function: interHaplotypeDistMatrix.r
Arlequin computation: Print distance matrix between haplotypes (see section 6.3.8.2)

7.6.4.6 Haplotype frequencies within population

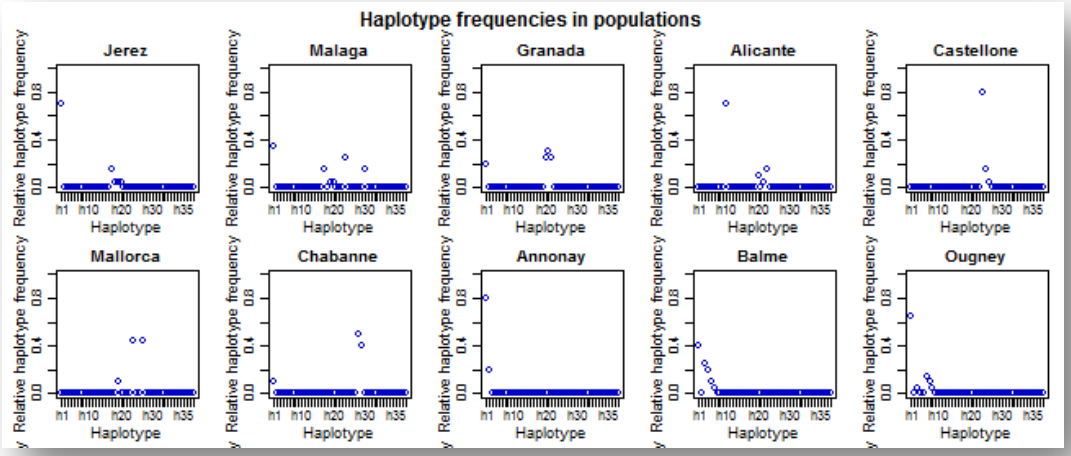
For each population, we produce graphs of the ordered distribution of allele frequencies and its neutral expectation as computed in the Ewens-Watterson neutrality test (see section 8.1.6.1)



XML tag in output file: expHapFreq
R-function: expectedHapFreq.r
Arlequin computation: Ewens-Watterson neutrality test (see section 6.3.8.6)

7.6.4.7 Haplotype frequencies in populations

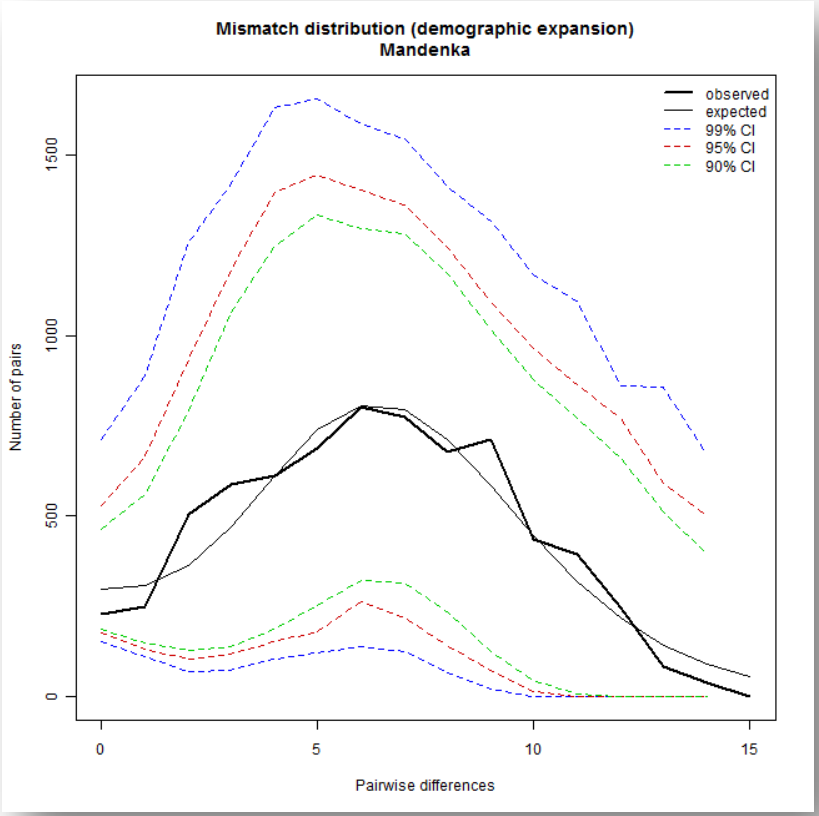
The frequency of all haplotypes (known phase is assumed) are plotted for each populations.



XML tag in output file: relHapFreq
R-function: relativeHapFreq.r
Arlequin computation: Search for shared haplotypes (see section 6.3.8.4.1)

7.6.4.8 Mismatch distribution

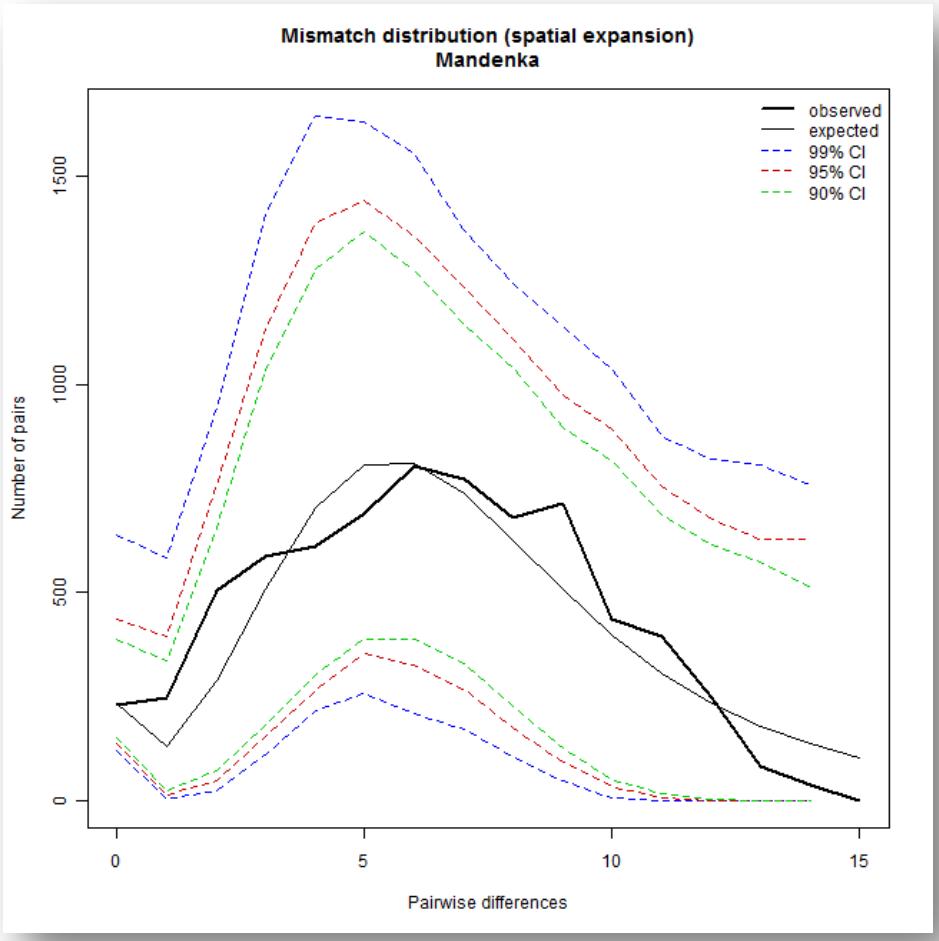
7.6.4.8.1 Demographic expansion



XML tag in output file: mismatchDemogExp
R-function: mismatch.r
Arlequin computation: Estimate parameters of demographic expansion (see section 6.3.8.3)

7.6.4.8.2 Spatial expansion

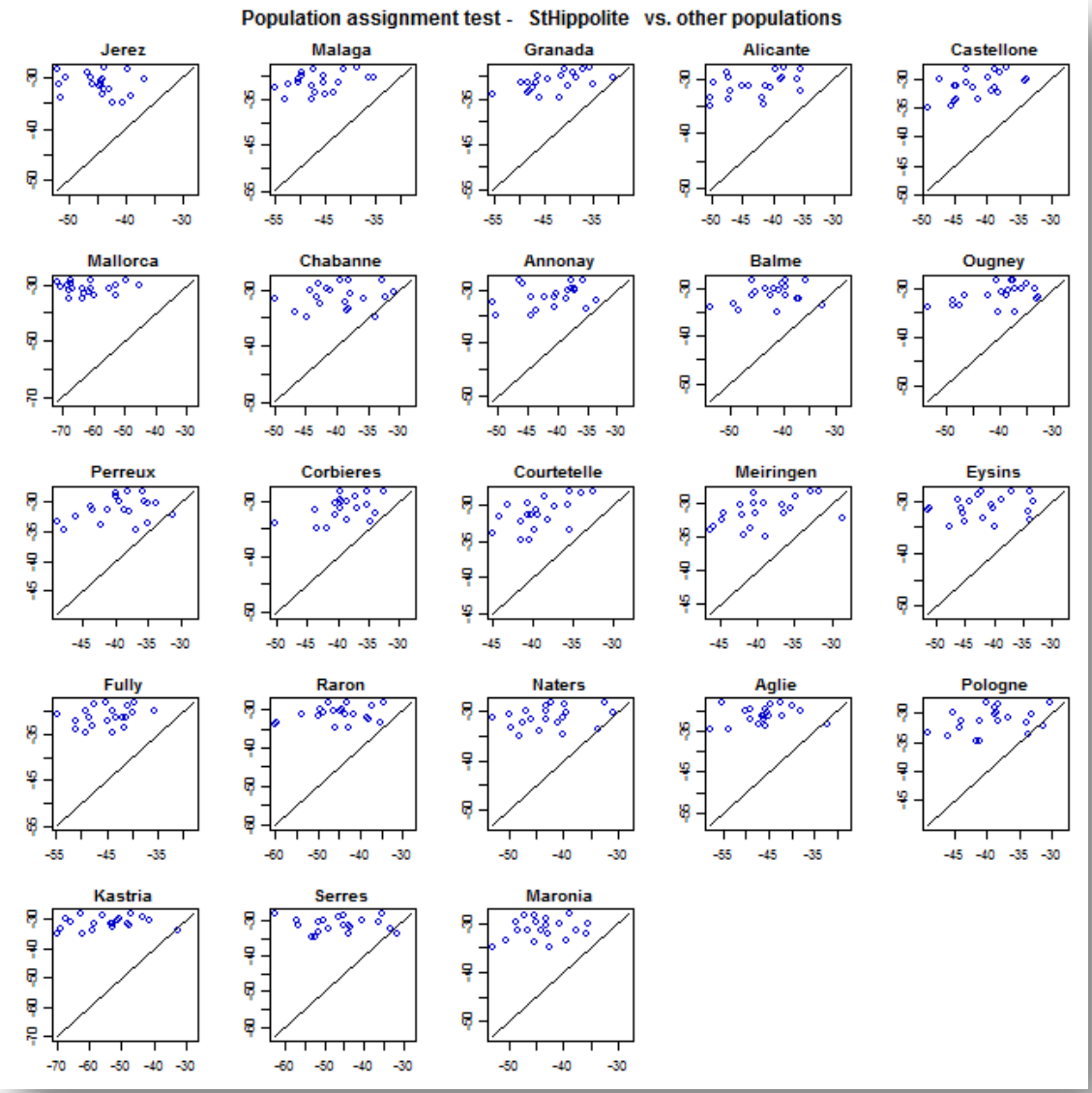
We produce plots of the observed mismatch distribution and its confidence interval



XML tag in output file: mismatchSpatialExp
R-function: mismatch.r
Arlequin computation: Estimate parameters of spatial expansion (see section 6.3.8.3)

7.6.4.9 Population assignment test

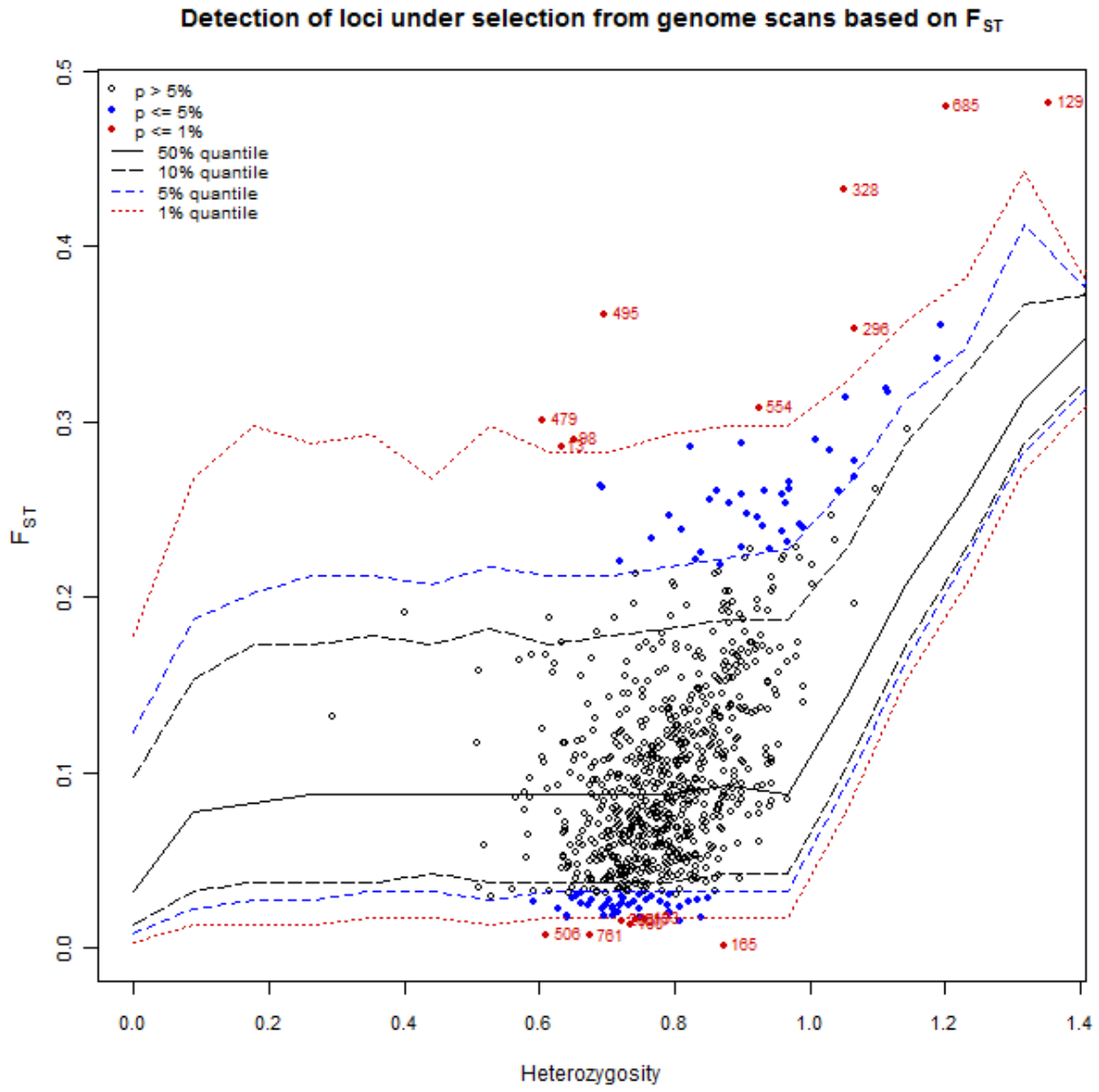
For each sample, we produce graph of the genotype likelihoods in the sampled population vs. that in all other populations.



