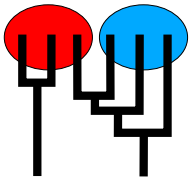
# *Migrate* Documentation



Version 0.7

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# Contents

**Abstract**

`Migrate` estimates population parameters (effective population size and migration rates) using genetic data (Electrophoretic markers, microsatellite markers, sequence data, and single nucleotide polymorphisms). It is a maximum likelihood estimator and uses a coalescent theory approach taking into account history of mutations and uncertainty of the genealogy. Currently there are two versions. (1) `migrate-0.4` uses a simple two population model with maximally 5 parameters: two population sizes, two migration rates, and a shape parameter for the variation of the mutation rates among loci and for sequence data it can also incorporate, but not estimate, rate heterogeneity among sites. (2) `migrate-n` estimates a full migration-matrix of $n$ populations. Allowing for likelihood ratio test and delivering profile likelihood curves. Development on (1) has stopped. I still fix bugs, of course. (2) is actively developed, therefore some features in (2) needs more testing.

# Theoretical considerations

## Maximum likelihood estimation of migration rates

Migrate calculates maximum likelihood estimates for migration rates and effective population sizes of two populations using genetic data (Fig 1). The parameters to estimate are $\Theta_1$, $\Theta_2$, $Nm_1$, $Nm_2$, which are 4 $\times$ effective population size $\times$ mutation rate per site per generation and effective population size $\times$ migration rate per generation in population 1 and 2, respectively. The estimation process uses an expansion of the coalescent theory (Kingman 1984a,b) which includes migration (Hudson 1990, Nath and Griffiths 1993, Notohara 1994). A likelihood estimate of the parameters $\mathcal{P}$ using genealogies $\mathcal{G}$ with data $\mathcal{D}$ would be

$$L(\mathcal{P}) = \sum_{G} \text{Prob}\,(\mathcal{D} \mid \mathcal{G})\ \text{Prob}\,(\mathcal{G} \mid \mathcal{P}).$$

This is the sum over the joint probability of the data given a genealogy (this is the conventional likelihood in a phylogenetic tree) and the probability of the coalescent. Unfortunately, this sum has an infinite number of summands; we have to sum over all genealogies and all possible branch length. We can solve this problem by using a Markov chain Monte Carlo approach with importance sampling due to Metropolis (1954) and Hastings (1974). For an introduction see Hammersley and Handscomb (1964) or Chib and Greenberg (1995), and see Kuhner et al. (1995) for its application to the coalescence theory). We bias the search path through all trees towards trees with higher likelihoods (Fig. 2) and have then to correct for this. The likelihood formula changes to

$$\frac{L(\mathcal{P})}{L(\mathcal{P}_0)} = \frac{1}{m} \sum_{i}^{m} \frac{\text{Prob}\,(D \mid g_i)\ \text{Prob}\,(g_i \mid \mathcal{P})}{\text{Prob}\,(D \mid g_i)\ \text{Prob}\,(g_i \mid \mathcal{P}_0)}.$$

This is very reasonable, because summands with low probabilities will almost not contribute to the final likelihood. For more information on the base model, you should read Beerli and Felsenstein (1999) and Kuhner et al. (1995). The approximation of the likelihood using a ratio makes it difficult to compare different runs of the program, if the program reports a likelihood then this is actually a ratio of likelihoods and since we recalculate the parameters for each chain, the values for $\mathcal{P}_0$ are different between runs, and therefore it is impossible to compare them. An escape of this problem is to run the program using the full model (e.g. $n \times n$ parameters and use the likelihood ratio test for specific scenarios.

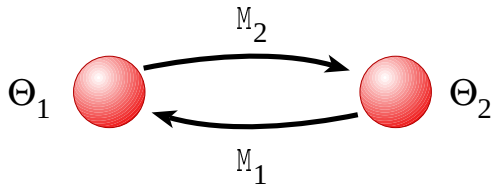


Figure 1: Populations exchanging migrants with rate $m_i$ per generations and with size $N_e$. The parameters are scaled by mutation rate $\mu$ which is with sequence data per site per generation. The estimated parameters are therefore: $\Theta_i$ which is $4N_e^{(i)}\mu$ and $\mathcal{M}_i$ which is $m_i/\mu$, the migration estimate is more common expressed as $4Nm$ which is just $\Theta\mathcal{M}$.

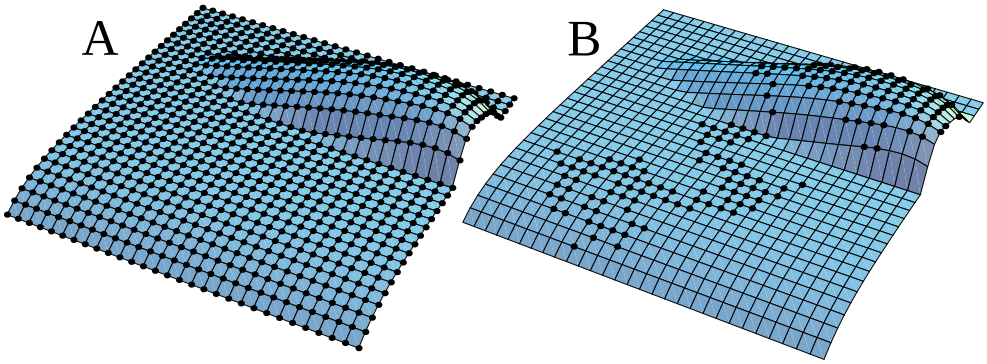


Figure 2: (A) On an imaginary, infinite likelihood surface we would need to sample every possible genealogy and sum all these values which is not possible, but trees with low probability will not contribute much to the final likelihood, (B) by biasing towards better trees we can sample effectively from those trees with high contribution to the final likelihood and can approximate the likelihood.

## Performance criteria

There is a difficulty with these kind of samplers that we do not know how long we have to run the sampler to get "accurate" estimates. Despite the huge literature about measures when to stop sampling, there is no good criteria available. Several ways exist to investigate if the results we get are good, we can check if

- the program is sampling form the right distribution: running the sampler with no data (e.g. sequence data with all "?????" data) should result in the distribution $\mathrm{Prob}\,(G|\mathcal{P}_0)\mathrm{Prob}\,(D|G)$, the one we sample from.
- simulation studies show that we can recover parameters and population structure that was used to create the data.
- comparison with other programs produce similar results. I compared `migrate` with `genetree` (Bahlo and Griffiths 1999) and with `fluctuate` (Kuhner et al. 1998). The comparison with `genetree` used two populations (England and Ghana: 2.5 kb sequence data for the beta-globin locus

[Harding et al. 1997]) and the results were very similar. For my paper on n-population I have worked out a 100-locus data set simulation that shows that `genetree` and `migrate`deliver the same estimates, and approximative confidence intervals, although `genetree` is very slow compared to `migrate`for that specific data set (Beerli and Felsenstein, in prep.). The comparison with `fluctuate` was for one population, yes you can run `migrate` with only one population, and for a data set simulated with a $\Theta = 0.01$ `migrate` delivered $\Theta = 0.0123$ with a 50% confidence interval of $0.08$ to $0.017$, while `fluctuate` delivered a point estimate of $\Theta = 0.0119$.

- the program is sampling many different genealogies; one can show this by plotting a curve showing on the x-axes all sampled trees and on the y-axis the likelihood of the genealogy (in our case this is $\mathrm{Prob}\,(D|G)$ , Figure 3). A plot of a sequence of $\mathrm{Prob}\,(\mathcal{P}|G_i)\mathrm{Prob}\,(D|G_i)$ is not useful because the genealogies contain different number of time intervals, and they are **not** comparable.

- One can show that starting from random start parameters, the estimates converge rather quickly after a few short chains, the updating of the start parameters over several short chains moves the estimates to the proper region and the remaining uncertainty is only driven by the often huge uncertainty about the parameter estimates in the data, aka the likelihood surface is flat for many parameter combinations and the data.

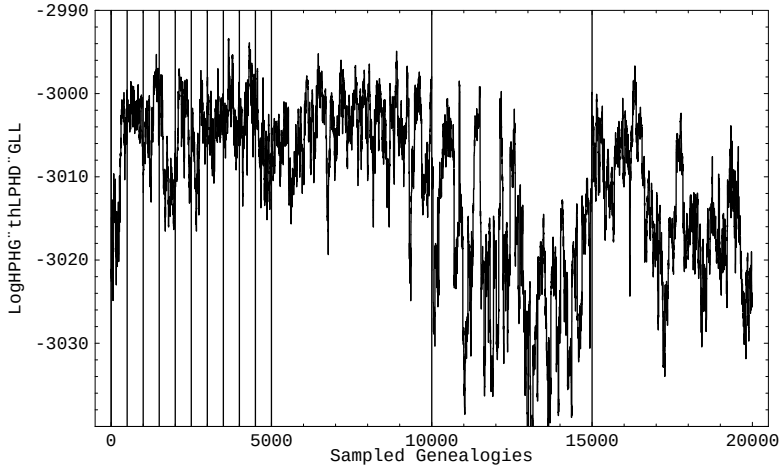


Figure 3: Data likelihood $\mathrm{Prob}\,(D|G)$ for all sampled genealogies: A sample run of migration estimation using 2 populations, the very long vertical lines mark chain boundaries (10 short and 3 long chains). Totally, $10$ short chains $\times 500$ sampled genealogies $+3$ long chains $\times$ sampled $5000$ genealogies were sampled out of total 400,000. The values for not recorded trees are not shown.

## Data models

### Infinite allele model

This assumes that every mutation will result in a new allele, there is no back mutation (Fig. 4). This model is used in all current implementations of electrophoretic data analyses packages (Biosys-1, GDA among

others) and perhaps is appropriate for this kind of data. *Migrate* is calculating the parameters for each locus independently and summarizes at the end taking the likelihood surfaces of each locus into account. These mean-parameters can be found by either assuming that the mutation rate has no variation (as all, at least those I know, other programs do) or uses a $\Gamma$ distributed mutation rate with shape parameter $\alpha$ which is in this case

$$\frac{1}{(\text{coefficient of variation})^2}.$$

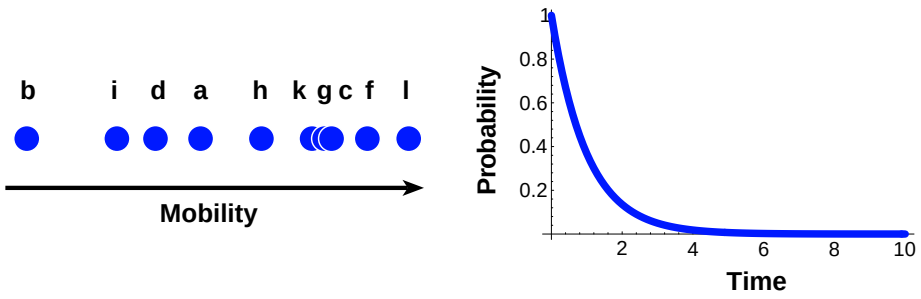


Figure 4: Left: Mobility of electrophoretic marker in an electric field. the alleles a,b,c,.. describe a possible sequence of mutation, their mobility is not correlated with the mutational history. Right: The probability that a given allele is not mutating during some time, this is a simple exponential relationship.

## Microsatellite model

### Ladder model

The ladder model was invented by Ohta and Kimura (1973, 1978) for electrophoretic markers, but was not as good as expected in describing real electrophoretic alleles. For microsatellites this model seems much more appropriate (e.g. Valdes et al. 1993, but see Di Rienzo et al. 1994), here obviously change happens mostly by slippage of the two DNA strands creating with higher probability a new allele which is only 1 step apart from the old than one which 2 steps apart (Fig. 5). Summarizing over loci can be done either by assuming the mutation rate is Gamma distributed or constant. This assumes, of course, independence between loci.

### Brownian motion approximation to the ladder model

This replaces the discrete stepwise mutation model with a continuous Brownian motion model The results are very similar to the exact stepwise mutation model, but the parameter estimation is several times faster. This is work still in progress (Felsenstein and Beerli, in prep.).

## Sequence model

Migrate implements the sequence model of Felsenstein (1984) available in `dnaml` (PHYLIP 4.0, Felsenstein 1997)(Fig. 6). The transition probabilities were published by Kishino and Hasegawa (1989). *Migrate* does not allow for recombinations and therefore is only well suited for mitochondrial sequences or other non-recombining DNA stretches. Summarizing over ”loci” assumes in addition that the loci are unlinked. The mutation rate among loci may be either constant or following a Gamma distribution.

