Manual for **Familias 3**

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Contents

i Preface

This document updates the documentation of the **Familias** software available at http://www.familias.no (previous versions can be found at http://familias.name) in connection with the 3.2 version released in February 2017. A complete list of changes and bug fixes appears on the home page. Additional material (lecture notes, exercises with solutions, videos etc.) are available at http://familias.name/book.html. Comments on the documentation or the program can be sent to daniel.l.kling@gmail.com. The book by Egeland, Mostad and Kling ((Egeland, Kling et al. 2015)contains complete details of the mathematical models and also provide more background and context to applications suited for **Familias**. Please help us improving this manual by sending suggestions whenever you cannot find an adequate description of the features.

A new section has been added (11) to cover some error handling and input checking performed by **Familias**.

ii News

Familias 3 (version 3.0 and above) includes a disaster victim identification module (DVI). In addition a blind search feature is implemented. Moreover a completely new interface to perform simulations is included. All is described in this manual.
Version 3.1.6 (and above) includes a Familial searching interface, briefly described in this manual. Several updates have been made so make sure to use the latest version. The paper (Kling and Füredi 2016) reports some real applications of **Familias** searching resulting in some serious crime cases being solved.

There is a separate software, **FamiliasPedigreeCreator**, freely available at http://www.familias.no (Download section) capable of preparing an R-script in turn producing plots for all **Familias** projects in a specific directory (and sub-directories). The plots are stored into png files that can be displayed in the software (version 3.2 and above) or inserted into a report.

iii Supported platforms

Familias runs on all Windows environments (tested on XP, 7, 8 and 10). For Mac users try a Windows emulator environment, see this site listing some commonly used emulators. Similar for users of other OS, the software should run on all Windows emulators.

1 Introduction

The **Familias** program may be used to compute probabilities and likelihoods in cases where DNA profiles of some people are known, but their family relationship is in doubt. Given several alternative family trees (or pedigrees) for a group of people, given DNA measurements from some of these people, and given a data base of DNA observations in the relevant population, the program may compute which pedigree is most likely, and how much more likely it is than others. Obviously, there are several other programs performing similar tasks. As far we know a distinguishing feature of **Familias** is its ability to handle complex cases where potential mutations, silent alleles and population stratification (θ-corrections) are accounted for, together with its ability to handle multiple pedigrees simultaneously. The program has been validated (Drabek 2009). The books(Buckleton, Triggs et al. 2005)and (Balding 2005)provide a general background to forensic genetics.

The original reference to **Familias** is (Egeland, Mostad et al. 2000) whereas (Kling, Tillmar et al. 2014) describes **Familias 3**. Several example data files, are available from the site http://familias.name. Online help including a short tutorial, is available directly from the help-function of the program.

Familias has been applied in a large number of cases, including identification following disasters, resolving family relations when incest is suspected and determining the most probable relation between a person applying for immigration and claimed relatives of the individual.

The contents of this document are as follows. Section 2 gives a brief introduction to program by means of a simple worked example. Next, Section 3 provides an overview of the options available in the program, along with suggestions for typical values for the various parameters. Some more theory and advanced options are presented in Appendix A1. Appendix A2contains links to old (**Familias** 2 or 1.97) and new (**Familias** 3) exercises with solutions. Appendices A3 and A4 describe advanced options. Finally, the file format of the input file is described in detail Appendix A5; this is only relevant for programmers as the purpose is to enable programmers to write code producing input files for the **Familias** program, on the "Familias format". There is open R version of the core of program http://cran.r-project.org/web/packages/Familias/ which facilitates extensions.

Regarding transferring data from GeneMapper® to **Familias,** Antonio Vozmediano (a.vozme@hotmail.es)and Lourdes Prieto (lourditasmt@gmail.com) have developed GeneMapperToFamilias which provides an alternative to more generic functionality described below. NB, **Familias** now accepts exported genotypes from GeneMapper as well.

2 Example – a preview of Familias

This section presents a very simple case. First, calculations are done by hand and then we demonstrate how the calculations are done using **Familias**.

We consider the following hypotheses concerning the relationship between a manAF and Child:

- *H_1: AF is the father of Child*
- *H_2: AF is not the father of Child*

An illustration of the hypothesised relationship is given in Figure 1. The mother is undisputed. Such illustrations denote men with squares and women with circles (Bennett, French et al. 2008).

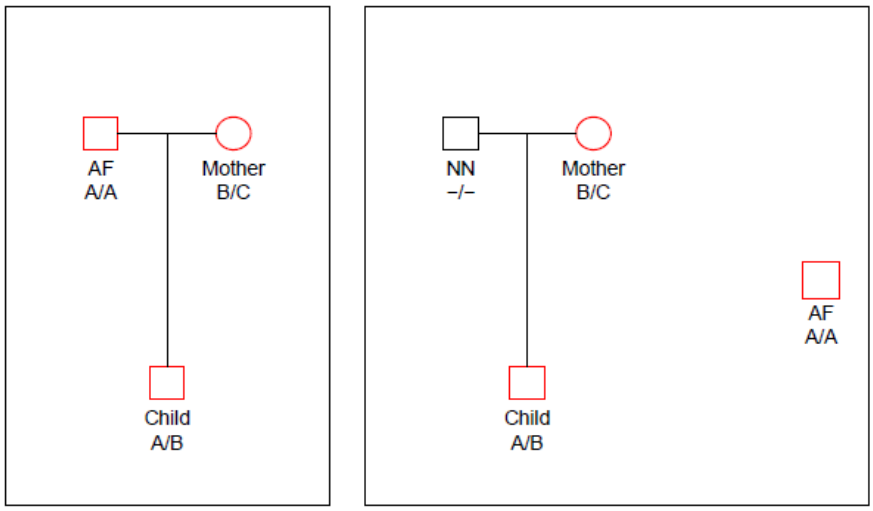


Figure 1. The pedigree corresponding to hypothesis H_1 (left) and H_2 (right)

The allele frequencies of A and B are $p_A = p_B = 0.05$ and Hardy-Weinberg equilibrium is assumed. The child has inherited the allele B from his mother and the allele A must be inherited from the father.

2.1 Calculation of the likelihood ratio (LR) by hand

The likelihood ratio is then given by

$$LR = \frac{P(\text{data} \mid H_1)}{P(\text{data} \mid H_2)} = \frac{P(\text{Child} = A/B \mid H_1)}{P(\text{Child} = A/B \mid H_2)} = \frac{1}{p_A} = \frac{1}{0.05} = 20.$$

2.2 Calculation of the likelihood ratio using Familias

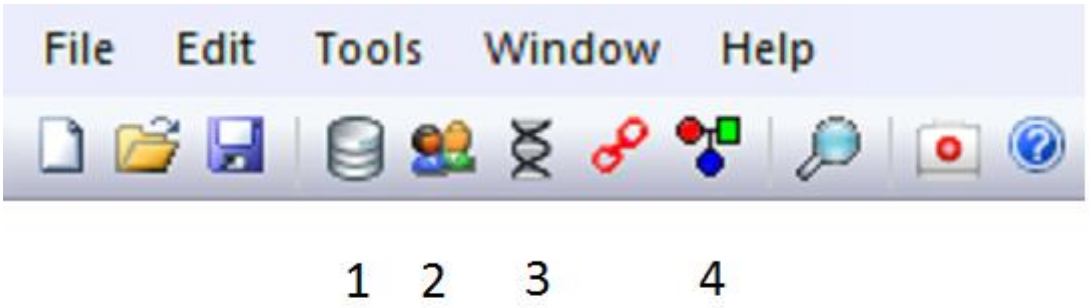


Figure 2. The main menu of Familias

The standard calculations in **Familias** are performed by going through the four steps indicated in Figure 2.

1. **General DNA data**. The window appearing after clicking  should be completed as shown in Figure 3 (only 'manual' is entered to indicate the database used; this has no impact on calculations).

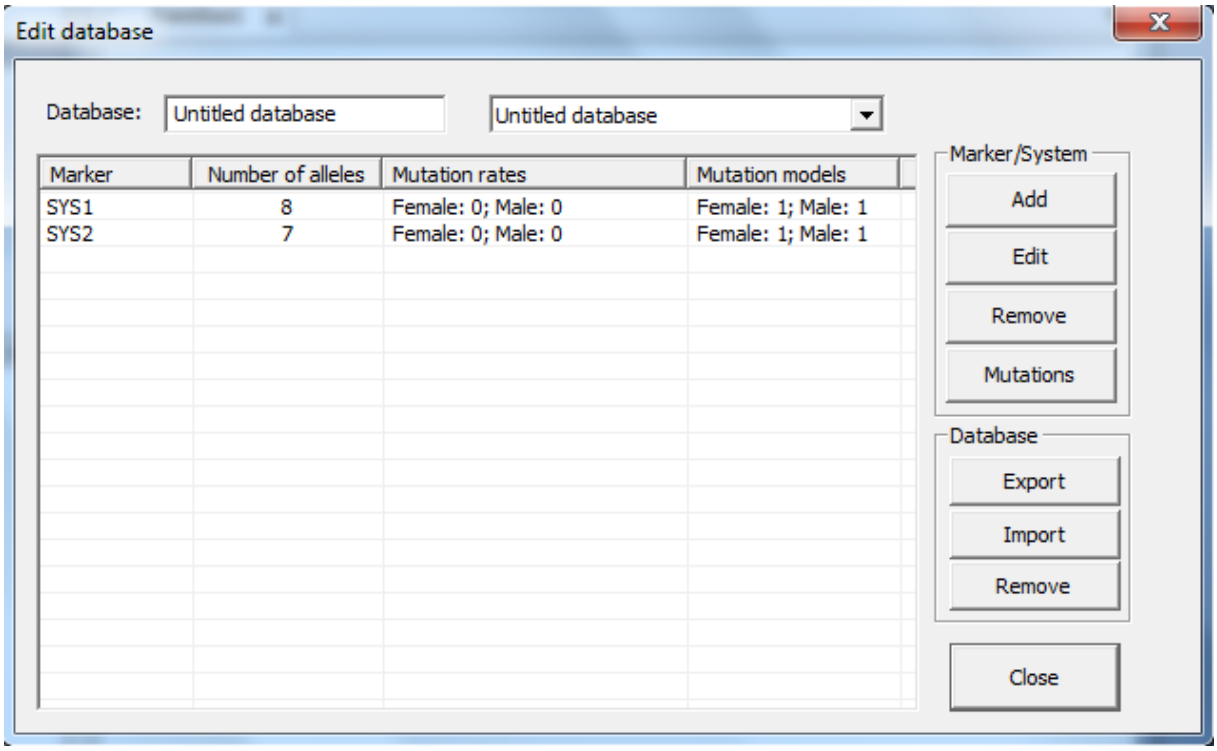


Figure 3. The General DNA data window.

2. **Allele system.** The window appearing after clicking Add should be completed as shown in Figure 4.

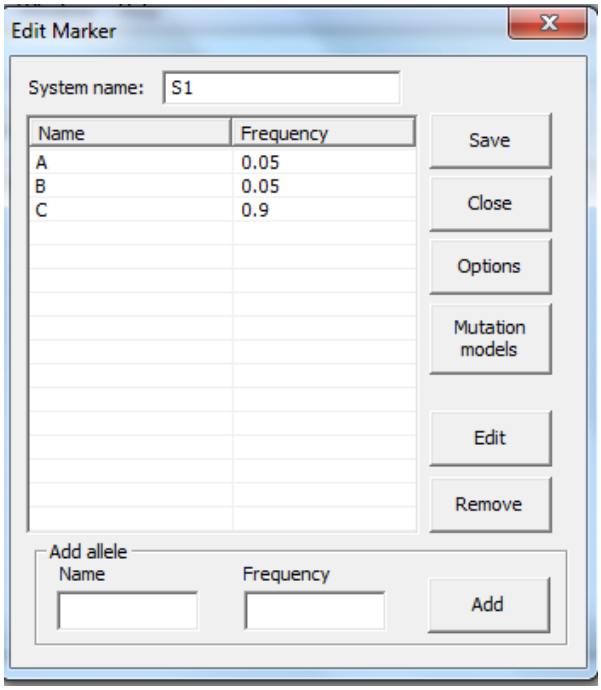


Figure 4. The Allele System window.

3. **Persons.** The window appearing after clicking  should be completed as shown in Figure 5 below.

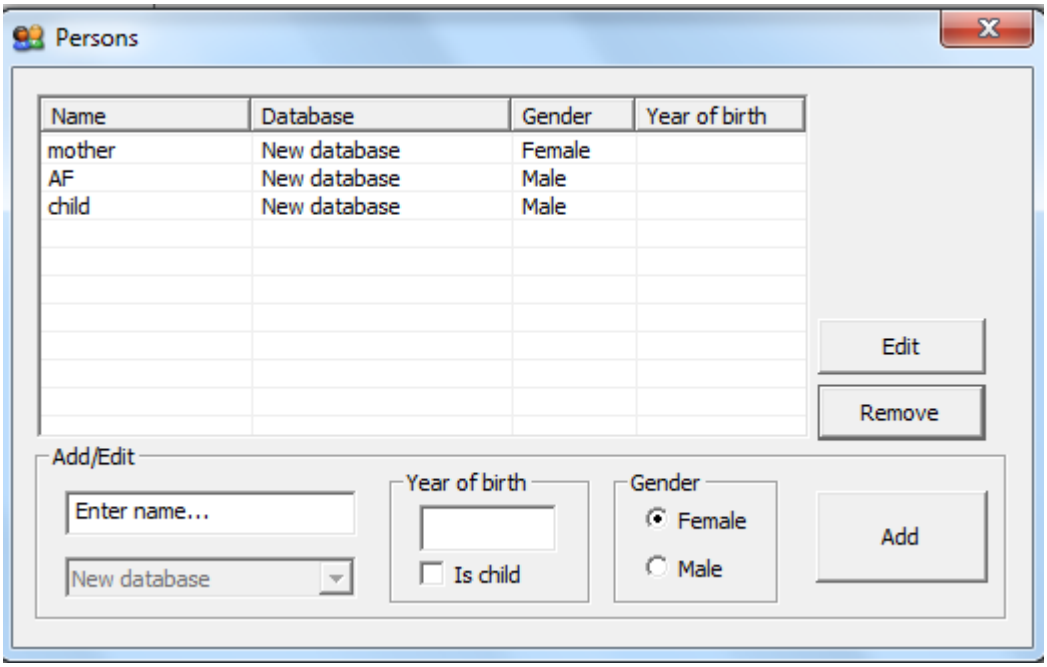


Figure 5. The Persons window.

4. **Case related DNA.** The window appearing after clicking  should be completed as shown in Figure 6below.

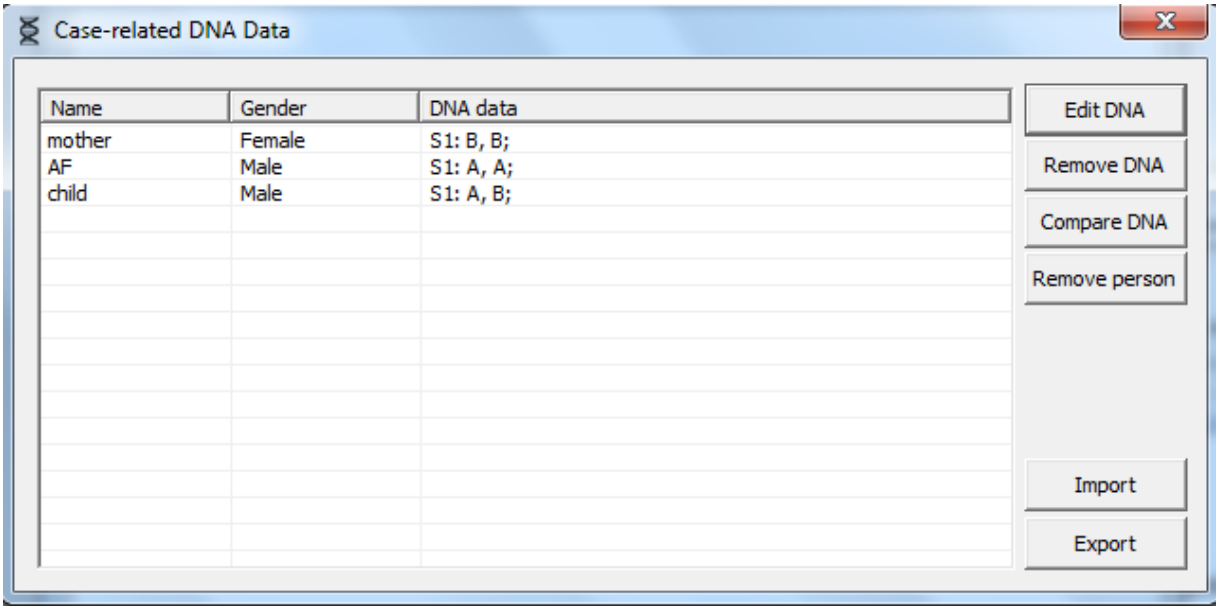


Figure 6. The Case related DNA window.

The data is entered by clicking the persons and using the menus.

4. **Pedigrees.** Click  . First the pedigree corresponding to hypothesis H_2 is entered by clicking Add. This should be completed as shown in Figure 7.

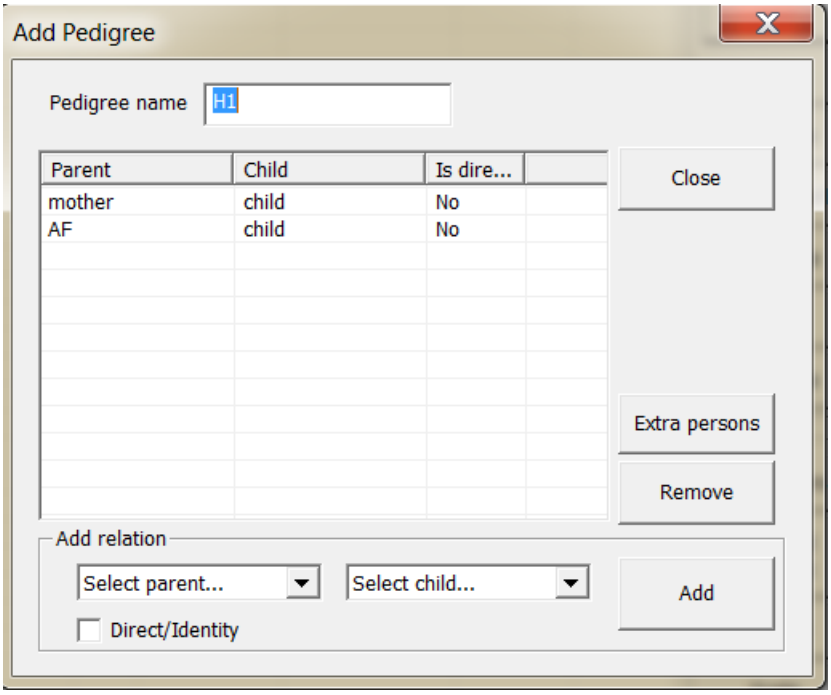


Figure 7. Defining the pedigree corresponding to hypothesis H_2.

The next pedigree is defined similarly. The answer, *LR = 20*, appears by clicking Calculate , results shown in Figure 8.

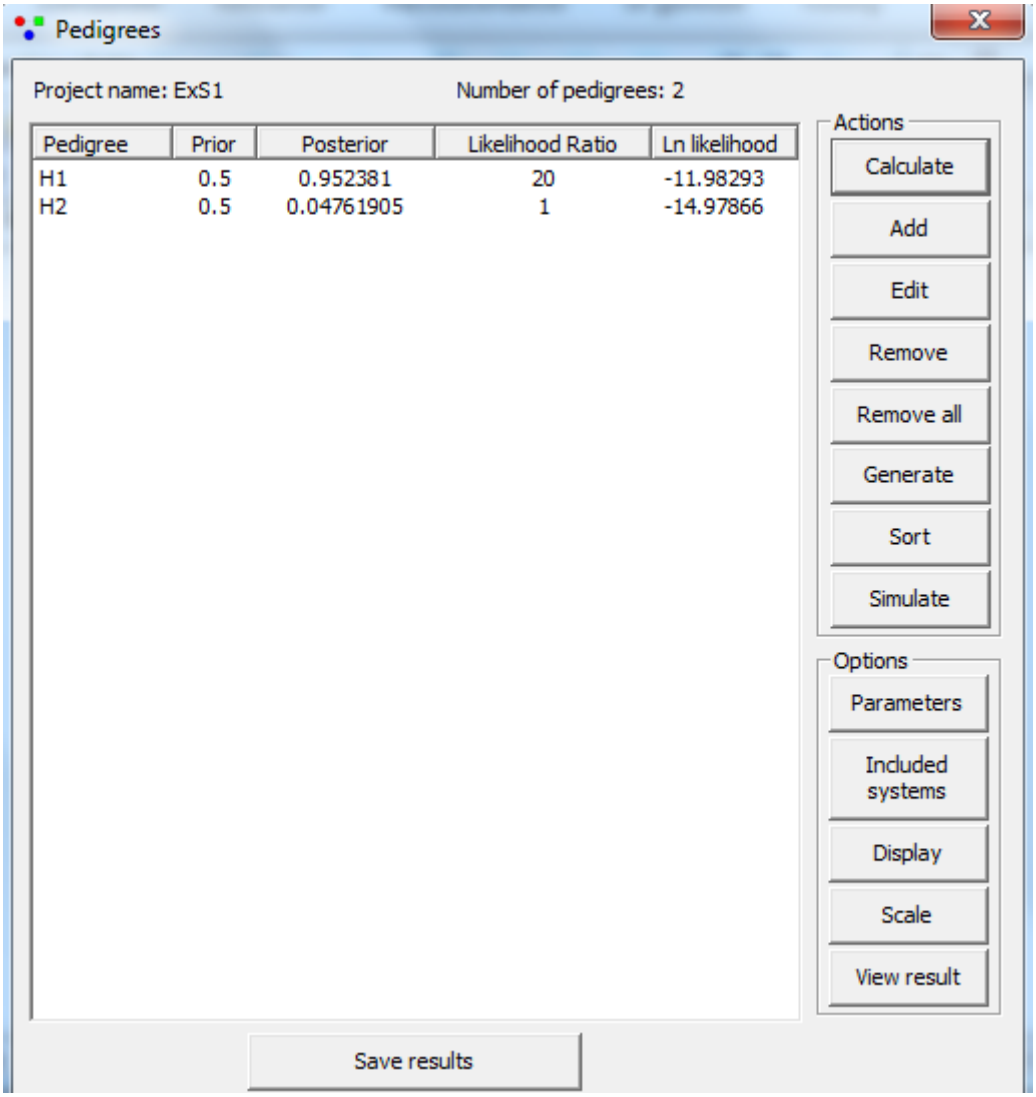


Figure 8. The result as shown in the Pedigrees window.