XML tag in output file: genotypeLikelihoodMatrix
R-function: genotLikelihoodMatrix.r
Arlequin computation: Perform genotype assignment for all pairs of populations (see section 6.3.8.8)

7.6.4.10 Detection of loci under selection

In this graph, we plot the joint distribution of F_{ST} and (heterozygosity within populations)/(1- F_{ST}) for the observed loci (small circles), as well as one-sided confidence interval limits obtained from simulated data (see section 8.2.8 for details) as dashed lines. Loci significant at the 5% level are shown as filled blue circles, while loci significant at the 1% level are shown as red filled circles. We also give the number of the loci under selection at the 1% level. If a hierarchical island model is used to detect loci under selection, we also output a plot for the joint distribution of F_{CT} and heterozygosity.



XML tag in output file: detSel_FStat_Pval & detSel_FST_CI
R-function: lociSelection.r
Arlequin computation: Detecting loci under selection from genetic structure analysis (see section 6.3.8.7.2)

8 METHODOLOGICAL OUTLINES

The following table gives a rapid overview of the methods implemented in Arlequin. A ✓ indicates that the task corresponding to the table entry is possible. Some tasks are only possible or meaningful if there is no recessive data, and those cases are marked with a ✕

	Data types									
	DNA & RFLP			**Microsat**			**Standard**			**Frequency**
Types of computations	**G+**	**G-**	**H**	**G+**	**G-**	**H**	**G+**	**G-**	**H**	
Standard indices ✕	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Molecular diversity ✕	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Mismatch distribution	✓		✓	✓		✓	✓		✓	
Haplotype (or allele) frequency estimation	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Linkage disequilibrium	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Hardy-Weinberg equilibrium ✕	✓	✓		✓	✓		✓	✓		
Tajima's neutrality test	✓		✓							
Fu's neutrality test	✓		✓							
Ewens-Watterson neutrality tests	✓		✓				✓		✓	✓
Chakraborty's amalgamation test	✓		✓				✓		✓	✓
Search for shared haplotypes between samples			✓			✓			✓	✓
AMOVA ✕	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Detection of selected loci ✕	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Minimum Spanning Network[1]	✓		✓	✓		✓	✓		✓	
Pairwise genetic distances ✕	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Exact test of population differentiation ✕	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Individual assignment test ✕	✓	✓		✓	✓		✓	✓		
Mantel test	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

G+: Genotypic data, gametic phase known
G- : Genotypic data, gametic phase unknown
H : Haplotypic data

[1] Computation of minimum spanning network between haplotypes is only possible if a distance matrix is provided or if it can be computed from the data.

8.1 Intra-population level methods

8.1.1 Standard diversity indices

8.1.1.1 Gene diversity

Equivalent to the expected heterozygosity for diploid data. It is defined as the probability that two randomly chosen haplotypes are different in the sample. Gene diversity and its sampling variance are estimated as

$$\hat{H} = \frac{n}{n-1}(1-\sum_{i=1}^{k} p_i^2)$$

$$\mathrm{V}(\hat{H}) = \frac{2}{n(n-1)}\left\{2(n-2)\left[\sum_{i=1}^{k} p_i^3 - (\sum_{i=1}^{k} p_i^2)^2\right] + \sum_{i=1}^{k} p_i^2 - (\sum_{i=1}^{k} p_i^2)^2\right\},$$

where *n* is the number of gene copies in the sample, *k* is the number of haplotypes, and p_i is the sample frequency of the *i*-th haplotype.

Note that Arlequin outputs the standard deviation of the Heterozygosity computed as $s.d.(\hat{H}) = \sqrt{V(\hat{H})}$.

Reference:
Nei, 1987, p.180.

8.1.1.2 Expected heterozygosity per locus

For each locus, Arlequin provides an estimation of the expected heterozygosity simply as

$$\hat{H} = \frac{n}{n-1}(1-\sum_{i=1}^{k} p_i^2)$$

8.1.1.3 Number of usable loci

Number of loci that show less than a specified amount of missing data. The maximum amount of missing data must be specified in the *General Settings* tab dialog .

8.1.1.4 Number of polymorphic sites (S)

Number of usable loci that show more than one allele per locus.

8.1.1.5 Allelic range (R)

For MICROSAT data, it is the difference between the maximum and the minimum number of repeats.

8.1.1.6 Garza-Williamson index (G-W)

Following Garza and Wlliamson (2001), the G-W statistic is given as $G-W=\frac{k}{R+1}$ where *k* is the number of alleles at a given loci in a population sample, and R is the allelic range. Originally, the denominator was defined as just *R* in Garza and Wlliamson (2001), but this could lead to a division by zero if a sample is monomorphic. This adjustment was introduced in Excoffier et al. (2005).

This statistic was shown to be sensitive to population bottleneck, because the number of alleles is usually more reduced than the range by a recent reduction in population size, such that the distribution of allele length will show "vacant" positions. Therefore the G-W statistic is supposed to be very small in population having been through a bottleneck and close to one in stationary populations.

Here we just report the statistics but do not provide any test.

8.1.2 Molecular indices

8.1.2.1 Mean number of pairwise differences (π)

Mean number of differences between all pairs of haplotypes in the sample. It is given by

$$\hat{\pi}=\frac{n}{n-1}\sum_{i=1}^{k}\sum_{j=1}^{k}p_i p_j \hat{d}_{ij},$$

where $\hat{d}_{ij}$ is an estimate of the number of mutations having occurred since the divergence of haplotypes *i* and *j*, *k* is the number of haplotypes, p_i is the frequency of haplotype *I*, and *n* is the sample size. The total variance (over the stochastic and the sampling process), assuming no recombination between sites and selective neutrality, is obtained as

$$\mathrm{V}(\hat{\pi})=\frac{3n(n+1)\hat{\pi}+2(n^2+n+3)\hat{\pi}^2}{11(n^2-7n+6)}. \qquad \text{(Tajima, 1993)}$$

Note that similar formulas are also used for *Microsat* and *Standard* data, even though the underlying assumptions of the model may be violated. Note also that Arlequin outputs the standard deviation computed as $s.d.(\hat{\pi})=\sqrt{V(\hat{\pi})}$.

References:

Tajima, 1983
Tajima, 1993

8.1.2.2 Nucleotide diversity or average gene diversity over L loci

It is computed here as the probability that two randomly chosen homologous (nucleotide or RFLP) sites are different. It is equivalent to the gene diversity at the nucleotide level for DNA data.

$$\hat{\pi}_n = \frac{\sum_{i=1}^{k} \sum_{j<i} p_i p_j \hat{d}_{ij}}{L}$$

$$\mathrm{V}(\hat{\pi}_n) = \frac{n+1}{3(n-1)L}\hat{\pi}_n + \frac{2(n^2+n+3)}{9n(n-1)}\hat{\pi}_n^2$$

Note that similar formulas are used for computing the average gene diversity over *L* loci for Microsat and Standard data, assuming no recombination and selective neutrality. As above, one should be aware that these assumptions may not hold for these data types. Note also that Arlequin outputs the standard deviation computed as $s.d.(\hat{\pi}_n) = \sqrt{V(\hat{\pi}_n)}$.

Note that for RFLP data this measure should be considered as the average heterozygosity per RFLP site, which is different from the true diversity at the nucleotide level, for which one would need to know the base composition of the restriction sites.

References:

Tajima, 1983
Nei, 1987, p. 257

8.1.2.3 Theta estimators

Several methods are used to estimate the population parameter $\theta = 2Mu$, where *M* is equal to 2 *N* for diploid populations of size *N* , or equal to *N* for haploid populations, and *u* is the overall mutation rate at the haplotype level.

8.1.2.3.1 Theta(Hom)

The expected homozygosity in a population at equilibrium between drift and mutation is usually given by

$$H = \frac{1}{\theta + 1}.$$

However, Zouros (1979) has shown that this estimator was an overestimate when estimated from a single or a few loci. Although he gave no closed form solution, Chakraborty and Weiss (1991) proposed to iteratively solve the following relationship between the expectation of $\hat{\theta}_H$ and the unknown parameter θ

$$\mathrm{E}(\hat{\theta}_H) = \theta\left(1 + \frac{2(1+\theta)}{(2+\theta)(3+\theta)}\right) \qquad \text{(Zouros, 1979)}$$

starting with a first estimate of $\hat{\theta}_H$ of $(1-H)/H$, and equating it to its expectation.

Chakraborty and Weiss (1991) give an approximate formula for the standard error of $\hat{\theta}_H$ as

$$\text{s.d.}(\hat{\theta}_H) \approx \frac{(2+\theta)^2(3+\theta)^2\,\text{s.d.}(H)}{H^2(1+\theta)\left[(2+\theta)(3+\theta)(4+\theta)+10(2+\theta)+4\right]},$$

where $\text{s.d.}(H)$ is the standard error of H given in section 8.1.1.1.

For MICROSAT data, Ohta and Kimura (1973) have shown that the expected homozygosity in stationary populations under a pure stepwise mutation model was equal to:

$$E(Hom) = \frac{1}{\sqrt{1+2\theta}}$$

where $\theta = 4N_e u$ for diploids and $\theta = 2N_e u$ for haploid systems. It follows that an estimator of θ can be obtained for microsatellite data as

$$\hat{\theta}_H = \frac{1}{(1-\hat{H})^2} - 1,$$

where $\hat{H}$ is the expected heterozygosity estimated as in section 8.1.1.2.

8.1.2.3.2 Theta(*S*)

$\hat{\theta}_S$ is estimated from the infinite-site equilibrium relationship (Watterson, 1975) between the number of segregating sites (*S*), the sample size (*n*) and θ for a sample of non-recombining DNA:

$$\theta = \frac{S}{a_1}$$

where

$$a_1 = \sum_{i=1}^{n-1} \frac{1}{i}.$$

The variance of $\hat{\theta}_S$ is obtained as

$$\text{V}(\hat{\theta}_S) = \frac{a_1^2 S + a_2 S^2}{a_1^2(a_1^2 + a_2)}, \qquad \text{(Tajima, 1989)}$$

where

$$a_2 = \sum_{i=1}^{n-1} \frac{1}{i^2}$$

8.1.2.3.3 Theta(*k*)

$\hat{\theta}_k$ is estimated from the infinite-allele equilibrium relationship (Ewens, 1972) between the expected number of alleles (*k*), the sample size (*n*) and θ :

$$\mathrm{E}(k) = \theta \sum_{i=0}^{n-1} \frac{1}{\theta + i}$$

Instead of the variance of $\hat{\theta}_k$, we give the limits ($\hat{\theta}_0$ and $\hat{\theta}_1$) of a 95% confidence interval around $\hat{\theta}_k$, obtained from Ewens (1972)

$$\Pr(\text{less than } k \text{ alleles} \mid \theta = \theta_0) = 0.025$$

$$\Pr(\text{ more than } k \text{ alleles} \mid \theta = \theta_1) = 0.025\,,$$

These probabilities are obtained by summing up the probabilities of observing *k*' alleles (*k*'=0,...,*k*), obtained as (Ewens, 1972)

$$\Pr(K = k \mid \theta) = \frac{\left|S_n^k\right| \theta^k}{S_n(\theta)}$$

where $\left|S_n^k\right|$ is a Stirling number of the first kind (see Abramovitz and Stegun, 1970), and $S_n(\theta)$ is defined as $\theta(\theta+1)(\theta+2)\ldots(\theta+n-1)$.

8.1.2.3.4 Theta(π)

$\hat{\theta}_\pi$ is estimated from the infinite-site equilibrium relationship between the mean number of pairwise differences ($\hat{\pi}$) and theta (θ):

$$\mathrm{E}(\hat{\pi}) = \theta \quad , \qquad \text{(Tajima, 1983)}$$

and its variance $\mathrm{V}(\hat{\pi})$ is given in section 8.1.1.1.

8.1.2.4 Mismatch distribution

It is the distribution of the observed number of differences between pairs of haplotypes. This distribution is usually multimodal in samples drawn from populations at demographic equilibrium, as it reflects the highly stochastic shape of gene trees, but it is usually unimodal in populations having passed through a recent demographic expansion (Rogers and Harpending, 1992; Hudson and Slatkin, 1991) or though a range expansion with high levels of migration between neighboring demes (Ray et al. 2003, Excoffier 2004).

8.1.2.4.1 Pure demographic expansion

If one assumes that a stationary haploid population at equilibrium has suddenly passed τ generations ago from a population size of N_0 to N_1, then the probability of observing *S* differences between two randomly chosen non-recombining haplotypes is given by

$$F_S(\tau,\theta_0,\theta_1) = F_S(\theta_1) + exp(-\tau\frac{\theta_1+1}{\theta_1})\sum_{j=0}^{S}\frac{\tau^j}{j!}\left[F_{S-j}(\theta_0) - F_{S-j}(\theta_1)\right], \qquad \text{(Li, 1977)}$$

where $F_S(\theta) = \frac{\theta^S}{(\theta+1)^{S+1}}$ is the probability of observing two random haplotypes with S differences in a stationary population (Watterson, 1975), $\theta_0 = 2uN_0$, $\theta_1 = 2uN_1$, $\tau = 2ut$, and u is the mutation rate for the whole haplotype.

Rogers (1995) has simplified the above equation, by assuming that $\theta_1 \to \infty$, implying there are no coalescent events after the expansion, which is only reasonable if the expansion size is large. With this simplifying assumption, it is possible to derive the moment estimators of the time to the expansion (τ) and the mutation parameter θ_0, as

$$\begin{aligned}\hat{\theta}_0 &= \sqrt{v-m} \\ \hat{\tau} &= m - \hat{\theta}_0\end{aligned}, \qquad \text{(Rogers, 1995)}$$

where m and v are the mean and the variance of the observed mismatch distribution, respectively. These estimators can then be used to plot $F_S(\tau,\theta_0,\infty)$ values. Note, however, that this estimation cannot be done if the variance of the mismatch is smaller than the mean.

However, Schneider and Excoffier (1999) find that this moment estimator often leads to an underestimation of the age of the expansion (τ). They rather propose to estimate the

parameters of the demographic expansion by a generalized non-linear least-square approach. This is the method we now use to estimate the parameters of the demographic expansion τ, θ_0, and θ_1.

Approximate confidence intervals for those parameters are obtained by a parametric bootstrap approach. The principle is the following: We computed approximate confidence intervals for the estimated parameters $\hat{\theta}_1, \hat{\theta}_0$ and $\hat{\tau}$ using a parametric bootstrap approach (Schneider and Excoffier, 1999) generating percentile confidence intervals (see e.g. Efron, 199, p. 53 and chap. 13).

- We generate a large number (*B*) of random samples according to the estimated demography, using a coalescent algorithm modified from Hudson (1990).
- For each of the *B* simulated data sets, we reestimate τ, θ_0, and θ_1 to get *B* bootstrapped values θ_0^*, θ_1^* and τ^*.
- For a given confidence level α, the approximate limits of the confidence interval were obtained as the $\alpha/2$ and $1-\alpha/2$ percentile values (Efron, 1993, p. 168).

It is important to underline that this form of parametric bootstrap assumes that the data are distributed according the sudden expansion model. In Schneider and Excoffier (1999), we showed by simulation that only the confidence interval (CI) for τ has a good coverage (i.e. that the true value of the parameter is included in a 100x(1-α)% CI with a probability very close to 1-α.). The CI of the other two parameters are overly large (the true value of the parameter was almost always included in the CI), and thus too conservative.