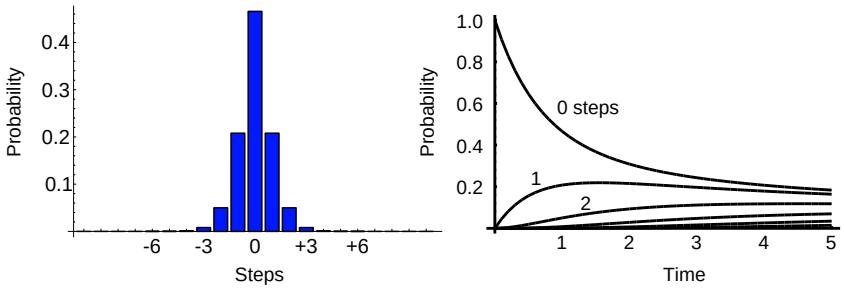


Figure 5: Left: Number of repeat changes of a microsatellite marker. The probability to have a slippage of only one repeat is higher than the slippage of more than one repeat, in a given time, here time=0.1. Right: The probability that a change of 0,1,2,.. steps is occurring during some time.

Like `dnaml`, *Migrate* also allows for different evolutionary rates, mutation categories and autocorrelation, although any use of these additional features can slow done to program to a crawl, but this may change in the future as computers double their speed roughly every 2 years.

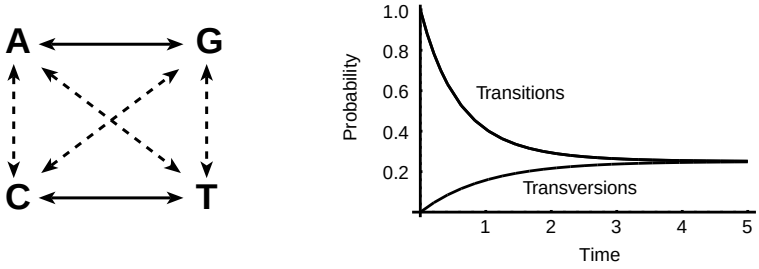


Figure 6: Left: Sequence mutation model. Transitions are are shown in black lines, transversion are shown with dotted lines. Right: The probability that a transition or transversion is occurring during some time. The shown graph uses equal base frequencies, but the used model does not need this restriction.

## Single nucleotide polymorphism data (SNP)[Work in progess]

We use a rather restrictive models for SNPs. Currently there are two versions implemented, but not fully tested. If you want to use the SNP options, please contact me before you run large scale analyses.

1. SNP were developed from a panel population of which we know the number of individuals, and that the markers developed were variable, but we do not know the actual nucleotides for the individuals.

2. We have found ALL variable sites and use them even if there are only a few members of another alleles present. In principal it is as you would sequence a stretch of DNA and then remove the invariant sites.

This is certainly not how people develop SNPs, but currently the closest we can come up with. The SNP coding is otherwise exactly the same as the coding for DNA data.
If you want to assume that the SNP are unlinked then you need to code each SNP like a sequence data locus with one nucleotide (see the examples for sequences), I have run successfully 50 SNP loci on a laptop with

40 MB of RAM. The SNP data is producing a huge upwards bias for Θ, for further explanations watch for a forthcoming paper (Kuhner, Beerli, Yamato, Dubb, and Felsenstein, in press).

# Program usage

If you want to know how to install or compile the program goto the sections at the end of this manual.
This manual is in a transition phase until the two-population program `migrate` and the $n$-population `migrate-n` are merged. Options only available with the one or other program are marked with either **(2-POP)** or **(N-POP)**.

## Data file specifications

The data needs to be in a certain form; for us, the following format was most convenient. Eventually we will include the NEXUS format (which is used in MacClade and Paup).
Syntax: a token is either a word, a collection of words, or a character or a number:

$< token >$ the token between the the "angle-brackets" is obligatory

$[token]$ in square brackets are optional.

$\{token\}$ are obligatory for some

$< token1|token2 >$ choose one of the token kind of data.

A range of numbers in a "word" token as in $<$`individual1 10-10`$>$ means that this token needs to be 10 characters long. The characters for any word token can normally include special characters, punctuation, and blanks, the token for the individual name `Ind1 02 @` is legal. The most common data file for enzyme electrophoretic data or microsatellite data would look like this (examples follow):

```
<Number of populations> <number of loci> {delimiter between alleles} [project ti-
tle 0-79]
<Number of individuals> <title for population 0-79>
<Individual 1 10-10> <data>
<Individual 2 10-10> <data>
....
<Number of individuals> <title for population 0-79>
<Individuum 1 10-10> <data>
<Individuum 2 10-10> <data>
....
```

The delimiter is needed for microsatellite data and the project title is optional. The `data` will be described in the following sections. The individual name has to be by default 10 characters (same as in PHYLIP), but can be changed to an other constant in the parmfile, even to a length of 0. For sequences or SNPs, the syntax is slightly different, the following case is for non-interleaved sequence data.

```
<Number of populations> <number of loci> [project title 0-79]
<number of sites for locus1> <number of sites for locus 2> ...
<Number of individuals> <title for population 0-79>
<Individuum 1 10-10> <data locus 1>
<Individuum 2 10-10> <data locus 1>
....
<Individuum 1 10-10> <data locus 2>
<Individuum 2 10-10> <data locus 2>
....
<Number of individuals> <title for population 0-79>
<Individuum 1 10-10> <data locus 1>
<Individuum 2 10-10> <data locus 1>
....
<Individuum 1 10-10> <data locus 2>
<Individuum 2 10-10> <data locus 2>
....
```

Interleaved sequence data:

```
<Number of populations> <number of loci> [project title 0-79]
<number of sites for locus1> <number of sites for locus 2> ...
<Number of individuals> <title for population 0-79>
<Individuum 1 10-10> <data locus 1 part 1>
<Individuum 2 10-10> <data locus 1 part 1>
....
<data ind1 locus 1 part 2>
<data ind2 locus 1 part 2>
....
<Individuum 1 10-10> <data locus 2>
<Individuum 2 10-10> <data locus 2>
....
<data ind1 locus 2 part 2>
<data ind2 locus 2 part 2>
....
etc.
```

The input for SNPs is the same as for sequence data.

## Examples of the different data types

The examples in this section look like real data, but they are only for the demonstration of the syntax, if you try run this “data” it will deliver often very strange values, I have added a “usable” test set of simulated data in the examples directory, see the file examples/README for more information.

### Enzyme electrophoretic data (infinite allele model)

The data is given in genotypes, any printable character with ASCII code bigger than 33 ('!') and smaller than 128 can be used. '?' is reserved for missing data. You can use multi-character coding when you use a delimiter (see the examples for microsatellites). If there is enough interest I can work on a input using gene frequencies, although I prefer to work on more interesting things than adjusting input files.

Example with 2 populations and 11 loci and with 3 and 2 individuals per population, respectively (this data set is only an example of syntax, analyzing this dataset would not make much sense).

```
 2 11 Migration rates between two Turkish frog populations
3 Akcapinar
PB1058    ee bb ab bb bb aa aa bb ?? cc aa
PB1059    ee bb ab bb bb aa aa bb bb cc aa
PB1060    ee bb b? bb ab aa aa bb bb cc aa
2 Ezine
PB16843   ee bb ab bb aa aa aa cc bb cc aa
PB16844   ee bb bb bb ab aa aa cc bb cc aa
```

## Microsatellite data

The third argument on the first line has to be a delimiter character, for example a **"."**. The data is given in genotypes, the number of repeats is the allele name separated by the specified spacer. '?' is reserved for missing data.

Example:

```
 2 3 . Rana lessonae: Seeruecken versus Tal
2   Riedtli near G\"undelhart-H\"orhausen
0         42.45 37.31 18.18
0         42.45 37.33 18.16
4   Tal near Steckborn
1         43.46 33.37 18.18
1         44.46 33.35 19.18
1         44.46 35.? 18.18
1         43.42 35.31 20.18
```

## Sequence data

After the individual name follows the base sequence of that species, each character being one of the letters A, B, C, D, G, H, K, M, N, O, R, S, T, U, V, W, X, Y, ?, or - . Blanks will be ignored, and so will numerical digits. This allows GENEBANK and EMBL sequence entries to be read with minimum editing. These characters can be either upper or lower case. The algorithms convert all input characters to upper case (which is how they are treated). The characters constitute the IUPAC (IUB) nucleic acid code plus some slight extensions. They enable input of nucleic acid sequences taking full account of any ambiguities in the sequence.

| Symbol | Meaning | |
|---|---|---|
| A | Adenine | |
| G | Guanine | |
| C | Cytosine | |
| T | Thymine | |
| U | Uracil | |
| Y | pYrimidine | (C or T) |
| R | puRine | (A or G) |
| W | ”Weak” | (A or T) |
| S | ”Strong” | (C or G) |
| K | ”Keto” | (T or G) |
| M | ”aMino” | (C or A) |
| B | not A | (C or G or T) |
| D | not C | (A or G or T) |
| H | not G | (A or C or T) |
| V | not T | (A or C or G) |
| X,N,? | unknown | (A or C or G or T) |
| O | deletion | |
| - | deletion | |

Example with 2 population with **2 loci**, the sequences are NOT interleaved:

```
   2 2 Make believe data set using simulated data (2 loci)
50 46
3    hinders wiesli
eis       ACACCCAACACGGCCCGCGGACAGGGGCTCGAGGGATCACTGACTGGCAC
zwo       ACACAAAACACGGCCCGCGGACAGGGGCTCGAGGGGTCACTGAGTGGCAC
drue      ATACCCAGCACGGCCGGCGGACAGGGGCTCGAGGGAGCACTGAGTGGAAC
eis       ACGCGGCGCGCGAACGAAGACCAAATCTTCTTGATCCCCAAGTGTC
zwo       ACGCGGCGCGAGAACGAAGACCAAATCTTCTTGATCCCCAAGTGTC
drue      ACGCGGCGCGAGAACGAAGACCAAATCTTCTTGATCCCCAAGTGTC
2    vorders wiesli
vier      CAGCGCGCGTATCGCCCCATGTGGTTCGGCCAAAGAATGGTAGAGCGGAG
fuef      CAGCGCGAGTCTCGCCCCATGGGGTTAGGCCAAATAATGTTAGAGCGGCA
vier      TCGACTAGATCTGCAGCACATACGAGGGTCATGCGTCCCAGATGTG
fuefLoc2  TCGACTAGATATGCAGCAAATACGAGGGGCATGCGTCCCAGATGTG
```

Same example with 2 population with **2 loci**, but the sequences are now interleaved:

```
   2 2 Make believe data set using simulated data (2 loci, interleaved)
50 46
3    hinders wiesli
eis       ACACCCAACACGGCCCGCGGACA
zwo       ACACAAAACACGGCCCGCGGACA
drue      ATACCCAGCACGGCCGGCGGACA
          GGGGCTCGAGGGATCACTGACTGGCAC
          GGGGCTCGAGGGGTCACTGAGTGGCAC
          GGGGCTCGAGGGAGCACTGAGTGGAAC
eis       ACGCGGCGCGCGAACGAAGACCA
```

```
zwo       ACGCGGCGCGAGAACGAAGACCA
drue      ACGCGGCGCGAGAACGAAGACCA
          AATCTTCTTGATCCCCAAGTGTC
          AATCTTCTTGATCCCCAAGTGTC
          AATCTTCTTGATCCCCAAGTGTC
2    vorders wiesli
vier      CAGCGCGCGTATCGCCCCATGTGGTTCGGCCAAAGAATG
fuef      CAGCGCGAGTCTCGCCCCATGGGGTTAGGCCAAATAATG
          GTAGAGCGGAG
  TTAGAGCGGCA
          TCGACTAGATCTG CAGCACATAC
          TCGACTAGATATG CAGCAAATAC
  GAGGGTCATGCGTCCCAGATGTG
  GAGGGGCATGCGTCCCAGATGTG
```

## Additional files

### Overview

I tried to make it simple and redundant, so that there are more than one way to set up things. There a several special file names, some of them can be changed others not:

| Filename | Description | Needed? | Name changeable |
|---|---|---|---|
| infile | holds you data | necessary | * |
| parmfile | holds options | optional | - |
| seedfile | holds a random number seed | optional | - |
| catfile | holds categories for mutation rate variation | optional | - |
| weightfile | holds weights for each site | optional | - |
| outfile | will be created and replace any file with the same name in the same directory | necessary | * |
| treefile | holds genealogies, this file will be created and will replace any file with the same name in the same directory | optional | - |
| mathfile | holds plot coordinates for the use in a mathematica notebook, this file will be created and will replace any file with the same name in the same directory | optional | * |
| sumfile | holds the summary statistic of the sampled genealogies for further analysis, this file will be created and will replace any file with the same name in the same directory | optional | * |

### Necessary input file

**infile** if this file is not present in the current directory than the program will ask for a data file, and you can give the path to it, you need to type the path, which is for Macintosh and Windows users probably rather uncomfortable. In the **menu** or **parmfile** you can specify an other default name for your datafile.