3 User's guide

In this section we explain how to use the **Familias** software with more details on the functionality. The main menu of **Familias** is illustrated in Figure 9below.

Figure 9. Main menus of Familias

The first four buttons are common to most windows programs: New file, Open file and Save file.. The next five buttons are specific to **Familias** and will be treated in the following sections. They are General DNA data, Persons, Case related DNA, Known relations, and Pedigrees . These buttons will make a window with the same title appear. In addition, there is functionality to do Blind search, and there is a DVI module. All windows can be accessed through the Tools (or File) menu where appropriate shortcuts can be found.

Usually, the user will go through some of the options in a particular fashion. First, the allele systems are defined under General DNA data (defining a population frequency database). This is sometimes done manually, but it is also possible to import such data from a database file (more common in case work). Secondly, the persons are defined by their name, gender and age under Persons (age is not mandatory). Next, under Case Related DNA Data, the genotypes of the relevant persons are entered for all or a subset of the available allele systems. Possible known relationships are entered under Known Relations. This last functionality is only used to save time in cases where some relationships should be fixed for all pedigrees and is therefore not really needed. Finally, the Pedigrees window is used to define pedigrees (either manually or automatically), and perform calculations of probabilities and likelihoods.

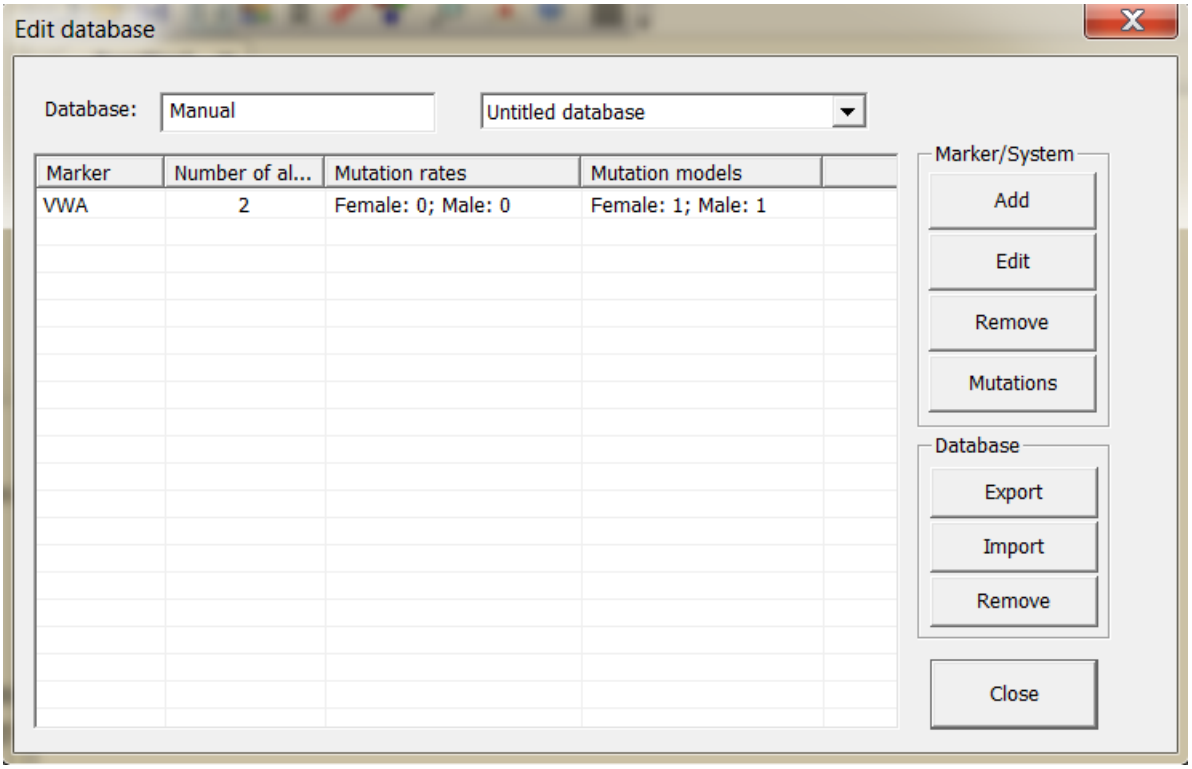


Figure 10. A system may be added, edited or removed manually.

3.1 General DNA data

This window provides options for adding, editing, removing, reading and writing allele systems/markers. An illustration of the window is given in Figure 10. Systems may be edited manually or by reading files.

3.1.1 Entering data manually

To enter a new allele system manually, press Add. Then the Allele system window, illustrated in Figure 11, appears. Here you enter the system name, the alleles and their respective frequencies. It is recommended to ensure that the allele frequencies sum to 1 (**Familias** will perform normalization if not). If necessary, an extra allele, called say 'Rest allele' can be added as demonstrated in Figure 11. A rest allele will be added automatically if the frequencies do not sum to 1. A technical note is that Familias uses the limit 0.00000001. In other words, if the allele frequencies sum to 1.000000001 they will not be adjusted.

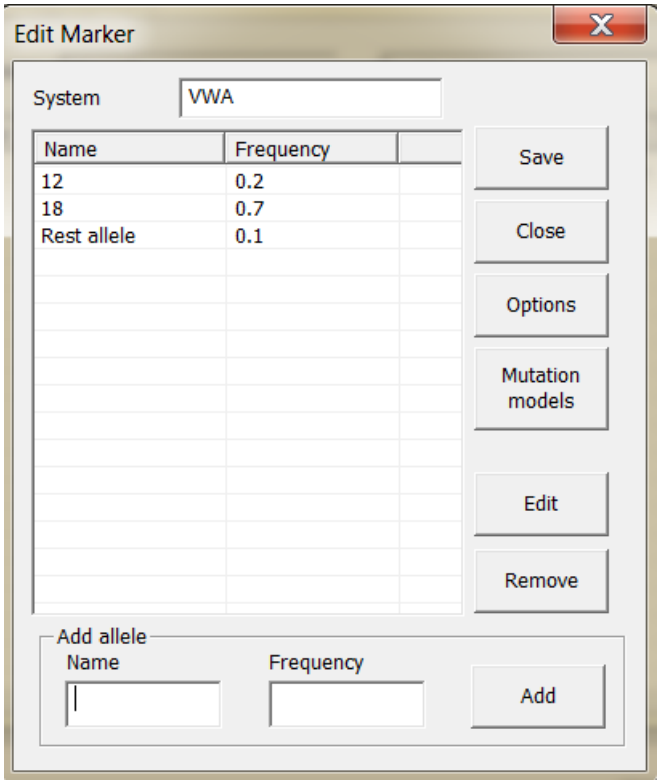


Figure 11. The window for entering an allele system.

3.1.2 Sorting

Alleles are sorted numerically according to name if possible, otherwise alphabetically. This is essential when using mutation models that depend on the ordering (the repeat number) of the alleles. Alleles with a repeat number below 10 do not need to be modified with 0 as the first digit in this version of **Familias** (In contrast to previous versions). If the first character of an allele name is a letter, it is probably wise to use small or capital letters consistently. For instance alleles a and E are sorted E, a whereas a, e are sorted a, e.

3.2 Import system data from file

The file below corresponds to the output from an Excel file, with tabs as separators. The different systems are listed below each other, separated by at least one blank line. The listing for each system starts with the name of the system, followed by a number of lines, each containing the name of the allele, and as the following item, the frequency. The alleles are sorted by the program, numerically if possible, otherwise alphabetically according to name, to correspond to the corresponding sorting when inputting alleles manually. The data is read in, and is added to the current allele systems. The name of the file read is recorded in the upper left corner adjacent to the field Database. This field can be edited to keep track of the database used or modified. If allele systems with the same names already exist, these are replaced. The systems are created with zero mutation rates and no silent alleles. If the

frequencies listed are not positive, an error is issued, and the reading of data stops. If the frequencies do not add to 1, they are adjusted to do so, with a warning. An example of input is given below.

Table 3.1: *Example of system data that can be read into **Familias** from the General DNA Data window. You can load the data between the lines below by cutting and pasting in an editor like Word or Excel. There should be a blank line before a new marker, i.e., before SYS2 below. It is important that you save the data as a text file, from excel you should use tab delimited text file, as mentioned previously. The allele frequencies of SYS2 do not sum to 1. On reading into **Familias** a warning will be given for this system before the allele frequencies are scaled to add to 1.*

SYS1	
A	0.002
B	0.096
C	0.119
D	0.225
E	0.326
F	0.163
G	0.056
H	0.013
SYS2	
6	0.056
7	0.073
8	0.190
9	0.192
10	0.253
11	0.143
12	0.089

3.3 Export system data to file

The system data can be written to a file on the same format as used for input. If you have problems importing system data, it is a good idea to first export data and check the file format.

3.4 Options

Some settings for the allele system/marker is found in the *Options* window, see Figure 12.

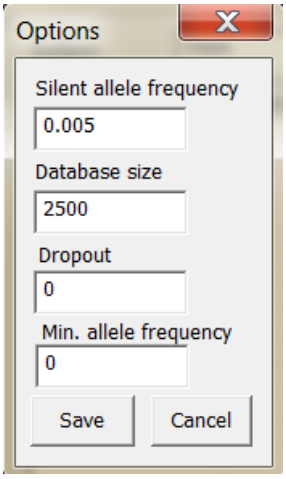


Figure 12. The window for changing allele system options.

3.4.1 Silent alleles

It is possible to specify a frequency for a silent allele. This refers to alleles that for some reason or other are not detected with the common methods. With a positive silent allele frequency, you cannot know whether an identified homozygote really is homozygote or if he is heterozygote with the other allele being a silent allele. The silent allele frequency and the other allele frequencies should add to 1. Further details on silent alleles are given in the solved exercises, see Appendix A2.

3.4.2 Database size

This option specifies the database size of the marker. This indicates the number of typed individuals that constitutes the populations frequency database. The value may be different for different markers. The value is used to compute frequencies of new (previously unobserved) alleles.

3.4.3 Dropout

Specifies marker specific dropout probability. Note, for dropout to be active you have to specify at least one profile you wish to model dropout for. This applies to kinship calculations, for dropout probabilities connected to direct matching see Advanced settings.

3.4.4 Min. allele frequency

Specifies the minor allele frequency (MAF) for the current marker. If a new allele is detected (or if an existing allele frequency is changed) a warning will be given if the specified frequency is lower than the MAF. The MAF may also be forced during the likelihood computations, see Advanced settings. Allele frequencies below the stipulated minimum are increased to the minimum value. The allele frequencies may then sum to more than 1 and scaling is required before saving. After the scaling it may happen that frequencies are slightly below the stipulated minimum.

3.5 Specifying mutation models

The default value for mutation rates are zero. However, if it is known or reasons to suspect that there is a non-zero mutation rate, it should be specified here. A reasonable mutation rate could be around 0.005. The program offers the possibility to distinguish between male and female mutation rates. The reason for this is that paternal alleles tend to mutate more often than maternal alleles. There are 5 different mutation models to choose from,

1) Equal probability (Simple)
2) Probability proportional to frequency (Stationary)
3) Step-wise (Unstationary)
4) Step-wise (Stationary)
5) Extended step-wise model (Unstationary)

A mutation model is defined by its mutation matrix. This mutation matrix can be viewed using the File > Advanced > View Mutation Matrix option. Mathematical details are provided in Appendix A1, along with an example of analytical calculations for the various models. However, to use the program all you really need to know regarding stationarity is the following: If a model is stationary this implies that adding irrelevant persons will not affect the result. Conversely, for unstationary models adding irrelevant persons may lead to slightly different results.

For models 3, 4 and 5 the probability of mutation depends on the size of the mutation. For example, if you have an allele with 14 repetitions, this allele will be more likely to mutate into an allele with 13 or 15 repetitions than to an allele with 12 or 16 repetitions. For models3, 4 and 5, **Familias** the user must supply a parameter. A typical Mutation range is 0.1.This value corresponds to a mutation probability that decreases by one tenth for each additional unit length difference between the parent allele and the offspring allele. Be aware that, for models 3 and 4, the "length" of the alleles is only decided by the order in which they are entered. The difference in length between two subsequent alleles is taken to be 1, which means that it in some circumstances it will be necessary to enter unobserved alleles. However, if using model 5, Extended stepwise model, the length of the alleles are taken to be the actual entered number. If using systems with base pair numbers as alleles (e.g. 300, 302 etc), this model will not work as intended. Then we should perhaps resort to one of the other models.

Consider next model 2, Probability proportional to frequency. Here the probability of mutating *to* an allele is proportional with this allele's frequency in the population. This means that if you have, e.g., an allele A with frequency 0.05 and another allele B with frequency 0.1, then the probability for a mutation leading to a new allele B is larger than one resulting in a new allele A.

In the model Equal probability (simple) the probability of mutating from one allele to another allele is the same independently of the frequency and the range of the alleles.

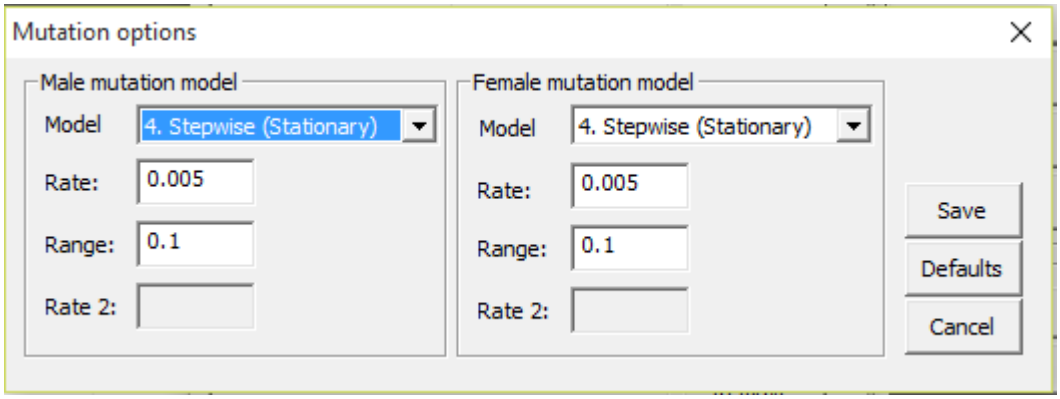


Figure 13. The mutation models and parameter options.

3.5.1 Mutation model dialog

There is a special window available to apply mutation parameters to all (or selected) systems at once. For instance to change the models of all systems or the rates. The dialog is accessed via File > Tools > Mutations. The dialog in in Figure 14 appears. The same options as in Figure 13 exist, but in addition a tick box to only change the models are available. This may be useful to keep marker specific rates and ranges and only change the models. In addition the user can choose to Apply to the selected systems/markers or Apply to all systems (regardless of selections).

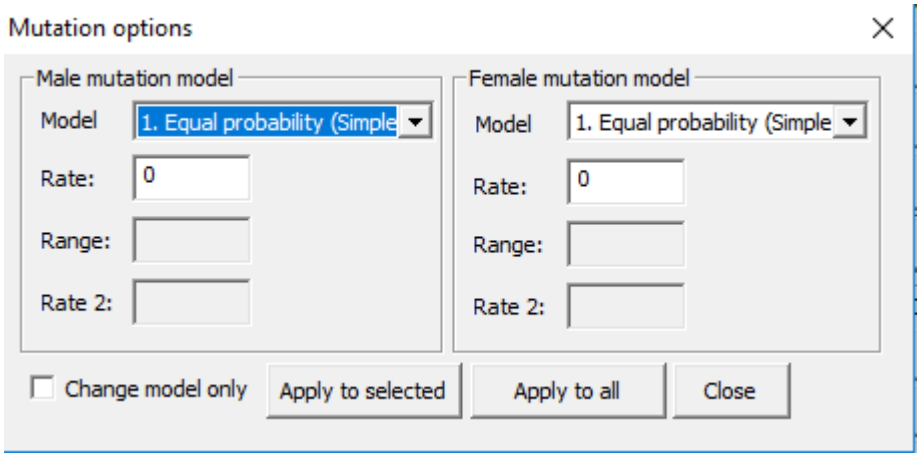


Figure 14. Mutation dialog.

3.6 Persons 

By pressing this button, the window shown in Figure 15appears. Here you define the persons involved in the case. For each person a name and gender must be specified. For most applications this is the only information needed and used. In addition, it is possible to enter a year of birth, and you may also specify if the person is a child or in effect has no children. Concerning the year-of-birth specification: as **Familias** only makes use of the relative dates, it is possible to use this option to specify age differences even when the exact year-of-birth is unknown. The "Is Child"-option is used to limit the number of possible pedigrees if the Generate option of the pedigree window is used to generate pedigrees automatically. Similarly, giving two persons the same year of birth also limits the number of pedigrees as

there will then be no parent-child relationships between these individuals. The list of persons is edited by means of Edit (or double clicking an item in the list) and Remove.

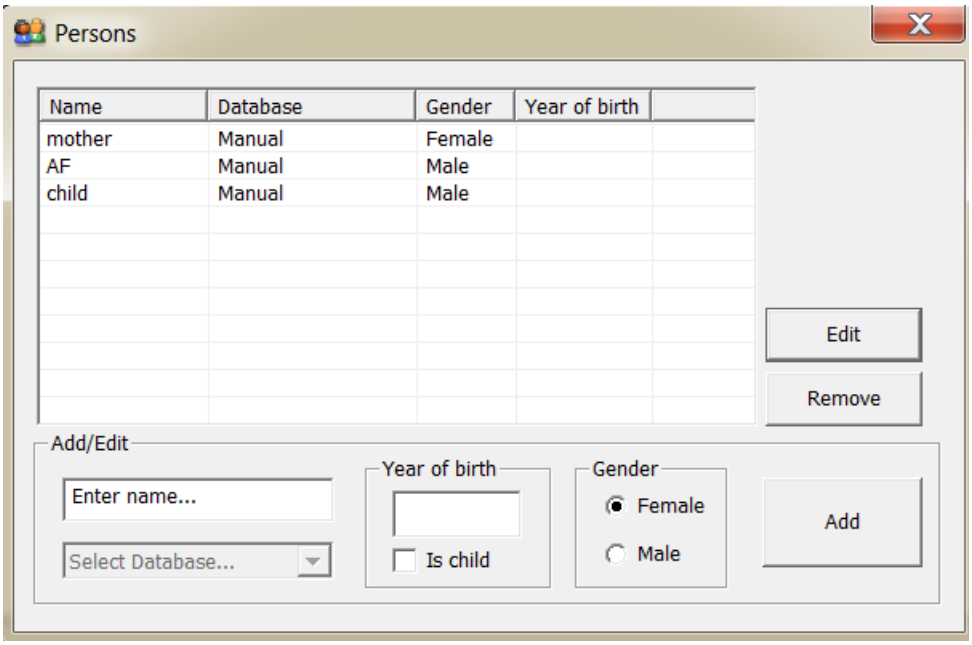


Figure 15. The window for entering the persons involved in a case.

3.7 Known relations

This is where known relations (fixed relations) are defined. You are advised to avoid the functionality in *Known relations* unless you intend to analyse a greater number of pedigrees. The functionality in this section is never really needed; it only simplifies input when many pedigrees are analyzed or generated.

If it is certain that, e.g., F is the father of D, then this could be specified here. It is only possible to define parent-child relations. This means that if, for example, two girls are known to be sisters, this cannot be defined straightforward, but through their relations with the common parents. The window is illustrated in Figure 16. The menu is not strictly needed as this information can be provided also when the pedigrees are defined. All relations defined in the Known relations window will appear in all pedigrees.

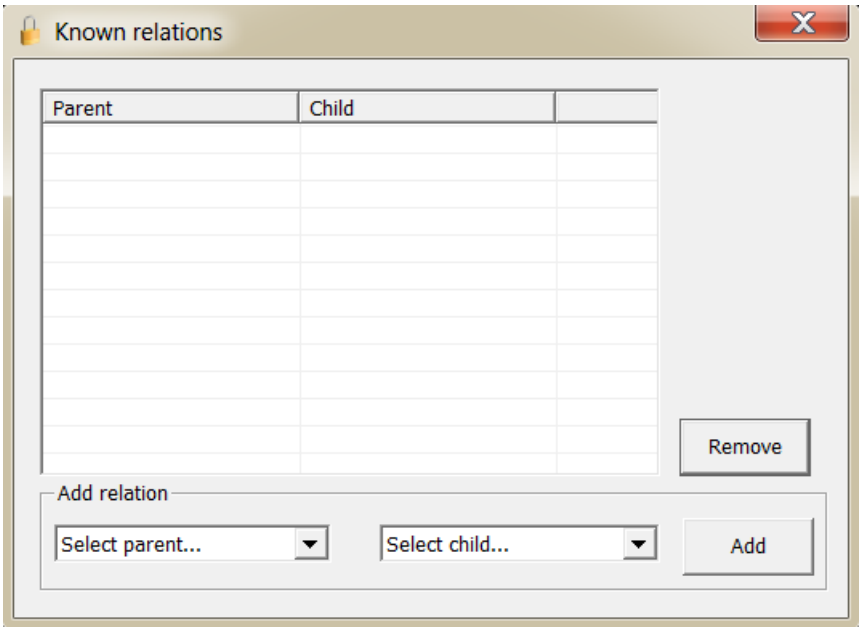


Figure 16. The window for entering known relations.

3.8 Case related DNA data

In this form you enter the DNA data for the persons for whom this information is available. This can be done manually or by reading from a file.