The validity of the estimated stepwise expansion model is tested using the same parametric bootstrap approach as described above. We used here the sum of square deviations (*SSD*) between the observed and the expected mismatch as a test statistic. We obtained its distribution under the hypothesis that the estimated parameters are the true ones, by simulating *B* samples around the estimated parameters. As before, we re-estimated each time new parameters θ_0^*, θ_1^* and τ^*, and computed their associated sums of squares SSD_{sim}. The P-value of the test is therefore approximated by

$$P = \frac{\text{number of } SSD_{sim} \text{ larger or equal to } SSD_{obs}}{B}.$$

For convenience, we also compute the raggedness index of the observed distribution defined by Harpending (1994) as

$$r = \sum_{i=1}^{d+1} (x_i - x_{i-1})^2 ,$$

where d is the maximum number of observed differences between haplotypes, and the x's are the observed relative frequencies of the mismatch classes. This index takes larger values for multimodal distributions commonly found in a stationary population than for unimodal and smoother distributions typical of expanding populations. Its significance is tested similarly to that of *SSD*.

References:

Rogers and Harpending (1991)

Rogers (1995)

Schneider and Excoffier (1999)

Excoffier (2004)

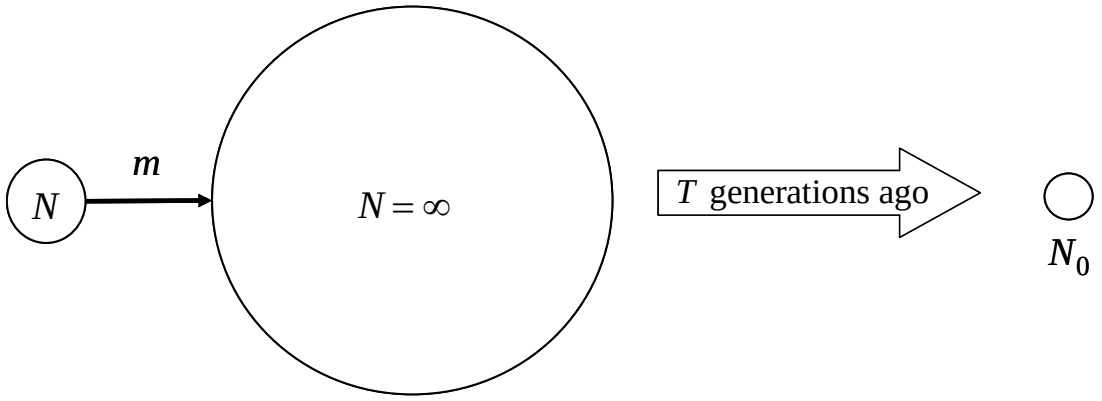
8.1.2.4.2 Spatial expansion

A population spatial expansion generally occurs if the range of a population is initially restricted to a very small area, and then the range of the population increases over time and over space. The resulting population becomes generally subdivided in the sense that individuals will tend to mate with geographically close individuals rather than remote individuals.

Based on simulations, Ray et al. (2003) have shown that a large spatial expansion can lead to the same signal in the mismatch distribution than a pure demographic expansion in a panmictic population, but only if neighboring sub-populations (demes) exchange many migrants (50 or more). The simulations performed in Ray et al. (2003) were performed in a two-dimensional stepping-stone model. *T* generations ago, a haploid population restricted to a single deme of size N, began to send migrants to neighboring demes at rate *m*, progressively colonizing the whole world. During the expansion, the size of each deme followed a logistic regulation with carrying capacity *K,* and intrinsic rate of growth *r*. During the whole process neighboring demes continue to exchange a fraction *m* of migrants.

While this model is difficult to describe analytically, Excoffier (2004) derived the expected mismatch distribution under a simpler model of spatial expansion. He assumed that one has sampled genes from a single deme belonging to a population subdivided into a infinite number of demes, each of size *N*, which would exchange a fraction *m* of migrants wirh other demes. This infinite-island model is actually equivalent to a continent-island model, where the sampled deme would exchange migrants at rate *m* with a unique population of infinite size. Some *T* generations in the past, the continent-island system would be reduced to a single deme of size N_0, like:

Continent-island model



After the expansion **Before the expansion**

Under this simple model, the probability that two genes currently sampled in the small deme of size *N* differ at *S* sites is given by

$$F_0(S;\ M,\ \theta_0;\ \theta_1,\ \tau) = \frac{\theta_1^S}{A^{S+1}} + \sum_{j=0}^{S}\left(\frac{(Me^{-\tau} + C)\theta_0^j\ \tau^{S-j}}{(M+1)(\theta_0+1)^{j+1}(S-j)!} - \frac{\tau^j \theta_1^{S-j} C}{j!A^{S-j+1}} \right)$$, Excoffier (2004)

where $\theta_0 = 2N_0\mu$, $\theta_1 = 2N_1\mu$, $\tau = 2T\mu$, and $A = \theta_1 + M + 1$, and $C = e^{-\tau A/\theta_1}$.

In Arlequin, we estimate the three parameters of a spatial expansion, τ, $\theta = \theta_0 = \theta_1$ (here we assume that $N = N_0$), and $M = 2Nm$, using the same least-square method as described in the case of the estimation of the parameters of a demographic expansion (see section 8.1.2.4.1). Like for the demographic expansion, we also provide the expected mismatch distribution and test the fit to the model by coalescent simulations of an instantaneous expansion under the continent-island model defined above.

References:

Ray et al (2003)

Excoffier (2004)

8.1.2.5 Estimation of genetic distances between DNA sequences

Definitions:

L:	Number of loci
Gamma correction:	This correction is proposed when the mutation rates cannot be assumed as uniform for all sites. It had been originally proposed for mutation rates among amino acids (Uzell and Corbin, 1971), but it seems also to be the case of the control region of human mtDNA (Wakeley, 1993). In such a case, a Gamma distribution of mutation rates is often assumed. The shape of this distribution (the unevenness of the mutation rates) is mainly controlled by a parameter a, which is the inverse of the coefficient of variation of the mutation rate. The smaller the a coefficient , the more uneven the mutation rates. A uniform mutation rate corresponds to the case where a is equal to infinity.
n_d :	Number of observed substitutions between two DNA sequences
n_s :	Number of observed transitions between two DNA sequences
n_v :	Number of observed transversions between two DNA sequences
ω	G+C ratio, computed on all the DNA sequences of a given sample

8.1.2.5.1 Pairwise difference

Outputs the number of loci for which two haplotypes are different

$$\hat{d} = n_d$$

$$\mathrm{V}(\hat{d}) = \hat{d}(L - \hat{d})/L$$

8.1.2.5.2 Percentage difference

Outputs the percentage of loci for which two haplotypes are different

$$\hat{d} = n_d / L$$

$$\mathrm{V}(\hat{d}) = \hat{d}(1 - \hat{d})/L$$

8.1.2.5.3 Jukes and Cantor

Outputs a corrected percentage of nucleotides for which two haplotypes are different.

The correction allows for multiple substitutions per site since the most recent common ancestor of the two DNA sequences. The correction also assumes that the rate of nucleotide substitution is identical for all 4 nucleotides A, C, G and T.

$$\hat{p} = n_d / L$$

$$\hat{d} = -\frac{3}{4}\log(1-\frac{4}{3}\hat{p})$$

$$\mathrm{V}(\hat{d}) = \frac{\hat{p}(1-\hat{p})}{(1-\frac{4}{3}\hat{p})^2 L}$$

Gamma correction:

$$\hat{d} = -\frac{3}{4}a\left[\ (1-\frac{4}{3}p)^{-1/a} - 1\ \right]$$

$$\mathrm{V}(\hat{d}) = p(1-p)\left[\ (1-\frac{4}{3}p)^{-2(1/a+1)}\ \right]/L$$

References:

Jukes and Cantor 1969
Jin and Nei 1990
Kumar et al. 1993

8.1.2.5.4 Kimura 2-parameters

Outputs a corrected percentage of nucleotides for which two haplotypes are different.

The correction also allows for multiple substitutions per site, but takes into account different substitution rates between transitions and transversions. The transition-transversion ratio is estimated from the data.

$$\hat{P} = \frac{n_s}{L}, \quad \hat{Q} = \frac{n_v}{L}$$

$$c_1 = 1/(1-2\hat{P}-\hat{Q}), c_2 = 1/(1-2\hat{Q}), c_3 = \frac{c_1 + c_2}{2}$$

$$\hat{d} = \frac{1}{2}\log(1-2\hat{P}-\hat{Q}) - \frac{1}{4}\log(1-2\hat{Q})$$

$$\mathrm{V}(\hat{d}) = \frac{c_1^2 P + c_3^2 \hat{Q} - (c_1\hat{P} + c_3\hat{Q})^2}{L}$$

Gamma correction:

$$c_1 = (1-2\hat{P}-\hat{Q})^{-(1/a+1)}, c_2 = (1-2\hat{Q})^{-(1/a+1)}, c_3 = \frac{c_1 + c_2}{2}$$

$$\hat{d} = \frac{a}{2}\left[(1-2\hat{P}-\hat{Q})^{-1/a} + \frac{1}{2}(1-2\hat{Q})^{-1/a} - \frac{3}{2} \right]$$

$$\mathrm{V}(\hat{d}) = \frac{c_1^2\hat{P} + c_3^2\hat{Q} - (c_1\hat{P} + c_3\hat{Q})^2}{L}$$

References:

Kimura (1980)

Jin and Nei (1990)

8.1.2.5.5 Tamura

Outputs a corrected percentage of nucleotides for which two haplotypes are different. The correction is an extension of Kimura 2-parameters method, allowing for unequal nucleotide frequencies. The transition-transversion ratios, as well as the overall nucleotide frequencies are computed from the original data.

$$\hat{P} = \frac{n_s}{L}, \quad \hat{Q} = \frac{n_v}{L}$$

$$c_1 = \frac{1}{1-\frac{\hat{P}}{2\omega(1-\omega)}}, \quad c_2 = \frac{1}{1-2\hat{Q}}, \quad c_3 = 2\omega(1-\omega)(c_1-c_2)+c_2$$

$$\hat{d} = -2\omega(1-\omega)\log(1-\frac{\hat{P}}{2\omega(1-\omega)}-\hat{Q}) - \frac{1}{2}(1-2\omega(1-\omega))\log(1-2\hat{Q})$$

$$\mathrm{V}(\hat{d}) = \frac{c_1^2\hat{P} + c_3^2\hat{Q} - (c_1\hat{P} + c_3\hat{Q})^2}{L}$$

References:

Tamura, 1992,

Kumar et al. 1993

8.1.2.5.6 Tajima and Nei

Outputs a corrected percentage of nucleotides for which two haplotypes are different. The correction is an extension of Jukes and Cantor method, allowing for unequal nucleotide frequencies. The overall nucleotide frequencies are computed from the data.

$$\hat{p} = \frac{n_d}{L}, \quad b = \frac{1}{2}(1-\sum_{i=1}^{4} g_i^2 + \frac{\hat{p}^2}{c}, \quad c = \sum_{i=1}^{3}\sum_{j=i+1}^{4} \frac{x_{ij}^2}{2g_i g_j},$$

where the g's are the four nucleotide frequencies, and x_{ij} is the relative frequency of the nucleotide pair i and j .

$$\hat{d} = -b\log(1-\frac{\hat{p}}{b})$$

$$V(\hat{d}) = \frac{\hat{p}(1-\hat{p})}{(1-\frac{\hat{p}}{b})^2 L}$$

References:

Tajima and Nei, 1984,

Kumar et al. 1993

8.1.2.5.7 Tamura and Nei

Outputs a corrected percentage of nucleotides for which two haplotypes are different.

Like Kimura 2-parameters, and Tajima and Nei distances, the correction allows for different transversion and transition rates, but a distinction is also made between transition rates between purines and between pyrimidines.

$$c_1 = \frac{2 g_A g_G}{g_R}, \quad c_2 = \frac{2 g_C g_T}{g_Y}, \quad c_3 = \frac{2 g_A g_G g_R}{2 g_A g_G g_R - g_R^2 \hat{P}_1 - g_A g_G \hat{Q}}$$

$$c_4 = \frac{2 g_T g_C g_Y}{2 g_T g_C g_Y - g_Y^2 \hat{P}_2 - g_T g_C \hat{Q}}$$

$$c_5 = \frac{2 g_A^2 g_G^2}{g_R (2 g_A g_G g_R - g_R^2 \hat{P}_1 - g_A g_G \hat{Q})}$$

$$+ \frac{2 g_T^2 g_C^2}{g_Y (2 g_T g_C g_Y - g_Y^2 \hat{P}_2 - g_T g_C \hat{Q})}$$

$$+ \frac{g_R^2 (g_T^2 + g_C^2) + g_Y^2 (g_A^2 + g_G^2)}{2 g_R^2 g_Y^2 - g_R g_Y Q}$$

$$\hat{P}_1 = n_s(A \leftrightarrow G), \quad \hat{P}_2 = n_s(C \leftrightarrow T), \quad \hat{Q} = \frac{n_s}{n_d}$$

$$\hat{d} = \quad -c_1 \log(1 - \frac{\hat{P}_1}{c_1} - \frac{\hat{Q}}{2 g_R}) - c_2 \log(1 - \frac{\hat{P}_2}{c_2} - \frac{\hat{Q}}{2 g_Y})$$

$$-2(g_R g_Y - c_1 g_Y - c_2 g_R)\log(1-\frac{Q}{2g_R g_Y})$$

$$\mathrm{V}(\hat{d}) = \frac{c_3^2\hat{P}_1 + c_4^2\hat{P}_2 + c_5^2\hat{Q} - (c_3\hat{P}_1 + c_4\hat{P}_2 + c_5\hat{Q})^2}{L}$$

Gamma correction:

$$\hat{d} = 2a\Big[\ c_1(1-\frac{\hat{P}_1}{c_1}-\frac{\hat{Q}}{2g_R})^{-1/a} + c_2(1-\frac{\hat{P}_2}{c_2}-\frac{\hat{Q}}{2g_Y})^{-1/a}$$

$$+(g_R g_Y - \frac{g_Y}{c_1}-\frac{g_R}{c_2})(1-\frac{\hat{Q}}{2g_R g_Y})^{-1/a} - 2g_A g_G - 2g_T g_C - 2g_R g_Y\ \Big]$$

$$\mathrm{V}(\hat{d}) = \frac{c_3^2\hat{P}_1 + c_4^2\hat{P}_2 + c_5^2\hat{Q} - (c_3\hat{P}_1 + c_4\hat{P}_2 + c_5\hat{Q})^2}{L}$$

References:

Tamura and Nei, 1994,
Kumar et al. 1993

8.1.2.6 Estimation of genetic distances between RFLP haplotypes

8.1.2.6.1 Number of pairwise difference

We simply count the number of different alleles between two RFLP haplotypes.

$$\hat{d}_{xy} = \sum_{i=1}^{L}\delta_{xy}(i)$$

where $\delta_{xy}(i)$ is the Kronecker function, equal to 1 if the alleles of the i-th locus are identical for both haplotypes, and equal to 0 otherwise.

When estimating genetic structure indices, this choice amounts at estimating weighted F_{ST} statistics over all loci (Weir and Cockerham, 1984; Michalakis and Excoffier, 1996).