## Optional input files

**parmfile** can hold specific menu options, this file and the possible options for the menu are explained in detail in section **menu and parmfile**.

**seedfile** holds a random number seed, this is just present for compatibility with PHYLIP, the random number seed can be set in various ways either in the menu or in the parmfile.

**catfile** hold the categories, for each locus you must give the number of categories, and the value of each category and then a string of category assignments for each site. You can use the # as a commentary character.

```
# Example catfile for two loci with 40 and 30 bp each
#
2 1 10 11111111111111111111222222222222222222
3 1 3 9 11111111112222333333333222222
```

**weightfile**, for each site and locus you need to give a weight, acceptable weights are integers from 0 - 9 and letters A-Z, A is the weight 10, B 11 and so on, in total there are 35 possible different weights possible. **You need a weight string for each locus**.

```
# Example weightfile for two loci with 40 and 30 bp each
#
11011011011011011011011011011011011011011101
33F33F22F22F22F22F22FHHHHHHHHHH
```

## Output file

**outfile** somewhere you want to read the results, that is it! The name outfile is the default, but can be changed either in the menu or the parmfile.

## Optional output files

**treefile** holds all, only those of the last chain or the best tree(s). The likelihood of each tree is given ($\mathrm{Prob}\,(\mathcal{D} \mid \mathcal{G})$) in a comment. The programs writes trees with migrations using the Newick format with extensions from the Nexus format, unfortunately I do not know yet a program who can print them nicely. Writing trees to a treefile adds some burden to the program and it will run slower, especially with the option BEST.

**mathfile** holds the raw likelihood surface data, if this was requested in the options. The name mathfile is the default, but can be changed in the menu or parmfile (see appendix).

**sumfile** holds the summaries of all genealogies, if this was requested in the parmfile or menu. The name sumfile is the default. His option allows to reanalyze a previous run for likelihood ratio test or profiles.

## How to run

If you have compiled and installed the program successfully (see Installation) and your data is in a good format (section data format) and perhaps has the name infile, just execute

`migrate-n` for 1 to n populations
`migrate-0.4` for 2 populations

Either by double clicking its icon (see on the title page) or for UNIX typing its name in a shell. Without any **parmfile**, *Migrate* will display a menu, in which you can change all the sensible options. For hints how to use the parmfile, look into section **Menu and Options** or the `parmfile.doc`. Once you know how to customize the options with the **parmfile** you will probably more often edit the parmfile than making the changes in the menu.

## Menu and Options

You can change the options in the menu (Fig. 7) using letters or in submenus numbers. In menu entry `Data type` you need to specify what kind of data you have and according to that type some other menu entries appear, for example: t/t ratio for sequences.

```
==========================================
MIGRATION RATE AND POPULATION SIZE ESTIMATION
using Markov Chain Monte Carlo simulation
==========================================
Version 0.7
Program started at   Thu May  6 23:20:28 1999


Settings for this run:
D       Data type
         (currently set: DNA sequence model)
I       Input/Output formats
P       Start values for the Parameters
S       Search strategy
W       Write a parmfile


Are the settings correct?
(Type Y or the letter for one to change)
```

Figure 7: Top menu of *Migrate*

Menu options can also be changed in the `parmfile`. I will show all possible options in the parmfile syntax, but the same items can be changed in the menu as well. All entries in the parmfile are not case sensitive and all options can be given only with the first letter, although I do not recommend that.

## Data type

**datatype=<Allele | Microsatellites | Brownian | Sequences | Nucleotide-polymorphisms | Panel-SNP | Genealogies >**
specifies the datatype used for the analyses, needless to say that if you have the wrong data for the chosen type the program will crash.

**Allele**: infinite allele model, suitable for electrophoretic markers, perhaps the "best" guess for codominant markers of which we do not know the mutation model.

**Microsatellite**: a simple electrophoretic ladder model is used for the change along the branches in genealogy.

**Brownian**: a Brownian motion approximation to the stepwise mutation model for microsatellites us used (this is MUCH faster than exact model, but is not a good approximation if population sizes are small (say below 10).

**Sequences**: Data are DNA or RNA sequences and the mutation model used is F84, first used by Felsenstein 1984 (actually the same as in `dnaml` (Phylip version 3.5), a description of this model can be found in Swofford et al. 1996.

**Nucleotide-polymorphism**:[SNP] the data likelihood is corrected for sampling only variable sites. We assume that the data was used to find the SNP.

**Panel-SNP**: the data likelihood is corrected for using a panel of SNP sites, that were polymorphic. The panel has to be population 1.

**G**enealogies: Reads the `sumfile` (see INPUT/OUTPUT section) of a previous run, with this options the genealogy sampling step will not be done and the genealogies provided in the `sumfile` are analyzed. This datatype makes it easy to rerun the program for different likelihood ratio test or different settings for the profile likelihood printouts.

### Sequence data

If you specified **datatype=Sequence** the following options have some meaning and will show up in the menu (see also details for these options in the main.doc and dnaml.doc of the PHYLIP distribution `http://evolution.genet`

**freq-from-data=< Yes | No:freqA freqG freqC freqT>**

**freq-from-data=Yes** calculates the base frequencies from the infile data, this will crash the program if in your data a base is missing, e.g. you try to input only transitions. The frequencies must add up at least to 0.9999.

**freq-from-data=No:0.2 0.2 0.3 0.3** Any arbitrary nucleotide frequency can be specified.

**ttratio=< r1 r2 .....>**
you need to specify a transition/transversion ratio, you can give it for each locus in the dataset, if you give fewer values than there are loci, the last ttratio is used for the remaining loci → if you specify just one ratio the same ttratio is used for all loci.

**interleaved=<Yes | No >**

If your data is interleaved you need to specify this here, the default is **interleaved=No**.

**categories=<Yes | No>**

If you specify **Yes** you need a file named "`catfile` in the same directory with the following Syntax: number_of_categories cat1 cat2 cat3 .. categorylabel_for_each_site for each locus, a # in the first column can be used to start a comment-line.
Example is for a data set with 2 loci and 20 base pairs each

```
# Example catfile for two loci
# in migrate you can use # as comments
2 1 10         1111111111222222222
5 0.1 2 5 23 3 11111122223333445555
```

**rates=< n : r1 r2 r3 ..rn>**

by specifying rates a hidden Markov model or rates is used for the sequences (Felsenstein and Churchill 1995), also see the PHYLIP documentation.

**prob-rates=< n : p1 p2 p3 ... pn>**

if you specify **rates** you need also the specify the probability of occurrence for each rate.

**autocorrelation=<Yes:value | No>**

if you assume hat the sites are correlated along the sequence, specify the block size, by assuming that only neighboring nucleotides are affected you would give a value=2.

**weights=<Yes | No>**

If you specify **Yes** you need a file `weightfile` with weights for each site, the weights can be the following numbers 0-9 and letters A-Z, so you have 35 possible weights available.

```
# Example weightfile for two loci
1111111111222222222
1111112222AAAA445XXXX5
```

**d**istfile=<Yes | No>

You can supply a distance file for each locus (using PHYLIP syntax). Each individual must have is own name. This option appears in the menu when you choose

```
0    Start genealogy is estimated using a UPGMA topology
```

The distance file is then used to create an UPGMA tree with a minimal number of migration events. For large trees this is options help to get better starting trees than the automatic tree generation which uses a rather unsophisticated distance method (differences). [Needs more testing, but works fine for me]

**usertree=<Yes | No>**

If you specify **Y**es you need a file `intree`. In this file you have starting trees for each locus, **BUT** these trees need to have migration events in them, currently only *Migrate* can write trees with migration events on it, if you inspect such a file you can see, how such a intree file is organized and could insert migration events by hand. If you need this options, please contact me at beerli@genetics.washington.edu

### Microsatellite data

If the **datatype=Microsatellite** is used, the following options have some meaning, please remark that if you use the Brownian motion option these restriction do not apply.

**micro-max=value**

specifies the maximal allowed number of repeats, this **M**UST be higher than your actual maximal repeat number in your dataset, if it is too high there is a penalty only on allocating to much space and perhaps in slight runtime degradation (the empty space has to be copied), but if it is too small your results will be **wrong!** The default is set to **micro-max=200**.

**micro-threshold=value**

specifies the window in which probabilities of change are calculated if we have allele 34 then only probabilities of a change from 34 to 35-44 and 24-34 are considered, the probability distribution is visualized in Figure 5 the higher this value is the longer you wait for your result, choosing it too small will produce wrong results. micro-threshold=10

### Electrophoretic data

No special variables, but see **Parmfile specific commands**.

### Nucleotide polymorphism

Similar to **sequence data**.

## Input/Output formats

This group of options specifies input file names and various output file options. Also, titles for the analysis can be specified. In addition, one can tailor the information the program is presenting during the execution. Some of the options in this manual are currently not implemented in the two population program (`migrate-0.4`, Beerli and Felsenstein 1999), the n-population version which will eventually replace the two-population version will contain all the mentioned options.

### Input formats

**infile=filename**

If you insist to have a datafile names other than `infile`, you can change this here, if you do not specify anything here, it will use any file with name `infile` present in the execution directory, if there is no `infile` than the program will ask for the datafile and you can specify the path to it (this may be hard on Macs and Wintel machines). If you use this option, do **N**OT use spaces or "/" or on Macs ":" in your filename. The default is obviously **infile=infile**

**random-seed=<Auto | Noauto | Own:seedvalue>**

The random number seed guarantees that you can reproduce a run exactly. I you do not specify the random number seed (**seed=Auto**) the program will use the system clock. With **seed=Noauto** the program expects to find a file named `seedfile` with the random number seed. With **random-seed=Own:seedvalue** you can specify the seed value in the parmfile (or in the menu).

Example for own seed:

**random-seed=Own:21465** If you want reproducible runs you should replace the **Auto** seed with your own starting number (best numbers are divisible by 4 + 1) The default is **r**andom-seed=Auto. I personally use always **random-seed=Own:seedvalue**. But then you need to change this for different run, otherwise the sequence of random numbers is always the same.

```
  INPUT FORMATS
  -------------
   1      Datafile name is infile
   2      Use automatic seed for randomiza-
tion?  No, seed=907711327
   3      Title of the analysis is <no title given>

  OUTPUT FORMATS
  --------------
   5      Print indications of progress of run?  Yes
   6      Print the data?                        No
   7      Outputfile name is outfile
   8      Plot likelihood surface? Yes, to out-
file and mathfile
   9      Profile-likelihood? Yes, tables and summary
                                      [Percentiles]
  10      Likelihood ratio test                  No
  11      Print genealogies?                     None
  12      Plot coordinates are saved in mathfile
  13      Summary of genealogies will not be saved

  Are the settings correct?
  (type Y to go back to the main menu or the let-
ter for the entry to change)
```

Figure 8: Input/Output menu of *Migrate*

**title=titletext**
if you wish to add an informative title to your analysis, you can do it here or in the infile, the infile will override the title specified here. The length of the title is maximal 80 characters. Example: **title=Migration parameter estimation of populations A and B of species X**.

### Output formats

**progress=**<**Yes**|**No**|**Verbose**> Show intermediate results and other hints that the program is running. Verbose adds more hints (at least for me) and information. The default is **progress=Yes**

**outfile=filename**
All output is directed into this file, the default name is outfile. If you use this option, do **NOT** use spaces or "/" or on Macs ":" in the filename. The default is obviously **outfile=outfile**

**print-data=**<**Yes**|**No**>
Print the data in the `outfile`. defaults is **print-data=No**.

**print-fst=**<**Yes**|**No**>
Print a table of an $F_{ST}$estimate for comparison (Beerli and Felsenstein 1999, Beerli 1998) [not recommended].