3.8.1 Manually

By marking one of the persons on the list in the window shown in Figure 17, and pressing Edit data, a new window appears (see Figure 18). Here you enter, for the selected person, the DNA data of all the investigated allele systems. For persons for whom there are no available DNA data, just leave it open. Apparent homozygotes are entered with two of the same allele, also in the cases where there could be silent alleles.

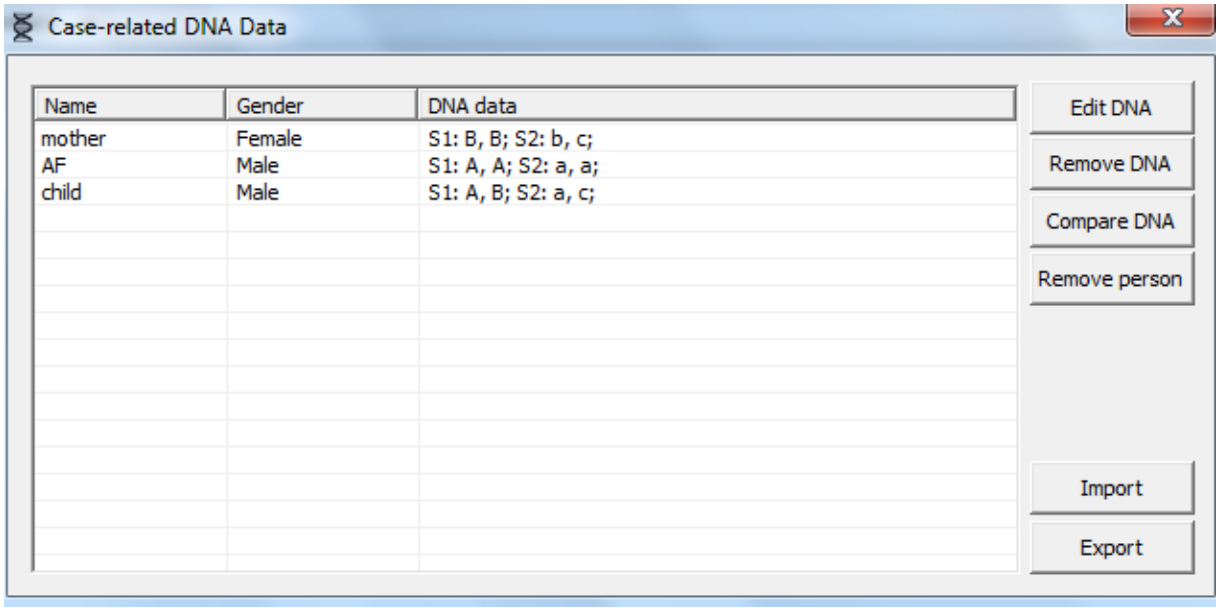


Figure 17. Selecting the persons to assign genotype data is done in this window.

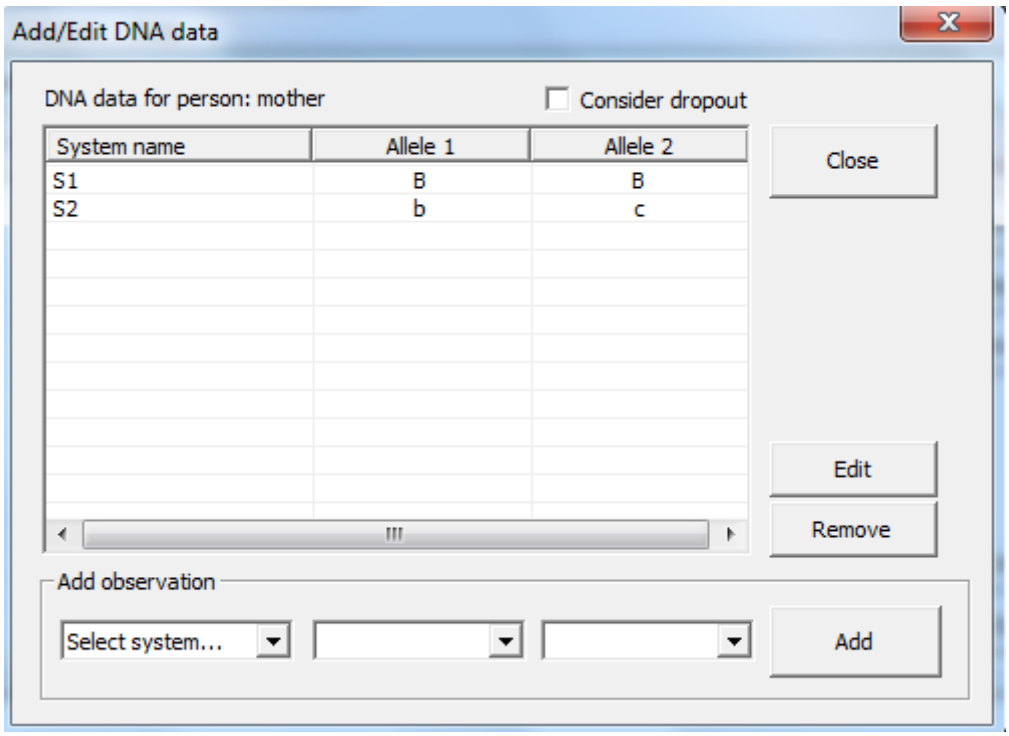


Figure 18. Adding DNA data for a selected person is done in this window. By ticking *Consider dropout* dropouts are considered. Note, you have to specify a dropout probability for each marker system or specify a profile specific dropout probability in the *Advanced*

3.9 Import case data

Data for specific samples can now also be read from files. The data for specific samples can be given as a table. There are four different format which can be read be **Familias**, listed below.

3.9.1.1 Tab separated file

The format can be outputted from Excel, using tabs as separators (from Excel, save as Text (Tab delimited)). The table should have a line with headings and the following lines should each represent a sample source, i.e., a person. Blank lines (i.e., lines where there is nothing in the first column) will be ignored. The first column should list the names of the sample sources, i.e., the persons. If the names correspond to names of persons already entered, the data will be added to the data for this person. Otherwise, the persons will be added as they are read in. The data for the systems must be provided prior to reading case data. There must be two columns specifying sex chromosomes in the table. These columns must be beside each other, the first must contain the letter "X" as all entries, and the second must contain either "X" or "Y", depending on the sex. (Remember that **Familias** is case sensitive. The X and Y should be in capitals.) When new persons are added, they will be given the sex specified by these columns. For existing persons, the data is ignored. Except for the three columns described above, all columns must come in pairs of two, beside each other, with the headings specifying the name of the allele system the columns contain data for. The headings for each pair must be identical, except for the last character (which could be, for example "1" and "2"). After the last character has been removed, the remaining name (removing blanks at the end) must correspond exactly to the name of an already entered allele system. The two columns below then contain the names of the alleles observed in this system, for the respective persons. Note that homozygotes must have alleles entered twice, once in each column. Missing data are coded with a '*'. Both (or none) alleles must be missing for a marker. An example of an input file is given below.

Table 3.2:*Example of case data that can be read into* **Familias** *from the Case Related DNA Data window. The system called SYS1 and SYS2 must be given on beforehand, for example by reading the data of Table 3.1 above. The names (na1, na2 and Jakob) may or may not be given. The loading of the data is explained previously.*

Name	Amel 1	Amel 2	SYS1 1	SYS1 2	SYS2 1	SYS2 2
Na1	X	X	F	G	8	9
Na2	X	Y	G	G	10	11
Jakob	X	Y	G	G	9	10

3.9.1.2 Tab separated (With commas between alleles)

The format can as previously be outputted from Excel, using tabs as separators (from Excel, save as Text (Tab delimited)). The table should have a line with headings, and the following lines should each represent a sample source, i.e., a person. Blank lines (i.e., lines where there is nothing in the first column) will be ignored. The first column should list the names of the sample sources, i.e., the persons. If the names correspond to names of persons already entered, the data will be added to the data for this person. Otherwise, the persons will be added as they are read in. The data for the systems must be provided prior to reading case data. There must be one columns specifying sex chromosomes in the table. For each person there must be either a X,X or X,Y in the specific column. When new persons are added, they will be given the sex specified by these columns. For existing persons, the data is ignored. For each system we have only one column where the header must correspond exactly to the name of an already entered

allele system. The column below then contains the names of the alleles observed in this system, for the respective persons with a separating comma between the alleles. Note that homozygotes must have alleles entered twice. Missing data are coded with a '*'. Both (or none) alleles must be missing for a marker. An example of an input file is given below.

Table 3.3:*Example of case data that can be read into* **Familias** *from the Case Related DNA Data window. The system called SYS1 and SYS2 must be given on beforehand, for example by reading the data of Table 3.1 above. The names (na1, na2 and Jakob) may or may not be given. The loading of the data is explained previously.*

name	amel	SYS1	SYS2
na1	X,X	F,G	8,9
na2	X,Y	G,G	10,11
Jakob	X,Y	G,G	9,10

3.9.1.3 GeneMapper file (Exported as tab separated file)

The analyzed data from GeneMapper should be outputted as shown in Table 3.4 below. The table should have four headings, with the order, Sample name, Marker name, Allele1 and Allele2.This can easily be specified creating a Table setting named **Familias**, e.g., where the Genotype tabs have exactly the specified setup. The first column should list the names of the sample sources, i.e., the persons. (Note that the same name may be listed on several rows, see Table 3.4) If the names correspond to names of persons already entered, the data will be added to the data for this person. Otherwise, the persons will be added as they are read in. The data for the systems must be provided prior to reading case data. There must be one rows specifying the sex chromosomes, i.e. the gender of the person in the table. When new persons are added, they will be given the sex specified by these columns. For existing persons, the data is ignored. For each system we have only one row where the second column of the row must correspond exactly to the name of an already entered allele system. The next two columns then contain the names of the alleles observed in this system.

Table 3.4:*Example of case data that can be read into* **Familias** *from the Case Related DNA Data window. The system called SYS1 and SYS2 must be given on beforehand, for example by reading the data of Table 3.1 above. The names (na1, na2 and Jakob) may or may not be given. The loading of the data is explained previously.*

Name	marker	allele1	allele2
na1	amel	X	X
na1	SYS1	F	G
na1	SYS2	8	9
na2	amel	X	Y
na2	SYS1	G	G
na2	SYS2	10	11
Jakob	amel	X	Y
Jakob	SYS1	G	G
Jakob	SYS2	9	10

3.9.1.4 CODIS xml format

Familias provides functionality to import data on the CODIS xml format. This file format is described elsewhere. One of the main point of this import function is the ability to easier exchange data between labs, as the CODIS format is fairly standardized. In addition **Familias** can import exported data from the CODIS software, exported to xml files (cmf format).

3.10 Compare data

In later versions of **Familias**, a Compare DNA button has been added. This button makes it easier to compare genotypes of several persons (if several persons are selected). If only one person is selected, 1/RMP, i.e., 1 divided by the random match probability, is calculated, see Figure 19. The user can convert this to the RMP. For the below example,

$$RMP = p_A^2 p_a^2 = 0.1^2 \times 0.05^2 = 0.000025$$
$$1/RMP = 40000$$

This calculation of RMP assumes Hardy-Weinberg equilibrium unless the kinship/theta parameter is non-zero.

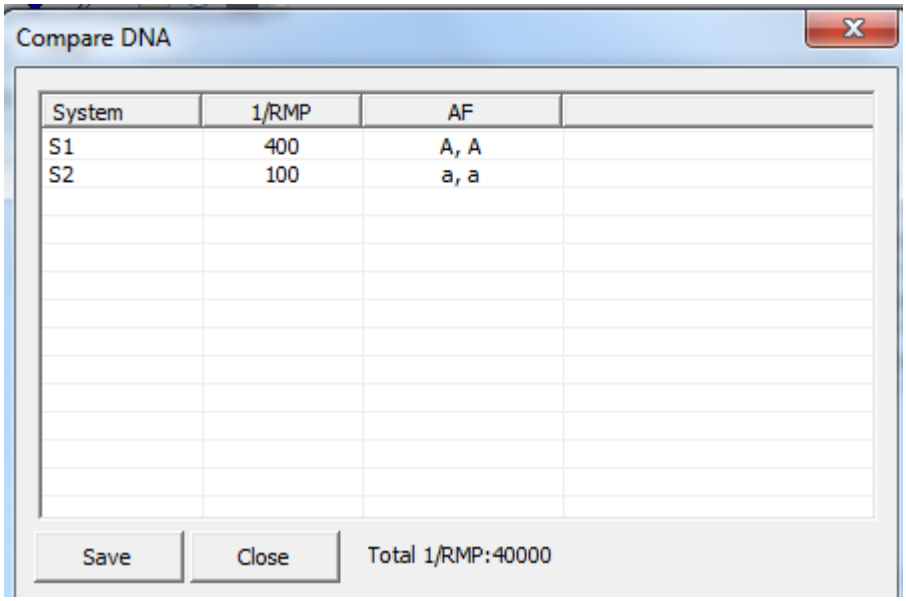


Figure 19. The compare DNA dialog, displaying the random match probability for a profile with two typed markers.

3.11 Pedigrees 

In this form you may add your own pedigrees or you may use **Familias** to generate pedigrees, this latter option is discussed in Appendix A3. After having generated the pedigrees, one can calculate probabilities and likelihoods ratios and produce reports. In the following we will go through the set of buttons and options of the window shown in Figure 20starting in the upper right corner.

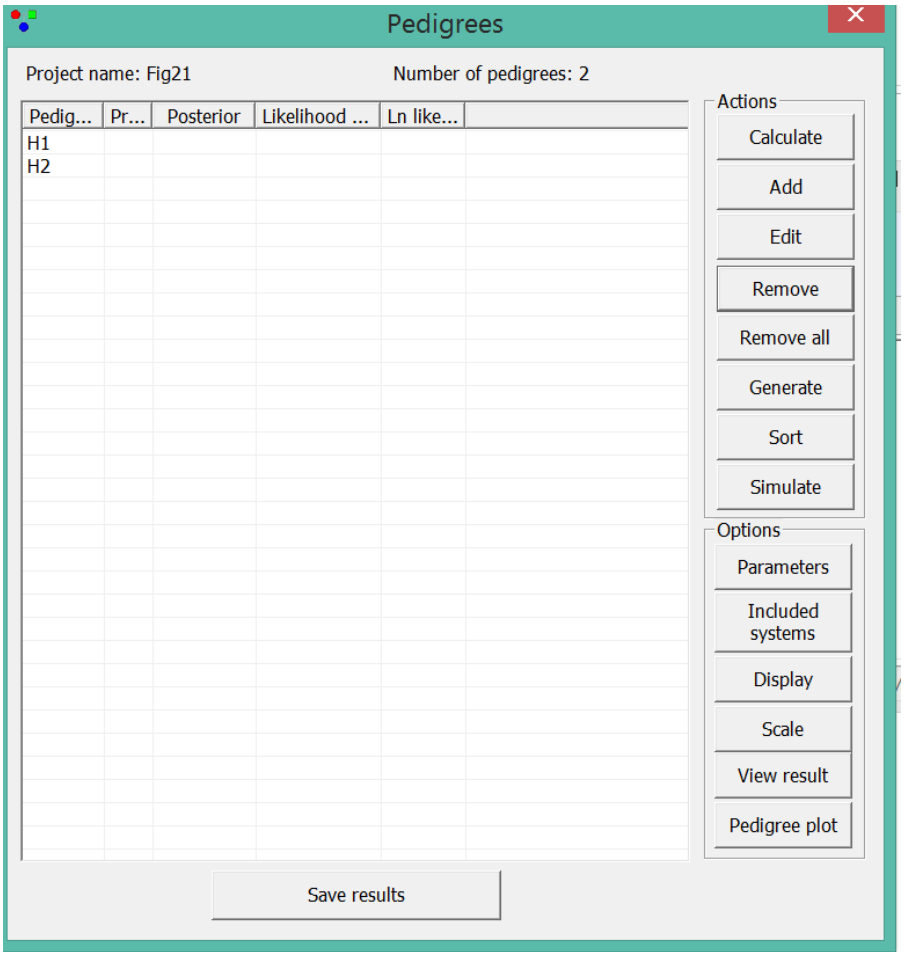


Figure 20. The Pedigrees window with two defined pedigrees.

Calculate
This button performs the calculations

Add: Creating pedigrees
Usually, the first thing to do is to create a set of pedigrees manually by clicking Add. The pedigree is defined by giving the parent child relations as exemplified in Figure 21. The Extra persons button is used to introduce individuals needed to define a pedigree. For instance, such extra persons may be needed to define cousin relations. There are various examples of pedigrees Available from http://familias.name/book.html.The pedigree name can be edited. Note that a relation can be defined as being direct/identity. This implies that the two samples/individuals are assumed to be from the same sample/individual and calculations are performed based on this assumption. For instance, for monozygotic twins the two individuals have a direct/identity relation in one of the hypotheses whereas a full sibling relation is defined in the alternative hypothesis. An R script for plotting in R is generated by Plot in R.

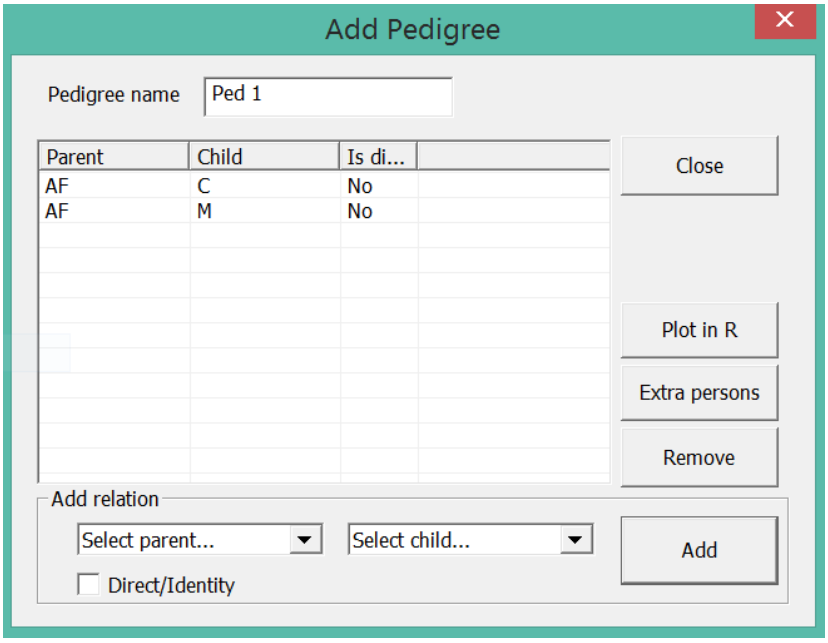


Figure 21. AF is defined as the father of C and M. C and M are thus half-sibs. An extra parent is needed to define full sibs.

Edit: Editing pedigrees
The pedigrees are edited using this button.

Remove
This button is used to remove all selected pedigrees.

Remove all
This button is used to remove all pedigrees.

Generate: Generate pedigrees automatically
See Appendix A3.

Sort
The results are sorting in according to decreasing LR.

Simulate
Starts the simulation interface, explained in Section 3.8.

Parameters
Various parameters can be set. The most frequently used is the kinship parameter ($\theta = F_{ST}$).
The remaining parameters are explained in Appendix A4.

Included systems
By default all systems are used for calculation. This option can be used to extract results for selected systems.

Display
Select what to display in the pedigree window. (Prior, Posterior, LR and ln likelihood).The natural logarithm displayed, i.e., ln can be converted to log10 by log10(x)=ln(x)/2.303

Scale
Select the pedigree to scale against, used when calculating LR.

View result
Select a pedigree and press to see genotypes and LR for all markers. This is a new functionality introduced in **Familias 3** that can be used to detect for instance mutation. The dialog appears in Figure 22, and there is most likely a mutation for the marker PENTA_E

Pedigree plot
Plots generated and saved as png files can be viewed along with the genotypes. The function is used in combination with the **FamiliasPedigreeCreator** software, mentioned in Section 11.

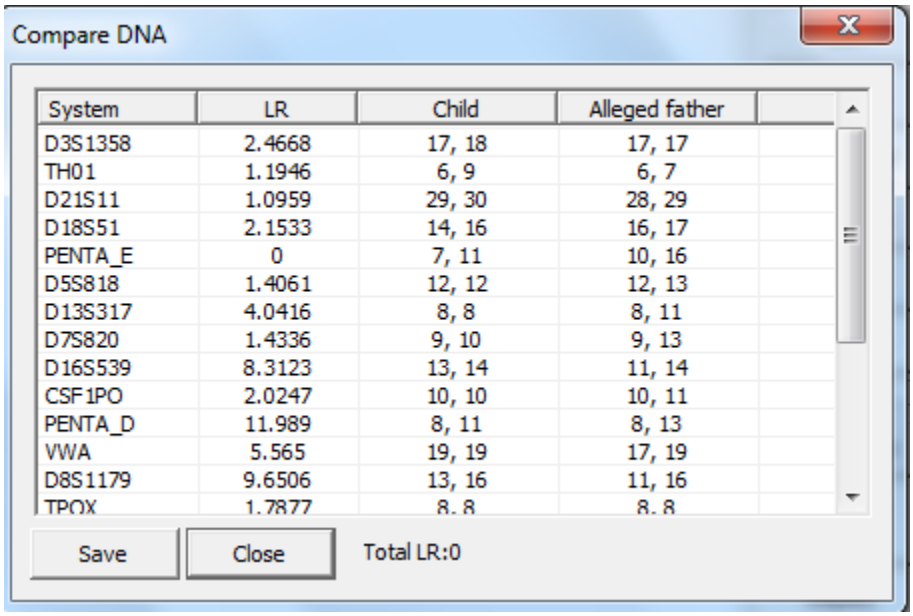


Figure 22. View of the results (LRs) for individual markers.

Save results
Brings up the Report dialog where results can be saved using several options

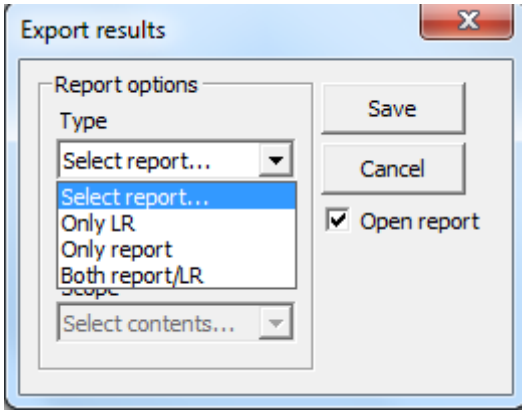


Figure 23. Creating a report.