8.1.2.6.2 Proportion of difference

We simply count the proportion of loci that are different between two RFLP haplotypes.

$$\hat{d}_{xy} = \frac{1}{L}\sum_{i=1}^{L}\delta_{xy}(i)$$

where $\delta_{xy}(i)$ is the Kronecker function, equal to 1 if the alleles of the i-th locus are identical for both haplotypes, and equal to 0 otherwise.

When estimating genetic structure indices, this choice will lead to exactly the same results as the number of pairwise differences.

8.1.2.7 Estimation of distances between Microsatellite haplotypes

8.1.2.7.1 No. of different alleles

We simply count the number of different alleles between two haplotypes.

$$\hat{d}_{xy} = \sum_{i=1}^{L} \delta_{xy}(i)$$

where $\delta_{xy}(i)$ is the Kronecker function, equal to 1 if the alleles of the i-th locus are identical for both haplotypes, and equal to 0 otherwise.

When estimating genetic structure indices, this choice amounts at estimating weighted F_{ST} statistics over all loci (Weir and Cockerham, 1984; Michalakis and Excoffier, 1996).

8.1.2.7.2 Sum of squared size difference

Counts the sum of the squared number of repeat difference between two haplotypes (Slatkin, 1995).

$$\hat{d}_{xy} = \sum_{i=1}^{L} (a_{xi} - a_{yi})^2 \quad ,$$

where a_{xi} is the number of repeats of the microsatellite for the i-th locus.

When estimating genetic structure indices, this choice amounts at estimating an analog of Slatkin's R_{ST} (1995) (see Michalakis and Excoffier, 1996, as well as Rousset, 1996 , for details on the relationship between F_{ST} and R_{ST}) .

8.1.2.8 Estimation of distances between Standard haplotypes

8.1.2.8.1 Number of pairwise differences

Simply counts the number of different alleles between two haplotypes.

$$\hat{d}_{xy} = \sum_{i=1}^{L} \delta_{xy}(i)$$

where $\delta_{xy}(i)$ is the Kronecker function, equal to 1 if the alleles of the i-th locus are identical for both haplotypes, and equal to 0 otherwise.

When estimating genetic structure indices, this choice amounts at estimating weighted F_{ST} statistics over all loci (Weir and Cockerham, 1984; Michalakis and Excoffier, 1996).

8.1.2.9 Minimum Spanning Network among haplotypes

We have implemented the computation of a Minimum Spanning Tree (MST) (Kruskal, 1956; Prim, 1957) between OTU's (Operational Taxonomic Units). The MST is computed

from the matrix of pairwise distances calculated between all pairs of haplotypes using a modification of the algorithm described in Rohlf (1973). The Minimum Spanning Network embedding all MSTs (see Excoffier and Smouse 1994) is also provided. This implementation is the translation of a standalone program written in Pascal called MINSPNET.EXE running under DOS, formerly available on http://anthropologie.unige.ch/LGB/software/win/min-span-net/.

8.1.3 Haplotype inference

8.1.3.1 Haplotypic data or Genotypic data with known Gametic phase

If haplotype i is observed x_i times in a sample containing n gene copies, then its estimated frequency ($\hat{p}_i$) is given by

$$\hat{p}_i = \frac{x_i}{n} \ ,$$

whereas an unbiased estimate of its sampling variance is given by

$$\mathrm{V}(p_i) = \frac{\hat{p}_i(1-\hat{p}_i)}{n-1} \ .$$

8.1.3.2 Genotypic data with unknown Gametic phase

8.1.3.2.1 EM algorithm

Maximum-likelihood haplotype frequencies can be estimated using an Expectation-Maximization (EM) algorithm (see e.g. Dempster et al. 1977; Excoffier and Slatkin, 1995; Lange, 1997; Weir, 1996). This procedure is an iterative process aiming at obtaining maximum-likelihood estimates of haplotype frequencies from multi-locus genotype data when the gametic phase is unknown (phenotypic data). In this case, a simple gene counting is not possible because several genotypes are possible for individuals heterozygote at more than one locus. Therefore, a slightly more elaborate procedure is needed.

The likelihood of the sample (the probability of the observed data **D**, given the haplotype frequencies - **p**) is given by

$$L(\mathbf{D}\,|\,\mathbf{p}) = \sum_{i=1}^{n} \prod_{j=1}^{g_i} G_{ij} \ ,$$

where the sum is over all n individuals of the sample, and the product is over all possible genotypes of those individuals, and $G_{ij} = 2p_i p_j$, if $i \neq j$ or $G_{ij} = p_i^2$, if $i = j$.

The principle of the EM algorithm is the following:

1) Start with arbitrary (random) estimates of haplotype frequencies.
2) Use these estimates to compute expected genotype frequencies for each phenotype, assuming Hardy-Weinberg equilibrium (The E-step).
3) The relative genotype frequencies are used as weights for their two constituting haplotypes in a gene counting procedure leading to new estimates of haplotype frequencies (The M-step).
4) Repeat steps 2-3, until the haplotype frequencies reach equilibrium (do not change more than a predefined epsilon value).

Dempster et al (1977) have shown that the likelihood of the sample could only grow after each step of the EM algorithm. However, there is no guarantee that the resulting haplotype frequencies are maximum likelihood estimates. They can be just local optimal values. In fact, there is no obvious way to be sure that the resulting frequencies are those that globally maximize the likelihood of the data. This would need a complete evaluation of the likelihood for all possible genotype configurations of the sample. In order to check that the final frequencies are putative maximum likelihood estimates, one has generally to repeat the EM algorithm from many different starting points (many different initial haplotype frequencies). Several runs may give different final frequencies, suggesting the presence of several "peaks" in the likelihood surface, but one has to choose the solution that has the largest likelihood. It may also arise that several distinct peaks have the same likelihood, meaning that different haplotypic compositions explain equally well the observed data. At this point, there is no way to choose among the alternative solutions from a likelihood point of view. Some external information should be provided to make a decision.

Standard deviations of the haplotype frequencies are estimated by a parametric bootstrap procedure (see e.g. Rice, 1995), generating random samples from a population assumed to have haplotype frequencies equal to their maximum-likelihood values. For each bootstrap replicate, we apply the EM algorithm to get new maximum-likelihood haplotype frequencies. The standard deviation of each haplotype frequency is then estimated from the resulting distribution of haplotype frequencies. Note however that this procedure is quite computer intensive.

Reference:

Excoffier and Slatkin (1995)

8.1.3.2.2 EM zipper algorithm

The EM zipper is a simple extension of the EM algorithm, aiming at speeding up the estimation process and allowing the handling of a much larger number of heterozygous sites per individual. The EM algorithm becomes indeed extremely slow when there are

more than 20 heterozygous sites per individual, and it is therefore not suited for the analysis of long stretches of DNA with hundreds of polymorphic sites.

The EM zipper therefore begins by estimating frequencies of two-locus haplotypes, and then adds another locus, to estimate 3-locus haplotype frequencies, and then adds another locus to get 4-locus haplotype frequencies, and so on until all loci have been added. At each stage, any n-locus genotype which incorporates a n-locus haplotype with estimated frequency equal to zero is prevented from being extended to n+1 loci, because it is likely that the frequency of an extended (n+1)-locus haplotype would have also been equal to zero. With this method, Arlequin does not need to build all possible genotypes for each individual, but it only considers the genotypes whose sub-haplotypes have non-null frequencies, and one can thus handle a much larger number of polymorphic sites than the conventional EM algorithm.

In Arlequin's tab dialog (see section 6.3.8.4.2.2), one can specify if the loci should be added in random order or not, and how many random orders to implement. After multiple trials, Arlequin outputs the locus order having led to the largest likelihood.

This version of the EM algorithm is equivalent to that implemented in the SNPHAP program (http://www-gene.cimr.cam.ac.uk/clayton/software/snphap.txt) by David Clayton.

8.1.3.2.3 ELB algorithm

Contrary to the EM algorithm which aims at estimating haplotype frequencies, the ELB algorithm attempts at reconstructing the (unknown) gametic phase of multi-locus genotypes. Phase updates are made on the basis of a window of neighbouring loci, and the window size varies according to the local level of linkage disequilibrium.

Suppose that we have a sample of *n* individuals drawn from some population and genotyped at *S* loci whose chromosomal order is assumed known. Adjacent pairs of loci are assumed to be tightly linked, but *S* may be large so that the two external loci are effectively unlinked. In this case, reconstructing the gametic phase in one step can be inefficient, because recombination may have created too many distinct haplotypes for their frequencies to be well estimated. Locally, however, recombination may be rare and to exploit this situation the updates in ELB of the phase at a heterozygous locus are based on “windows” of neighboring loci. The algorithm adjusts the window sizes and locations in order to maximize the information for the phase updates.

ELB starts with an arbitrary phase assignment for all individuals in the sample.

Associated with each heterozygous locus is a window containing the locus itself and neighboring loci

At each iteration of the algorithm, an individual is chosen at random and its heterozygous loci are successively visited in random order. At each locus visit, two attempts are then made to update that window, by proposing, and then accepting or rejecting, (i) the

addition of a locus at one end of the window, and (ii) the removal of a locus at the other end. The locus being visited is never removed from the window, and each window always includes at least one other heterozygous locus. The two update proposals are made sequentially so that the window can either grow by one locus, shrink by one locus, or, if both changes are accepted, the window "slides" by one locus either to the right or the left. If both proposals are rejected, the window remains unchanged. Next, the phase at the locus being visited is updated based on the current haplotype pairs, within the chosen window, of the other individuals in the sample.

8.1.3.2.3.1 Phase updates

Let h_{11} and h_{22} denote the two haplotypes within the window given the current phase assignment, and let h_{12} and h_{21} denote the haplotypes which would result from the alternative phase assignment at the locus being visited. Ideally, we would wish to choose between the two haplotype assignments, h_{11}/h_{22} and h_{12}/h_{21}, with probabilities proportional to their (joint) population frequencies. These are unknown, and in practice they are too small for direct estimation to be feasible. To overcome the latter problem we assume HWE, so that we now seek to choose between h_{11}/h_{22} and h_{12}/h_{21} with probabilities proportional to $p_{11}p_{22}$ and $p_{12}p_{21}$, where p_{ij}, $i,j=1,2$, denotes the population frequency of h_{ij}. Although the p_{ij} are also unknown, we can estimate them using the n_{ij}, the haplotype counts among the other n-1 individuals in the sample, given their current phase assignments within the window.

Adopting a Bayesian posterior mean estimate of p_{ij} $p_{i'j'}$, based on a symmetric Dirichlet prior distribution for the p_{ij} with parameter $\alpha > 0$, and hence we propose

$$\Pr\left(h_{11}/h_{22}\,\middle|\,\{n_{ij}\}\right)=\frac{(n_{11}+\alpha)(n_{22}+\alpha)}{(n_{11}+\alpha)(n_{22}+\alpha)+(n_{12}+\alpha)(n_{21}+\alpha)}. \qquad (1)$$

Larger values of α imply a greater chance of choosing a haplotype pair that includes an unobserved haplotype. A small values of α=0.01 has been show to perform well by simulation in most circumstances.

Current phase in selected window

ACCT**C**GCCT
GCTA**T**CTAG

Switch phase update

ACCT***T***GCCT
GCTA***C***CTAG

8.1.3.2.3.2 Recombination update

Instead of performing a switch update as before, we can also update the phase using a recombination update, like:

Current phase in selected window
ACCT**C**GCCT
GCTA**T**CTAG

Right recombination phase update
ACCT**T***CTAG*
GCTA**C***GCCT*

In that case, we choose to change the phase of all sites either located on the right or on the left of the focal site. The proportion of updates being recombination steps can be set up in ELB tab dialog as shown in section 6.3.8.4.2.1. A small value is in order (less than 5%) since it implies a large change which may often be rejected, and cause the chain not to mix properly. The rationale for this kind update (initially not described in Excoffier et al (2003) is to more largely explore the set of possible gametic phase by provoking a radical change from time to time.

8.1.3.2.3.3 Handling mutations

Increasing α thus allows more flexibility to choose new haplotypes, but this is a "noisy" solution: all unobserved haplotypes are treated the same. However, a recent mutation event can create haplotypes that are rare, but similar to a more common haplotype, whereas haplotypes that are very dissimilar to all observed haplotypes are highly implausible. This phenomenon is particularly prevalent for STR loci, with their relatively high mutation rates.

To encapsulate the effect of mutation, when making a phase assignment we give additional weight to an unobserved haplotype for each observed haplotype that is "close" to it. Here, we define "close" to mean "differs at one locus", and in the phase update we choose h_{11}/h_{22} rather than h_{12}/h_{21} with probability

$$\Pr\left(h_{11}/h_{22}\,\middle|\,\{n_{ij}, n_{ij\_1}\}\right) = \frac{(n_{11}+\alpha+\varepsilon\, n_{11\_1})(n_{22}+\alpha+\varepsilon\, n_{22\_1})}{(n_{11}+\alpha+\varepsilon\, n_{11\_1})(n_{22}+\alpha+\varepsilon\, n_{22\_1})+(n_{12}+\alpha+\varepsilon\, n_{12\_1})(n_{21}+\alpha+\varepsilon\, n_{21\_1})}, \qquad (2)$$

where n_{ij_1} is the sample count of haplotypes that are close to h_{ij} within the current window. Since ε is a parameter reflecting the effect of mutation, it should for example be larger for STR than for SNP or DNA data. By simulation we have found that a value of ε=0.1 gave good results for STR (microsatellite) data, and a value of ε=0.01 for other data types worked well.

8.1.3.2.3.4 Sliding window size updates

The value of $R = \max\{r, 1/r\}$, where $r = p_{11}p_{22}/p_{12}p_{21}$, gives a measure of linkage disequilibrium (LD) within the window. Broadly speaking, at each choice between two windows, we would generally prefer the window that gives the largest value to *R*. Based on (2), a natural estimate of *r* is

$$\left[(n_{11}+\alpha+\varepsilon\, n_{11\_1})(n_{22}+\alpha+\varepsilon\, n_{22\_1})\right] \Big/ \left[(n_{12}+\alpha+\varepsilon\, n_{12\_1})(n_{21}+\alpha+\varepsilon\, n_{21\_1})\right],$$

but this estimate leads to difficulties, since larger windows tend to have smaller counts and hence more extreme estimates, amounting to a "bias" towards larger windows. This bias could be counteracted by increasing α but we prefer to adjust α to optimize the phase updates probability (2). Instead, we add a constant (γ) to both numerator and denominator leading to:

$$\hat{r} = \frac{(n_{11}+\alpha+\varepsilon\, n_{11\_1})(n_{22}+\alpha+\varepsilon\, n_{22\_1})+\gamma}{(n_{12}+\alpha+\varepsilon\, n_{12\_1})(n_{21}+\alpha+\varepsilon\, n_{21\_1})+\gamma} \qquad (3)$$

Thus, at each attempt to update the length of a window in step 3) above, we choose between windows according to their $\hat{R} = \max\left\{\hat{r}, \frac{1}{\hat{r}}\right\}$ values: window 2 replaces window 1 with probability

$$\hat{\rho} = \frac{\hat{R}_2}{\hat{R}_1 + \hat{R}_2}. \qquad (4)$$

Even a large value for γ can fail to prevent a window from growing too large when two consecutive heterozygous loci in an individual are separated by many homozygous loci. The window must then be large in order to contain the necessary minimum of two heterozygous loci. To circumvent the problem of small haplotype counts which may then result, when updating an individual's phase allocation, we can ignore homozygous loci that are separated from the nearest heterozygous locus by more than an given number of intervening homozygous loci. This is the parameter called "*Heterozygous site influence zone*" to be chosen in ELB tab dialog in section 6.3.8.4.2.1.