**plot=**<**No** | **Yes**>**[:**<**Outfile**|**Both**>**[:**<**std**|**log**>**:**{**mig-axis-start,mig-axis-end,theta-axis-start,theta-axis-end**}<**:printpos**<**M** | **Nm**>>**]]**

if **plot=No** then no plot of the parameter space is shown in the `outfile`, if **Yes** then you can specify whether you want to have the accurate numbers in a separate file (`mathfile`) using *printpos* "pixel" in each direction,or only the ASCII-graphics plot in the `outfile`. The last option (M or N)let you define wether you want the plot in $\mathcal{M} \times \Theta$ or (default) $4Nm \times \Theta$. Default is `plot=Yes:Outfile`, Example of a more complicated statement: `plot=Yes:Both:std:0,10,0,0.025:100N`

After a run `mathfile` will contain the following

2-pop **l**ocus1=((x11,x12,...,x1n),(x21,..x2n),...,(xn1,..xnn)); locus2=... the combination of all estimates is the last locus = locus(n+1) the syntax of this file is so that you can import it directly into Mathematica by using <<**mathfile** (see in the example directory of this distribution for more material on this issue). The default is **plot=Yes** which is equivalent to **plot=Yes:Both**.

n-pop In **(N-POP)** the `mathfile` will print only all summed up emmigration and immigration from/into a population, and the format changed to printing only raw numbers: there are *printpos* × *printpos* cells for each plot (default for *printpos* is 36), so for 2 loci and and 3 populations you get a total of 1552 numbers, you can read these into mathematica using

```
rows=cols=36;
pop=3;
data=ReadList[''mathfile'',Table[Table[Table[Table[Number, {cols}],
{rows}], {2}],{pop}]; loci=Length[data]
```

**profile=<No|Yes<:<Fast|Percentile|Spline|Discrete|Quick >><:M | Nm >(N-POP)**

Print profile likelihood. See section **Likelihood ratio tests and profile likelihood**. Default is **profile=Yes:Fast:N**.

**No**: No profile likelihoods are evaluated.

**Y**es, All: Evaluate profile likelihoods and print tables for each parameter and also a summary table with the approximative percentiles for each variable.

**Percentile** evaluates the profiles at the percentiles (0.01, 0.05, 0.10, 0.25, 0.50, 0.75, 0.90, 0.95, 0.99). This will need a LOT of time: (1) it has to find the percentiles evaluating a full maximization for n-1 parameters each.

**Quick** [means quick and dirty] Evaluates the profiled parameter assuming that the parameters ($\Theta_I$ and $\mathcal{M}_{ji}$ are uncorrelated. This is equal to fixing all parameter at the maximum likelihood and evaulate the likelihood for the profiled parameters. This is very fast and often rather close to the *Percentile* option.

**Fast** A mixture of *Quick* and *Percentile*. This is the default. The percentiles are found using *Quick* and then one final full maximization of all other parameters is done.

**Discrete** Evaluate the profile likelihood at specific points which are ML-estimate × (0.02, 0.10, 0.20, 0.5, 1, 2, 5, 10, 50).

**Spline** Evaluates *Discrete* and then uses a cubic spline routine to evaluate the percentiles. This is the one version I would prefer, but I have difficulties to get a spline that has its peak at the ML-estimate. Currently, if you use this method make sure that the 0.5 percentiles has the highest log likelihood. [This needs to be fixed]

**M or N** The profiles are evaluated using $\Theta$ and $\mathcal{M}$, with the option N (the default) the migration values are printed as $\Theta\mathcal{M}$ (for most data this is $4Nm$, but for mtDNA this could mean it is $Nm$). With **M** the $\mathcal{M} = m/\mu$ are printed instead of the $4Nm$.

**l-ratio=<None | <Mean|Loci>:testparam> (N-POP)**

Likelihood ratio tests. See section **Likelihood ratio tests and profile likelihood**. Default is **l-ratio=None**.

**print-trees=<All | None | Last | Best>**

print genealogies into `treefile`. Remember these trees contain migration events, although I followed the NEXUS rules (Maddison 1998) and the migration events are in comment brackets, I do not know of any program being able to read this kind of trees. I would like to hear from you if you know any other program who can read such a tree.

**None**: `treefile` is not initialized and no trees are printed, this is the fastest and the one I recommend.

**All**: will print all trees (you want to do that only for ridiculously small datasets with too short chains or you have **Gigabytes** of free storage).

**Last**: Only the trees of the last long chain are printed, Still you will need lots of space.

**Best**: Prints the tree with the highest data-likelihood for each locus. This is slow! And give not very much information, except if you are more interested in the best tree than in the best parameter estimate.

Default is **print-trees=None**

**mathfile=filename**

the plotcoordinates are directed into this file. If you use this option, do **N**OT use spaces or "/" or on Macs ":" The default is obviously **mathfile=mathfile**.

**sumfile=<No | Yes | Yes:filename >**

Intermediate results of the genealogy sampling process are save into a file named `sumfile` or into the file for that you specify the filename. You can use this `sumfile` to rerun the program for further analysis, e.g. calculating likelihood ratios or profile likelihoods, see **datatype=Genealogy**.

## Start values for the Parameters

**theta=<Fst | Own:value1,value2>**

With **Fst** the programs tries to use an $F_{ST}$ based measure (Maynard Smith 1970, Nei and Feldman 1972) for the estimation of $\Theta_1$ and $\Theta_2$ which are the 4 $\times$ effective population size $\times$ mutation rate for each population. **Own: value1, value2** defines arbitrary start values. The default is **theta=Own:1.0,1.0**, which is inappropriate for sequence data where values around 0.01 are more common.

**migration=<Fst|Own:value1,value2> (2-POP)**

With **Fst** the programs tries to use an $F_{ST}$ based measure (Maynard Smith 1970, Nei and Feldman 1972, Beerli 1998, Beerli and Felsenstein 1999) for the estimation of $m_1/mu$ and $m_2/mu$. The values for **Own** are given in terms of $4N_em$ which is 4 $\times$ effective population size $\times$ migration rate per generation. The default is **migration=FST**

```
START VALUES FOR PARAMETERS
---------------------------
1       Use a simple estimate of theta as start?
           Estimate with FST (Fw/Fb) measure
2       Use a simple estimate of migration rate as start?
           Estimate with FST (Fw/Fb) measure
3       Mutation rate is constant? Yes


FST-CALCULATION (for start value)
---------------------------------
4       Variable Theta, M symmetric


MIGRATION MODEL
---------------
5       Model is set to Full migration matrix model

Are the settings correct?
(Type Y to go back to the main menu or the letter for an en-
try to change)
```

Figure 9: 'Start value for the parameter' menu of *Migrate*

**migration=<Fst|Own:Migration matrix > (N-POP)**

The **mi**gration matrix is a $n$ by $n$ table with **-** on the diagonal and can look like this for four populations

```
migration=OWN:{ - 1.0 1.1 1.2 0.9 - 0.8 0.7 2.1 2.2 - 2.3 1.4 1.5 1.6
- }
```

or like this

```
migration=OWN:{ -   1.0 1.1 1.2
                0.9 -   0.8 0.7
                2.1 2.2 -   2.3
                1.4 1.5 1.6 - }
```

**mutation=<Gamma | NoGamma>**

If there are more than one locus the program summarizes over all loci. The Gamma flag allows for the variation of the mutation rate of each locus according to a Gamma distribution with shape parameter $\alpha$ (alpha) (which is the inverse of the square of the coefficient of variation (CV) of the mutation rate, CV=standard deviation / mean). This computationally daunting mostly for numerical reasons: the program is maximizing a product of integrals over all possible mutation rates for each locus likelihood. With **Nogamma** the summarizing step is simply finding the best parameters by maximizing the sum of the log-likelihoods of each locus. The default is **mutation=Nogamma**

## $F_{ST}$ calculation

*Migrate* is using the $F_{ST}$ calculation only to generate starting values for the MCMC runs, when you did not wnat to give your guess-values for the parameters. With two population and one locus we can only calculate 3 quantities from the data for $F_{ST}$: the homozygosity within each population and between them. Therefore we only can estimate 3 parameters, either both populations have the same size and different migration rates or the sizes can be different, but the migration rates are the same.
**fst-type=**$<$**Theta** | **Migration** $>$

**fst-type=Theta**
: $\Theta$ for each population is variable, and the migration rate is fixed.

**fst-type=Migration**
: Migration rate for each population is variable, and $\Theta$ is fixed. If the number of populations in the **(N-POP)** program is bigger than 2 only the option **fst-type=Theta** is available. All pairwise Theta estimates are averaged.

## Migration model

If you do not specify anything the joint maximum likelihood estimate of all $n \times n$ parameters are found.

**custom-migration=**$< NONE|migration - matrix >$
The migration matrix contains the migration rates from j to i on row i, and the $\Theta$ are on the diagonal. The migration matrix can consist of connections that are

- 0: not estimated
- m: mean value of either $\Theta$ or $\mathcal{M}$.
- s: symmetric migration
- c: constant value (toghether with `migration=OWN..` or `theta=OWN..`) [does not work yet]
- *: no restriction

The values can be spaced by blanks, newlines A few examples for 4 populations: Full model: **custom-migration=**{********
********
********
********}
N-island model: **custom-migration=**{**m m m m**
**mm mm**
**m mmm**
**mmmm**}
Stepping Stone model with symmetric migrations, and unrestricted $\Theta$ estimates:
**custom-migration=**{***s00 s*s0 0s*s 00s***}
Source-Sink (the first population is the source):
**custom-migration=**{***000**000**0*000**}

```
SEARCH STRATEGY

1       Number of short chains to run?                    10
2       Short sampling increment?                         20
3       Number of recorded genealogies in short chain?   500
4       Number of long chains to run?                      3
5       Long sampling increment?                          20
6       Number of recorded genealogies in long chain?   5000
7       Number of genealogies to discard at
        the beginning of each chain? [Burn-in]           200

------------------------------------------------------------
Obscure options (consult the documentation on these)

8       Tempering (Heating) during increment:   No
9       Sample at least a fraction of new genealogies? No
10      Epsilon of parameter likelihood
        [please read the manual for this!]  100.00000


Are the settings correct?
(Type Y to go back to the main menu or the letter for an en-
try to change)
```

Figure 10: 'Search strategy' menu of *Migrate*

## Search strategy

This section is the key to good results and you should not just use the defaults, for guidance how I would do this see in the section **how long to run**.
The terminology of **short** or **long** chains is arbitrary, actually you could choose values so that short chains are longer than the "long" chains. Anyway, Markov chain Monte Carlo (MCMC) approaches tend to give better results when the start parameters are close to the maximum likelihood values. One way to achieve this is running several short chains and use the result of the last chain as starting value for the new chain. This should produce better and better starting values, if the short chains are not too short.

**Number of short chains to run? (short-chains=value**
: we run most of the time about 10 short chains, which is enough if the starting parameters are not too bad. Default is **short-chains=10**.

**Short sampling increment? (short-inc=value)**
: The sampled genalogies are correlated to reduce the correlation between genealogies and to allow for a wider search of the genealogy space (better mixing), we sample not every genealogy, the default is **short-inc=20** means that we sample a genealogy and step through the next 19 and sample then again.

**Number of steps along short chains? (short-steps=value)**
: The default number of genealogies to sample for short chains is about 200. But this may be to few genealogies for your problem. If you big data sets it needs normally bigger samples or higher increments to move around in the genealogy space.

**Number of long chains to run? (short-chains=value**

we run most of the time 2 long chains. The first equlibibrates and the last is the one we use to estimate the paramters. Default is **long-chains=2**.

**Long sampling increment? (long-inc=value)**

The default is the same as for short chains.

**Number of steps along long chains? (long-steps=value)**

The default number of genealogies to sample for long chains is about 2000. I often choose the "long" chains about 10 times longer than the "short" chains.

**Number of genealogies to discard at the beginning of each chain?**
**(burn-in=value)**

Each chain inherits the last genealogy of the last run, which was created with the old parameter set. Therefore the first few genealogies are biased towards the old parameter set. When **burn-in** is bigger than 0, the first few genealogies in each chain are discarded. The default is **burn-in=200**.

## Obscure options

If you are not experienced with MCMC or run *Migrate* for the first, second,... time, do not bother about the options here.

**Tempered transitions**: [to come and not fully test yet]

**Sample at least a fraction of new genealogies? ( moving-steps=<Yes:ratio | No >)**

With some data the acceptance ratio is very low, for example with sequence data with more than 5000 bp the accpetance ratio drops below 10% and one should increase the length of the chains. One can do this either by increasing the **long-inc**, or **long-steps** or by using **moving-steps**. The ratio means that at least that ratio of genealogies specified in **long-steps** have to be new genealogies and if that fraction is not yet reached the sampler keeps on sampling trees. In unfortunate situation this can go on for a rather long period of time. You should always try first with the default **moving-steps=No**. An example:
You specified **long-steps=2000**,and **long-inc=20** and the acceptance-ratio was only 0.02, you have visited 40,000 genealogies of which only 800 are new genealogies so that you have maximally sampled 800 different genealogies for the paramter estimation. In a new run you can try **moving-steps=Yes:0.1**, the sampler is now extending the sampling beyond the 40000 genealogies and finally stopping when 4000 new genealogies were visited.