Only LR just gives precisely only the LR (total). Next format (rtf, csv, xml or txt) can be selected and finally the extent of detail (Simple, Moderate, Complete).

4 DVI module

Disaster victim identification is a term describing the event where a number of unidentified samples are compared with a number of reference samples, commonly with known origin. The latter could be personal belongings such as profiles from tooth brushes etc., while it is also common to obtain data from relatives of the missing person, so called reference family members.

The DVI module is divided into three steps, first adding the unidentified individuals/samples and their genotypes, second the reference families and the alleged pedigrees and last the DVI search. There are also several functions that may be additionally carried out in each step, see below for detailed description.

4.1 Add unidentified persons

Open the DVI interface by clicking the button , or from *Tools > DVI module > Add Unidentified Persons*. By pressing this button, the window shown in Figure 24appears (Note, the exact appearance may vary slightly depending on what version of the **Familias** you are using).

For each person/element/remain a name and gender must be specified. See below for a description of each button.

Edit person
Edit the general information for a person such as gender and name.

Edit DNA
By marking one of the persons on the list, in the window shown in Figure 1, and pressing Edit data, a new window appears (see Figure 25). Here you enter, for the selected person, the DNA data of all the investigated allele systems. For persons for whom there are no available DNA data, just leave it empty. Apparent homozygotes are entered with two of the same allele, also in the cases where there could be silent alleles. More commonly we import genotype data from file, see below.

Remove
Removes the selected persons from the list.

Move
Used to place an individual in a reference family (after or before identification).

Sort
Sorts the list alphabetically by ID.

Compare
By selecting one (or more) of the persons in the list in Figure 24and pressing Compare, a comparative view of the person's DNA will appear.

Search selected
Includes only the selected persons in the DVI search. The selected list is only stored for one search and is restored upon returning to this window.

Blind search
Opens up the blind search interface for the list of unidentified persons. NB! Not used to blindly compare the unidentified remains with the reference families. Such functionality is described in Section 4.4 below.

Import/Export
Imports/Exports data.

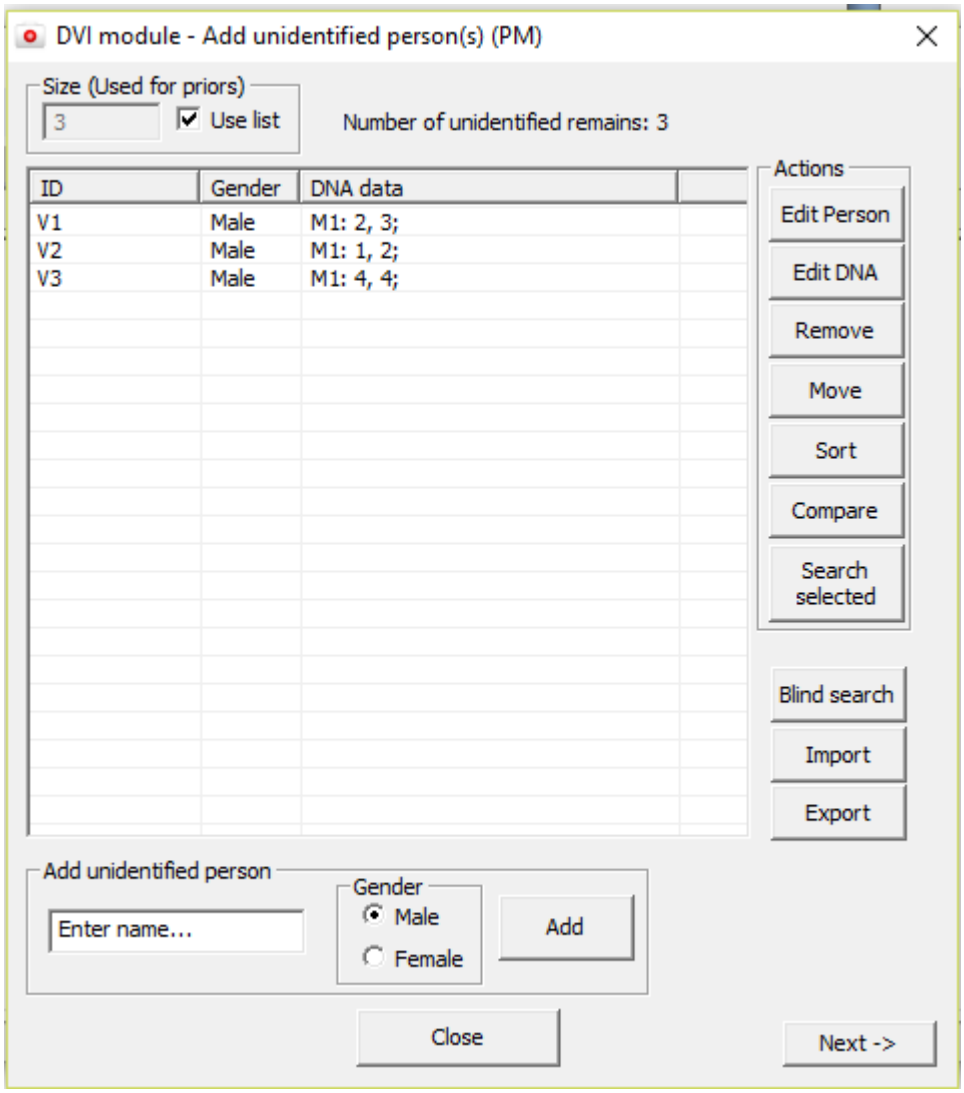


Figure 24. The Add unidentified persons window for entering the persons involved and their genotypes.

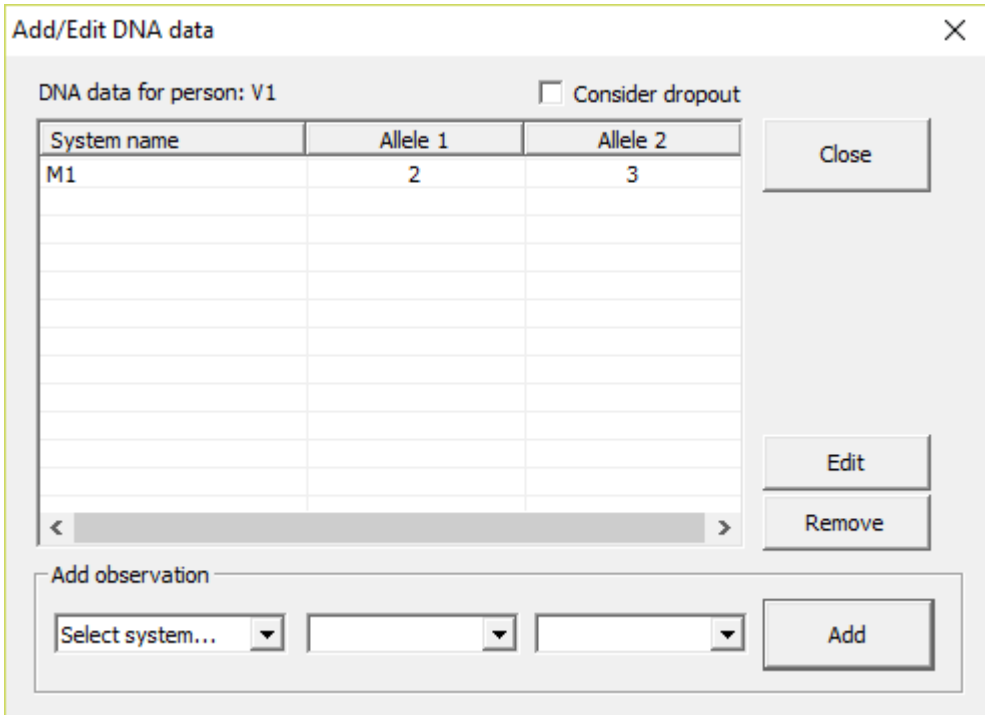


Figure 25. Adding DNA data for a selected person is done in this window.

4.2 Add reference family

Once the persons are defined in the *Add unidentified persons* window click Next (or click Add Reference Families from Tools > DVI module.

See below for a comprehensive description of each of the buttons on the dialog that appears, see Figure 26 for an illustration.

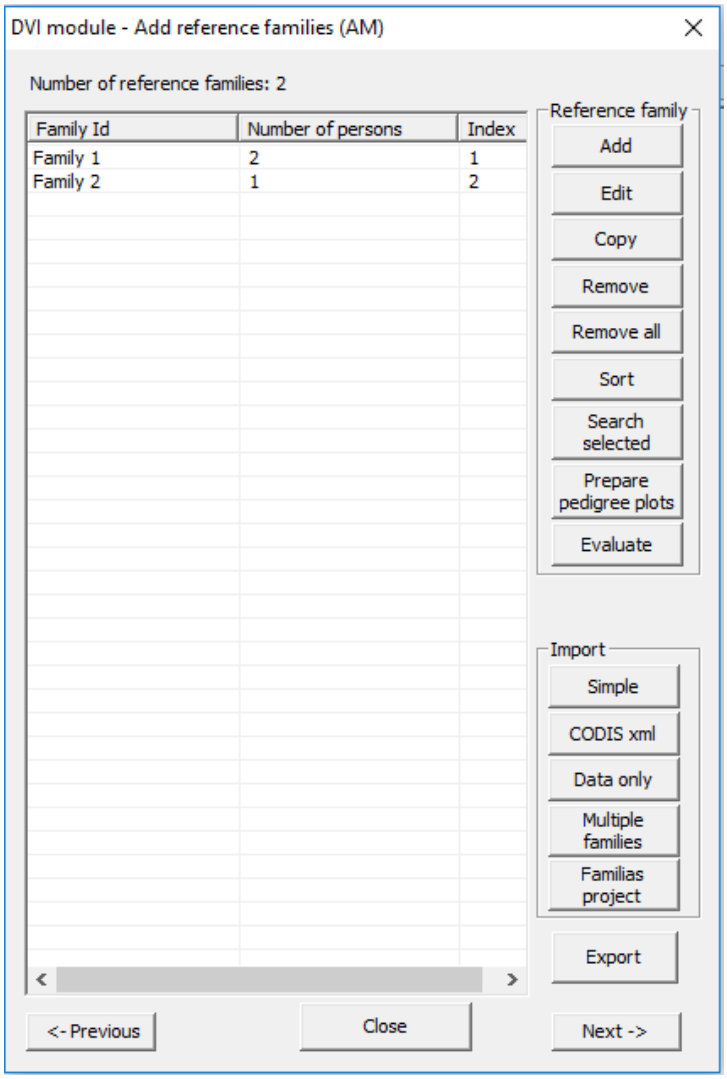


Figure 26. Window for defining and importing reference families.

Add

Adds a new family. Define the persons in each family in the window illustrated in Figure 27, and specify the relation between the individuals in the family and the missing person in the pedigree window shown inFigure 28. The buttons appearing in the figure are more or less self-explanatory. The button Check may be used to check the pedigrees for any inconsistencies/mutations.

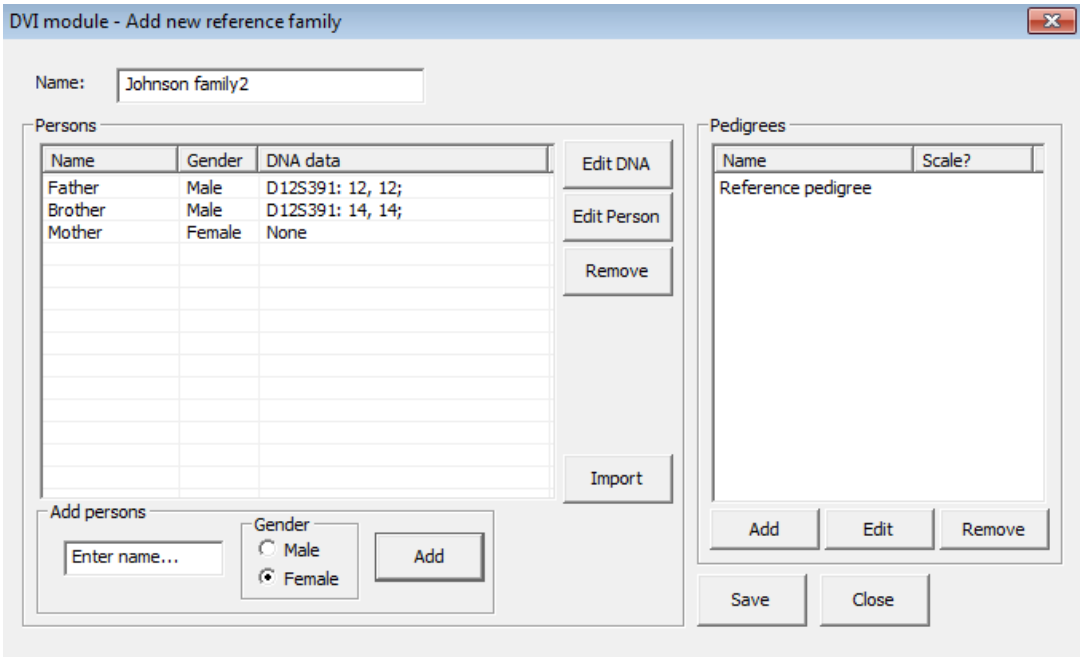


Figure 27. Adding persons to the family and their genotypes is done to the left in this window, the pedigrees are generated by clicking the Add button to the right in the window. The 'Reference pedigree' in the upper right part is always included by default to include the possibility that a victim belongs to none of the families.

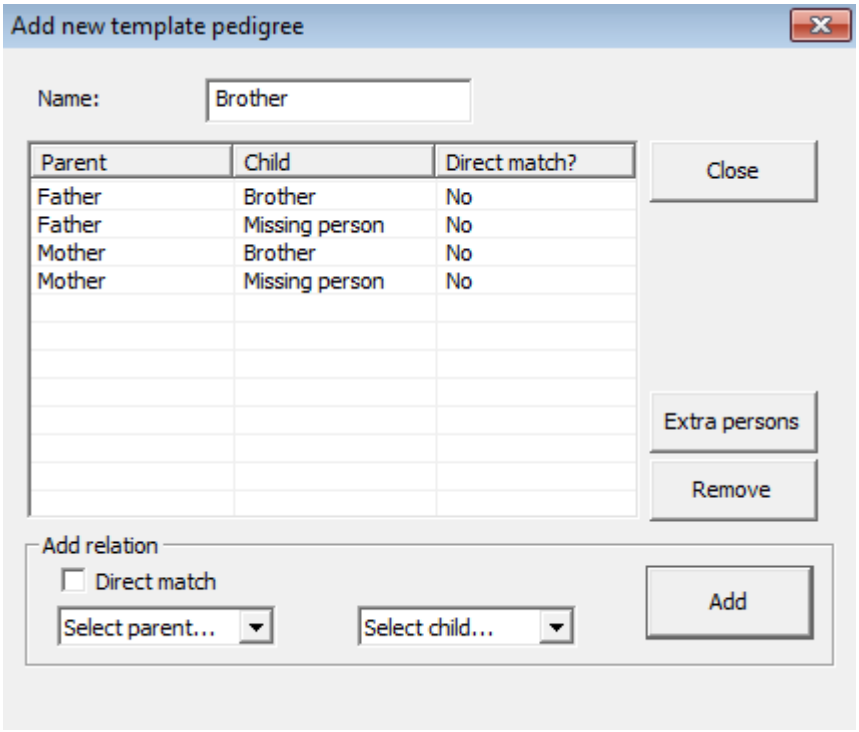


Figure 28. Defining a pedigree in the DVI module. The individual named Missing person is used to indicate a link to each of the unidentified person in the subsequent search.

Edit
Open the selected family for edit.

Copy

Copy a selected family including persons and pedigrees. Useful to define several missing persons within the same family.

Remove/Remove all
Remove selected families.

Sort
Sorts the list alphabetically by ID.

Search selected
Includes only the reference families in the DVI search. The selected list is only stored for one search and is restored upon returning to this window.

4.2.1 Prepare pedigree plots

This feature is new in **Familias 3.2** and will create an R-script for the selected reference families. Running this R-script will generate plots for all the selected families and store them in a folder connected to the DVI project. The plots may be viewed outside **Familias** or using the Evaluate feature described next

4.2.2 Evaluate

This will open a new dialog to evaluate the selected reference families. See detailed description in Section 4.3 below.

4.2.3 Import data from file

For larger DVI cases or missing person operations it is convenient to import data from a file instead. **Familias** supports a number of different formats, described below.

Simple
This format corresponds to the normal **Familias** format, described in Section 3.9.1.1. A (optional) relationship indicator may precede the line describing the data for the person. **Familias** will try to automatically generate the pedigrees. All imported families should be checked such that the relationships have been correctly identified. See Table 3.5 for a comprehensive list of relationships. See below for file format.

Sample id	*D12S391 1*	*D12S392 2*
[Brother]		
Per	*12*	*14*

CODIS xml
The CODIS xml format is a format used in the CODIS software and also by some other systems. The format is described elsewhere but allows for simple transfer of data. **Familias** can easily read a complete export file from the CODIS software and can interpret some standard relationships.

Data only

Similar to the Simple format but in this file an additional column describing the family id (preceding sample id) is included. This makes it in turn possible to include also several reference families in a single file. See below for file format:

Family id	*Sample id*	*D12S391 1*	*D12S392 2*
Family 1	*Per*	*12*	*14*
Family 2	*Daniel*	*13*	*14*

The following format should also be accepted:

Family id	*Sample id*	*D12S391*
Family 1	*Per*	*12,14*
Family 2	*Daniel*	*13,14*

Multiple families
Same as the previous format but in addition include a column (preceding sample id), describing the relationship, see Table 3.5.
See below for file format:

Family id	*Relationship*	*Sample id*	*D12S391 1*	*D12S392 2*
Family 1	*[Brother]*	*Per*	*12*	*14*
Family 2	*[Father]*	*Daniel*	*13*	*14*

Familias project
Recognizes and imports files on the standard **Familias** (.fam or .txt) format. Imports persons and pedigrees as well as known relations. Extra persons are turned into ordinary family members. Recognizes the identifier "missing person" or "MISSING PERSON" as the missing person.

Table 3.5: *Relationships recognized by* **Familias**

Relationship
[Brother]
[Sister]
[Sibling]
[Father]
[Mother]
[Parent]
[Son]
[Daughter]
[Child]
[Aunt]
[Uncle]
[Niece]
[Nephew]
[Half-sister]
[Half-brother]
[Grandmother]
[Grandfather]

[Direct]
[Identity]

4.3 Evaluate reference families

The functionality described here relates to the dialog in the DVI module (Add Reference Families > Evaluate). The dialog is a versatile tool to thoroughly evaluate the performance of each of the reference families in an identification. The dialog below will appear.

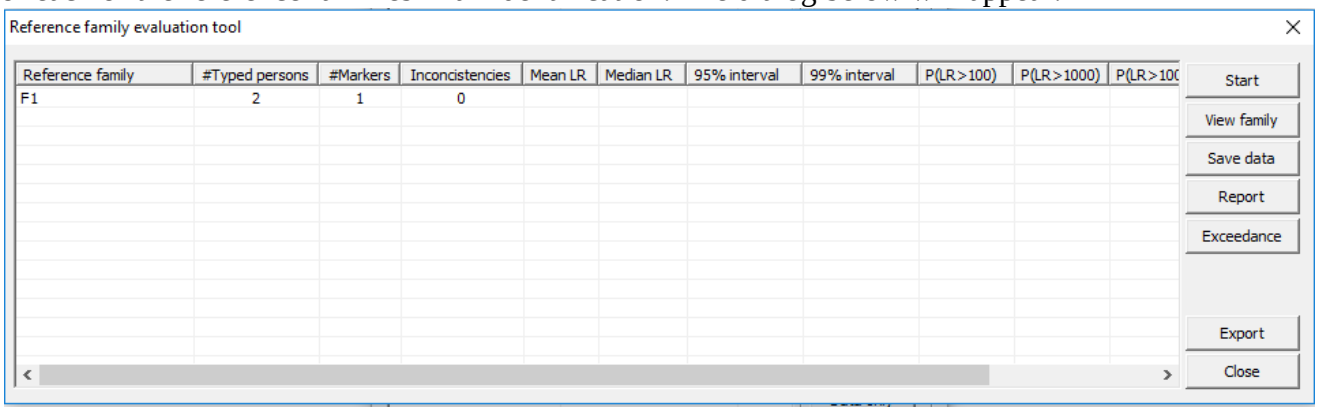


Figure 29. Reference family evaluation interface.

All selected reference families are listed. The following also appears in the table,

1. Number of typed persons (information on which markers not provided)
2. Number of typed markers (combined for all typed persons in the family)
3. Number of inconsistent markers (can be used to locate persons/markers that may cause problems in the search)
4. Summary statistics parameters (mean/median, intervals, exceedance probabilities, exclusion probability) that will be available once simulations have been performed, see below.

The buttons are described below.

4.3.1 Start

Pressing Start will initiate a simulation process. The window below will appear with some options. Selecting *Conditional simulations* will cause **Familias** to generate an R script. This script may be run in R making use of the library *fam2r*, which is a wrapper for the library *paramlink*, to conditionally simulate data. In short this simulation approach uses the available genotypes (i.e. typed reference family members) to obtain summary statistics.

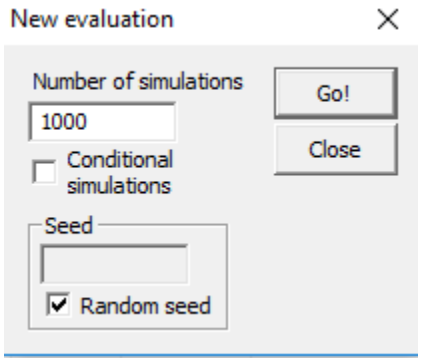


Figure 30. Starting a new evaluation/simulation.