8.1.3.2.3.5 Handling missing data

In handling missing data, the philosophy underpinning ELB is to ignore the affected loci rather than to impute missing data or to augment the space of possible genotypes. In the presence of missing data, the haplotype "counts" n_{ij} and n_{ij_1} are not necessarily integers: individuals with missing data at *m* loci within a current window of length *L* contribute 1-*m*/*L* to n_{ij} (or n_{ij_1}) for each haplotype at which the remaining *L*-*m* loci match h_{ij} exactly (or with one mismatch).

Reference:

Excoffier et al. (2003)

8.1.4 Linkage disequilibrium between pairs of loci

Depending on whether the haplotypic composition of the sample is known or not, we have implemented two different ways to test for the presence of pairwise linkage disequilibrium between loci.

We describe in detail below how the two tests are done.

8.1.4.1 Exact test of linkage disequilibrium (haplotypic data)

This test is an extension of Fisher exact probability test on contingency tables (*Slatkin*, 1994a). A contingency table is first built. The k_1xk_2 entries of the table are the observed haplotype frequencies (absolute values), with k_1 and k_2 being the number of alleles at locus 1 and 2, respectively. The test consists in obtaining the probability of finding a table with the same marginal totals and which has a probability equal or less than the observed table. Under the null-hypothesis of no association between the two tested loci, the probability of the observed table is

$$L_0 = \frac{n\ !}{\prod_{i,j} n_{ij}!} \prod_i (n_{i*}/n)^{n_{i*}} \prod_j (n_{*j}/n)^{n_{*j}} ,$$

where the n_{ij}'s denote the count of the haplotypes that have the i-th allele at the first locus and the j-th allele at the second locus, n_{i*} is the overall frequency of the i-th allele at the first locus (i=1,... k_1) and n_{*i} is the count of the i-th allele at the second locus (i=1,... k_2).

Instead of enumerating all possible contingency tables, a Markov chain is used to efficiently explore the space of all possible tables. This Markov chain consists in a random walk in the space of all contingency tables. It is done is such a way that the probability to visit a particular table corresponds to its actual probability under the null hypothesis of linkage equilibrium. A particular table is modified according to the following rules (see also Guo and Thompson, 1992; or Raymond and Rousset, 1995) :

1) We select in the table two distinct lines i_1, i_2 and two distinct columns j_1, j_2 at random.
2) The new table is obtained by decreasing the counts of the cells (i_1, j_1) (i_2, j_2) and increasing the counts of the cells (i_1, j_2) (i_2, j_1) by one unit. This leaves the marginal allele counts n_i unchanged.
3) The switch to the new table is accepted with a probability equal to

$$R = \frac{L_1}{L_0} = \frac{(n_{i_1,j_2}+1)(n_{i_2,j_1}+1)}{n_{i_1,j_1}\, n_{i_2,j_2}},$$

where R is just the ratio of the probabilities of the two tables.

The steps 1-3 are done a large number of times to explore a large amount of the space of all possible contingency tables having identical marginal counts. In order to start from a random initial position in the Markov chain, the chain is explored for a pre-defined number of steps (the dememorization phase) before the probabilities of the switched tables are compared to that of the initial table. The number of dememorization steps should be enough (some thousands) such as to allow the Markov chain to "forget" its initial state, and make it independent from its starting point. The *P*-value of the test is then taken as the proportion of the visited tables having a probability smaller or equal to the observed contingency table.

A standard error on *P* is estimated by subdividing the total amount of required steps into B batches (see Guo and Thompson, 1992, p. 367). A *P*-value is calculated separately for each batch. Let us denote it by P_i (i=1,...,B). The estimated standard error is then calculated as

$$s.d.(P) = \sqrt{\frac{\sum_{i=1}^{B}(P-P_i)^2}{B(B-1)}}.$$

The process is stopped as soon as the estimated standard deviation is smaller than a pre-defined value specified by the user.

Reference:

Raymond and Rousset (1995)

8.1.4.2 Likelihood ratio test of linkage disequilibrium (genotypic data, gametic phase unknown)

For genotypic data where the haplotypic phase is unknown, the test based on the Markov chain described above is not possible because the haplotypic composition of the sample is unknown, and is just estimated. Therefore, linkage disequilibrium between a pair of loci is tested for genotypic data using a likelihood-ratio test, whose empirical distribution is obtained by a permutation procedure (Slatkin and Excoffier, 1996). The likelihood of the data assuming linkage equilibrium (L_{H*}) is computed by using the fact that, under this hypothesis, the haplotype frequencies are obtained as the product of the allele frequencies. The likelihood of the data *not* assuming linkage equilibrium (L_H) is obtained by applying the EM algorithm to estimate haplotype frequencies. The likelihood-ratio statistic given by

$$S = -2\log(\frac{L_{H^*}}{L_H})$$

should in principle follow a Chi-square distribution, with (k_1-1) (k_2-1) degrees of freedom, but it is not always the case in small samples with large number of alleles per locus. In order to better approximate the underlying distribution of the likelihood-ratio statistic under the null hypothesis of linkage equilibrium, we use the following permutation procedure:

1) Permute the alleles between individuals at one locus only.
2) Re-estimate the likelihood of the data L_H' by the EM algorithm. Note that $L_{H*}$is unaffected by the permutation procedure.
3) Repeat steps 1-2 a large number of times to get the null distribution of L_H , and therefore the null distribution of S.

Note that this test of linkage disequilibrium assumes Hardy-Weinberg proportions of genotypes, and the rejection of the test could be also due to departure from Hardy-Weinberg equilibrium (see Excoffier and Slatkin, 1998)

Reference:

Excoffier and Slatkin (1998)

8.1.4.3 Measures of gametic disequilibrium (haplotypic data)

- ***D*, *D'* , and r^2 coefficients**:

  Note that these coefficients are computed between all pairs of alleles at different loci, and that their computation assumes that the gametic phase between alleles at different loci is known .

  1) D: The classical linkage disequilibrium coefficient measuring deviation from random association between alleles at different loci (Lewontin and Kojima, 1960) is expressed as

  $$D_{ij} = p_{ij} - p_i p_j ,$$

  where p_{ij} is the frequency of the haplotype having allele i at the first locus and allele j at the second locus, and p_i and p_j are the frequencies of alleles i and j, respectively.

  2) D'_{ij} : The linkage disequilibrium coefficient D_{ij} standardized by the maximum value it can take ($D_{ij,\max}$), given the allele frequencies (Lewontin 1964), as

  $$D'_{ij} = \frac{D_{ij}}{D_{ij,\max}} ,$$

  where $D_{ij,\max}$ takes one of the following values:

  $$\min(p_i p_j , (1-p_i)(1-p_j)) \quad \text{if} \quad D_{ij} < 0$$

  $$\min((1-p_i) p_j , p_i (1-p_j)) \quad \text{if} \quad D_{ij} > 0$$

  3) r^2 : Another conventional measure of linkage disequilibrium between pairs of alleles at two loci is the square of the correlation coefficient between allele frequencies, which can be expressed as a function of the linkage disequilibrium measure *D* as

  $$r^2 = \frac{D^2}{p_i(1-p_i) p_j (1-p_j)} .$$

8.1.5 Hardy-Weinberg equilibrium.

To detect significant departure from Hardy-Weinberg equilibrium, we follow the procedure described in Guo and Thompson (1992) using a test analogous to Fisher's

exact test on a two-by-two contingency table, but extended to a triangular contingency table of arbitrary size. The test is done using a modified version of the Markov-chain random walk algorithm described Guo and Thomson (1992). The modified version gives the same results than the original one, but is more efficient from a computational point of view.

This test is obviously only possible for genotypic data. If the gametic phase is unknown, the test is only possible for each locus separately. For data with known gametic phase, it is also possible to test for the non random association of haplotypes into individuals. Note that this test assumes that the allele frequencies are given. Therefore, this test is not possible for data with recessive alleles, as in this case the allele frequencies need to be estimated.

A contingency table is first built. The kxk entries of the table are the observed allele frequencies and k is the number of alleles. Using the same notations as in section 8.2.2, the probability to observe the table under the null-hypothesis of no association is given by Levene (1949)

$$L_0 = \frac{n!\prod_{i=1}^{k} n_{i*}!}{(2n)!\prod_{i=1}^{k}\prod_{j=1}^{i} n_{ij}!}\,2^H ,$$

where H is the number of heterozygote individuals.

Much like it was done for the test of linkage disequilibrium, we explore alternative contingency tables having same marginal counts. In order to create a new contingency table from an existing one, we select two distinct lines i_1, i_2 and two distinct columns j_1, j_2 at random. The new table is obtained by decreasing the counts of the cells (i_1, j_1) (i_2, j_2) and increasing the counts of the cells (i_1, j_2) (i_2, j_1) by one unit. This leaves the alleles counts n_i unchanged. The switch to the new table is accepted with a probability R equal to :

1) $$R = \frac{L_{n+1}}{L_n} = \frac{n_{i_1 j_1} n_{i_2 j_2}}{(n_{i_1 j_2}+1)(n_{i_2 j_1}+1)} \frac{(1+\delta_{i_1 j_1})(1+\delta_{i_2 j_2})}{(1+\delta_{i_1 j_2})(1+\delta_{i_2 j_1})}, \text{ if } \; i_1 \neq j_1 \text{ or } i_2 \neq j_2$$

2) $$R = \frac{L_{n+1}}{L_n} = \frac{n_{i_1 j_1} n_{i2\,j2}}{(n_{i_1 j_2}+1)(n_{i_2 j_1}+2)} \frac{4}{1}, \text{ if } \; i_1 = j_1 \text{ and } i_2 = j_2$$

3) $R = \frac{L_{n+1}}{L_n} = \frac{n_{i_1 j_1}(n_{i_2 j_2} - 1)}{(n_{i_1 j_2} + 1)(n_{i_2 j_1} + 1)} \frac{1}{4}$, if $i_1 = j_2$ and $i_2 = j_1$.

As usual δ denotes the Kronecker function. R is just the ratio of the probabilities of the two tables. The switch to the new table is accepted if R is larger than 1.

The P-value of the test is the proportion of the visited tables having a probability smaller or equal to the observed (initial) contingency table. The standard error on the P-value is estimated like in the case of linkage disequilibrium using a system of batches (see section 8.1.4.1).

Reference:

Guo and Thomson (1992)

8.1.6 Neutrality tests.

8.1.6.1 Ewens-Watterson homozygosity test

This test is based on Ewens (1972) sampling theory of neutral alleles. Watterson (1978) has shown that the distribution of selectively neutral haplotype frequencies could be conveniently summarized by the sum of haplotype (allele) frequencies (F), equivalent to the expected homozygosity for diploids. This test can be performed equally well on diploid or haploid data, as the test statistic is not used for its biological meaning, but just as a way to summarize the allelic frequency distribution. The null distribution of F is generated by simulating random neutral samples having the same number of genes and the same number of haplotypes using the algorithm of Stewart (1977). The probability $p = \Pr(F_{sim} \leq F_{obs})$ of observing random samples with F values identical or smaller than the original sample is recorded and output. Note that this probability is not a p-value, as observed data having very large F value will be associated with a high p but can still be considered as significant (i.e. if p>0.95). This tests is currently limited to sample sizes of 2000 genes or less and 1000 different alleles (haplotypes) or less. It can be used to test the hypothesis of selective neutrality and population equilibrium against either balancing selection or the presence of advantageous alleles.

References:

Ewens (1972)

Watterson (1978)

8.1.6.2 Ewens-Watterson-Slatkin exact test

This test is essentially similar to that of Watterson (1978) test, but instead of using F as a summary statistic, it compares the probabilities of the random samples to that of the observed sample (Slatkin 1994b, 1996). The probability of obtaining a random sample

having a probability smaller or equal to the observed sample is recorded. The results are in general very close to those of Watterson's homozygosity test. Note that the random samples are generated as explained for the Ewens-Watterson homozygosity test.

References:

Ewens (1972)

Slatkin (1994b, 1996)

8.1.6.3 Chakraborty's test of population amalgamation

This test is also based on the infinite-allele model, and on Ewens (1972) sampling theory of neutral alleles. By simulation, Chakraborty (1990) has noticed that the number of alleles in a heterogeneous sample (drawn from a population resulting from the amalgamation of previously isolated populations) was larger than the number of alleles expected in a homogeneous neutral sample. He also noticed that the homozygosity of the sample was less sensitive to the amalgamation and therefore proposed to use the mutation parameter inferred from the homozygosity (θ_{Hom}) (see section 8.1.2.3.1) to compute the probability of observing a random neutral sample with a number of alleles similar or larger than the observed value ($\Pr(K \geq k_{obs})$ (see section 8.1.2.3.3 to see how this probability can be computed). It is an approximation of the conditional probability of observing some number of alleles given the observed homozygosity.

References:

Ewens (1972)

Chakraborty (1990)

8.1.6.4 Tajima's test of selective neutrality

Tajima's (1989a) test is based on the infinite-site model without recombination, appropriate for short DNA sequences or RFLP haplotypes. It compares two estimators of the mutation parameter theta ($\theta = 2Mu$, with M=2N in diploid populations or M=N in haploid populations of effective size N). The test statistic D is then defined as

$$D = \frac{\hat{\theta}_\pi - \hat{\theta}_S}{\sqrt{Var(\hat{\theta}_\pi - \hat{\theta}_S)}},$$

where $\hat{\theta}_\pi = \hat{\pi}$ and $\hat{\theta}_S = S / \sum_{i=0}^{n-1} (1/i)$, and S is the number of segregating sites in the sample. The limits of confidence intervals around D may be found in Table 2 of Tajima's paper (Tajima 1989a) for different sample sizes.

The significance of the *D* statistic is tested by generating random samples under the hypothesis of selective neutrality and population equilibrium, using a coalescent simulation algorithm adapted from Hudson (1990). The P value of the *D* statistic is then obtained as the proportion of random F_S statistics less or equal to the observation. We also provide a parametric approximation of the *P*-value assuming a beta-distribution limited by minimum and maximum possible D values (see Tajima 1989a, p.589). Note that significant *D* values can be due to factors other than selective effects, like population expansion, bottleneck, or heterogeneity of mutation rates (see Tajima, 1993; Aris-Brosou and Excoffier, 1996; or Tajima 1996, for further details).

References:

Tajima *(*1993)

Aris-Brosou and Excoffier (1996)

Tajima (1996)

8.1.6.5 Fu's F_S test of selective neutrality

Like *Tajima's* (1989a) test, Fu's test (Fu, 1997) is based on the infinite-site model without recombination, and thus appropriate for short DNA sequences or RFLP haplotypes. The principle of the test is very similar to that of Chakraborty described above. Here, we evaluate the probability of observing a random neutral sample with a number of alleles similar or smaller than the observed value (see section 8.1.2.3.3 to see how this probability can be computed) given the observed number of pairwise differences, taken as an estimator of θ. In more details, Fu first calls this probability $S' = \Pr(K \geq k_{obs} \mid \theta = \hat{\theta}_\pi)$ and defines the F_S statistic as the logit of *S*'

$$F_S = \ln\left(\frac{S'}{1-S'}\right) \qquad \text{(Fu, 1997)}$$

Fu (1997) has noticed that the F_S statistic was very sensitive to population demographic expansion, which generally lead to large negative F_S values.