**Epsilon of parameter likelihood (long-chain-epsilon=value)**

The likelihood values are ratios

$$\frac{L(\mathcal{P})}{L(\mathcal{P}_0)} = \frac{1}{n}\sum_i \frac{\text{Prob }(G_i|\mathcal{P})}{\text{Prob }(G_i|\mathcal{P}_0)} \qquad \text{(Beerli and Felsenstein, 1999)}$$

When the Likelihood values are very similar then the ratio will be close to 1, or 0 when we use logarithms. This means that the sampler is not improving drastically between chains: (a) it found the maximum likelihood estimate or (b) it is so far from the maximum likelihood estimate that the surface is so flat that all likelihood values are equally bad. using a smaller value than the default **long-chain-epsilon=100.00** for example a value of 1.0 would guarantee that the sampler keeps on sampling new

long chains as long as that log-likelihood-difference drops below 1.0. In some cases this will never happen and the program will not stop.

## Parmfile specific commands

### Important parmfile options

**menu=<Yes|No>**
defines if the program should show up the menu or not. The default is **menu=Yes**.

**end**
Tells the parmfile reader that it is at the end of the parmfile. **THIS IS NEEDED!**

### Options to change the lengths of words and texts

If you change these, you should understand why you want to do this.

**nmlength=number**
defines the maximal length of the name of an individuum, if for a strange reason you need longer names than 10 characters (e.g. you need more than 10 chars to characterize an individual) and you do not need this very often then set it to a higher value, if you have no individual names you can set this to zero (0) and no Individual names are read. the default is **nmlength=10**, this is the same as in PHYLIP.

**popnmlength=number**
Is the length of the name for the population. The default is **popnmlength=100**

**allelenmlength=number**
This is only used in the infinite allele case. Length of an allele name, the default should cover even strange lab-jargons like `Rvf` or `sahss` (*Rana ridibunda* very fast, *Rana saharica* super slow) The default is **allelenmlength=6**

## Likelihood ratio tests and profile likelihood

▷ ONLY with **N-POP**: This section is still incomplete ◁.

## Likelihood ratio test

The parameter estimation is done with a maximum likelihood method, this gives the opportunity to easily test different hypotheses against others, when the hypotheses are hierarchical (e.g. Casella and Berger 1996). For example, we wish to test that the migration rates are the same in a two population model with 4 parameters:

$$H_0 : \mathcal{M}_{21} \neq \mathcal{M}_{12} \qquad \Theta_1 = \hat{\Theta}_1, \Theta_2 = \hat{\Theta}_2, \tag{1}$$

$$H_1 : \mathcal{M}_{21} = \mathcal{M}_{12} \qquad \Theta_1 = \hat{\Theta}_1, \Theta_2 = \hat{\Theta}_2, \tag{2}$$

and then can test using the test statistics

$$-2\log\left(\frac{\mathrm{L}(\Theta_x)}{\mathrm{L}(\hat{\Theta})}\right) \leq \chi^2_{df,\alpha} \tag{3}$$

In the example the degrees of freedom would be two: we are changing two parameters. We need to run `migrate` with the full model: all parameter can vary independently. We get parameter estimates $\hat{\Theta}_1$, $\hat{\Theta}_2$, $\hat{\mathcal{M}}_{21}$, and $\hat{\mathcal{M}}_{12}$. We compare this maximum likelihood with the likelihood when we restrict the migration rate to be the same for example the mean of both estimates. The ratio between these two likelihoods is in the limit (if there is a huge amount of data) $\chi^2$ distributed (Formula 3, Figure 11).
If you have mtDNA data this methods is theoretically not applicable, because you cannot increase the data beyond the full sequence of the mitochondrion, but I am pretty sure that for most situations the test will be still appropriate. There is a problem due to the implementation of the program that we can not allow that parameters go to 0.0. A parameter of 0.0 has a 0.0 probability. Tests against 0.0 need a halfed significance level, because we truncate at 0.0, and therefore are testing only one-sided (...cit...).

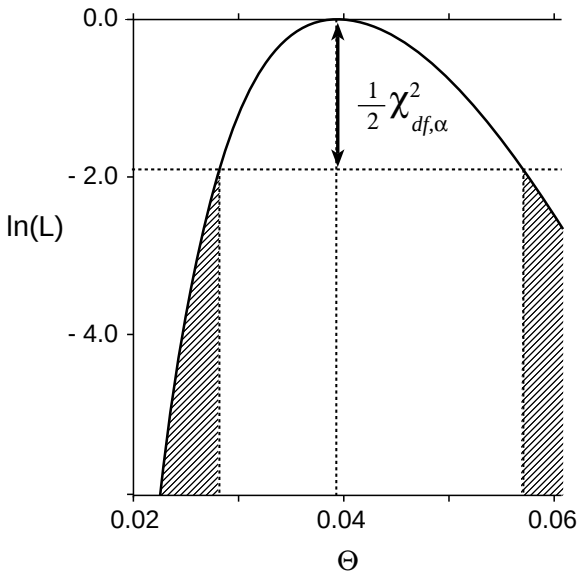


Figure 11: Likelihood ratio test: dashed areas are outside of the 95% confidence limit. $\Theta$ is $4N_e\mu$; $df = 1$, $\alpha = 0.05$

Do not forget that these likelihoods are only approximations. Comparison with exact likelihoods for genealogies with 3 tips and no migration show that the MCMC curves are exactly the same as the "exact" curves. When the program is not run long enough the MCMC curves tend to be wider than the "exact" curves and have their maximum biased towards the parameter value at which we run the chains. We expect when there are many sampled individuals that it is likely that you run the program not long enough and therefore will get wrong confidence interval estimates and will stick too close to the start parameters. (Figure 12). You can check for this by running the program several times from very different start values. Just looking at the point estimates, is probably not enough, you need to inspect the profile likelihoods too. Most of the time it seems that real single locus data is not very great for the estimation of migration rates and the "confidence" intervals are huge.

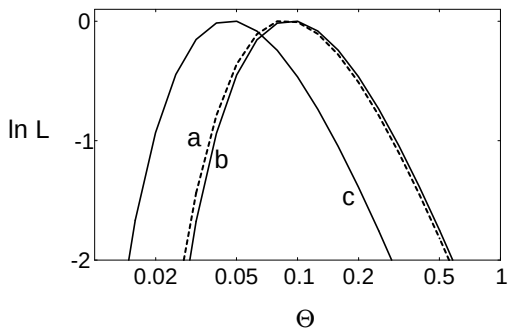


Figure 12: Log likelihood curves from (a) the exact likelihood calculation for a genealogy with 3 samples, (b) an MCMC based estimator with only one (1) sampled genealogy with start value $\Theta_0 =$ Watterson estimate, (c) with one acceptance using a $\Theta_0 = 0.00001$. The data are 3 sequences each 1000 bp long and generated with a $\Theta = 0.1$, running the program some 1000 genealogies delivers a likelihood curve indistinguishable from the exact likelihood curve.

For the `parmfile` there is an option **l-ratio** which you can use to define a hypothesis against the program run (Null-hypothesis). You can repeat the statement for testing more than one hypothesis, but you may need to correct your significance level for multiple tests. The syntax is:
**l-ratio:**<**Means**|**Loci**><**:param1,param2,param3,....paramn*n**>

**Means** over all loci

**loci** for each locus, this may not be valid for sequences, the likelihood ratio test assumes convergence if the sample size goes to infinity, but with a finite sites model and one locus this can not be achieved, so the the $\chi^2$ statistic may not be appropriate.

The syntax for each **param1, param2,...** is rather complicated: **param1 =** <***** |**x** | **m** | **value**>

***** the value is the same as the one from the estimate ($= H_0$)

**x** the value will be maximized.

**m** the value is the mean of the parameters, either $\Theta$ or $4N_e m$.

**value** is any arbitrary value you want to test against the $H_0$.

Examples for two populations for the parmfile entries:
**l-ration=Means:0.01,0.011,1.0,1.1;**
**l-ratio=Means:*,*,m,m;**
**l-ratio=Means:x,m,*,0;**

The parameters are ordered according to the following rule:
$\Theta_1$, $\Theta_2$, ..., $\Theta_n$, $4N_e^{(1)}m_{2,1}$, $4N_e^{(1)}m_{3,1}$, ..., $4N_e^{(1)}m_{n,1}$,$4N_e^{(2)}m_{1,2}$, $4N_e^{(2)}m_{3,2}$, ..., $4N_e^{(2)}m_{n,2}$, ...,$4N_e^{(n)}m_{(n-1),n}$

Although you specify $4Nm$ the program evaluates $\mathcal{M}$ for the test and prints $4Nm = \Theta\mathcal{M}$. This seems more accurate, then the parameters $\Theta$ and $\mathcal{M}$ are uncorrelated.

Example with 3 populations based on the following migration matrix:

$$\begin{array}{ccc} - & 2 & 1 \\ 1.8 & - & 1 \\ 0.5 & 0.6 & - \end{array}$$

results in the string
**l-ratio=Loci:*,*,*,2,1,1.8,1,0.5,0.6;**
Do not forget the semicolon, the current program is picky and needs it ▷ the program should be more forgiving ◁.

## Profile likelihood

Parameter estimation in high dimensions causes serious problems in the presentation of results: for 2 population we have 4 parameters, with 8 population 64, etc. One would like to show the high dimensional surface but we are crudely limited to 3 and perhaps can understand graphs up to five. Showing one parameter at a time only shows us a transection through the solution space, but is perhaps the best we can do. By using profile likelihoods we can trace a parameter and also see how the other parameter change at given values for our profile parameter. Instead of finding the parameters at the maximum likelihood, we fix the profile parameter at some arbitrary value and then maximize the other parameters at that profile likelihood. This constructs a path through the solution space, which we can use to construct approximate confidence limits using the likelihood ratio test criteria (Fig 13) with a degree of freedom of 1 (well, this is true in "asymptopia" but may produce very tight confidence intervals (see Beerli and Felsenstein 2000). Several advanced statistic textbooks discuss the use of likelihood ratio and the related profile likelihoods (e.g. Casella 1996), but I like the compact, and in my opinion, very readable, short text of Meeker and Escobar (1995).

## Monitoring progress

The program will show additional information if the **progress** flag is set (**progress=Yes** is the default). You can even see more with **progress=verbose**. The progress is report similar to the following screen dump fragment for each chain and each locus. I added a line number which is not part of the output (Y means standard progress report, V are the additional lines in verbose mode).

```
01Y 11:49:01   Start conditions: theta={811.90959,0.03487}, M={140.99436,0.00000},
02Y           Start-tree-log(L)=-93.678120
03Y 11:49:01   Equilibrate tree (first 200 trees are not used)
04Y 11:49:03   Long chain   1: lnL=0.21525   ,
05Y            theta={0.04026,0.05527}, M={83.96647,45.78351}
06V            Sampled tree-log(L)={-98.760356 .. -93.035062}, best in group =-93.019453
07V            log(P(g|Param))  -20 to  -18  -16  -14  -12  -10   -8   -6   -4   -2    0   All
08V            Counts                    0    0    0    0    0    0    0    0  144   56   200
09V            Maximization steps needed:   134
10V            Coalescent nodes:  0  1  2  3
11V              population   0:  *  -  -  -
12V              population   1:  -  -  -  *
13Y            Acceptance-ratio = 1095/2000 (0.547500)
.....
```

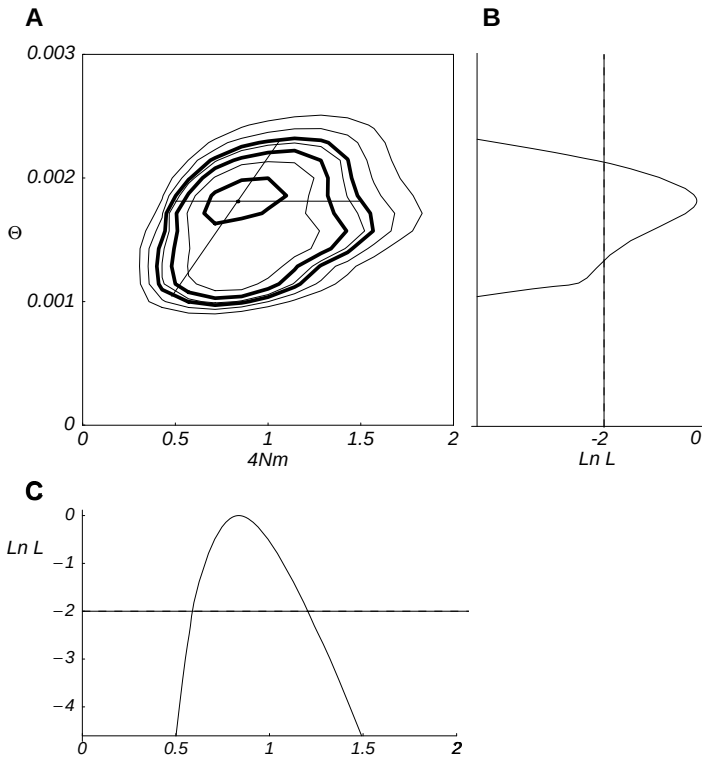


Figure 13: Profile likelihood, for a series of values of a parameter, the other parameter are maximized and the likelihood given that parameter is highest along the straight lines in A. (A) Contour plots for a run with two variables, the thick lines are the 50%, 95%, and 99% confidence contours. (B) is the profile likelihood curve for $\Theta$ and (C) is the profile likelihood curve for 4Nm (based on $\mathcal{M}$). The 95% confidence range for B and C are for values with log likelihood values above -2.

```
14Y 11:49:09   Final parameter estimation over all loci
15Y
16Y          <paste in correct part>
17Y
18Y 11:49:09   Program finished
```