4.3.2 View family

Brings up a view to watch the pedigree (provided plotting has been done using functionality described previously in the section "Prepare pedigree plots") as well as genotypes and any inconsistent markers.

4.3.3 Save data

This function will save the raw LR output from the simulations. Useful to further study the reference families.

4.3.4 Report

This will generate a report for the families with all the results from the evaluations. Not yet implemented.

4.3.5 Exceedance

This will export exceedance probabilities for a range of thresholds. Useful for plotting and other purposes.

4.3.6 Export

Writes all the elements of the displayed table to a text file.

4.4 Search

When the persons and pedigrees in each family are defined click Next (or click Search from Tools > DVI module. The functions, see Figure 31is described below.

Search

Perform a search. It is recommended to save all changes before conducting the DVI search. Prior to the search, a dialog will ask for a match threshold, pick 0 (zero) to obtain results for all comparisons. NB! If the *Quick search* feature is enabled in the *Advanced* dialog (which is default), only matches with less mismatches (i.e. markers with inconsistencies) will be reported, see also Section Advanced options8.

Quick scan

Performs a quick scan. This option brings up the blind search interface, see Figure 32, and will blindly search the reference family members against the unidentified persons using specified parameters. In other words, this function disregards any specified family relations and performs a blind search. The scan will perform pairwise matching, i.e. each family

member is tried separately against each unidentified person. This will mitigate problems resulting from unknown “false” relationships in the reference families.

Sort
Sorts the match list. The user selects the sort key.

Apply threshold
Apply a new LR threshold, possibly decreasing the number of matches in the list.

Display
Select what columns to display in the match list.

View match
View the specifics of a match, i.e., the LR for individual markers.

Confirm match
Confirms a match and create a report on a specific match. In addition it is possible to move an unidentified person to a reference family thus effectively removing him/her from the list of unidentified individuals.

Remove
Remove a selected match from the list.

Create report
Create a comprehensive report of the search. The same options as described in Section 3.11 are available when creating the report.

Export list
Exports the list to a tab-separated text file. The file can be easily edited and manipulated in a software such as **Excel**.

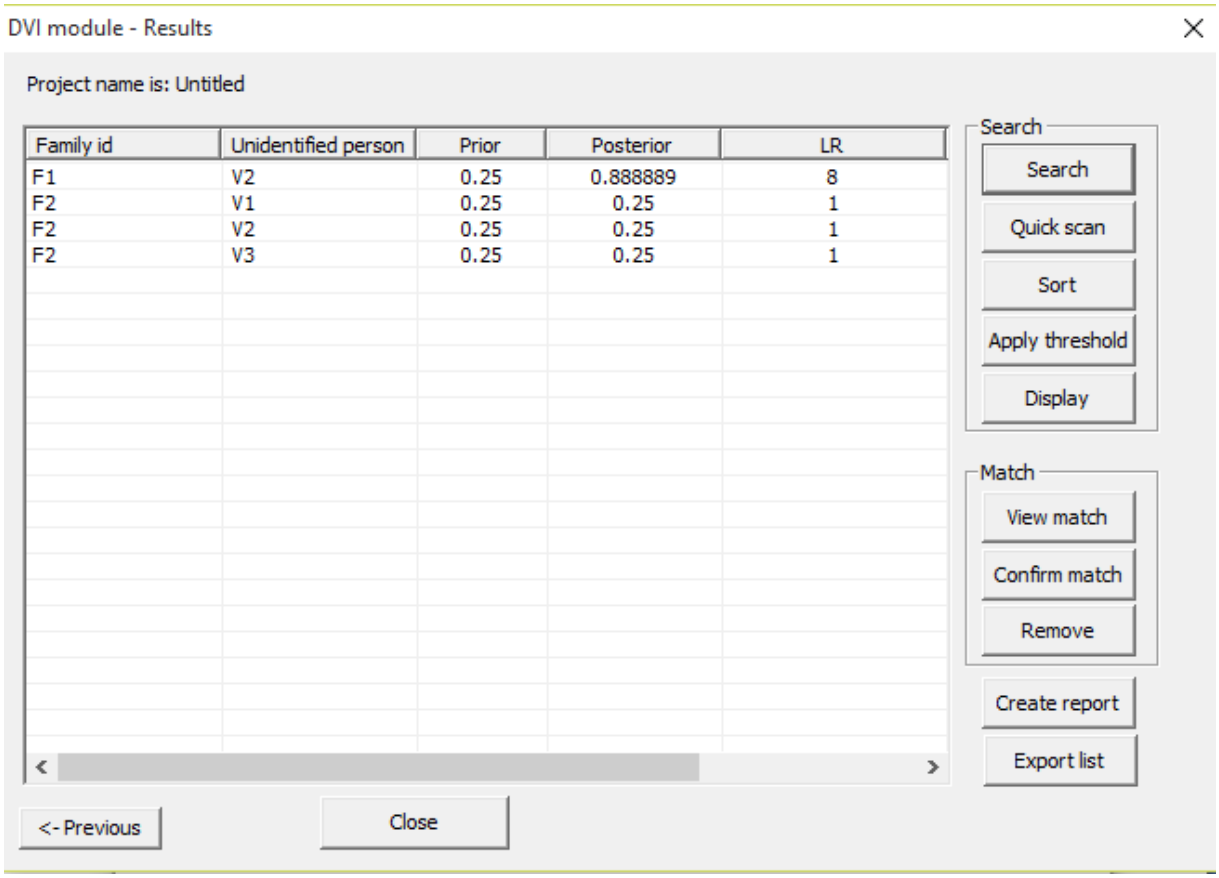


Figure 31. The results from a DVI search. Both LR and posterior probability are displayed.

5 Blind Search

5.1 The blind search

The Blind Search module is a new tool (in **Familias 3**) used to perform an unspecific relationship search for a set of person with some DNA data. Consider for example a list of persons with DNA data for which we want to know about any undefined relations. Using the module we may perform a search for any of the relationships, Parent-Child, Siblings, Half-siblings, Cousins, 2nd cousins and Direct-matches. (See figure below for the search options dialog.)

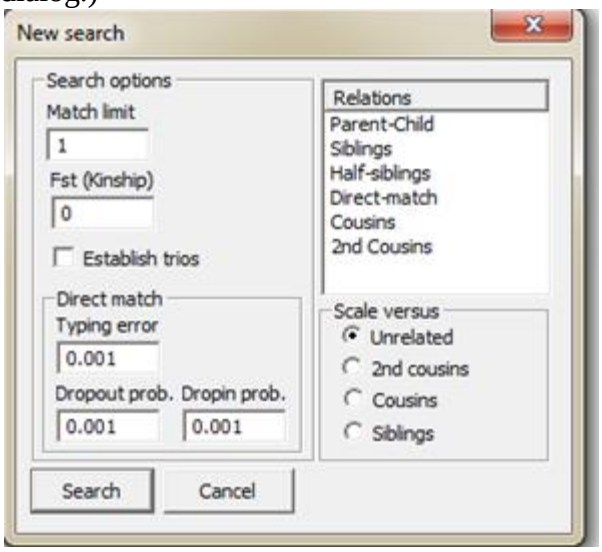


Figure 32. Starting a new blind search.

The search will perform a pair-wise comparison with all persons against each other person and calculate an LR for each selected relationship. Keep in mind that we cannot distinguish between for instance half-siblings and uncle-nephew, which is why the above relationships should rather be considered by their identical by descent sharing coefficients (IBD). We consider, k_0, k_1 and k_2 corresponding to the probability of sharing 0, 1 or 2 alleles IBD. For the relationships mentioned above the corresponding values (k_0,k_1,k_2) are (0,1,0), (0.25,0.5,0.25), (0.5,0.5,0), (0,0,1), (0.75,0.25,0) and (0.9375,0.0625,0), where several relationships may fit into the same IBD sharing pattern. Mutations are only modeled for the Parent-Child relation and disregarded for the other relationships.

Furthermore, the value *Match limit* corresponds to the threshold which a certain match will have to exceed in order to be reported. The *Fst (Kinship)* corresponds to the subpopulation correction parameter. The direct-matching feature contains a specific algorithm described in Kling et al. (2014) and needs three different parameters, *Typing error*, *Dropout probability* and *Dropin parameter*. A more complete description including formulae appears in Section 2.3 of Kling et al. (2014). We may addition scale the LR versus some different relationships, Unrelated, 2nd cousins, Cousins or Siblings, i.e. what likelihood appears in the denominator of the LR. The figure below illustrates the results from a search.

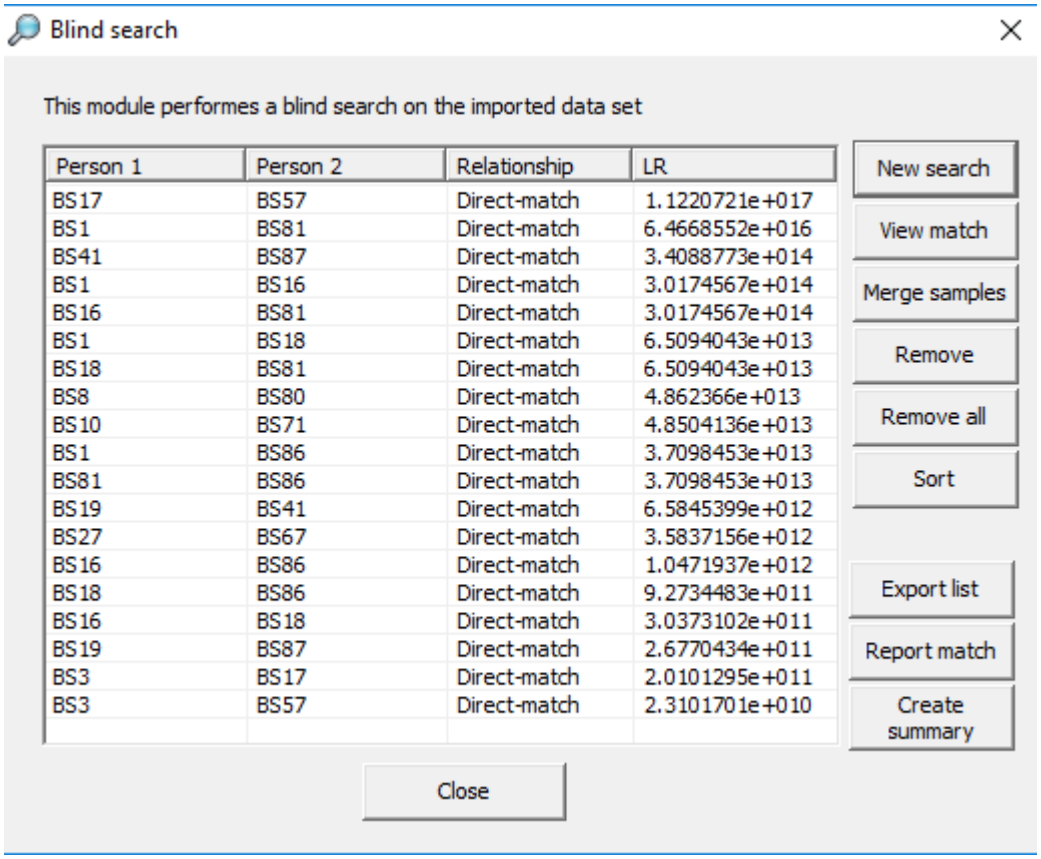


Figure 33. Blind search results.

Below follows a description of each of the buttons in Figure 33.

New Search
Brings up the dialog displayed in Figure 32 to start a new search and to define the parameters.

View match
Starts a dialog to view the individual marker results as well as displaying the profiles of the individuals in the match.

Merge samples
Brings up the dialog in Figure 34 where two samples can be merged. This only applies if the match if based on a Direct-match, see description above. Some information about the number of overlapping markers as well as matching markers is displayed. By pressing Merge, one of the samples is stored in the other as a merged profile.

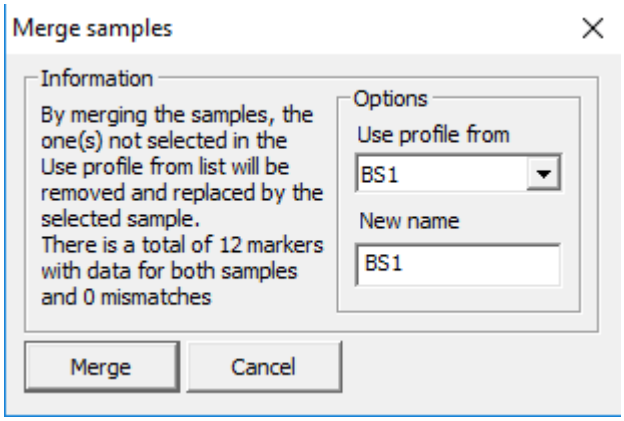


Figure 34. Merging samples.

Remove (and Remove all)
Removes the selected match(es) from the list.

Sort
Sort the list

Export list
Will export the list as displayed in Figure 33 to a tab separated text file.

Report match
Will create a specific report for a selected match.

Create summary
Creates a summary report of the search.

The blind search module is also implemented in the DVI module (see Section 4) and the Familial searching (see Section 7) module.

5.2 Viewing merged profiles

In **Familias**, version 3.2 and above, a new tool is accessible via Tools > Merged profiles, see Figure 35. First select the type of persons, *Normal casework*, *DVI – Unidentified* or *Familial searching* in the dropdown list. In Figure 35 we are viewing all merged profiles in the category *DVI – Unidentified* persons. Selecting a specific profile from the list to the left brings up further information about the profiles. Below is a brief descriptions of the buttons.

Create Report
This will generate report including information about the groups of merged profiles as well as the profiles themselves.

Print list
This will export the list displayed in the left window of Figure 35.

Unmerge
This will separate the profiles of a previously merged profiles, adding the merged profiles in the end of the selected category of persons.

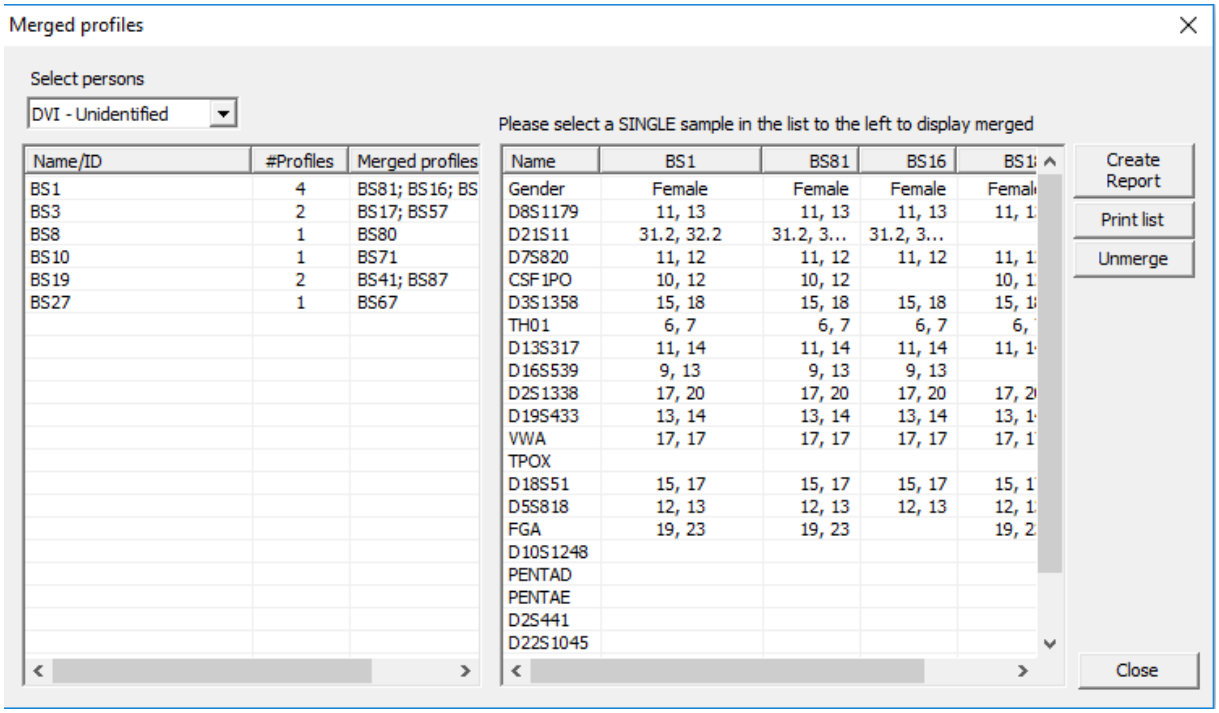


Figure 35. Viewing merged profiles.

6 Simulation interface

The simulation interface (appearing in Figure 36) is a tool to simulate genetic data and compute statistical summaries, e.g. mean/median/stdev information of the LR. This can be performed prior to obtaining a case, to assess what can be expected on a given case, but also following a computation on a specific case, to assess whether the results are expected.

6.1.1 Simulate

This button (found in the *Pedigree* window) brings up the simulation interface. An example with input is shown below for the introductory example (see Section 2) with the first marker.

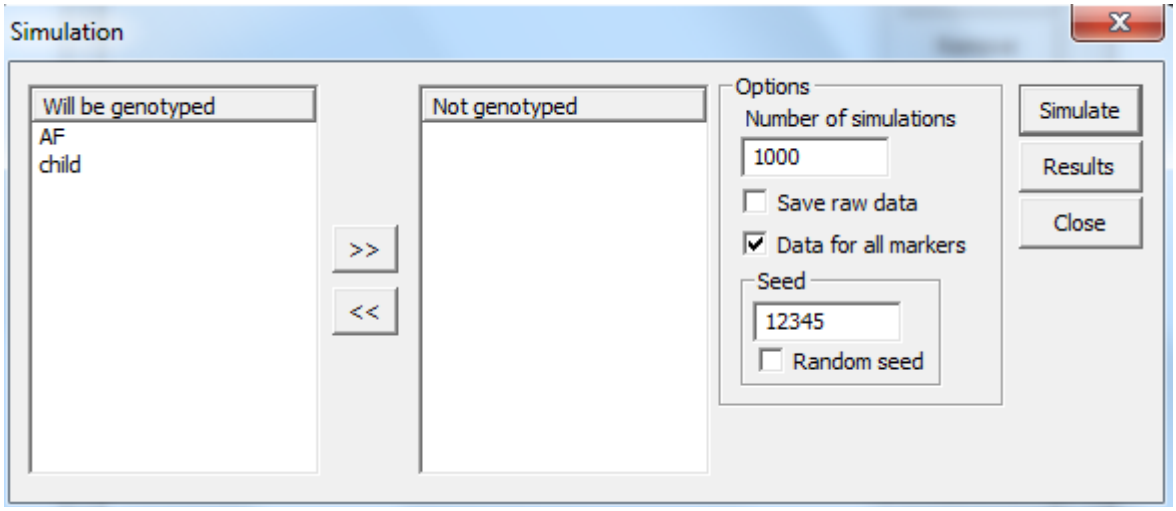


Figure 36. Starting a new simulation. Selecting the individuals that will be/is genotyped and some other options.

The above will give thousand simulations of AF and the child for all markers defined, in this case only one. The seed set to 12345 so repeated simulations will give the same results. The results appear in **Figure 37**. Below follows a description of the buttons appearing in **Figure 36**.

Simulate
Start the simulations with the specified settings. **Familias** may appear to "hang"/temporarily be unresponsive, but is in fact working hard to complete the simulations. If mutations are considered, considerable computation times can be expected.

Results
This will open the *Results* window, see Figure 37. The window will be opened automatically if a simulation process is started.

Number of simulations
Specifies the number of simulations to be performed. It is recommended to perform at least 1000 simulations to obtain reasonable values. Keep in mind that for each simulation we will simulate data for each of the pedigrees and do computations for all pedigrees. In other words, the total number of computations will be #Simulations * #nPedigrees * #nPedigrees.

Save raw data

This will save the raw data from the simulations. Two different types of data may be saved

1. Genotypes
2. Likelihoods

This is defined in the *Advanced* dialog.

Data for all markers
Default is on. This option allows the simulations to be performed with either all markers in the frequency database or only the ones selected in the *Pedigrees* dialog (see *Included systems* in Section 3.11)

Seed
Used to specify where the simulations will start. If the same seed is used each time the simulations are started, we expect exactly the same results, given all other values/parameters remain unchanged. Random seed will use a different seed each time.

Will be genotyped
Specify which persons will be genotyped. In a case of siblings (without data from the parents), the two siblings should appear as genotyped while the parents should appear as not genotyped. The simulation will then simulate data for all persons but only include genotypes for the siblings in LR calculations.

Continuing on our example, we have used a stepwise mutation model (stationary) and used the Display button to select the output in **Figure 37**.

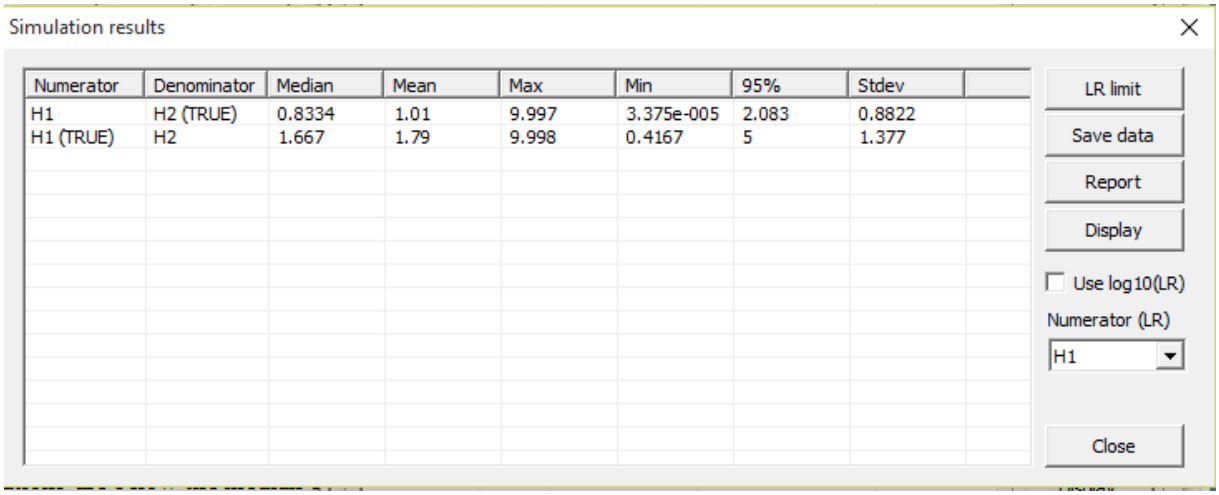


Figure 37. Simulation results with summary statistics.