The significance of the F_S statistic is tested by generating random samples under the hypothesis of selective neutrality and population equilibrium, using a coalescent simulation algorithm adapted from Hudson (1990). The P-value of the F_S statistic is then obtained as the proportion of random F_S statistics less or equal to the observation. Using simulations, Fu noticed that the 2% percentile of the distribution corresponded to the 5% cutoff value (i.e. the critical value of the test at the 5% significance level). We indeed confirmed this behavior by our own simulations. Even though this property is not fully understood, it means that a F_S statistic should be considered as significant at the 5% level, if its P-value is below 0.02, and not below 0.05.

Reference:

Fu (1997

8.2 Inter-population level methods

8.2.1 Population genetic structure inferred by analysis of variance (AMOVA)

The genetic structure of population is investigated here by an analysis of variance framework, as initially defined by Cockerham (1969, 1973), and extended by others (see e.g. Weir and Cockerham, 1984; Long 1986). The Analysis of Molecular Variance approach used in Arlequin (AMOVA, Excoffier et al. 1992) is essentially similar to other approaches based on analyses of variance of gene frequencies, but it takes into account the number of mutations between molecular haplotypes (which first need to be evaluated).

By defining groups of populations, the user defines a particular genetic structure that will be tested (see the input file notations for more details). A hierarchical analysis of variance partitions the total variance into covariance components due to intra-individual differences, inter-individual differences, and/or inter-population differences. See also Weir (1996), for detailed treatments of hierarchical analyses, and Excoffier (2000) as well as Rousset (2000) for an explanation why these are *covariance* components rather than *variance* components. The covariance components (σ_i^2's) are used to compute fixation indices, as originally defined by Wright (1951, 1965), in terms of inbreeding coefficients, or later in terms of coalescent times by Slatkin (1991).

Formally, in the haploid case, we assume that the *i*-th haplotype frequency vector from the *j*-th population in the *k*-th group is a linear equation of the form

$$\mathbf{x}_{ijk} = \mathbf{x} + \mathbf{a}_k + \mathbf{b}_{jk} + \mathbf{c}_{ijk} .$$

The vector **x** is the unknown expectation of $\mathbf{x}_{ijk}$, averaged over the whole study. The effects are **a** for group, **b** for population, and **c** for haplotypes within a population within a group, assumed to be additive, random, independent, and to have the associated covariance components σ_a^2, σ_b^2, and σ_c^2, respectively. The total molecular variance (σ^2) is the sum of the covariance component due to differences among haplotypes within a population (σ_c^2), the covariance component due to differences among haplotypes in different populations within a group (σ_b^2), and the covariance component

due to differences among the *G* populations (σ_a^2). The same framework could be extended to additional hierarchical levels, such as to accommodate, for instance, the covariance component due to differences between haplotypes within diploid individuals. Note that in the case of a simple hierarchical genetic structure consisting of haploid individuals in populations, the implemented form of the algorithm leads to a fixation index F_{ST} which is absolutely identical to the weighted average *F*-statistic over loci, $\hat{\theta}_w$, defined by Weir and Cockerham (1984) (see Michalakis and Excoffier 1996 for a formal proof). In terms of inbreeding coefficients and coalescence times, this F_{ST} can be expressed as

$$F_{ST} = \frac{f_0 - f_1}{1 - f_1} = \frac{\bar{t}_1 - \bar{t}_0}{\bar{t}_1}, \qquad \text{(Slatkin, 1991)}$$

where f_0 is the probability of identity by descent of two different genes drawn from the same population, f_1 is the probability of identity by descent of two genes drawn from two different populations, $\bar{t}_1$ is the mean coalescence times of two genes drawn from two different populations, and $\bar{t}_0$ is the mean coalescence time of two genes drawn from the same population.

The significance of the fixation indices is tested using a non-parametric permutation approach described in Excoffier et al. (1992), consisting in permuting haplotypes, individuals, or populations, among individuals, populations, or groups of populations. After each permutation round, we recompute all statistics to get their null distribution. Depending on the tested statistic and the given hierarchical design, different types of permutations are performed. Under this procedure, the normality assumption usual in analysis of variance tests is no longer necessary, nor is it necessary to assume equality of variance among populations or groups of populations. A large number of permutations (1,000 or more) is necessary to obtain some accuracy on the final probability. A system of batches similar to those used in the exact test of linkage disequilibrium (see end of section 8.1.4.1) has been implemented to get an idea of the standard-deviation of the P values.

We have implemented here 6 different types of hierarchical AMOVA. The number of hierarchical levels varies from two to four. In each of the situations, we describe the way the total sum of squares is partitioned, how the covariance components and the associated *F*-statistics are obtained, and which permutation schemes are used for the significance test.

Before enumerating all the possible situations, we introduce some notations:

SSD(*T*) : Total sum of squared deviations.

SSD (*AG*) : Sum of squared deviations Among Groups of populations.

SSD (*AP*) : Sum of squared deviations Among Populations.

SSD (*AI*) : Sum of squared deviations Among Individuals.

SSD (*WP*) : Sum of squared deviations Within Populations.

SSD (*WI*) : Sum of squared deviations Within Individuals.

SSD (*AP*/*WG*) : Sum of squared deviations Among Populations, Within Groups.

SSD (*AI*/*WP*) : Sum of squared deviations Among Individuals, Within Populations.

G : Number of groups in the structure.

P : Total number of populations.

N : Total number of individuals for genotypic data or total number of gene copies for haplotypic data.

N_p : Number of individuals in population p for genotypic data or total number of gene copies in population p for haplotypic data.

N_g : Number of individuals in group g for genotypic data or total number of gene copies in group g for haplotypic data..

8.2.1.1 Haplotypic data, one group of populations

Source of variation	Degrees of freedom	Sum of squares (SSD)	Expected mean squares
Among Populations	*P* - 1	SSD(*AP*)	$n\sigma_a^2 + \sigma_b^2$
Within Populations	*N* - *P*	SSD(*WP*)	σ_b^2
Total	*N* - 1	SSD(*T*)	σ_T^2

Where *n* and F_{ST} are defined by

$$n = \frac{N - \sum_p \frac{N_p^2}{N}}{P-1},$$

$$F_{ST} = \frac{\sigma_a^2}{\sigma_T^2}.$$

- We test σ_a^2 and F_{ST} by permuting haplotypes among populations.

8.2.1.2 Haplotypic data, several groups of populations

Source of variation	Degrees of freedom	Sum of squares (*SSD*)	Expected mean squares
Among Groups	*G* - 1	SSD(*AG*)	$n''\sigma_a^2 + n'\sigma_b^2 + \sigma_c^2$
Among Populations / Within Groups	*P* - *G*	SSD(*AP*/*WG*)	$n\sigma_b^2 + \sigma_c^2$
Within Populations	*N* - *P*	SSD(*WP*)	σ_c^2
Total:	*N* - 1	SSD(*T*)	σ_T^2

Where the *n*'s and the *F*-statistics are defined by:

$$S_G = \sum_{g \in G} \sum_{p \in g} \frac{N_p^2}{N_g}, \quad n = \frac{N - S_G}{P - G},$$

$$n' = \frac{S_G - \sum_{p \in P} \frac{N_p^2}{N}}{G - 1}, \quad n'' = \frac{N - \sum_{g \in G} \frac{N_g^2}{N}}{G - 1}$$

$$F_{CT} = \frac{\sigma_a^2}{\sigma_T^2}, \quad F_{SC} = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_c^2} \text{ and } F_{ST} = \frac{\sigma_a^2 + \sigma_b^2}{\sigma_T^2}$$

- We test σ_c^2 and F_{ST} by permuting haplotypes among populations among groups.
- We test σ_b^2 and F_{SC} by permuting haplotypes among populations within groups.
- We test σ_a^2 and F_{CT} by permuting populations among groups.

8.2.1.3 Genotypic data, one group of populations, no within- individual level

Source of variation	Degrees of freedom	Sum of squares (SSD)	Expected mean squares
Among Populations	*P* - 1	SSD(*AP*)	$n\sigma_a^2 + \sigma_b^2$
Within Populations	2*N* - *P*	SSD(*WP*)	σ_b^2
Total:	2*N* - 1	SSD(*T*)	σ_T^2

Where *n* and F_{ST} are defined by

$$n = \frac{2N - \sum_{P} \frac{2N_p^2}{N}}{P - 1},$$

$$F_{ST} = \frac{\sigma_a^2}{\sigma_T^2}.$$

If the gametic phase is know:

- We test σ_a^2 and F_{ST} by permuting haplotypes among populations.

If the gametic phase is unknown:

- We test σ_a^2 and F_{ST} by permuting individual genotypes among populations.

8.2.1.4 Genotypic data, several groups of populations, no within- individual level

Source of Variation	Degrees of freedom	Sum of squares (SSD)	Expected mean squares
Among Groups	*G* - 1	SSD(*AG*)	$n''\sigma_a^2 + n'\sigma_b^2 + \sigma_c^2$
Among Populations / Within Groups	*P* - *G*	SSD(AP/*WG*)	$n\sigma_b^2 + \sigma_c^2$
Within Populations	2*N* - *P*	SSD(*WP*)	σ_c^2
Total:	2*N* - 1	SSD(*T*)	σ_T^2

Where the *n*'s and the *F*-statistics are defined by:

$$S_G = \sum_{g \in G} \sum_{p \in g} \frac{2N_p^2}{N_g}, \quad n = \frac{2N - S_G}{P - G},$$

$$n' = \frac{S_G - \sum_{p \in P} \frac{2N_p^2}{N}}{G - 1}, \quad n'' = \frac{2N - \sum_{g \in G} \frac{2N_g^2}{N}}{G - 1},$$

$$F_{CT} = \frac{\sigma_a^2}{\sigma_T^2}, \quad F_{ST} = \frac{\sigma_a^2 + \sigma_b^2}{\sigma_T^2} \quad \text{and} \quad F_{SC} = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_c^2}.$$

If the gametic phase is known:

- We test σ_c^2 and F_{ST} by permuting haplotypes among populations and among groups.
- We test σ_b^2 and F_{SC} by permuting haplotypes among populations but within groups.

If the gametic phase is not known:

- We test σ_c^2 and F_{ST} by permuting individual genotypes among populations and among groups.
- We test σ_b^2 and F_{SC} by permuting individual genotypes among populations but within groups.

In all cases:

- We test σ_a^2 and F_{CT} by permuting whole populations among groups.

8.2.1.5 Genotypic data, one population, within- individual level

Source of variation	Degrees of freedom	Sum of squares (SSD)	Expected mean squares
Among Individuals	*N* - 1	SSD(*AI*)	$2\sigma_a^2 + \sigma_b^2$
Within Individuals	*N*	SSD(*WI*)	σ_b^2
Total:	2*N* - 1	SSD(*T*)	σ_T^2

Where F_{IS} is defined as:

$$F_{IS} = \frac{\sigma_a^2}{\sigma_T^2}.$$

- We test σ_a^2 and F_{IS} by permuting haplotypes among individuals.

8.2.1.6 Genotypic data, one group of populations, within- individual level

Source of Variation	Degrees of freedom	Sum of squares (SSD)	Expected mean squares
Among Populations	*P* - 1	SSD(*AP*)	$n\sigma_a^2 + 2\sigma_b^2 + \sigma_c^2$
Among Individuals / Within Populations	*N* – *P*	SSD(*AI*/*WP*)	$2\sigma_b^2 + \sigma_c^2$
Within Individuals	*N*	SSD(*WI*)	σ_c^2
Total	2*N* – 1	SSD(*T*)	σ_T^2

Where *n* and the *F*-statistics are defined by:

$$n = \frac{2N - \sum_{p \in P} \frac{2N_p^2}{N}}{P - 1}$$

$$F_{ST} = \frac{\sigma_a^2}{\sigma_T^2}, \quad F_{IT} = \frac{\sigma_a^2 + \sigma_b^2}{\sigma_T^2} \quad \text{and} \quad F_{IS} = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_c^2}.$$

- We test σ_c^2 and F_{IT} by permuting haplotypes among individuals among populations.
- We test σ_b^2 and F_{IS} by permuting haplotypes among individuals within populations.
- We test σ_a^2 and F_{ST} by permuting individual genotypes among populations.

8.2.1.7 Genotypic data, several groups of populations, within- individual level

Source of Variation:	Degrees of freedom	Sum of squares (SSD)	Expected mean squares
Among Groups	*G* - 1	SSD(*AG*)	$n''\sigma_a^2 + n'\sigma_b^2 + 2\sigma_c^2 + \sigma_d^2$
Among Populations / Within Groups	*P* – *G*	SSD(*AP*/*WG*)	$n\sigma_b^2 + 2\sigma_c^2 + \sigma_d^2$
Among Individuals / Within Populations	*N* - *P*	SSD(*AI*/*WP*)	$2\sigma_c^2 + \sigma_d^2$
Within Individuals	*N*	SSD(*WI*)	σ_d^2
Total:	2*N* – 1	SSD(*T*)	σ_T^2

Where the *n*'s and the *F*-statistics are defined by:

$$n = \frac{2N - \sum_{g \in G} \sum_{p \in g} \frac{2N_p^2}{N_g}}{P - G} \quad , \quad n' = \frac{\sum_{g \in G} \frac{(N - N_g)}{N_g} \sum_{p \in g} 2N_p^2}{N(G-1)} \quad , \quad n'' = \frac{2N - \frac{\sum_{g \in G} 2N_g^2}{N}}{G-1}$$

$$F_{CT} = \frac{\sigma_a^2}{\sigma_T^2}, \quad F_{IT} = \frac{\sigma_a^2 + \sigma_b^2 + \sigma_c^2}{\sigma_T^2} \quad , \quad F_{IS} = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_d^2} \quad \text{and} \quad F_{SC} = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_c^2 + \sigma_d^2}.$$

- We test σ_d^2 and F_{IT} by permuting haplotypes among populations and among groups.
- We test σ_c^2 and F_{IS} by permuting haplotypes among individuals within populations.
- We test σ_b^2 and F_{SC} by permuting individual genotypes among populations but within groups.
- We test σ_a^2 and F_{CT} by permuting populations among groups.

8.2.2 Minimum Spanning Network (MSN) among haplotypes

It is possible to compute the Minimum Spanning Tree (MST) and Minimum Spanning Network (MSN) from the squared distance matrix among haplotypes used for the calculation of F-statistics in the AMOVA procedure. See section 8.1.2.9 for a brief description of the method and references.

8.2.3 Locus-by-locus AMOVA

AMOVA analyses can now be performed for each locus separately in the same way it was performed at the haplotype level. Variance components and F-statistics are estimated for each locus separately and listed into a global table. The different variance components from different levels are combined to produce synthetic estimators of F-statistics, by summing variance components estimated at a given level in the hierarchy in the numerator and denominator to produce F-statistics as variance component ratios. Therefore the global F-statistics are not obtained as an arithmetic average of each locus F-statistics (see e.g. Weir and Cockerham 1984, or Weir 1996).

If there is no missing data, the locus-by-locus and the haplotype analyses should lead to identical sums of squares, variance components, and F-statistics. If there are missing data, the global variance components should be different, because the degrees of freedom will vary from locus to locus, and therefore the estimators of F-statistics will also vary.

8.2.4 Population pairwise genetic distances

The pairwise F_{ST}'s can be used as short-term genetic distances between populations, with the application of a slight transformation to linearize the distance with population divergence time (Reynolds et al. 1983; Slatkin, 1995).

The pairwise F_{ST} values are given in the form of a matrix.

The null distribution of pairwise F_{ST} values under the hypothesis of no difference between the populations is obtained by permuting haplotypes between populations. The *P*-value of the test is the proportion of permutations leading to a F_{ST} value larger or equal to the observed one. The *P*-values are also given in matrix form.