The values reported should give some hints how the program progresses through the sample space. The tree likelihoods (line 06V) should go steadily up until a peak in the likelihood surface has been reached. It can go down through a valley of bad values and either recover on the same peak or another one. If this process runs long enough it is guaranteed that it will find the global maximum. But the program is not searching the tree-likelihood maximum, it searches through the space defined by $\mathrm{Prob}\,(\mathcal{D} \mid \mathcal{G})\mathrm{Prob}\,(\mathcal{G} \mid \mathcal{P})$ and its maximum is not necessarily at the highest tree likelihood. The "histogram" (07V, 08V) of the $\mathrm{Prob}\,(\mathcal{G} \mid \mathcal{P})$ reflects this. the histogram is scaled so that the best value is 0. If most of the values are in the topmost class the estimate is probably in good accordance with the trees, otherwise the process should run longer. Of course if all genealogies are in the topmost class one could wonder if the process is sampling different trees at all, but this can be checked with the acceptance ratio. If the Acceptance ratio (13Y) drops below 10% consider to run the program with ten time longer chains just to sample enough different genealogies, so that the parameter estimates are not governed by a few genealogies only.

If the single locus maximization step needs more than 200 iterations (09V), please send a report, then it should find most of the time the maximum in fewer than 50 iterations.
If you have chosen to discard the first few trees using **burn-in=value**, you will see line (3Y).

# Accuracy of results

## Run time and accuracy

▷ Not complete ◁

If you have looked in the menu `Search Strategy` then you saw that we distinguish between short and long chains. Since the MCMC process is going from a not so good estimate (the first guess, you specify in `Start values for Parameters`) to a better estimate along a "gradient" on the likelihood surface, the success in recovering the best parameters is driven by the steepness of this surface. This means if there is few information in the data, the likelihood surface will be flat and the estimation process need a long time to wander to a peak (if at all) . The short chains allow for a burn-in period in which the the trees and the parameters can equilibrate, for the final estimate we use only the last of the long chains. The necessary length of these chains is specified by the number of individuals, length of sequences and variability of the data. There are no good estimates what a good length for the final chains should be, but watch for a paper of Joe Felsenstein discussing power calculations in a single population case.

For *Migrate* it seems that in simulated datasets with around 20 individuals and 10 "electrophoretic" loci the truth can be recovered.

During my simulations for the paper on `Migrate`, I detected problems with the accurate estimation of the migration rate with start to be obvious with very long sequences (say above 1000bp). The first tree is constructed using an UPGMA topology and a Fitch algorithm to insert the migrations. This process will insert a minimum of migrations onto the tree. If now the sequences define a good topology for your guessed start parameters the program will tend to be stuck with this starting tree. This is fine for estimating the population size, but the migrations are not well distributed on the tree. I am currently working on some extension that the program is searching long enough through the genealogy space even in these cases. At the moment I recommend that you run longer chains and watch the acceptance-rejection, if the program finds about 200 new trees for short chains and about 2000 trees for long chains or more then the estimation process should be fine. If in your initial run you see acceptance ratios of only 2% you should definitely increase the length of the chains, or use the option **moving-steps** or consider heating (tempering) ▷ implemented but not test yet ◁.

## Quick guide for achieving "good" results with `migrate`

Of course this is not a fool proof guide, then it's easy to give advice with data simulated using the same sequence model as the inference program. But, besides monitoring progress, I would:

- Run *Migrate* with the default values using $F_{ST}$ to find the start parameters.
- Check the log, if the data-likelihood of the start tree for each chain is walways improving then consider to supply your own distance matrix (`distfile` option), or give own starting values or run more short chains.
- Rerun, using the obtained parameter estimates of the last run.
- If the results do not change much , perhaps you can stop. Otherwise increase the length of the chains, increasing the increment (**short-inc** and **long-inc** is not increasing the memory usage, but you can also increase the number of sampled genealogies (**short-sample** or **long-sample**). For example increase by a factor of 10.
- Change the random number seed and check if you get similar results.

## How to avoid conflicts with other computer users

The run time of `migrate` is highly dependent on the number of populations, the length of the chains, and the number of loci. It is common that a single data set can run for many hours even very fast machines. For some users this can produce a problem, either the system administrator or other users gets mad about you consuming "all" resources, this is mostly CPU and for large data sets also memory.
**For UNIX systems** the immediate, but perhaps wrong, answer to this people is that these demanding programs are one of the reasons to use these fast computers; a run of `migrate` does normally not compromise any editing, mail reading, word processing on shared machines. To free a terminal you can put migrate into background and log out.

1. Run `migrate-n`
2. Change the menu as you think is apropriate.
3. In the main menu use **(W)rite a parmfile**.
4. Kill the program (Control-c) or use **(Q)uit**.
5. Edit the `parmfile` and change the entry `menu=YES` to `menu=NO` and any other option you want to change. If you intend to run the program several times you should change for each run the the `random-seed=OWN:somenumber`.
6. Rerun the program with
   `nohup (nice migrate-n > migrate.log ; date | \ mail -s ''migrate finished'' youremailaddress) &`
   the `nohup` allows you to logout without stopping the program, additionally potential output is logged into nohup.out. The `nice` causes to program to run slower when other users are using the machine "unniced". On servers the nicing often happens automatically after some time or they have a specific batch system, ask you system administrator what's best for a long run.

7. logout or do something else, you will get mail when migrate has finished, if you are curious and want to known when approximately it will finish peek into the file `migrate.log`, but do not save it.

**For Windows and especially Macintosh systems** the program is unfortunately not a so good citizen and is disturbing other programs. To run long `migrate` on these machines the best way is to run this on a private machines, where you have the control.

# Presentation of results

Contents of the output in `outfile`: Some of the output options vary according to the datatype. + = always present, o = optional, Default = $\star$

| Item | Description | Status |
|---|---|---|
| List of options | all used options are specified | + |
| Summary of data | (Too) short data summary | + |
| Dataset | Print of the dataset | o |
| MCMC estimates | List of the estimated parameters for each locus and the mean | + |
| Shape $\alpha$ | Estimation of the shape parameters $\alpha$ for the variation of the mutation rate | o |
| $F_{ST}$table | Table of the possible start values generated with a $F_{ST}$estimator | o |
| plots | plot of the likelihood surface in outfile | o$\star$ |
| | plot of the likelihood surface into mathfile | o |
| $\alpha$-histogram | Table of shape values versus log(likelihood), $\alpha$ is varying whereas the other parameters are held constant at the maximum of the surface. | o |
| Profiles | Profile likelihood tables | o$\star$ |
| Percentiles | Percentiles table, summary of profile tables | o$\star$ |

The $F_{ST}$ calculations are based on mean differences in populations compared to mean differences between populations, for more information you should consult Maynard Smith (1970) and Hudson et al. (1989). In the Appendix you can find a sample outfile with some comments.

## Walk through an outfile

The following output pieces are from `outfile.seq` in the `example` directory.

### Title and Options

```
============================================
Example for sequence data
```

```
  ============================================
  MIGRATION RATE AND POPULATION SIZE ESTIMATION
  using Markov Chain Monte Carlo simulation
  ============================================
  Version 0.7

  Program started at Sun May 22 23:40:38 1998
         finished at Mon May 23 00:25:32 1998



Options in use:
---------------
Datatype:                                  DNA sequence data
Random number seed (with internal timer)             674365543
Start parameters:
   Theta values were generated  from the FST-calculation
   M values were generated from the FST-calculation
Migration model: Migration matrix model with variable Theta
Gamma-distributed mutation rate is not used
Markov chain parameters:
   Short chains (short-chains):                          10
      Trees sampled (short-inc*samples):              10000
      Trees recorded (short-sample):                    500
   Long chains (long-chains):                             3
      Trees sampled (long-inc*samples):              100000
      Trees recorded (long-sample):                    5000
   Number of discard trees per chain:                   200
Print options:
   Data file:                                        infile
   Output file:                                     outfile
   Print data:                                           No
   Print genealogies:                                    No
   Plot data:                  Yes, to outfile and mathfile
   Profile likelihood:            Yes, tables and summary
```

This is the title and options part. Don’t cut away the options, so you will still know a few weeks later with what kind of options and how long you run the program.

### Summary of the data

```
Summary of data:
---------------
Datatype:                                    Sequence data
Number of loci:                                          1

Population                                        Individuals
-------------------------------------------------------------
  1 population_number_0                                  25
  2 population_number_1                                  21
Total of all populations                                 46

Empirical Base Frequencies
-----------------------------------------------------------
Locus     Nucleotide                        Transition/
```

```
          ------------------------------  Transversion ratio
          A       C       G       T(U)
-----------------------------------------------------------
   1      0.2461  0.2450  0.2497  0.2591       0.60000
```

The data summary is (too) short, and self explanatory, you can also print the data (not shown). Print the data the first time you use the program with your data and check if it was read correctly: I control the first and the last individual in a population and check a few sites at both ends of the sequence. If the program crashes shortly after the start almost certainly the data contains some trouble. The most common error is having the wrong number of individuals and/or number of sites.

### Parameter estimates

```
=============================================================================
MCMC estimates
=============================================================================
Population [x] Loc. Log(L)   Theta    4Nm
                             [4Ne mu]  1,x     2,x
-------------- ---- -------- -------- ---------------------------------------
 1: population    1  2.88   0.04567 ------- 4.03909
 2: population    1  2.88   0.02857 7.80435 -------

Comments:
 There were 10 short chains (500 used trees out of sampled 10000)
and 3 long chains (5000 used trees out of sampled 100000)
```

This is the main output of the program. For each population there is a list of all loci and the estimates and if there are more than one locus, there is also an estimate over all loci. The ln(L) is the maximum log likelihood. This value is a ratio $ln(L) = ln(L(\mathcal{P})/L(\mathcal{P}_0))$. The parameter $\mathcal{P}_0$ are different between different runs of the program and therefore you cannot simply compare between different runs.
The column marked Theta ($\Theta$) gives the population sizes for each population and each locus, of course the number of individuals in that population $N_e$ is for all loci the same, and the variance you see is (a) the variance of the sampler, (b) stochastic variance due to the coalescence process, (c) variance of the mutation rate. The migration parameter $4Nm$ is to read the following way: in population 1, the **2,x** means that the immigration from population two into one is $4N_1m_{21} = 4.039$. in population 2 the **1,x** means that the immigration from population one into two is $4N_2m_{12} = 7.804$ If the program is also allowing for variable mutation rate (you don't want to use that with one locus), then you will get also an estimate for the shape parameter alpha ($\alpha$) for the distribution of the mutation rates.

### $F_{ST}$ table

This will not be shown as a default, anymore. It is merely used as a starting value for the Maximum likelihood estimates. The table are similar to the table of the MCMC estimates.