Consider the first line above. The simulations are conditioned on H2 (denominator hypothesis), AF and child are unrelated. Data is simulated for AF and child. We expect mostly small LR values, but not 0 as a stepwise stationary mutation model with mutation rate 0.001 and range 0.1 is used. 50% of the simulations are below the median 0.8334, the mean is 1.01. The maximum value is 9.997. Line two gives the same result, but now conditioned on that H1 is true, AF being the father and larger LR-s are expected, but not hugely so as only one marker with four alleles is considered.

The LR limit button is used to find the fraction of simulations exceeding a prescribed level, see figure below.

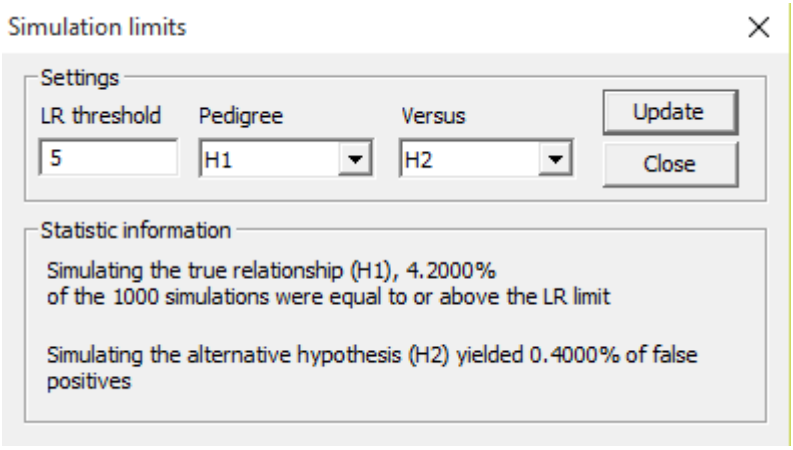


Figure 38. The simulation limit window.

The buttons in Figure 37 is explained below.

LR limit
The dialog in Figure 38 appears. This is used to use the simulation data to estimate true positive rate and false positive rate. In other words, to display results in a way that may be easier to understand.

Save data
Save the output from the simulations (LR:s) The data can be read into R after slight editing of the output file.

Report
Save a comprehensive report of the simulation results.

Display
Used to select which statistics to display.

If the *Use log10(LR)* box is ticked, all results are displayed on a logarithmic scale instead.

Further details on simulations are provided in Section 2.2 of Kling et al. (2014).

7 Familial searching

This section briefly describes functionality included in the Familial searching module of **Familias** (version 3.1.6 and above).Familial searching is a concept where we search a database of convicted offenders and traces against reference profiles or traces from crime scenes to find relatives. In other words, we compare each element of the database with each profile of interest and compute a LR comparing the hypotheses that the two profiles are related or not.

The interface is opened in Tools > Familial searching. The action opens up the *Import database* dialog where the database of persons/traces is defined. (Preferably imported from a file). Note, the Familial searching interface can handle mixtures. The buttons appearing in Figure 39 is explained below.

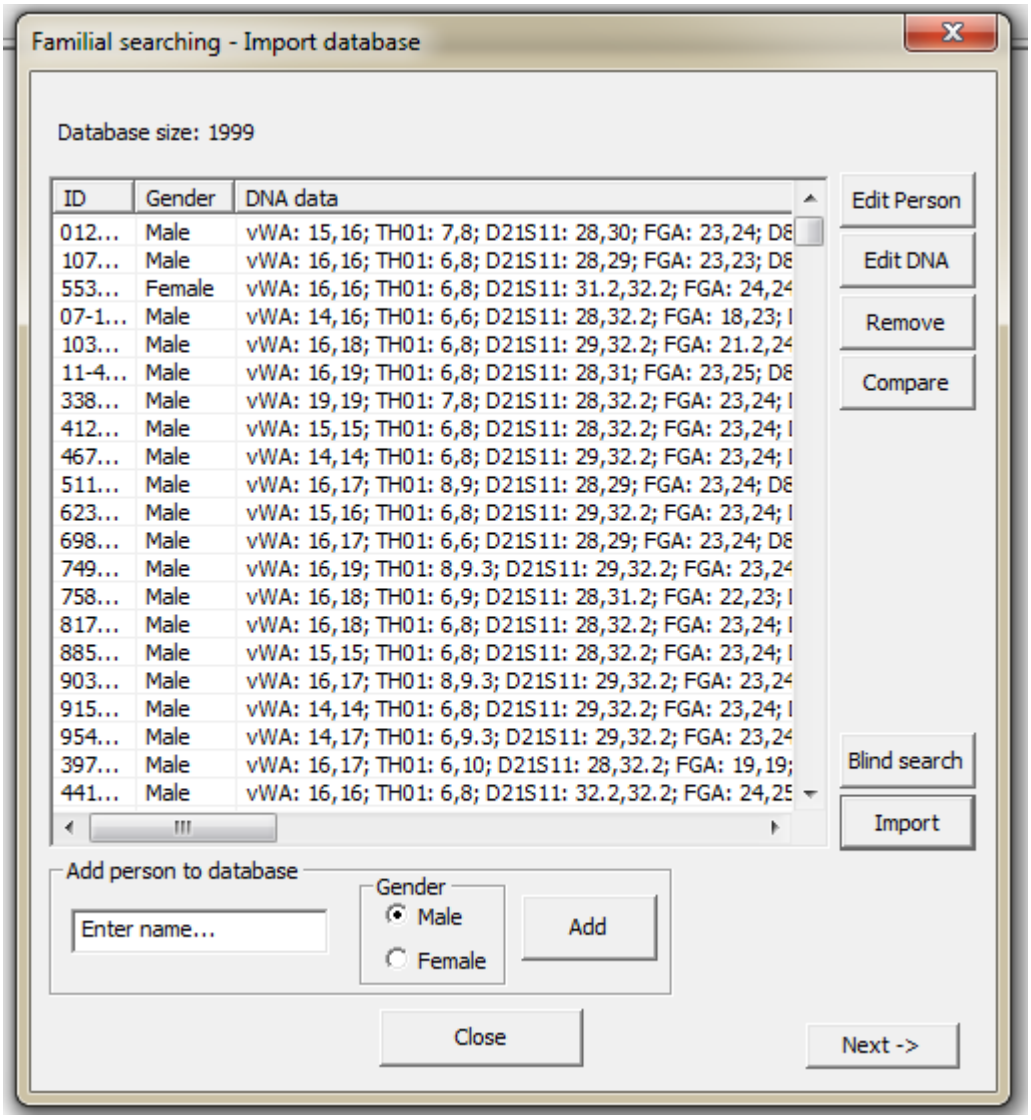


Figure 39. The Familias searching interface - Importing database with convicted offenders.

Edit Person
Edit a person/database element

Edit DNA
Edit the DNA data of a selected person/database element.

Remove
Removes a selected person/database element

Compare
Compare the DNA data for a number of selected persons/database elements. If only one person is selected, the random match probability for the profile is displayed instead.

Blind search
Perform a blind search in the database. Find direct matches and/or related elements.

Import
Import a database of persons/traces using any of the import option described previously. It is common to have a CODIS database. The CODIS format is the only allowing for the import of mixtures.

The next dialog is the *Options* dialog. The dialog a combination of defining/importing the profiles/traces to search for and to define the search parameters.

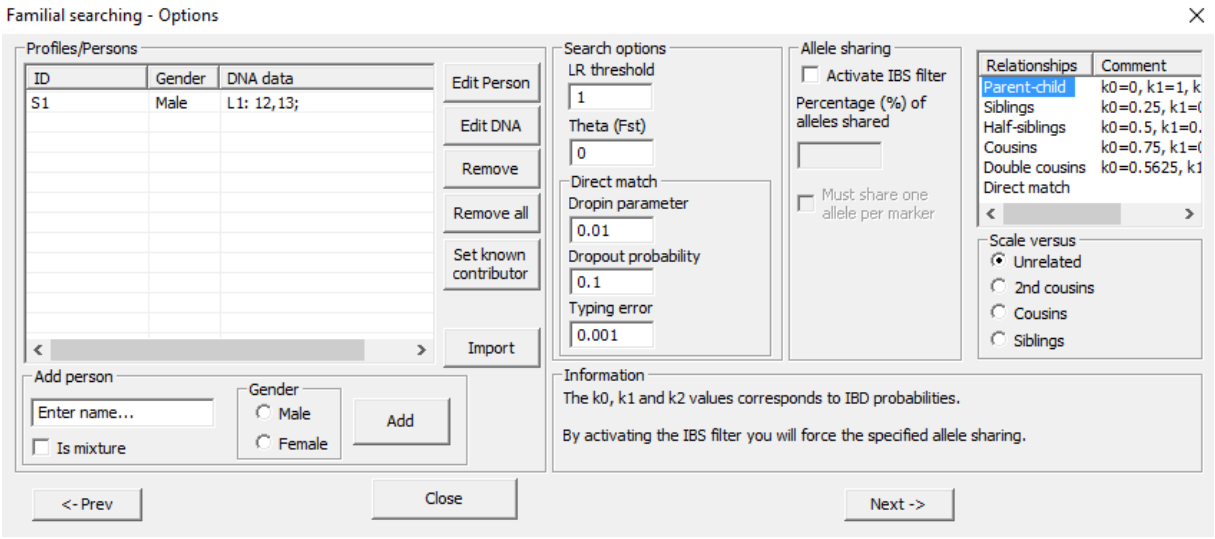


Figure 40. Familial searching interface – Defining traces/profiles and search parameters.

7.1 Profiles/Persons

Edit Person
Edit a person/trace

Edit DNA
Edit the DNA data of a selected person/trace

Remove
Remove a selected person/trace

Remove all
Remove all persons/traces

Set known contributor
Set known contributors of a profile/trace. Used to e.g. distinguish the profile of the perpetrator from the victim.

Import
Import a set of persons/traces using any of the import options described previously.

7.2 Search options

LR threshold
Threshold for the a match to be reported, i.e. all matches with a LR above the threshold will be saved for further processing.

Theta (Fst)
Correction for subpopulation effects. Positive value between 0 and 1.

Drop-in parameter (Direct matching)
This function relates to the direct matching feature, described in detail in Kling et al (2014). Briefly the drop-in parameter describes the probability that an allele is in the profile as an artifact.

Dropout probability (Direct matching)
This function relates to the direct matching feature, described in detail in Kling et al (2014).Briefly the parameter describes the probability that an allele has failed to amplify in the PCR, causing a homozygote genotype, whereas the true genotype is heterozygote.

Typing error (Direct matching)
This function relates to the direct matching feature, described in detail in Kling et al (2014). Briefly the parameter describes the probability that a genotype has been erroneously called in the analysis, also known as any error caused in the laboratory procedure.

Activate IBS filter
Activates a filter that will remove matches that do not meet the specified IBS thresholds, see below.

Percentage (%) of alleles shared
A filter that removes matches where the number of shared alleles (total number shared IBS/total number possible shared for the overlapping markers) is below this threshold.

Must share one allele per marker
Certifies that all matches share at least one allele per marker

Relationships
Select the relationships to search for.

Scale versus

Select the relationship you wish the search to scale against. Normally "Unrelated", unless there is a suspicion that the persons in the database are related to some degree, e.g. in a smaller population individuals may be related as 2nd cousins.

7.3 Search

The next step is the *Perfom search* dialog, see **Fel! Hittar inte referenskälla.** below. An xplanation of the output is given at the end. Below follows a description of the buttons.

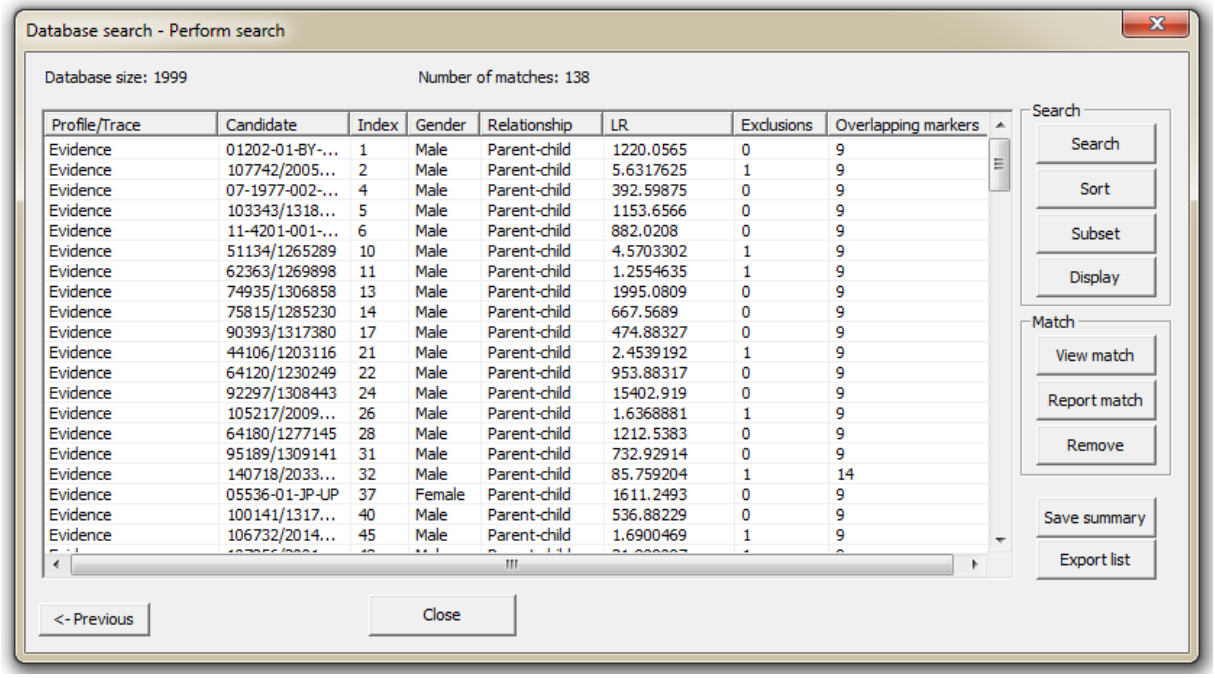


Figure 41. Familias searching interface – Performing a search

Search
Perform a search using the specified parameters in the previous dialog. The profiles/traces will be searched against all the database elements and matches will be listed for all hits exceeding the LR threshold.

Sort
Sort the matches according to LR

Subset
Select a subset of the matches using specific methods. Explanations are given for each method.

Display (Unused)
Select which things to display in the search window. Not implemented yet.

View match
Brings up a window where the user obtains a detailed view of the match with LR for each marker.

Report match
Create a report for a specific match.

Remove
Removes a selected match from the list.

Save summary
Save a summary of the search as a report

Export list
Export the search list to a tab-separated text file.

Explanation of the result

Profile/trace	**Candidate**	**Index**	**Gender**
The ID of the trace	The ID of the database match	Index of the candidate in the database	Gender of the candidate

Relationship	**LR**	**Exclusions**
The indicated relationship for the candidate and the trace	The likelihood ratio given the Relationship and the alternative hypothesis (usually unrelated)	The number of markers where the trace and the candidate do not share any markers (only applies to parent-child relationship)

Overlapping markers	**Shared alleles**	**IBS=0,1,2**
The number of overlapping markers between the trace and the candidate	The percentage of shared alleles.	Percentage of markers with 0,1 or 2 alleles shared IBS.

8 Advanced options

Some miscellaneous options are available by accessing File > Advanced, see Figure 42 below.

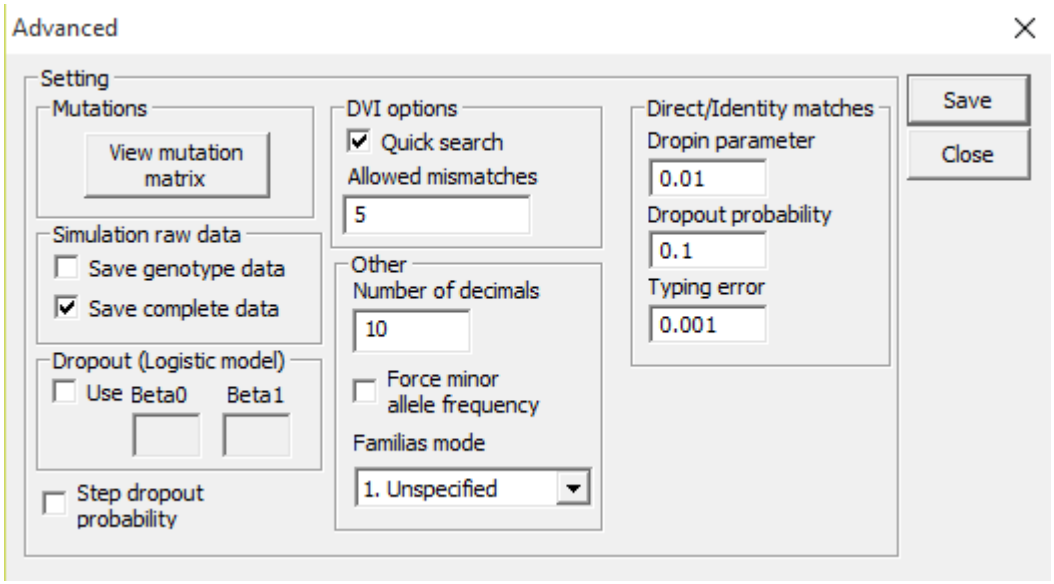


Figure 42. Advanced settings dialog.

View mutation matrix

The mutation matrix for a selected marker is displayed, see figure below. The results can be exported to a tab-separated file using the Export button.

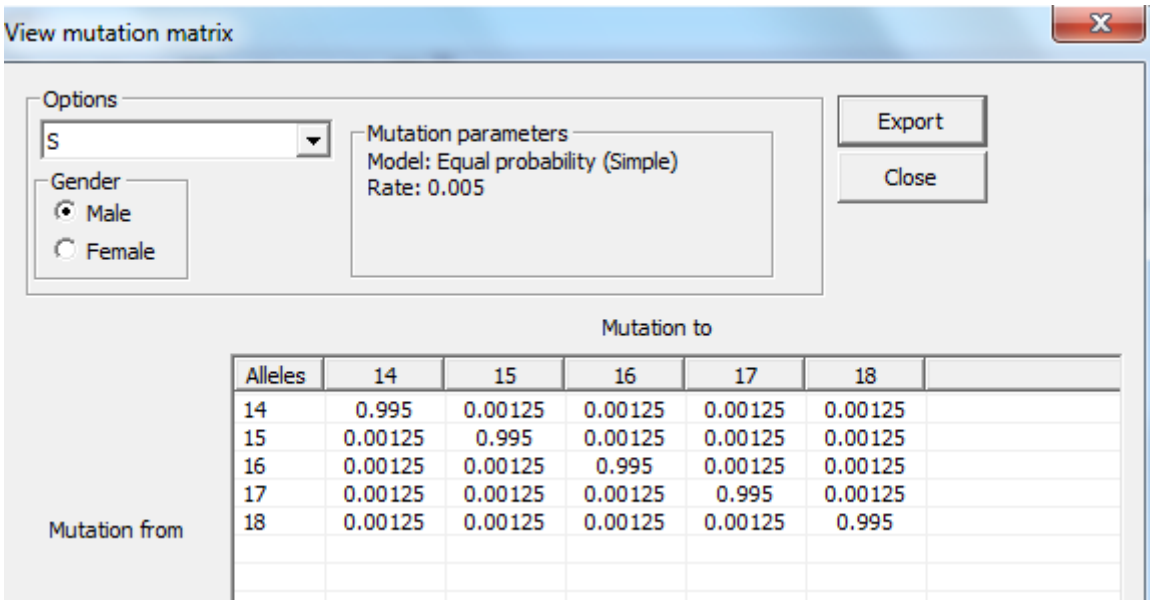


Figure 43. View mutation matrix dialog.

Simulation raw data

In connection with simulations (see Section 6), the user can specify that the simulation data is to be saved for potential further use, e.g. plotting using other programs. The amount of data to

be saved (complete data or only genotype data) can be specified. Different default options for names of output files are given depending on the choice of the user.

Dropout (logistic model)
Allelic dropout and its implication in relationship calculations is described by Dørum et al (2015). The user may here select to use profile specific dropout probabilities, instead of only marker specific.

A logistic model may be used to model dropouts. This option is for advanced users only! We specify the model as: $\log\left(\frac{d}{1-d}\right) = \beta_0 + \beta_1 \log(H)$, where d is the dropout probability, H is the peak height of the surviving allele (measured in RFU) and the β:s are estimated through regression, see for instance STRvalidator, Hanson et al. (2015)

Step dropout probability
Instead of specifying a static dropout probability the user may desire to see the LR for a number of values. By ticking the Step dropout probability feature, a dialog will appear when performing LR calculations asking the user to specify a range of dropout probabilities.

Quick search
The quick search feature is implemented to perform a faster search in the DVI module. If ticked, a fast search disregarding mutations will be performed first. For matches with mutations (specifically markers where the LR=0), calculations will be undertaken if the number of **Allowed mismatches** is above the number of markers with LR=0. It is recommended to allow quick searches for speed but setting the allowed mismatches fairly high, e.g. 4-5 to allow for possible mutations. In other words, the allowed mismatches corresponds to the number of inconsistencies we allow.

Number of decimals
The idea is that the user can specify the number of digits (for floats) displayed in different windows, e.g. the *Pedigree* window.

Force minor allele frequency
This options forces the minor allele frequency to be used in LR calculations. (Only applies to computations in the *Pedigree* window.) Be aware that by allowing this the sum of the allele frequencies for a system/marker may exceed 1.

Familias mode
Specifies the project type, *Normal* (Casework), *DVI* or *Familial searching*. Note that if selecting for instance DVI, only DVI data will be saved.