Three other matrices are computed from the F_{ST} values:

8.2.4.1 Reynolds' distance (Reynolds et al. 1983):

Since F_{ST} between pairs of stationary haploid populations of size N having diverged t generations ago varies approximately as

$$F_{ST} = 1-(1-\frac{1}{N})^t \approx 1-e^{-t/N}$$

The genetic distance $D = -\log(1-F_{ST})$ is thus approximately proportional to t/N for short divergence times.

8.2.4.2 Slatkin's linearized F_{ST}'s (Slatkin 1995):

Slatkin considers a simple demographic model where two haploid populations of size N have diverged τ generations ago from a population of identical size. These two populations have remained isolated ever since, without exchanging any migrants. Under such conditions, F_{ST} can be expressed in terms of the coalescence times $\bar{t}_1$, which is the mean coalescence time of two genes drawn from two different populations, and $\bar{t}_0$ which is the mean coalescence time of two genes drawn from the same population. Using the analysis of variance approach, the F_{ST}'s are expressed as

$$F_{ST} = \frac{\bar{t}_1 - \bar{t}_0}{\bar{t}_1} \qquad \text{(Slatkin, 1991, 1995)}$$

Because, $\bar{t}_0$ is equal to *N* generations (see e.g. Hudson, 1990), and $\bar{t}_1$ is equal to τ + *N* generations, the above expression reduces to

$$F_{ST} = \frac{\tau}{\tau + N}.$$

Therefore, the ratio $D = F_{ST}/(1-F_{ST})$ is equal to τ / N, and is therefore proportional to the divergence time between the two populations.

8.2.4.3 M values (M = Nm for haploid populations, M = 2Nm for diploid populations).

This matrix is computed under very different assumptions than the two previous matrices. Assume that two populations of size *N* drawn from a large pool of populations exchange a fraction *m* of migrants each generation, and that the mutation rate *u* is negligible as compared to the migration rate *m*. In this case, we have the following simple relationship at equilibrium between migration and drift,

$$F_{ST} = \frac{1}{2M+1}$$

Therefore, *M*, which is the absolute number of migrants exchanged between the two populations, can be estimated by

$$M = \frac{1-F_{ST}}{2F_{ST}}.$$

If one was to consider that the two populations only exchange with each other and with no other populations, then one should divide the quantity *M* by a factor 2 to obtain an estimator *M'* = *Nm* for haploid populations, or *M*'= 2*Nm* for diploid populations. This is because the expectation of F_{ST} is indeed given by

$$F_{ST} = \frac{1}{\frac{4Nmd}{(d-1)}+1}$$ (e.g. Slatkin 1991)

where *d* is the number of demes exchanging genes. When d is large this tends towards the classical value 1/(4*Nm* +1), but when *d*=2, then the expectation of F_{ST} is 1/(8*Nm*+1).

8.2.4.4 Nei's average number of differences between populations

As additional genetic distance between populations, we also provide Nei's raw (*D*) and net (D_A) number of nucleotide differences between populations (Nei and Li, 1979). *D* and net D_A are respectively computed between populations 1 and 2 as

$$D = \hat{\pi}_{12} = \sum_{i=1}^{k} \sum_{j=1}^{k'} x_{1i} x_{2j} \delta_{ij}$$, and

$$D_A = \hat{\pi}_{12} - \frac{\hat{\pi}_1 + \hat{\pi}_2}{2},$$

where k and k' are the number of distinct haplotypes in populations 1 and 2 respectively, x_{1i} is the frequency of the *i*-th haplotype in population 1, and δ_{ij} is the number of differences between haplotype *i* and haplotype *j*.

Under the same notation concerning coalescence times as described above, the expectation of D_A is

$$D_A = 2\mu \quad (\overline{t_1} - \overline{t_0}) = 2\mu\tau ,$$

where μ is the average mutation rate per nucleotide, τ is the divergence time between the two populations. Thus D_A is also expected to increase linearly with divergence times between the populations.

8.2.4.5 Genetic distance $(\delta\mu)^2$ (microsatellite data only)

For microsatellite data, Goldstein et al. (1995) have introduced $(\delta\mu)^2$, a measure of genetic distance between pairs of populations based on the Stepwise Mutation Model (SMM). The distance betweenpopulations A and B is simply defined as:

$$(\delta\mu)^2 = \left(\mu_A - \mu_B\right)^2 ,$$

where μ_A and μ_B are the average number of allelic size differences within populations A and B respectively, computed over all loci. Of course, the computation of these distances assumes that alleles are coded as a measure that is proportional to the number of motif repeats in the microsatellite.

Goldstein et al. (1995) have shown that, if one can assume that the two populations having diverged *T* generations ago are now at mutation drift equilibrium, and that they had the same mean repeat size at the time of their divergence, then the expected value of $(\delta\mu)^2$ is equal to 2*uT*, where *u* is the mutation rate per generation. $(\delta\mu)^2$ thus increases linearly with divergence time. Note however, that non-linearities arise in case of recent population size change.

8.2.4.6 Relative population sizes - Divergence between populations of unequal sizes

We have implemented a method to estimate divergence time between populations of unequal sizes (Gaggiotti and Excoffier 2000). The model assumes that two populations have diverged from an ancestral population of size N_0 some *T* generations in the past, and have remained isolated from each other ever since. The sizes of the two daughter populations can be different, but their sum adds up to the size of the ancestral population.

From the average number of pairwise differences between and within populations, we try to estimate the divergence time scaled by the mutation rate ($\tau = 2Tu$), the size of the ancestral population size scaled by the mutation rate ($\theta_0 = 2N_0u$ for haploid populations and $\theta_0 = 4N_0u$ for diploid populations), as well as the relative sizes (*k* and [1-*k*]) of the two daughter populations.

The estimated parameters result from the numerical resolution of a system of three non-linear equations with three unknowns, based on the Broyden method (Press et al. 1992, p.389).

The significance of the parameters is tested by a permutation procedure similar tot that used in AMOVA. Under the hypothesis that the two populations are undifferentiated, we permute individuals between samples, and re-estimate the
three parameters, in order to obtain their empirical null distribution. The percentile value of the three statistics is obtained by the proportion of permuted cases that produce statistics larger or equal to those observed. It thus provides a percentile value of the three statistics under the null hypothesis of no differentiation.

The values of the estimated parameters should be *interpreted with caution*. The procedure we have implemented is based on the comparison of intra and inter-population diversities (π's) which have a large variance, which means that for short divergence times, the average diversity found within population could be larger than that observed between populations. This situation could lead to negative divergence times and to daughter population relative size larger than one or smaller than zero (negative values). Also large departures from the assumed pure-fission model could also lead to observed diversities that would lead to aberrant estimators of divergence time and relative population sizes. One should thus make those computations if the assumptions of a pure fission model are met and if the divergence time is relatively old. Simulation results have shown that this procedure leads to better results than other methods that do not take unequal population sizes into account when the relative sizes of daughter populations are indeed unequal. According to our simulations (Table 4 in Gaggiotti and Excoffier 2000) conventional methods such as described above lead to better results for equal population size (k=0.5) and short divergence times (T/N_0<0.5). However, the fact that the present method leads to clearly aberrant results in some cases is not necessarily a drawback. It has the advantage to draw the user attention to the fact that some care has to be taken with the interpretations of the results. Some other estimators that would be grossly biased but whose values would be kept within reasonable bounds would often lead to misinterpretations.

Note that the numerical method we have used to resolve the system of equation may sometimes fail to converge. An asterisk will indicate those cases in the result file that should be discarded because of convergence failure.

8.2.5 Exact tests of population differentiation

We test the hypothesis of a random distribution of k different haplotypes or genotypes among r populations as described in Raymond and Rousset (1995). This test is analogous to Fisher's exact test on a 2x2 contingency table extended to a $r \times k$ contingency table. All potential states of the contingency table are explored with a Markov chain similar to that described for the case of the linkage disequilibrium test (section 8.1.4.1). During this random walk between the states of the Markov chain, we estimate the probability of

observing a table less or equally likely than the observed sample configuration under the null hypothesis of panmixia.

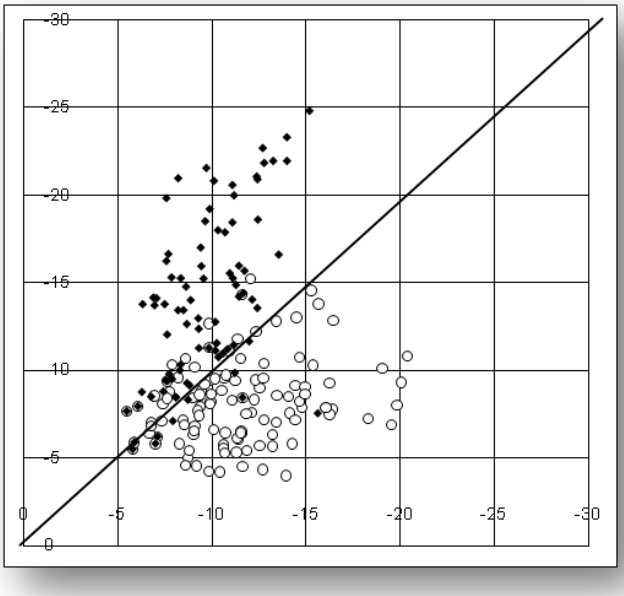
For haplotypic data, the table is built using sample haplotype frequencies (Raymond and Rousset 1995).

For genotypic data with unknown gametic phase, the contingency table is built from sample genotype frequencies (Goudet et al. 1996).

As it was done previously, an estimation of the error on the *P*-value is done by partitioning the total number of steps into a given number of batches (see section 8.1.4.1).

8.2.6 Assignment of individual genotypes to populations

It can be of interest to try to determine the origin of particular individuals, knowing a list of potential source populations (e.g. Rannala and Montain, 1997; Waser and Strobeck, 1998; Davies et al. 1999). The method we have implemented here is the most simplest one, as it consists in determining the log-likelihood of each individual multi-locus genotype in each population sample, assuming that the individual comes from that population. For computing the likelihood, we simply use the allele frequencies estimated in each sample from the original constitution of the samples. We also assume that all loci are independent, such that the global individual likelihood is obtained as the product of the likelihood at each locus. The method we have implemented is inspired from that described in Paetkau et al. (1995, 1997) and Waser and Strobeck (1998). The resulting output tables can be used to represent log-log plots of genotypes for pairs of populations likelihood (see Paetkau et al. 1997 and Waser and Strobeck 1998), to identify those genotypes that seem better explained by belonging to another population from that they were sampled.



For instance we have plotted on this graph the log-likelihood of individuals sampled in Algeria (white circles) for two HLA class II loci versus those of Senegalese Mandenka individuals (black diamonds). The overlap of the two distribution suggests that two loci are not enough to provide a clear cut separation between these two populations. One also sees that there is at least one Mandenka individual whose genotype would be much better explained if it came from the Algerian population than if it came from Eastern Senegal. Note that interpreting these results in terms of gene flow is difficult and hazardous.

8.2.7 Mantel test

The Mantel test consists in testing the significance of the correlation between two or more matrices by a permutation procedure allowing getting the empirical null distribution of the correlation coefficient taking into account the auto-correlations of the elements of the matrix. In more details, the testing procedure proceeds as follows:

Let's first define two square matrices $\mathbf{X} = \{x_{ij}\}$ and $\mathbf{Y} = \{y_{ij}\}$ of dimension N. The N^2 elements of these matrix are not all independent as there are only N-1 independent contrasts in the data. This is why the permutation procedure does not permute the elements of the matrices independently. The correlation of the two matrices is classically defined as

$$r_{XY} = \frac{SP(\mathbf{X},\mathbf{Y})}{\sqrt{SS(\mathbf{X}) \,.\, SS(\mathbf{Y})}},$$

the ratio of the cross product of **X** and **Y** over the square root of the product of sums of squares. We note that the denominator of the above equation is insensitive to permutation, such that only the numerator will change upon permutation of rows and columns. Upon closer examination, it can be shown that the only quantity that will actually change between permutations is the Hadamard product of the two matrices noted as

$$Z_{XY} = \mathbf{X} * \mathbf{Y} = \sum_{i=1}^{N} \sum_{j=1}^{i} x_{ij}\, y_{ij}$$

which is the only variable term involved in the computation of the cross-product.
The Mantel testing procedure applied to two matrices will then consist in computing the quantity Z_{XY} from the original matrices, permute the rows and column of one matrix while keeping the other constant, and each time recompute the quantity Z^*_{XY}, and compare it to the original Z_{XY} value (Smouse et al. 1986).
In the case of three matrices, say **Y**, $\mathbf{X_1}$ and $\mathbf{X_2}$, the procedure is very similar. The partial correlation coefficients are obtained from the pairwise correlations as,

$$r_{Y X_1 . X_2} = \frac{r_{YX_1} - r_{X_1 X_2} r_{YX_2}}{\sqrt{(1 - r^2_{X_1 X_2})(1 - r^2_{YX_2})}} .$$

The other relevant partial correlations can be obtained similarly (see e.g. Sokal and Rohlf 1981). The significance of the partial correlations are tested by keeping one matrix constant and permuting the rows and columns of the other two matrices, recomputing each time the new partial correlations and comparing it to the observation (Smouse et al. 1986). Applications of the Mantel test in anthropology and genetics can be found in Smouse and Long (1992).

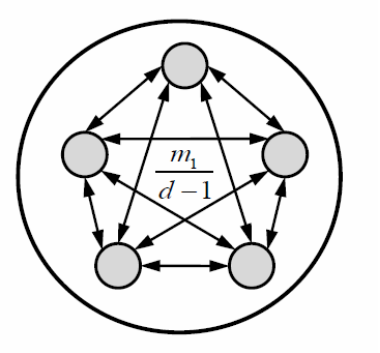
8.2.8 Detection of loci under selection from *F-statistics*

Several procedures have been proposed to detect loci under selection based on the patterns of genetic diversity found in a population, based on the observed pattern of diversity within populations, and several tests are indeed implemented in Arlequin (see e.g. section 8.1.6).
But, selection can also affect genetic diversity between populations, since a locus under balancing selection should show too even allele frequencies across populations and loci under local directional selection should show large differences between populations (Cavalli-Sforza 1966; Lewontin and Krakauer 1973). This observation has recently led to the development of several methods comparing levels of genetic diversity and differentiation within and between populations (see e.g. Beaumont and Nichols 1996; Schlotterer 2002; Beaumont and Balding 2004; Foll and Gaggiotti 2008; Excoffier et al. 2009).

8.2.8.1 Island model (FDIST approach)

Beaumont and Nichols(1996) proposed to obtain the distribution of F_{ST} across loci as a function of heterozygosity between populations by performing simulations under an finite island-model, and to specifically identify outlier loci as being those present in the tails of

the generated distribution. They have shown that this simple island model led to F_{ST} distributions that were very similar to those expected under alternative models, like scenarios of recent divergence and growth (colonisation), of isolation by distance (2-D stepping stone) or of heterogeneous levels of gene flow between populations. Their approach was implemented in the FDIST computer program, with some modifications.