### Likelihood surface plots

```
Log-Likelihood surfaces for each of the   2 populations
-----------------------------------------------------

Legend:
   X = Maximum likelihood
   * = in approximative 50% confidence limit
   + = in approximative 95% confidence limit
```

```
   - = in approximative 99% confidence limit


Locus 1
    x-axis= 4Nm [effective population size * migration rate],
   y-axis = Theta,
   units = log10
Maximum log likelihood on plot
Population 1: population_number_0
  Immigration: 4Nm=5.179470, Theta=0.051795, log likelihood=2.678661
  Emmigration: 4Nm=13.895000, Theta=0.051795, log likelihood=2.749731

                Immigration                             Emmigration

     -3     -2     -1      0      1      2     -3     -2     -1      0      1      2
     ++------+------+------+------+------++    ++------+------+------+------+------++
   2 +                                    +    +                                    +
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
   1 +                                    +    +                                    +
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
   0 +                                    +    +                                    +
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
  -1 +                          ---       +    +                          --        +
     |                        ++*+        |    |                        -+++++      |
     |                       -+*X*-       |    |                       +*X*+*+      |
     |                      ++**+-        |    |                      -+**+*+       |
     |                      +++-          |    |                      -++-+         |
     |                                    |    |                                    |
     |                                    |    |                                    |
  -2 +                                    +    +                                    +
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
     |                                    |    |                                    |
  -3 +                                    +    +                                    +
     ++------+------+------+------+------++    ++------+------+------+------+------++
     -3     -2     -1      0      1      2     -3     -2     -1      0      1      2
```

For each population and each locus there will be a summary contour plot for all immigrations and all emmigrations. These plots give some information about the confidence you should have in the estimates. Keep in

mind that even with two populations there are 4 parameters and the likelihood. A plot is a kind of diagonal through this high dimensional space (in this example: 5 dimensions);

### Profile likelihoods

```
Profile likelihood for parameter Theta_1
Parameters are evaluated at percentiles
using cubic splines of profiled parameter
(faster, but not so exact).
--------------------------------------------------------------------------------
Per.  Ln(L)     Theta_1     *Theta_1*   Theta_2     M_21       M_12
--------------------------------------------------------------------------------
0.01  -3.645      0.0223     0.0223     0.0297    81.9303   293.8230
0.05  -2.065      0.0240     0.0240     0.0297    81.9441   294.2779
0.10  -1.329      0.0250     0.0250     0.0297    81.9766   294.5011
0.25  -0.284      0.0266     0.0266     0.0296    82.0709   294.7953
0.50   2.878*     0.0457     0.0457     0.0286    88.4385   273.2104
0.75   0.324      0.0789     0.0789     0.0279    96.2738   252.5011
0.90  -1.065      0.0900     0.0900     0.0277    97.4555   251.1683
0.95  -1.910      0.0966     0.0966     0.0277    98.0544   250.5631
0.99  -3.884      0.1119     0.1119     0.0276    99.2213   249.4557
--------------------------------------------------------------------------------
- = not possible to evaluate, most likely value either 0.0 or Infinity
    in the parameter direction, the likelihood surface is so flat
    that the calculation of the percentile(s)  failed.
```

The profile likelihood table give you some idea how the parameters vary when we hold one constant. In the default setting the program tries to find the parameter values that are at percentiles. How this is done for $\Theta_1$: (1) calculate the likelihood value for a few values smaller and bigger than the ML-estimate. (2) calculate a spline function. (3) find the $\Theta_1$ that is at the percentile $x$ using the splines. (4) recalculate the likelihood and maximize the other parameter again using the full formula. In the example, $\Theta_1$ varies almost independently from the others, looking more closely it seems that $\Theta_2$ slightly shrinks while $\Theta_1$ grows.

### Summary of profile likelihood tables

```
================================================================================
Summary of profile likelihood percentiles of all parameters
================================================================================

Parameter                          Lower percentiles
             ------------------------------------------------------------------
                0.01          0.05          0.10          0.25          0.50
--------------------------------------------------------------------------------
Theta_1         0.02228       0.02399       0.02497       0.02664       0.04567
Theta_2         0.00946       0.01188       0.01331       0.01567       0.02857
 M_21          30.53718      36.49126      39.97529      46.64759      88.43845
 M_12         114.08445     132.49441     143.22648     163.32323     273.21045


Parameter                          Upper percentiles
             ------------------------------------------------------------------
                0.50          0.75          0.90          0.95          0.99
--------------------------------------------------------------------------------
Theta_1         0.04567       0.07889       0.09003       0.09660       0.11190
Theta_2         0.02857       0.05709       0.07586       0.09833       0.15052
```

```
 M_21          88.43845     201.06595     215.26333     225.18048     245.85767
 M_12         273.21045     805.85153     896.08361     957.07762    1083.66503
-------------------------------------------------------------------------------
- = not possible to evaluate, most likely value either 0.0 or Infinity
    in the parameter direction, the likelihood surface is so flat
    that the percentiles cannot be calculated.
```

This summarizes only the likelihood and profile parameter column in the profile likelihood tables and can be used to give some idea about the confidence you should have into the estimates. $\Theta_1$ has a approximative 90%-confidence interval from 0.02399 to 0.09660 with a best estimate of 0.04567. (the data was simulated with a $\Theta_1 = 0.05$, for further "true" values see the `README` in the `example` directory.

# Frequently asked questions, errors and warnings, and troubleshooting

This section will increases when I get more feedback. The order of the questions/answers is probably random or historical.

## Questions

1. How can I code haploid data for *Migrate*?
2. I have haploid data, what is $\Theta$?
3. I have mtDNA sequence data what is $\Theta$?
4. Why are the Likelihood values different between runs?
5. It run with the default number of chains etc. Has it run long enough?
6. How long does it run?
7. Can I use haplotype frequencies as input?

## Answers

1. **I have haploid allelic data, how should I structure my infile**
   Unfortunately, I was biased towards diploid data for microsatellite and enzyme electrophoretic data and you need to fake diploids for the infile. Your microsatellite exampled data look like this:

```
      Locus1 Locus2 Locus3 Locus4 Locus5
Ind1   11      45     14     15     89
Ind2   11      47     13     15     67
```

```
Ind3   11      43     13     15     67
Ind4   12      47     13     15     73
Ind5   11      45     13     15     89
```

And your `infile` should look like this

```
   2  5  . Example input for haploid microsatellite data
5  Fake diploid population 1
Ind1   11.?      45.?     14.?     15.?     89.?
Ind2   11.?      47.?     13.?     15.?     67.?
Ind3   11.?      43.?     13.?     15.?     67.?
Ind4   12.?      47.?     13.?     15.?     73.?
Ind5   11.?      45.?     13.?     15.?     89.?
4 Fake diploid population 2
..data not shown..
```

Or

```
   2  5  . Example input for haploid microsatellite data
3  Fake diploid population 1
Ind1Ind2   11.11 45.47 14.13 15.15 89.67
Ind3Ind4   11.12 43.47 13.13 15.15 67.73
Ind5????   11.?  45.?  13.?  15.?  89.?
4 Fake diploid population 2
..data not shown..
```

The "?" are removed for the analysis (But recognize that in sequence data the ? are not removed.

2. **I have haploid data, do I have to multiply my $\Theta$, $\mathcal{M}$ and $4Nm$?**
   The $\Theta$ you get with haploid data is $\Theta = 2N_e\mu$. Comparing with other values for haploid data should be fine, but you need to multiply when you compare it with a $Theta$ from diploid data.

3. **I have mtDNA data, do I have to multiply my $\Theta$, $\mathcal{M}$ and $4Nm$?**
   See question above, but in most vertebrates mtDNA is only passing through the maternal lineages and is haploid, for a comparison with diploid data you should probably multiply by 4.

4. **Why are the likelihoods between runs different?**
   The likelihoods are really ratios

$$\frac{L(\mathcal{P})}{L(\mathcal{P}_0)} = \frac{1}{m}\sum_{i}^{m}\frac{\text{Prob}\,(D \mid g_i)\,\text{Prob}\,(g_i \mid \mathcal{P})}{\text{Prob}\,(D \mid g_i)\,\text{Prob}\,(g_i \mid \mathcal{P}_0)}.$$

   and we run several chains and update the $\mathcal{P}_0$ between chains. For a comparison we would need that the second last chain of each run delivers exactly the same parameters, which we then would use for the comparison. A possibility is to run only one long chain in each run with some given parameters $\mathcal{P}_0$. This not really recommended if the start values are not very close to the true parameters.

5. **It run with the default number of chains etc. Has it run long enough?**
   this depends on the number of populations you want to analyze. If you have one it will be almost certainly enough. But if you try to analyze 6 or more it almost certainly will not. You need to experiment a little with the length of chains. See chapter 3 (Accuracy of results).

6. **How long does it run?**
   With `progress=Yes` the program tries to estimate the length of a run from the work it has done so far, after the first short chain ithis may be rather imprecise, but you may realize that you need to wait minutes or days. The time calculated is only based on the genealogy search, and does not include the time to create the plots for each locus and population. Therefore, if you have many populations and many loci you can expect to wait longer than the time stamp indicates. There is an addtional time estimate for the profile-likelihoods.

7. **Can I use haplotype frequencies as input?** No, input formats are a rather arbitrary matter, and I decided that you need to input each single sequence of genotype. I principle it would be easy to add a "frequency" input mode, but currently I have not time to do that. But keep asking for it, if this is so important to you.

## Errors and warnings displayed by `migrate`

The errors are in no particular ordering, but I will move more important ones to the beginning of their sections.

### Errors

The program aborts when it encounters one of the following conditions. Of course there are certainly conditions I have not thought of.

**SEVERE ERROR: ....** Most often your `infile` contains a problem (e.g. number of sites does not match the number actual sites given, number of individuals does not match). If you fail to correct the problem. please contact me.

**ERROR: Datatype is wrong, please use a valid data type!**
**ERROR: the program will crash anyway, so I stop now**
You probably specified a wrong letter for the data type in the `parmfile`

**ERROR: Wrong datatype, only the types a, m, s, n ERROR: (electrophoretic alleles,**
**ERROR: microsatellite data,**
**ERROR: sequence data,**
**ERROR: SNP polymorphism) are allowed.**
You probably specified a wrong letter for the data type in the `menu`

**ERROR: The parmfile contains an error on line XX**
There was a wrong entry or even more likely wrong values in the `parmfile` on line xx.

**ERROR: Inconsistency between your Menu/Parmfile and your datafile**
Most likely your parmfile assumes there are n subpopulations and you assume m subpopulations. Problems

with the migration matrix are likely.

**ERROR: There is a conflict between your menu/parmfile**
**ERROR: and your datafile: number of populations are not the same**
Most likely your parmfile assumes there are n subpopulations and you assume m subpopulations.

**ERROR: cannot find seedfile**
You specified that the random number is in `seedfile`, but the file is not present in the directory `migrate` is running.

**ERROR: Failure to read seed method, should be**
**ERROR: seed=auto or seed=seedfile or seed=own:value**
**ERROR: where value is a positive integer**
Either seed specification in `seedfile` or `parmfile` is wrong.

**ERROR: Failure to read start theta method, should be**
**ERROR: theta=FST or theta=Own:x.x**
**ERROR: or theta=Own:{x.x, x.x , x.x, .....}**
**ERROR: migration=Own:migration value**
the start parameters are not correctly specified.

**ERROR: Failure to read start migration method**
the start parameters are not correctly specified.

**ERROR: Custom migration matrix was completely set to zero?!**
the custom migration matrix was not correctly specified.

## Warnings

**WARNING: migration limit (xx) exceeded: yy**
**WARNING: results may be underestimating migration rates**
**WARNING: for this chain**
If this happens only a few times in short chains, don't worry. If it happens in the last chain or very often, then your migraiton estimates will be most likely underestimated, but the migration rates between these populations will be very high, anyway. It means that there is an upper limit of possible migration events on the genealogies, and this is set as a default to number_of_populations $\times$ 1000.

**WARNING: Migration forced**
**WARNING: results may overestimate migration rates**
**WARNING: for this chain**
Migration rate is essentially 0.0, the program proposes sometimes a migration event even so the probabilities would foce a coalescence, this heuristic helps to escape the fatal attraction to 0.0. If 4Nm is smaller than 0.1 the program will propose randomly every tenth event a migration event. This genealogy has then still to be

accepted. Hitting this boundary can produce an upwards bias, but it should be only be recognizable when your populations are barely connected, if at all.

**WARNIN‘G: This does look like sequence data**
**WARNING: I just read a number of sites=0**
**WARNING: If you use the wrong data type, the program will abort**
Check your datatype!

**WARNING: ————————————**
**WARNING: Target branch problems with time=xx**
**WARNING: ————————————**
If you encounter this, abort the program, and try to find the error in the `infile`, but if the data prints conrrectly, please contact me. Probably I should declare this a severe error and abort.

**WARNING: proposed and new likelihood differ: xx != yy**
**WARNING: abort the program and try to find the errors**
**WARNING: there could be a wrong datatype, or infile**
**WARNING: to check the data you can print it (see menu)**
If you have problems to resolve this error (check for errors in infile), please contact me and try to give as much information as you can (including your dataset).

**WARNING: Inappropiate entry in parmfile:** *keyword* **ignored**
The *keyword* of a parmfile entry was wrong, often misspelled.

**WARNING: You forgot to add your guess value:**
**WARNING: Theta=Own:pop1,pop2, ...**
**WARNING: or Theta=Own:guess_pop (same value for all)**
You probably specified Theta=Own and forgot to say what values.

**WARNING: You forgot to add your guess value, use either:**
**WARNING: migration=FST**
**WARNING: or migration=Own:{guess_4Nm} (same value for all)**
**WARNING: or migration=Own:{ - 4Nm21 4Nm31 .... 4Nm12 - 4Nm23 ...}**
You probably specified migration=Own and forgot to say what values. See the parmfile section, about how to give the migration values.