Dropin parameter
Used in the direct matching functionality, e.g. in DVI searches and blind searching. Specifies the parameter used to assess the probability that an allele drops in.

Dropout probability
Used in the direct matching functionality, e.g. in DVI searches and blind searching. Specifies the probability that an allele drops out.

Typing error
Used in the direct matching functionality, e.g. in DVI searches and Blind searching. Specifies the probability that a genotype is typed wrong.

9 Create database

Feature will be described in detail in future versions of the manual. Briefly, the feature is accessed in File > Create database. Fundamentally, the feature can import genotype data for a number of individuals, for instance in the GeneMapper format described in 3.9.1.3, without prior specification of a frequency database. The functionality will create a database from these individuals and produce some statistic information, such as important forensic parameters. See Figure 44 below.

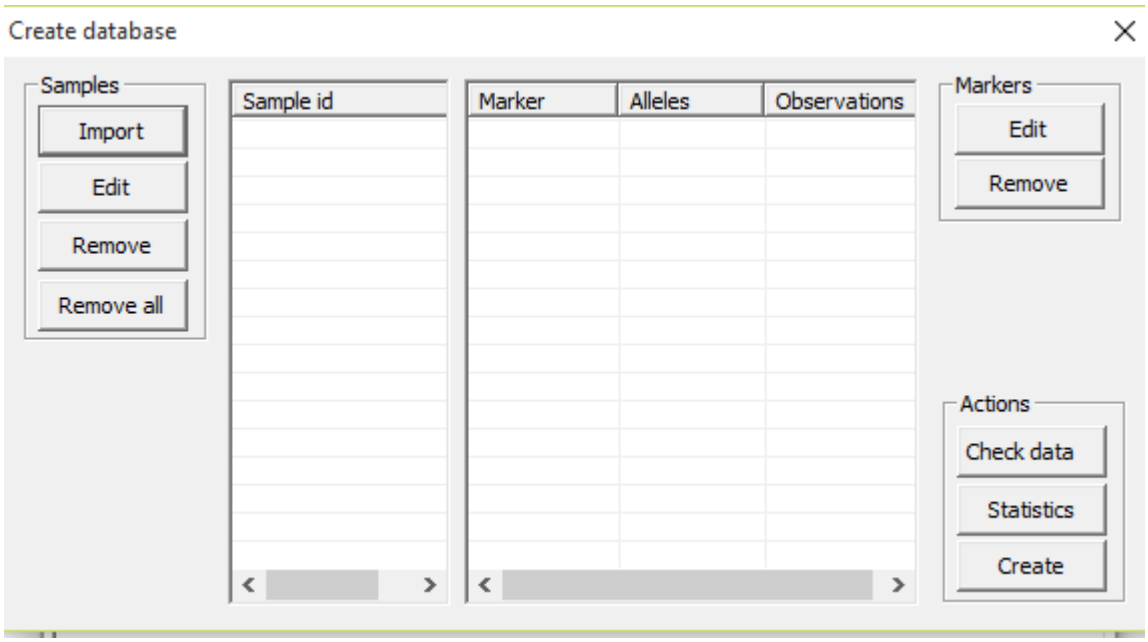
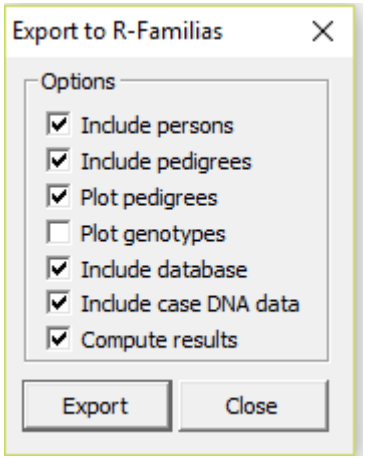


Figure 44. The create database tool

10 Export to R-Familias

The feature is accessed in File > Export to R-Familias. This brings up the window displayed below and lets the user export a complete **Familias** project to the R version of the software. This includes functionality to plot the pedigrees as well as the genotypes of the involved persons (requires the R library *paramlink* to be installed). Further instructions appear on http://familias.name/openfamilias.html and some useful links on http://familias.name/book.html.



11 Plotting

The later versions of **Familias** (3.1.9.6 and above) allows plotting of pedigrees. There are several ways to achieve this; they are briefly described below.

1. Use the software **FamiliasPedigreeCreator**, briefly mentioned in the Preface. This software is freely available at http://www.familias.no (Downloads section) and creates an R-script which in turn will create png files for all **Familias** projects in a specified directory (and subdirectories). The png files may then be displayed in the software via the *Pedigrees* dialog and View Result button or inserted directly in a report.
2. Use the option found in File > Export to R-Familias (Select to plot). This will generate an R-script and plots will be displayed. These are not automatically stored but the user can decide to if necessary.
3. Use the option located in the *Pedigrees* dialog via Add/Edit pedigree and the Plot button. This will generate an R-script that can be run to plot the pedigree only. No files are stored.
4. In the *DVI module*, either use the plotting function described in 3. to plot individual pedigrees, or
5. Plot all pedigrees using the function located in the *Add Reference Families* window and the Prepare pedigree plots button. This will generate an R-script that will plot and store the figures as png files (for all the selected families). These can be displayed in the software by selecting the same families and pushing the Evaluate button and in the next dialog pushing the View Family button. The missing person will be indicated with red.

There are several ways to alter the plots, we refer to the R-package paramlink (https://cran.r-project.org/web/packages/paramlink/index.html) implementing functions of the kinship2 package. A useful parameter is the cex that will effectively increase or decrease the size and the text. Try decreasing the parameter if the text/pedigree is to large, good values should be 0.4-1.

12 Error handling and input checking

This section contains some basic information about what checks **Familias** performs to look for errors in input data (both files and manual input). Here is a list of some common errors, remember the list is not exhaustive. In addition, very little checking is performed on reading from file.

Description	**Handled**
Input markers/systems with an extra blank/space before or after the name	This is generally handled in **Familias**, but any characters are otherwise accepted.
Input alleles with an extra blank/space after or before the name	The same as above.
Names of persons/individuals/pedigrees/families.	Duplicate names and empty names are not allowed. Otherwise, all names are allowed with any characters.
Input numbers out of bounds	Generally a check is performed to ensure all frequencies or probabilities are in the range 0 to 1. Other numbers are normally checked to be within reasonable ranges.
Relations	Generally whenever a relation is added to a pedigree or as a known relation a check is performed to find if the relation is ok. Checks include number of parents, gender of parents and year of birth of individuals as well as some other consistency controls.

13A Appendices

13.1 A1 Theory and methods

The method **Familias** is based on may be divided into the following stages: First, we describe the set of possible pedigrees involving the relevant persons. This may sometimes be a very large number. Secondly, we assign a prior probability distribution to this set of pedigrees, based on non-DNA evidence. Finally, we introduce DNA measurements and mutation parameters, obtaining a posterior probability distribution on the pedigree set. Likelihood ratios (LR-s) may also be calculated and then prior distributions are not needed.

Familias determines relationships between persons through parent-child relations. When you define persons in **Familias,** you distinguish persons based on those who may have children and those you know do not have children. This distinction will typically be made based on age. It is thus possible to define a person as a child. If no such information is available, then the safest alternative is to classify all the persons as adults. Next, the persons involved are characterized according to gender.

Based on the information above, one may generate all possible pedigrees containing only these individuals. However, one will frequently be interested in pedigrees involving persons not included in the original group. For example, to describe that a woman has three children with the same man, it is necessary to include this man in the pedigree, even though his DNA is unavailable. The implemented approach introduces a number of "extra" men and "extra" women and generates all possible, different pedigrees.

13.2 A1.1 Prior model

The set of pedigrees generated should contain the pedigrees we consider probable given the background information, but will also contain a large number of pedigrees that are unlikely for different reasons. For example, many very incestuous pedigrees will be generated; in most cases, they should not be considered a priori as likely as non-incestuous pedigrees. Similarly, most pedigrees will indicate a more promiscuous behavior than is usual in most cultures.

Familias generates a probability distribution on the set of pedigrees reflecting such considerations. Starting with an equal probability distribution on the pedigree set, we may choose to modify the prior probabilities of different pedigrees using the three options *inbreeding*, *promiscuity* and *generations*. The first parameter may be used to increase or decrease the probabilitiesof pedigrees involving inbreeding. A similar comment applies to promiscuity, while generations allude to the modification of probabilities of pedigrees extending over several generations. The prior distribution is proportional to

$$M_I^{b_I} M_P^{b_P} M_G^{b_G} \tag{1.1}$$

where M_I, M_P and M_G are non-negative parameters provided by the user of the program. The subscripts refer to the three mentioned options. The corresponding integer exponentials b_I, b_P and b_G explained next are calculated by **Familias**. b_I is the number of children whose parents have a common ancestor in the pedigree. For *promiscuity*, the number of pairs having

precisely one parent in common is calculated and denoted b_P. The number of persons in the longest chain of generations starting with a named person and ending in another named person is calculated and assigned the value b_G. In addition, it is possible to discard automatically all pedigrees where the number of generations b_G exceeds a prescribed level.

Letting $M_I = 0$, the prior probability of all incestuous pedigrees is 0. A value of the parameter between 0 and 1 decreases the probability of incestuous alternatives in comparison to non-incestuous ones, while a value exceeding 1 increases the probability of incestuous constellations. There is apriori no maximally incestuous pedigree as $M_I$may be arbitrarily large. Similar comment applies to the other options. A small, artificial example illustrates some of the concepts above. Assume three men, M1, M2 and M3 are found dead and two alternatives are considered: *H_1: M1 is the father of M2 who is the father of M3* and *H_2: M1 is the father of M2, while M3 is unrelated to M1 and M2.* The ratio of the priors corresponding to alternatives H_1 and H_2 follows from Equation (1) as

$$\frac{M_I^0 M_P^0 M_G^3}{M_I^0 M_P^0 M_G^2} = M_G$$

We emphasize that this prior is but one pragmatic suggestion among many others possible; in many cases they are not needed. The default of the parameters M_I, M_G and M_P is by **Familias** set to equal 1 and therefore implies that all pedigrees have, a priori, the same probability. Further details on the prior model including examples appear in Egelandet al. (2000).

13.3 A1.2 Posterior model

According to Bayes' theorem the *posterior probability ratio* (PPR) may be written as

$$\text{Posterior probabilility ratio} \;=\; \text{Likelihood ratio} \times \text{Prior probability ratio}$$

In a more mathematical terminology

$$\frac{\Pr(H_p \mid E, I)}{\Pr(H_d \mid E, I)} = \frac{\Pr(E \mid H_p, I)}{\Pr(E \mid H_d, I)} \times \frac{\Pr(H_p \mid I)}{\Pr(H_d \mid I)} \qquad (1.2)$$

where*E* typically stands for evidence, more precisely DNA-data, and *I* is some conditioning information like for example age. Relating to a forensic evidence interpretation, the term H_p is the prosecution hypothesis and the defendant hypothesis is denoted H_d. Usually it is the likelihood ratio (LR) that is reported in court.

It remains to explain the calculation of the likelihood $\Pr(E \mid H, I)$. A version of the Elston-Stewart algorithm is implemented (Elston and Stewart 1971). The algorithm is extended to account for possible substructure, silent alleles and mutations and these extensions are explained in the coming sections.

13.4 A1.3 Subpopulation corrections

The probability of a set of DNA-data is calculated by looking at the different loci separately before multiplying the results. For all individuals, a locus of the DNA consists of two alleles, which can be either equal, constituting a homozygous locus, or different, giving a heterozygous locus. The probability of a particular combination of alleles (the genotype) is in the simplest cases calculated by means of Hardy-Weinberg's law. This law states that the probability of being either heterozygote $A_i A_i$ or homozygote $A_i A_j$ is given by

$$\Pr(A_i A_j) = \begin{cases} P_{ii} = p_i^2 & \text{if } i = j \\ P_{ij} = 2 p_i p_j & \text{if } i \neq j \end{cases} \qquad (1.3)$$

Wherep_i is the frequency of allele A_i in the population.

Assuming the following conditions are satisfied:

i. random mating,
ii. no selection,
iii. no mutation,
iv. no migration,

the population in question is at so-called Hardy-Weinberg equilibrium, and Equation (1.4) is valid.

In situations where mutations and non-random mating occur, the assumptions in Hardy-Weinberg's law are no longer necessarily satisfied. As mentioned, Hardy-Weinberg's law may not apply in the presence of population stratification and relatedness. To handle this, **Familias** incorporates a kinship parameter, which is set by the user. The parameter corresponds to the traditional F_{ST} known from population genetics (see, e.g., [1]). It takes into consideration that within a subpopulation there tends to be a higher frequency for homozygosis than if Hardy-Weinberg equilibrium is obtained.

If p_i is the frequency of A_i in the population, then the genotypic frequencies are described by

$$\Pr(A_i A_j) = \begin{cases} F_{ST} p_i + (1 - F_{ST}) p_i^2 & \text{if } i = j \\ 2(1 - F_{ST}) p_i p_j & \text{if } i < j \end{cases}. \qquad (1.4)$$

Generally, the complete correction (sometimes referred to as θ-correction) described in (Balding and Nichols 1994) is implemented.

The differences between probabilities calculated with and without incorporating kinship can be quite large. For example, the probability of a genotype (*A*, *A*) when p_A = 0.05, is 0.00250. However, using a kinship parameter of 0.01, this probability becomes 0.00298.

It can be problematic to decide an appropriate value for the kinship parameter. One suggestion is to use 0.01-0.05 for Europeans while the value may be even higher for more divergent populations.

13.5 A1.4 Mutation models

There are fivedifferent mutation models available in **Familias** (Egeland et al. (2000)). The mutation model is specified for each allele system, and can be different for males and females. The alternative models are:

1) Equal probability (Simple)
2) Probability proportional to frequency (Stationary)
3) Stepwise (Unstationary)
4) Stepwise (Stationary)
5) Extended stepwise (Unstationary)

We provide details of the models below in an order deviating from the above for practical reasons.A mathematical note, the following section requires an understanding of basic statistics and linear algebra.

A1.4.1 Step-wise (unstationary).

It is convenient to first describe model 3. In the decreasing model we assume that the list of alleles is expanded to include all “possible” alleles, and that they are listed by increasing lengths. The probability of mutation from allele a to allele b decreases in this model as a function of the difference in length between the alleles. This property is illustrated in Figure A.1, where the thickness of the arrows illustrates the probability of the transitions. The transition matrix *M* for this model is given by:

$$M = \begin{bmatrix} 1-R & k_1 r^{|1-2|} & . & . & k_1 r^{|1-N|} \\ k_2 r^{|2-1|} & 1-R & . & . & k_2 r^{|2-N|} \\ . & . & . & . & . \\ . & . & . & . & . \\ k_N r^{|N-1|} & . & . & . & 1-R \end{bmatrix},$$

Where*R* is the overall mutation rate, *r* is a constant between 0 and 1 (0<r<1). The *r* parameter is provided by the user and is Mutation range in Familias. k_i is chosen such that $\sum_{j=1}^{N} m_{ij} = 1$.

A calculation gives $k_i = \frac{R(1-r)}{r(2-r^{i-1}-r^{N-i})}$.

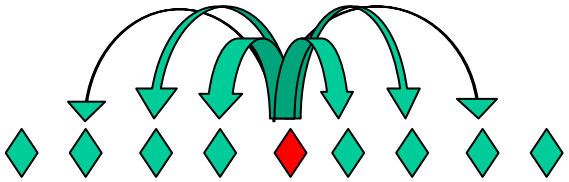


Figure A.1: *Mutation model*

13.5.1 A1.4.2 Step-wise (stationary)

This model is explained in(Dawid, Mortera et al. 2002) and is a stationary version of the previously described model. Below we provide some details beyond those presented in the mentioned paper. The current implementation may give a somewhat unreasonable mutation matrix for some particular combinations of parameter settings. We hope to rectify this problem in future releases. In the meantime, the unstationary version may be a safer version.

We want to generate stationary mutation models. Recall that a mutation model can be represented as a square matrix $M = \left[m_{ij} \right]$ where m_{ij} is the probability of mutating from allele *i* to allele *j*. The fact that these values are probabilities is contained in the requirement $M1 = 1$ where 1 is the column vector of ones, and in the requirement that all elements of *M* are non-negative. Let *p* be the column vector of allele population frequencies and *p'* the transposed(row) vector. Then *M* is stationary iff (if and only if) *p'M = p'*

How can one modify a mutation model so that it becomes stationary? Clearly this can be done in many ways, but an attractive alternative would be to adjust, for each allele, the probability that a mutation occurs, while keeping unchanged the relative probabilities of the identities of the resulting mutated alleles after a mutation. In terms of a mutation model matrix, this corresponds to adding (or subtracting) various values along the diagonal, while adjusting the remaining values so that the numbers on each line still sum to 1. Technically, let *A* be a mutation model, i.e., *A1 = 1* and all elements non-negative. Then we will find a stationary version of it by writing *M = DA + I – D*, where *D* is a diagonal matrix. We get *M1 = DA1 + 1 – D1 = 1*, so *M* is a mutation matrix, as long as *D* is defined so that the elements of *M* are non-negative: This means that $d_{ii} \geq 0$ and $d_{ii} \leq 1/(1-a_{ii})$. *M* is also stationary iff *p'M = p'*, that is, if *p'DA + p' – p'D = p'*, i.e., iff*p'DA = p'D*, i.e., iff*v = Dp* is a right eigenvector of *A'* belonging to the eigenvalue 1.

Assume *A* is symmetric, as it is in our examples. Then 1 is such an eigenvector, and we get a solution by defining *D* such that *1b = Dp*, where *b* is some positive scalar. Note that *b* must be small enough so that

$$d_{ii} \le 1/(1-a_{ii}),\ \text{i.e.,}\ b \le \min_i \left(p_{ii}/(1-a_{ii})\right).$$

Thus we can always generate a stationary mutation model from a symmetric mutation model matrix, in the manner above.

Define *A* by defining $a_{ij} = c^{|i-j|}$ for $i \neq j$ for some constant *c*, and define a_{ij} for *i=j*so that *A1 = 1*. Then the stabilized matrix *M* becomes defined by $m_{ij} = ba_{ij} / p_i$ for $i \neq j$ and m_{ij} for $i =$ *j*again computed so that *M1=1*. We get

$$m_{ii} = 1-[bc / p_i(1-c)](2-c^{i-1}-c^{n-i})$$

The parameter *c* is assumed input from biological knowledge, while *b* is computed from the overall mutation rate *R*, using the following relation:

$$1-R = p_1 m_{11} + p_2 m_{22} + \ldots + p_n m_{nn}$$

giving

$$b = R(1-c)^2/[2c(n-cn-1+c^n)] \qquad (1.5)$$

With the user giving as input *R* and *c*, the program computes the mutation model *M* by first computing *b* as above, then computing the off-diagonal elements of *M*, and then the diagonal by requiring the rows to sum to 1. Note that the requirement that b cannot be too large translates to the requirement that for all *i*

$$R \le 2(n-cn-1+c^n)/[(1-c)(2-c^{i-1}c^{n-i})]p_i .$$

As another example, define *A = 1p'*. then clearly *A1 = 1*. To stabilize it, we choose a *D* such that *p'DA = p'D*. We may choose *D = kI* for some constant *k*. We get that we must have $k \le 1/(1-p_i)$ for all i, and, defining *R* as above, we get that

$$R \le \frac{\sum_i (1-p_i)\, p_i}{(1-p_i)}$$

for all *i*.

A1.4.3 Extended step-wise model (unstationary).

The model is described in Kling et al. (2014) and a slightly revised version appears below. There is a need for a new mutation model capable of handling transitions to and from microvariants, e.g. between 9 to 9.3. Some current models treat such *microvariant mutations* (MVM) in the same way as *integer mutations*(IM) or neglect them as the mentioned transitions are considered improbable. This is biologically unreasonable and the problem has become more pronounced as MVM are more common in the latest STR kits.

We specify the model by letting *M* be the mutation matrix, with elements m_{ij}, where $i,j=1,\ldots,N$ and where *N* is the number of alleles. Each element m_{ij} is the probability of a transition from allele A_i to allele A_j. The current model separates the overall mutation rate, denoted μ , into two parts, one corresponding to integer mutations, *R*, and one to the micro-variants α , i.e., $\mu = R + \alpha.$ Biologically *R* is often explained by slippage error during DNA replication (Ellegren 2000) while α is connected to insertions/deletions and point mutations. The last parameter, the mutation range *r*, is defined as for previous IM models; it is the value with which the probability decreases for each further step away from the original allele mutates.

Next the model is specified precisely by the transition probabilities m_{ij} .There are three different alternatives:

1. $m_{ij} = 1 - \mu, if\ i = j$,i.e. the probability that an allele does not mutate.
2. $m_{ij} = k_i R r^{|i-j|}, if\ i \neq j$, for integer mutations.
3. $m_{ij} = \frac{\alpha}{N_i}, if\ i \neq j$, for micro variant mutations and N_i is the number of MVM-s from allele *i*.

The rows must sum to unity and therefore the normalizing constants k_i are determined by the constraints $\sum_{j=1}^{N} m_{ij} = 1.$

Example 1. Consider a marker containing the alleles 9, 9.3, 10, 10.3 and 15. The transition matrix *M* is then given by:

$$M = \begin{bmatrix} 1-\mu & \alpha/2 & k_1 R r^1 & \alpha/2 & k_1 R r^6 \\ \alpha/3 & 1-\mu & \alpha/3 & k_2 R r^1 & \alpha/3 \\ k_3 R r^1 & \alpha/2 & 1-\mu & \alpha/2 & k_3 R r^5 \\ \alpha/3 & k_4 R r^1 & \alpha/3 & 1-\mu & \alpha/3 \\ k_5 R r^6 & \alpha/2 & k_5 R r^5 & \alpha/2 & 1-\mu \end{bmatrix}$$

In this case, k_1 is found as follows

$$1 = 1 - (R + \alpha) + \frac{\alpha}{2} + k_1 R r + \frac{\alpha}{2} + k_1 R r^6 \leftrightarrow k_1 = \frac{1}{r + r^6}$$

Similar calculations can be shown for the other k_i. Note that, the matrix M is not symmetric, meaning that the probability of observing a mutation from 9 to 9.3 is not the same as observing a mutation from 9.3 to 9. This is a consequence of the definition of *M*. Further note

that for transitions from allele 9 for example, N_i=2 as there are two MVM:s given allele 9 as starting point. NB! There is a small deviation of this model from the description that appears in Kling et al. (2014).