The approach of Excoffier et al. (2009) implemented in Arlequin is similar to that in FDIST, where coalescent simulations are used to get a null distribution and confidence intervals around the observed values, and see if observed locus-specific F_{ST} values can be considered as outliers F_{ST} conditioned on the global observed F_{ST} value. The approach also assumes a finite island model where *d* demes of size *N* receive on average *Nm* new immigrant genes per generation, randomly chosen from all the other demes. Under this model, one expects the following relationship between the parameters of the island model and F_{ST}, as

$$F_{ST} = \frac{1}{1+\frac{4Nmd}{d-1}}$$ (Slatkin 1991)

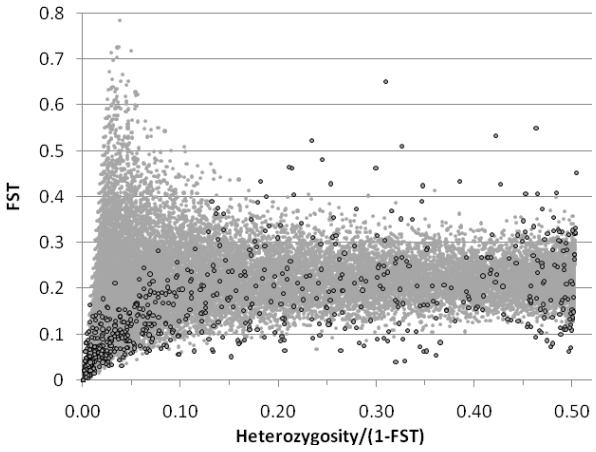
allowing one to estimate *m* from the above equation for a fixed number of simulated demes d and a fixed deme size. Mutations are then added under a given mutation model on top of the simulated coalescent tree to create genetic diversity, and to obtain the joint distribution of F_{ST} and heterozygosity between populations. In Arlequin, mutation models other than the finite site model are used, and for instance a specific SMM model is used for microsatellite data, and a specific SNP model is used for DNA sequences, with the possibility in the latter case to define a minimum frequency for the derived allele (DAF_{min}). It is also possible in Arlequin to compute Rho-statistics for microsatellite data (see Michalakis and Excoffier, 1996) instead of conventional F-statistics, which have been shown to lead to an unbiased distribution of F_{ST}. A final difference between the FDIST approach and that implemented in Arlequin is that the heterozygosity between populations $\hat{H}_1$ is inferred from the average heterozygosity within population $\hat{h}_0$ as

$$\hat{H}_1 = \hat{h}_0 / \left(1 - \hat{F}_{ST}\right)$$ (Excoffier et al, 2009).

Loci with variable heterozygosities are generated by modeling different mutation rates. For each simulation, we obtain a different mutation rate by drawing a target heterozygosity at random from a uniform distribution and use classical relationships between heterozygosity and scaled mutation rate $\theta = 4kdNu$ as $\theta = \left(1-H\right)^{-1} - 1$ under the IAM (Wright 1931), and $\theta = \frac{1}{2}\left[\left(1-H\right)^{-2} - 1\right]$ under the SMM model (Ohta and Kimura, 1973).

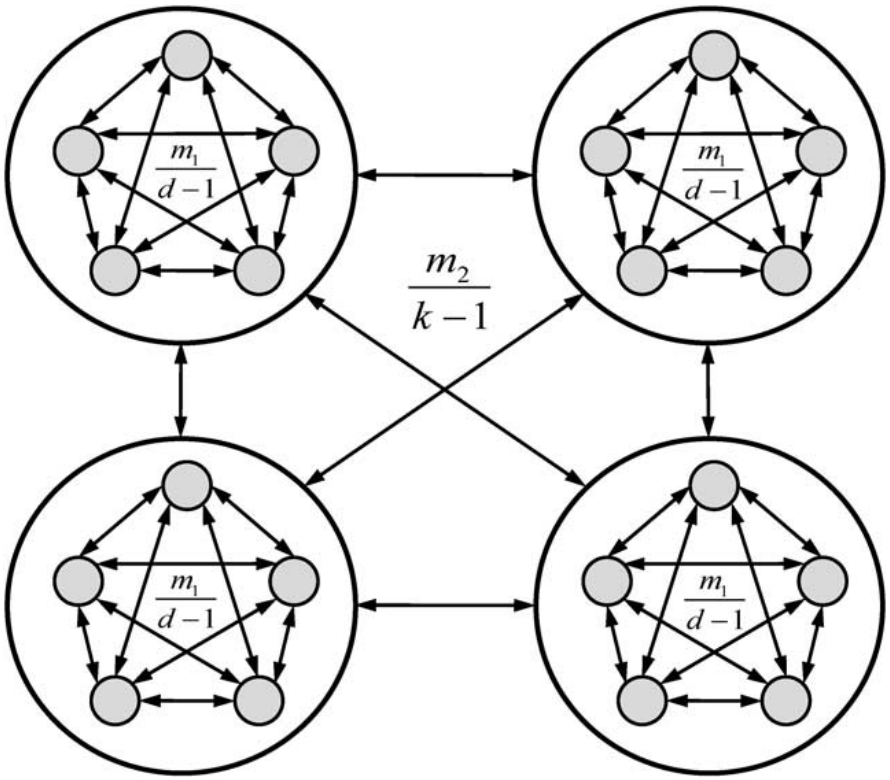
8.2.8.2 Hierarchical island model

The finite island model has been recently shown to lead to a large fraction of false positives, if populations samples belong to a hierarchically subdivided population or if some population samples have a recent shared history, such as after some range expansion over different continents (Excoffier et al. 2009). Intuitively, this can be understood by realizing that the precision in estimating FST should increase with the number of sampled populations, and therefore the confidence intervals around a given FST value should become narrower with the number of sampled populations. So, if some sampled populations are not independent units, but share a very recent common ancestry with some others, confidence intervals estimated by assuming all populations are equally related would be too narrow, and some loci will be false positives.



Excess of false positives (taken from Excoffier et al. 2009):
The excess of false positives occurring when samples drawn from a hierarchically structured population are analyzed under a finite island model is illustrated in the above figure. The diversity of 1,000 SNP loci (open circles) was simulated under a hierarchical island model with 10 groups of 100 demes. The joint null distribution of F_{ST} and Heterozygosity (30,000 grey dots) was then obtained under a finite island, leading to a large number of outlier loci.

In other to overcome this problem and to reduce the number of false positive loci, a hierarchical island model of population (as defined by Slatkin and Voelm, 1991) was used to model some heterogeneity in population affinities.



This model shown above, where demes within groups exchange migrants at rate $m_1/(d-1)$ and demes between groups exchange migrants at rate $m_2/(k-1)$ (where d is the number of demes within each group, and k is the number of groups) has been studied by Slatkin and Voelm (1991). They inferred relationship between the model parameters and expected G-statistics. Similar relationship can be inferred with hierarchical *F*-statistics (Excoffier et al. 2009) as shown below:

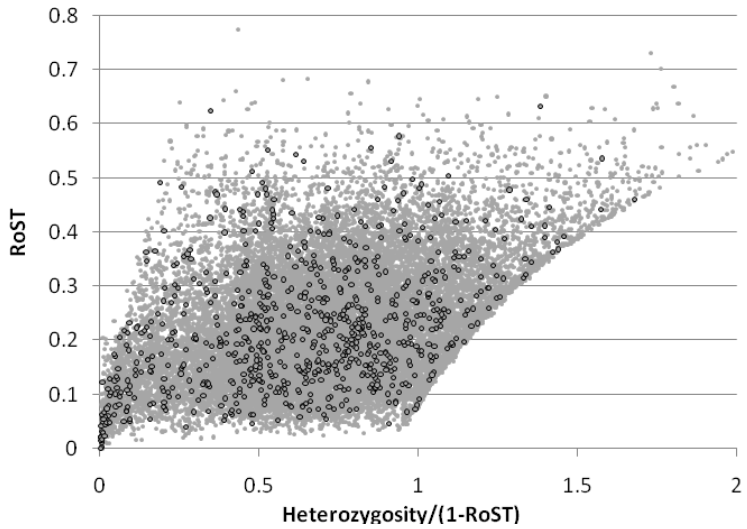
$$F_{SC} = \frac{1}{1+4Nm_1\frac{d}{d-1}}$$

$$F_{CT} = \frac{1}{1+4Nd\frac{k}{k-1}m_2+(d-1)\frac{k}{k-1}\frac{m_2}{m_1}}$$

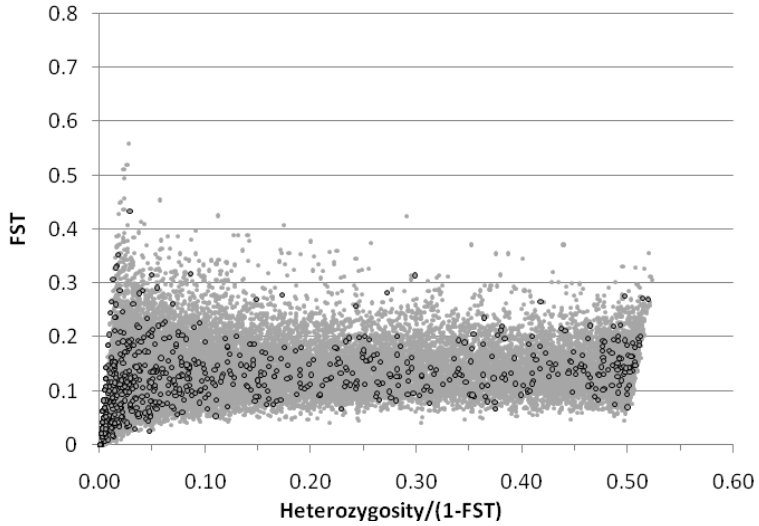
$$F_{ST} \approx \frac{1}{1+4Nd\frac{k}{(k-1)}m_2},$$

It follows that the parameters of a hierarchical island-model can be specified such as to have in expectation the observed *F*-statistics, and therefore that coalescent simulations can be used to simulate the null distribution of these statistics under the hierarchical island model.

For detecting outlier loci, **we advocate the use of F_{ST} and not F_{CT} as a test statistic**.



Example of F_{ST} distributions obtained for microsatellite loci (taken from Excoffier et al. 2009). The diversity of 1000 STR loci (open circles) was simulated under a hierarchical island model with 10 groups of 100 demes. The migration rates within and between groups were adjusted such as to have F_{SC} =0.05 and F_{CT}=0.2, implying an F_{ST} of 0.240. The joint null distribution of F_{ST} and Heterozygosity (20,000 grey dots) was then obtained under a hierarchical island based on F-statistics computed assuming a stepwise mutation model (RoST). Note that the x-axis representing the heterozygosity between populations can be larger than 1 since it is computed as the heterozygosity within population divided by (1-F_{ST}).



Example of F_{ST} distributions obtained for SNP data (taken from Excoffier et al. 2009). The diversity of 1,000 SNP loci (open circles) was simulated under a hierarchical island model with 10 groups of 100 demes. The joint null distribution of F_{ST} and Heterozygosity (30,000 grey dots) was then obtained under a hierarchical island.

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10 APPENDIX

10.1 Overview of input file keywords

Keywords	Description	Possible values
[Profile]		
Title	A title describing the present analysis	A string of alphanumeric characters within double quotes
NbSamples	The number of different samples listed in the data file	A positive integer larger than zero
DataType	The type of data to be analyzed (only one type of data per project file is allowed)	STANDARD, DNA, RFLP, MICROSAT, FREQUENCY
GenotypicData	Specifies if genotypic or gametic data is available	0 (haplotypic data), 1 (genotypic data)
LocusSeparator	The character used to separate adjacent loci	WHITESPACE, TAB, NONE, or any character other than "#", or the character specifying missing data Default: WHITESPACE
GameticPhase	Specifies if the gametic phase is known (for genotypic data only)	0 (gametic phase not known), 1 (known gametic phase) Default: 1
RecessiveData	Specifies whether recessive alleles are present at all loci (for genotypic data)	0 (co-dominant data), 1 (recessive data) Default: 0
RecessiveAllele	Specifies the code for the recessive allele	Any string within quotation marks This string can be explicitly used in the input file to indicate the occurrence of a recessive homozygote at one or several loci. Default: "null"
MissingData	A character used to specify the code for missing data	"?" or any character within quotes, other than those previously used Default: "?"
Frequency	Specifies the format of haplotype frequencies	ABS (absolute values), REL (relative values: absolute values will be found by multiplying the relative frequencies by the sample sizes) Default: ABS

Keywords	Description	Possible values
[Data]		
[[HaplotypeDefinition]]	(facultative section)	
HaplListName	The name of a haplotype definition list	A string within quotation marks
HaplList	The list of haplotypes listed within braces ({...})	A series of haplotype definitions given on separate lines for each haplotype. Each haplotype is defined by a haplotype label and a combination of alleles at different loci. The Keyword EXTERN followed by a string within quotation marks may be used to specify that a given haplotype list is in a different file

Keywords	Description	Possible values
[Data]		
[[DistanceMatrix]]	(facultative section)	
MatrixName	The name of the distance matrix	A string within quotation marks
MatrixSize	The size of the matrix	A positive integer larger than zero (corresponding to the number of haplotypes listed in the haplotype list)
LabelPosition	Specifies whether haplotypes labels are entered by row or by column	ROW (the haplotype labels will be entered consecutively on one or several lines, within the MatrixData segment, before the distance matrix elements), COLUMN (the haplotype labels will be entered as the first column of each row of the distance matrix itself)
MatrixData	The matrix data itself listed within braces ({...})	The matrix data will be entered as a format-free lower-diagonal matrix. The haplotype labels can be either entered consecutively on one or several lines (if LabelPosition=ROW), or entered at the first column of each row (if labelPosition=COLUMN). The special keyword EXTERN may be used followed by a file name within quotation marks, stating that the data must be read in an another file

Keywords	Description	Possible values
[Data]		
[[Samples]]		
SampleName	The name of the sample. This keyword is used to	A string within quotation marks

	mark the beginning of a sample definition	
SampleSize	Specifies the sample size	An integer larger than zero. For haplotypic data, it must specify the number of gene copies in the sample. For genotypic data, it must specify the number of individuals in the sample.
SampleData	The sample data listed within braces ({...})	The keyword EXTERN may be used followed by a file name within quotation marks, stating that the data must be read in a separate file. The SampleData keyword ends a sample definition

Keywords	Description	Possible values
[Data]		
[[Structure]]	(facultative section)	
StructureName	The name of a given genetic structure to test	A string of characters within quotation marks
NbGroups	The number of groups of populations	An integer larger than zero
Group	The definition of a group of samples, identified by their SampleName listed within braces ({...})	A series of strings within quotation marks all enclosed within braces, and, if desired, on separate lines

Keywords	Description	Possible values
[Data] **[[Mantel]]**	(facultative section) Allows computing the (partial) correlation between *YMatrix* and *X1* (*X2*).	
MatrixSize	The size of the matrix entered into the project	An integer larger than zero
YMatrix	Specifies which matrix is used as *YMatrix*.	"fst", "log_fst", "slatkinlinearfst", "log_slatkinlinearfst", "nm", "custom"
MatrixNumber	Number of matrices to be compared with the *YMatrix*.	1 :we compute the correlation between *YMatrix* and *X1* 2 :we compute the partial correlation between *YMatrix, X1*and *X2*
YMatrixLabels	Labels to identify the entries of the *YMatrix*. In case of *YMatrix*="fst", these labels should correspond to population names in the sample.	A series of strings within quotation marks all enclosed within braces, and, if desired, on separate lines
DistMatMantel	A keyword used to define a matrix, which can be either the Ymatrix, or another matrix that will be compared with the Ymatrix.	The matrix data will be entered as a format-free lower-diagonal matrix.
UsedYMatrixLabels	Labels defining the sub-matrix of the YMatrix on which the correlation is computed.	A series of strings within quotation marks all enclosed within braces, and, if desired, on separate lines