## Troubleshooting

If you think you have found a bug please report this to
`beerli@genetics.washington.edu`. I would like to know every warning you see while you compile the program, if you send me bug-reports please include your hardware and system specifications, your `infile`, your `parmfile` (if any), and a “printout” of the warnings or errors.
BUT, mostly, the problem is that the data in the infile is in a wrong format: you can expect the program to

crash when you try to use the `datatype=Allelic` and your infile contains sequence data. I am trying to reduce the number of strange error messages, but this has lower priority than adding new features/improving code.

**Please, before you report a bug, compare your infile with the examples**.

# Program distribution and installation

## Program availability

*Migrate* can be fetched from our www-site (`http://evolution.genetics.washington.edu/lamarc.html`) and is free for non commercial use. Currently we have the following packages available:

| | |
|---|---|
| migrate.tar.gz | Source |
| migdoc.pdf | Documentation |
| migrate.src.pm.sea.hqx | Source for powermac (Metrowerks) |
| migrate.powermac.sea.hqx | Powermac binaries |
| migrate.alpha.tar.gz | Dec Alpha DUNIX binaries |
| migrate.openstep.tar.gz | OPENSTEP/NeXT [Intel] binaries |
| migrate.linux.tar.gz | LINUX binaries |
| migrate.solaris.tar.gz | Solaris 2.6 binaries |
| migwin.exe | WindowsNT/95 self extracting archive |

## Installation

### Binaries

On UNIX system unpack with `tar xvfz migrate.[system].tar.gz` or `gunzip -c migrate.[system].tar.gz | tar xf -`. This builds a directory `migrate` with a subdirectory `examples`, the files `README, HISTORY`, and the programs `migrate` and `migrate-n`. The program can be moved to a location like `/usr/local/bin` and the documentation (HTML files are in documentation/migratedoc) to your HTML directory (e.g. `/usr/local/etc/httpd/htdocs`). On Powermacs or Windows machines double click the archive and a folder system similar the UNIX directories above will be created.

## Source

### UNIX

1. **gunzip -c migrate.tar.gz — tar xf -** or
   **tar xfz migrate.tar.gz** this creates a directory ”migrate” with ”src” and ”examples” in it.
2. `cd migrate`
3. `configure`
   (this scripts checks your system and will report functions the program needs, if a function is not, it will report an error, which I need to know.
4. `make`
   (please report warnings and especially errors) the result should be a binary `migrate` in the migrate directory.
5. `make install`
   (this will install the program and man-page into usr/local/bin, /usr/local/man/man1 ; you need to be root to do this; this step is not necessary)

### Powermac

The source code for the Powermac is the same as the general source code but it is packaged with a minimal graphical interface file and a Metrowerks Codewarrior project, which should make it very easy to compile (if you have a very recent Metrowerks compiler).

1. Unpack (it is a self extracting archive).
2. Open the migrate.$\mu$ file and use the submenu `Make` (I compiled with Metrowerks CodeWarrior Pro 4)

# Miscellaneous

## Wish list

- Send me a reprint if you used `Migrate` for your publication.
- Cite the documentation and our paper ▷ Once it's published ◁, see below.
- Report problems to `beerli@genetics.washington.edu`
- Suggestions (if you need these improvements very soon, add a check so that I can hire a programmer to implement all those  )

## How to give credit

Please cite:

**Beerli, P.** 1997. MIGRATE 0.7: documentation and program, part of LAMARC. Revised May 19, 1999. Distributed over the Internet,
`http://evolution.genetics.washington.edu/lamarc.html`
[Downloaded: ...date....]

**Beerli, P., and J. Felsenstein.** 1999. Maximum likelihood estimation of migration rates and population numbers of two populations using a coalescent approach. Genetics 152(2): 763-773.

**Beerli, P.** 1998. Estimation of migration rates and population sizes in geographically structured populations. In Advances in molecular ecology (Ed. G. Carvalho). NATO-ASI workshop series. IOS Press, Amsterdam. Pp. 39-53.

## Copyright

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And also all people who thought it worth to report errors and foggyness in menu and explanation: Mats Bjorklund, Allen Rodrigo, Carol Reeb, Byron Adams, Tony Metcalf, Toby Hay, Peter Galbusera, Scott Edwards, Reinaldo Brito [List is ordered by date and certainly incomplete].

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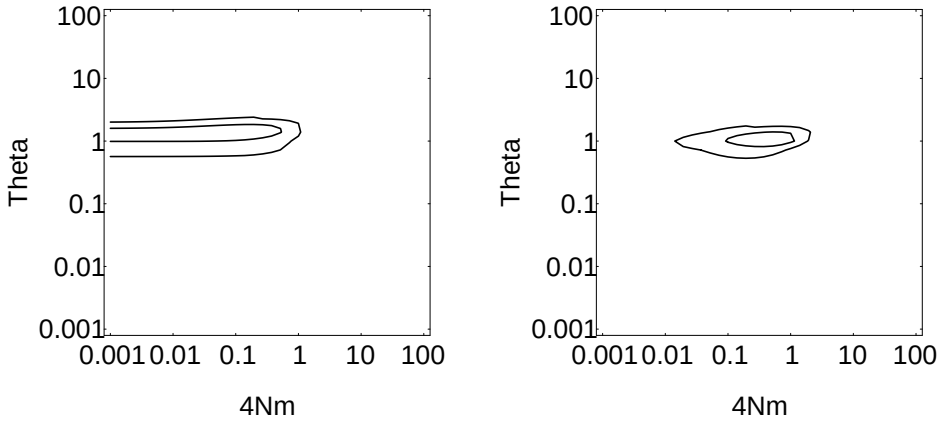
# Appendix

## Mathematica plot package

If you have access to the program `Mathematica`, you can open the `lamarc.example.ma` in the `example` directory. With it you can create nicer likelihood surface plots than the ones you see in the outfile. ▷ the syntax used is only valud of migrate-0.4, I need to clean the approriate tools for migrate-n for public consumption ◁

Example:




## History and persistent problems

[people] in brackets helped to find bugs/problems.

May 19, 1999 MIGRATE-N 0.7 Updated documentation, several minor things, warnings and error reporting should be more consistent, I am adding a section to the manual that describes all error/warning messages [partly done], the plotting graphics are more flexible now, but still need more work. You can specify the range and type of axes (log-scale, std-scale), and if the migration parameter shall be plotted as M=m/mu or 4Nm. Fix of inconsistency in migration value menu input [Reinaldo Brito]. Fix of an error in the profile-method=FAST (it will need now more time to finish, because it is doing the final maximization over all other parameters), if you want its old behavior, that assumes that Theta and M are not correlated [not a too bad assumption], then use profile=YES:QUICK.

Feb 14, 1999 MIGRATE-N 0.6.3 Updated documentation (fixed errors in description of `random-seed` options, added important material to profile-likelihood) , inclusion of improved man page, fixed configure for SGI's with out gcc.

Oct 11, 1998 MIGRATE-N 0.6 Addition of datatype=n that is for single nucleotide polymorphism data, no simulation with this kind of data is yet done, so I do not know about biases etc. Profile tables now report 4Nm instead of m/mu for the migration parameters. Documentation contains now more about what you can and cannot do with the reported log(likelihood) values [Mats Bjorklund]. Binaries for OPENSTEP available [thanks to Magnus Nordborg giving me an account on his machine]. Registered users: 206

Sep 1, 1998 MIGRATE-N 0.4/0.5 [was not released, was too busy with other things] FST start values work now also for microsatellite data but I still need to check the correctedness of the FST table when the data are microsatellites. Fixed wrong emmigration plots. Fixed wrong start calculations for allelic data when a delimiter was used, and several minor bug fixes. Profile-method "uncorrelated" from version alpha.1 recovered. Registered users: 197

June 14, 1998 MIGRATE-N alpha.3 and MIGRATE-0.4.2 Several minor changes in migrate-n: menu addition for -profile method: profile-method=¡Spline — Percentiles — Discrete¿ Spline: uses 1-dimensional splines to find percentiles, faster than the "Percentiles" option but not so accurate, "Discrete" evaluates at "fixed" (0.02, 0.1, 0.2, 0.5, 1, 2, 5, 10, 50) * MLE of parameter. -with progress=yes you can see now a rough prognosed time of end of sampling genealogies and if you use profiles an estimated time of finishing. -Fix of reading in intermediate results (sumfile). -Most importantly a (hopefully) stable compile for Windows, I failed to find the cause why the program compiled with WATCOM failed to finish with "bigger" data sets, it is now compiled with mingw32/gcc-win32, this is a windows port of the same system I am using on my workstation. Please report failures, I can only try a limited set of examples. Migrate-0.4.2: new windows binary (using mingw32/gcc-win32) Registered users: 163

May 30, 1998 MIGRATE-N alpha.2 and MIGRATE-0.4.1 With more than 2 sequence loci, there was a problem with the T/T-ratio, when the ratio was not specified for each locus. Start parameter problems with microsatellite data fixed [Mats Bjorklund]. Persistent problems with Windows executable sometimes I get floating point errors, on all other systems this does not occur. Registered users: 153

May 29, 1998 MIGRATE-N alpha.1 and MIGRATE-0.4 Memory bug in FST calculation found and fixed [Daniel Yeh] No change of Migrate-0.4 Registered users: 148.

May 26, 1998 MIGRATE-N and MIGRATE-0.4 This release has the two population version (Migrate-0.4) and an alpha-version of Migrate-n that can solve migration matrix population model with unequal population sizes and unequal migration rates for n populations, I tried up to 10 and the results where

fine, but I am pretty sure that if you try to feed in all your date of 100 subpopulation it will (a) probably crash, but more importantly (b) will need TERRIBLY long to run. I would like to get some feedback about what you want to see in the outfile, menu etc. Registered Users: 138.

March 18, 1998: MIGRATE 0.4

Update of the manual, but still not complete. More complex sequence evolution models (categories, weights, autocorrelation etc.) should work now, it was broken. Cleanup of some output file lines, and some menu entries. The FST estimation (Remember FST is only used to generate start parameter values) is in pre 0.4 versions logically flawed. It estimates 2 parameters per population using F_within and F_between, but there is only 1 F_between. Correctly, we can only estimate maximally 3 parameters with 1 locus for two populations. I added an option into the MENU and into the PARMFILE (fst-type=<Theta | Migration >) with which you can decide which parameter is considered the same for both populations. Registered users: 103

August 20, 1997: MIGRATE 0.3.1

Confusing menu entries for start theta and 4Nm values fixed [Carol Reeb], the start migration values are now 4Nm and *not* m/mu values as before. Automatic Random number seed on Macs and perhaps on other Systems delivered sometimes negative values, now fixed [Carol Reeb], although I would recommend to use your own random number seeds: best values are 4n + 1 in the range of 5 .. 2147483647, so there are plenty of start random number seeds. Menu entry for usertree options should be no more clear, the usertree options needs a genealogy with migration events on it [Tony Metcalf]. Currently MIGRATE can construct those, or you have to do it by hand, if you need to do this send me email, because the doc is not updated. Registered users:52

June 20, 1997: MIGRATE 0.3.0

Brownian motion approximation to stepwise mutation model for microsatellites added. Solved problems: Input problems with microsatellites data, major memory allocation problem for datasets with more than 100 gene copies fixed [Carol Reeb]. Update of some citation and FST output tables [Byron Adams]. Persistent problems: Long sequences AND high number of individuals need much longer chains than the proposed default. Try ten times longer ”long” chains. Or use the option ”moving-steps”. Registered users:38

May 12, 1997: MIGRATE 0.2.1a

Fixed problems: Interleaved sequence data should work now, last character of individual names is now printing, and printing of second population data should work, too, although the EP data printout is still ugly. [Allen Rodrigo]. Memory problem with some Allelic data fixed. Registered users: 30

April 30, 1997: MIGRATE 0.2a

Fixed problems or changes: Corrections of several minor problems, Printing of the data fixed, but still ugly; Memory problem with large sequences fixed. Options: treefile added, can write now a genealogy with migrations; the option progress=Verbose for more information during a run, the progress=Yes gives now less information than before. Output: covariance matrix for combined loci now prints, too. Persistent problems: -Long sequences need very long chains to remove the starting conditions for the migration rate from the first tree (see documentation). -Microsatellites still have probably a bias downwards in Theta, but I need more simulations to make this more clear. Registered users: 8

March 4, 1997: First trial release of MIGRATE 0.1a

This release is not announced widely, because I have to test, almost everything including all HTMLs, registration, and the program itself: simulations need time. Registered users: 1