A1.4.4 Probability proportional to frequency (stationary)

In this model the probability of mutating to an allele is proportional to that allele's frequency. This model is as mentioned stationary. The transition matrix *M* for this model is given by:

$$M = \begin{bmatrix} 1-k+kp_1 & kp_2 & . & . & kp_N \\ kp_1 & 1-k+kp_2 & . & . & kp_N \\ . & . & . & . & . \\ . & . & . & . & . \\ kp_1 & . & . & . & 1-k+kp_N \end{bmatrix}$$

where k is a constant. This model satisfies the stationarity condition $\sum_{i=1}^{N} p_i m_{ij} = p_j$. The overall mutation rate becomes $R = k\sum_{i=1}^{N} p_i(1-p_i)$, therefore we must set the constant to be

$$k = \frac{R}{\sum_{i=1}^{N} p_i(1-p_i)}.$$

Note that if the frequency of the entered alleles do not sum to 1, **Familias** will assume there is a single extra allele making up for the rest of the probability when computing k . If this is not the case, *k*will be slightly wrong. Thus the frequencies of all the alleles in the system should be entered when using the proportional model.

13.5.2 A14.5 Equal probability (simple)

In this model we assume that there are *Q* different alleles observed in a database and that $N \geq Q$ is the number of "possible" alleles. The model can best be described by means of a transition matrix M, where the elements m_{ij} denote the probabilities that alleles i are inherited as alleles j (*i, j = 1,…,N*). For this model, the probability of not mutating is for each allele $1-R$, where *R* is the overall mutation rate. The probability of mutating to any of the possible other alleles is the same $(= R/(N-1))$. This model is in fact stationary if and only if the allele probabilities are equal. So the transition matrix *M* is given by:

$$M = \begin{bmatrix} 1-R & \frac{R}{N-1} & . & . & \frac{R}{N-1} \\ \frac{R}{N-1} & 1-R & . & . & . \\ . & . & . & . & . \\ . & . & . & . & . \\ \frac{R}{N-1} & . & . & . & 1-R \end{bmatrix}$$

Note that the "frequency" of an allele entered into **Familias** is in fact interpreted as the probability of observing that allele. Thus, if the entered frequencies sum to 1, there is a zero probability of observing any other alleles, and the program requires that $N = Q$. To use $N > Q$, you need to make sure the probabilities input sum to (slightly) less than 1.

13.5.3 A1.4.6 An example illustrating the mutation models

This example is a paternity case with an alleged father (AF) with genotype (*A*, *B*) and a child (CH) with genotype (*C*, *D*).The population properties of the allele system (S1) are given in Table A1.

Table A1: *Properties of allele system S1.*

Allele label	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Repeat number	14	15	16	17	18	19	20	21
Count	44	49	127	175	133	58	12	2
Proportion	0.073	0.082	0.212	0.292	0.222	0.097	0.019	0.003

We consider the following hypotheses:

- H_0: AF is the father of CH.
- H_1: AF and CH are unrelated.

We use a mutation rate of R = 0.005, and calculate likelihood ratios assuming the various mutation models.

The likelihood assuming H_0 is $p_A p_B (p_C (m_{AD} + m_{BD}) + p_D (m_{AC} + m_{BC}))$. The likelihood assuming the alternative hypothesis is $4 p_A p_B p_C p_D$. So the likelihood ratio is then

$$LR = \frac{\Pr(E \mid H_0)}{\Pr(E \mid H_1)} = \frac{p_C (m_{AD} + m_{BD}) + p_D (m_{AC} + m_{BC})}{4 p_C p_D}$$

a) For the equal probability model (model 1) we set the number of possible alleles to 8, which leads to $m_{AC} = m_{AD} = m_{BC} = m_{BD} = 0.005/7$. The likelihood ratio then becomes

$$LR = \frac{0.212 \cdot 0.01/7 + 0.292 \cdot 0.01/7}{4 \cdot 0.212 \cdot 0.292} = 0.0029.$$

b) For the proportional model (model 2) $m_{AD} = m_{BD} = kp_D$ and $m_{AC} = m_{BC} = kp_C$. Hence,

$$LR = \frac{2p_C p_D k + 2p_C p_D k}{4p_C p_D} = k.$$

Furthermore, the constant k is equal to

$$k = \frac{R}{\sum_{i=A}^{H} p_i(1-p_i)} = \frac{0.005}{0.800} = 0.0063.$$

c) For the decreasing model (model 3) we use a mutation range $r = 0.5$. The individual mutation probabilities are

$$m_{AD} = k_1 r^3, m_{BD} = k_2 r^2, m_{AC} = k_1 r^2, m_{BC} = k_2 r,$$

where

$$k_1 = \frac{R(1-r)}{r(1-r^7)} = \frac{0.0025}{0.496} = 0.005, k_2 = \frac{R(1-r)}{r(2-r-r^6)} = \frac{0.0025}{0.742} = 0.003.$$

This leads to

$$LR = \frac{0.212 \cdot (0.005 \cdot 0.5^3 + 0.003 \cdot 0.5^2) + 0.292(0.005 \cdot 0.5^2 + 0.003 \cdot 0.5)}{4 \cdot 0.212 \cdot 0.292} = 0.0047$$

d) For model 4, we calculate $b = R(1-c)^2/[2c(n-cn-1+c^n)] = 0.0004161$, matrices A and Mas explained in Appendix 4.2 and find

$$m_{AD} = 0.00071,\ m_{BD} = 0.0012,\ m_{AC} = 0.0014,\ m_{BC} = 0.0025,\ LR = 0.0064$$

The different models lead to very small likelihood ratios as expected. However, the relative differences are considerable and the choice of model might well influence the overall LR considerably. Usually it will be a good idea to check the robustness of the conclusions by incorporating different mutation models.

13.6 A2 Solved excercises

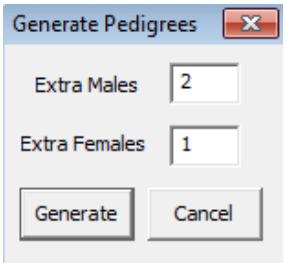
The **Familias 2.0** (or 1.97) exercises remain available from http://familias.name. New exercises with solutions for **Familias 3** are now available from http://familias.name/book.html

13.7 A3 Generating pedigrees automatically

The Generate button of the Pedigrees window can be used to generate pedigrees automatically. All possible pedigrees involving parent child relationships are generated. Keep in mind that as more persons are introduced, the number of generated pedigrees increases almost explosively. Often, as in the cases where only two pedigrees are to be compared, it is preferable to construct them manually. So far the largest number of pedigrees generated in a

case is about 10000 (in test examples). There is no limit to the number of pedigrees produced, however, extreme cases may cause the program to "hang" [2].

When generating pedigrees, the program uses the information that some persons are designated as children (i.e., having no children) and the Year of Birth information. No pedigrees will be generated that imply a generation length of less than 12 years. The generated pedigrees are named Ped1, Ped2, … etc. To view the details of a pedigree, double-click it; and the window in the figure below appears.



This is the same window that appears when pressing Add for manual construction of pedigrees. The pedigree is defined by the list of parent-child relations, and is thus altered by adding or removing these relations. You use the Persons-button to add the extra men and women that are necessary to define the wanted pedigree.

Figure A1.*Adding extra persons.*

As an alternative to adding anonymous extra persons here, the extra persons could have been defined in the Persons window described above. This is especially useful if one wants to put constraints on the number and types of possible pedigrees generated automatically, by introducing, e.g., extra persons that are of a certain age. Note that this may influence the computation of the Generationsparameter.

13.8 A4 Implementation of prior distribition

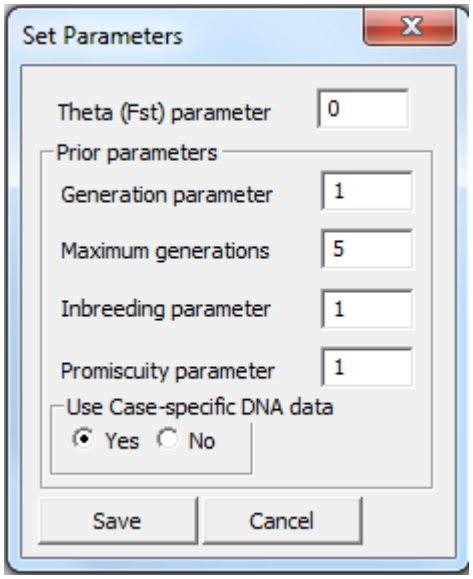


Figure A2 Parameter settings

After having entered the interesting pedigrees, one can calculate posterior probabilities for the various alternatives. By pressing Parameters(see Figure 20), the window shown in Figure A2 appears. Here you are supposed to specify parameters that are used in the calculations of posterior probabilities, including the parameters defining the prior. The default corresponds to a non-informative prior, that is, where all the pedigrees get the same prior probability. After a

possible change in the parameters the pedigrees' posterior probabilities appear. The pedigrees are now listed by decreasing probability.

The Generations parameter gives you the opportunity to modify the likelihood of pedigrees extending over several generations. More precisely, the calculated number is the number of persons in the longest chain of generations starting with a named person (not an "extra" person) and ending in an adult (not a "child"). For example, a pedigree consisting of a father and an adult son has generations value $b_G = 2$, while if the son is marked as "child", the generations value is $b_G = 1$. By setting the generations parameter to a number between 0 and 1, short pedigrees are emphasized, and by using a number larger than 1, *long* pedigrees are emphasized. In addition, it is possible to define a cut-off length for the generation chain. By specifying Max generations to, e.g., 2, you give a prior probability of zero to all pedigrees extending over more than two generations.

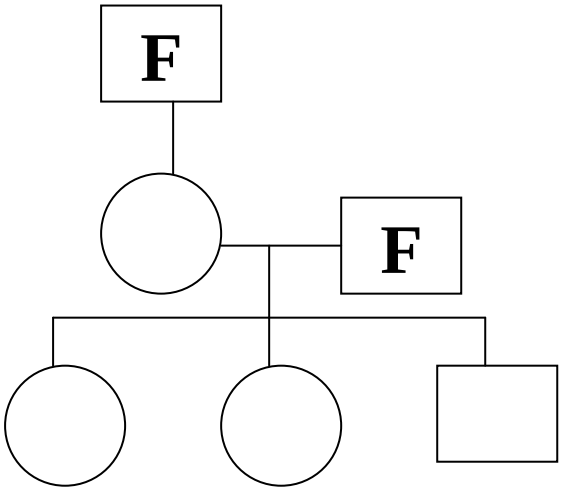
The Inbreeding parameter is used to alter the prior probability of incestuous pedigrees. More precisely, the value b_I computed is the number of persons in the pedigree such that its parents have a common ancestor. Thus, for example, a pedigree where cousins have three children together gets a value $b_I = 3$, while one where siblings have one child gets a value $b_I = 1$. Setting the inbreeding parameter to zero is equivalent to giving a zero prior probability to all incestuous constellations. A value between 0 and 1 decreases the prior probability of incestuous alternatives relative to the non-incestuous ones, while a value exceeding 1 increases the probability of incestuous constellations.

The Promiscuity parameter is used to alter the prior probability of pedigrees involving "promiscuous" behavior. More precisely, the value b_P computed is the number of pairs of half-siblings. Again: a value between 0 and 1 suppresses such pedigrees, while a value superseding 1 enhances them. Setting the parameter to zero gives a zero prior probability to all pedigrees where any person has children with more than one partner.

Example: Given the pedigree in Figure A3 and parameters provided by the user $M_G = M_I = M_P = 0.5$. The program will evaluate the pedigree and calculate the corresponding b – factors, which in this case are $b_G = 3$, $b_I = 3$ and $b_P = 3$, giving a value $M_G^{b_G} M_I^{b_I} M_P^{b_P} = 0.5^3 \cdot 0.5^3 \cdot 0.5^3 = 0.00195$. (Note that each of the children are half-siblings with their mother, giving $b_P = 3$). This means that this pedigree will be weighted down in comparison to a pedigree where the generations-, inbreeding- and promiscuity-factors are smaller, giving the pedigree in Figure A3 a smaller prior.

You may choose to calculate the posterior probabilities *without* the use of case-specific DNA data, by selecting "No" for the relevant variable. This will show you the prior probability distribution on the pedigrees, and is useful whenever you use a non-flat (non-default) prior.

The Kinship parameter takes values between 0 and 1.



13.8.1

Figure A3: *Pedigree*

By pressing OK in the Probability-window, the posterior probabilities for all pedigrees appear, and the list is also sorted from the most probable to the least probable pedigree.

13.8.2

Note that, when computing probabilities, the program will remove all pedigrees found to be equivalent to a previous pedigree in the list. For example, if the user has entered the pedigree without any relationships several times, only the first of these will remain.

13.9 A5 Description of general input files for Familias

The purpose of this document is to enable programmers to write code producing input files for the Familias program, on the "Familias format". This format was designed to store all information contained in the user interface between runs of Familias. Thus it was never designed to be readable or practical to use as a data exchange format. Nevertheless, it is released now as possibly the simplest way to exchange data between Familias and other computer applications.

13.9.1 Versions

The Familias format can often change for each release. However, backwards compatibility is ensured in that any version of Familias is able to read the format output from all previous versions. The key here is that the version number of the program producing the file is always given in the third line of the file. The format described in this document is the one output from version 1.81. Thus it will not be readable by previous versions of Familias. But it will be readable by future versions.

13.9.2 Simplified format

The "Familias format" stores some information which is irrelevant when importing data from other programs in order to do computations with Familias. For example, it is possible to store already computed probabilities, or the order in which pedigrees are listed in the pedigree list. In the table given below, lines specifying such information have been simplified and set to

default values. However, the completely general use of these lines is explained in the notes below the table.

13.9.3 Format verification

As the format was not really intended to be produced by other programs, very little verification is done at the input stage that the input file really conforms to the correct format. For example, it is not checked that indices of persons and alleles are within the bounds given by the sizes of the lists, that pedigrees are legal as pedigrees, and so on. Run-time errors, or even faulty results, are likely to occur if the input files are incorrect.

13.9.4 How to interpret the table

The table below has four columns. The first contains line numbers: These are included for reference only; the actual format does not contain such numbers. The second column describes the information each line should contain. Information inside comparison signs,

<like this >

is meta-information, and should be substituted with the corresponding content. Information without such signs should be included as written.

The third and fourth columns of the table indicate which lines should be repeated, and how they should be repeated.

13.9.5 File format

1	<Any text, in quotes; may describe the file >		
2	<Any text, in quotes; may describe the file >		
3	1.81		
4	< Number of persons involved in pedigrees >		
5	<Name of person >		}
6	#FALSE#		}
7	-1		} Repeated
8	#FALSE#		} for each
9	<#TRUE# if male, #FALSE# if female >		} person
10	< Number of allele systems with data for this person >		}
11	< Index of first allele, starting at zero >	} Repeated	}
12	< Index of second allele, starting at zero >	} for each	}
13	< Index of system, starting at zero >	} allele system	}
14	< Any text , in quotes>		
15	0		
16	0		
17	0		
18	< Number of pedigrees >		
19	< Index of pedigree, starting at 0 >		}
20	< Name of pedigree >		}
21	0		} Repeated
22	0		} for each
23	< Number of relations in pedigree >		} pedigree
24	< Index of parent in relation, starting at zero >	} Repeated for	}
25	< Index of child in relation, starting at zero >	} each relation	}

```
26  #FALSE#
27  < Number of allele systems >
28  #FALSE#
29  < Name of allele system, in quotes >                                  }
30  < Female mutation rate >                                              }
31  < Male mutation rate >                                                }
32  < Female mutation model >                                             }
33  < Male mutation model >                                               }
34  < Number of possibilities >                                           } Repeated
35  < Female mutation range >                                             } for each
36  < Male mutation range >                                               } allele
37  < #TRUE# or #FALSE#: system has a silent allele >                     } system
38  < Silent allele frequency >                                           }
39  < Number of alleles in the system >                                   }
40  < Allele name >                                  } Repeated for       }
41  < Allele frequency >                             } each allele        }
```

13.9.6 Explanation by line numbers

LINE NUMBERS	EXPLANATION
1-2	We encourage that the first two lines are used to describe the program producing the file. They are not read by the familias program.
3	The version number for the format: The file will be readable by familias with a version number higher than or equal to this number.
4	In the simplified format, we assume that all persons involved in the pedigrees have a name, and are listed in the list of persons further down. However, in general in familias, some pedigrees could be described using “extra persons” without names. These persons would then not be included in the total number of persons given on this line.
5	The name should be given in quotes, like “Mother”.
6	This line should be #TRUE# if year of birth data is included for this person, otherwise it should be #FALSE#. Note that familias uses the year of birth for a person only when automatically generating new pedigrees, so it should rarely be necessary to input such information.
7	When the previous line is #FALSE#, this line should contain -1. Otherwise, it should contain the year of birth for the person.
8	This line should be #TRUE# if the person is specified as a “child”, otherwise, it should be #FALSE#. The default value is #FALSE#. Note that familias only uses this information when automatically generating new pedigrees, so it should rarely be necessary to use anything but the default setting here.
10	Persons included just to describe the pedigrees properly, and for which no DNA data is available, should have 0 here. Note that it is not necessary that all the persons who do have DNA data have data from exactly the same allele systems.
11	The index of the first allele for this person in this allele system should be given: It is the index (when counting from zero) of the allele in the list of alleles for this system given further down in the file.

12	The index of the second allele; see line 11. Note that even homozygote persons must be input with two (equal) alleles.
13	The index of the allele system in which the two alleles above are contained: It is the index (when counting from zero) of the allele system in the list of such systems given further down in the file.
14	This line will generally contain the text “Known relations” in files produced by familias: The line is not used when reading in data.
15 - 17	In general, these lines are used to specify fixed relations, i.e., relations which occur in all pedigrees. In our simplified format, we assume that all relations are specified directly in the pedigrees. Thus these lines should just contain zeroes. In general, the format is as follows: Line 15 contains the number of “extra females” used to specify the fixed relations. Line 16 contains the number of “extra males”. Line 17 contains the number of fixed relations. Then follow pairs of lines, with one pair for each relation. The first line of each pair specifies the index of the parent of the relationship, and the second line the index of the child. The indices start at zero in the list of available persons: This list starts with the list of named persons, continues with the extra females, and ends with the extra males.
18	The number of pedigrees. For most applications, this number should be 2.
19	In our simplified format, this should just be 0 for the first pedigree, 1 for the second pedigree, and so on. In general, these numbers are used for the following purpose: The order in which the pedigrees are listed in the pedigree window can change after probabilities are computed; then they are listed by decreasing probability. When a familias file containing already computed probabilities is stored, the line indicates the place (starting at zero) of this pedigree in that ordered list.
20	The name of the pedigree. The name should be included in quotes, like “Ped1”. The actual name can be chosen freely, as long as two pedigrees do not have identical names.
21	This indicates the number of “extra females” in the pedigree, i.e., females that are included the pedigree in order to describe it correctly, but for whom we have no DNA data. For most applications, we would recommend using named persons instead, and include these in the list of persons above.
22	The number of “extra males” in the pedigree: See line 21.
23	The number of relations in the pedigree.
24	The index (when counting from zero) of the parent in the relation, in the list of persons given above. When “extra persons” are included, the list is extended, first with the extra females, and then with the extra males.
25	The index (when counting from zero) of the child in the relation, in the list of persons given above. When “extra persons” are included, the list is extended, first with the extra females, and then with the extra males.
26	This line should be #TRUE# when already computed probabilities are included in the file. The default is #FALSE#. If computed probabilities are included, a number of lines are inserted between lines 27 and 28: For each pedigree, the following is listed: First the probability for the pedigree, and then, with one line for each allele system, the likelihood for the data in this system with this pedigree. Then follows a line with #TRUE# or #FALSE# according to whether DNA data was used to compute the probabilities (i.e., whether they are prior or posterior probabilities), a line with the kinship parameter used in computations, and finally lines specifying each of the

	following parameters used in the computations: The generations parameter, the max generations parameter, the inbreeding parameter, and the promiscuity parameter.
27	The number of allele systems
28	This should be #TRUE# if "database information" is included in the data. The default is #FALSE#. (This line is NOT repeated for each allele system.) If database information is included, an extra line is inserted below this line: It contains, in quotes, the string specifying the database information. (This is the string specified in the top field of the "General DNA data" box of the user interface).
29	The name of the allele system, in quotes, like "Allele system". Note that the name can be anything, but that different allele systems must have different names.
30-31	These are the female and male mutation rates; default is 0. The numbers must be non-negative and less than 1. If the mutation model is 1 or 3, there are also some (high) theoretical limits to how high the mutation rate can be; consult the manual for details.
32-33	Indices in the list of possible mutation models: 0 means "Equal probability (simple and fast)" 1 means "Probability proportional to frequency (stationary)" 2 means "Prob. decreasing with range (equal)" 3 means "Prob. decreasing with range (stationary)" Default is 3. See the allele system window in the user interface, and the manual, for further explanation.
34	The total number of alleles, including the silent allele.
35-36	The "range" of the mutation model: This is the same number that is input in the corresponding box in the user interface. It must be a positive number less than 1. For more detailed information, consult the manual. Default is .1.
37	Use #TRUE# if the system has a silent allele, use #FALSE# otherwise.
38	When there is no silent allele, use 0 as a default; otherwise use the frequency of the silent allele.
39	The number of alleles in the system, NOT including a possible silent allele. There must be at least 2 alleles in each system.
40	The name of the allele, in quotes, like "A1". Note that the names can be anything, but that different allele systems must have different names. Note also that for mutation models 2 and 3 (se lines 32-33) the order of the alleles matter. Thus the alleles are always assumed to be ordered alphabetically, and should be input in alphabetical order.
41	The frequency of the allele. Note that the frequencies of alleles in a system, including any silent allele, must add up to exactly 1. All frequencies must be positive numbers.

13.10 References

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