

SUMMARY

Manual for VAREFF package: R software

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This package works with R software (version 2.10.0 or later) and the `library(mcmc)`.
Load VarEff and mcmc.

Functions	Description	File used	Files created
<code>Theta()</code>	Estimates the mean of current (Theta0), intermediate (Theta1) and ancestral (Theta2) theta values, asks for parameters and builds a script to run VarEff ("job.R").	infile.txt	job.Theta job.R
<code>VarEff()</code>	Model VAREFF. Applies the mathematical resolution to get the posterior distribution of N_e at past times, and create a .Batch file	infile.txt	job.Batch (core of the estimates)
<code>NatSizeDist()</code>	Calculates estimates of effective size (N_e) at a number of times from 0 to a certain time ago (given by the user), plots and saves results on files.	job.Batch	job.Nstat job.Ndist job_mmm.pdf (Files with the results in terms of N_e)
<code>LogSizeDist()</code>	Same as NatSizeDist() in the $\text{Log}(N_e)$ scale.	job.Batch	job.Lstat job.Ldist job_LLL.pdf (Files with the results in terms of $\text{Log}(N_e)$)
<code>NTdist()</code>	Graphical summary of the posterior distribution of estimates of $\text{Log}(N_e)$ in the past (a 2D plot).	job.Batch	job.2D job82D.pdf figure in R graphics
<code>plotNdistrib()</code>	Plots the posterior distributions of the estimates of N_e (or $\text{Log}(N_e)$) at a number of times in the past.	job.Ndist	job_Ndist.pdf or job_Ldist.pdf figure in R graphics

Examples in the following made use of data drawn from the simulation of a sharp bottleneck in a population of size 1000, reduced to size 100 since 200 generations. Estimations made use of 20 microsatellite markers submitted to a (very) high rate of mutation ($\mu = 0.01$).

A. DEFINITIONS OF VARIABLES

Variables used by VarEff

- **parafile** (Name that you give to the job and to the output files created by the model)
- **infile** (Name of the data file)
- **NBLOC** (Number of Loci)
- **JMAX** (Number of time when the effective size has changed, used to generate step functions simulating the pas demography. Ex: , JMAX=2, if we think that the population took 3 different effective sizes in the past)
- **MODEL** (choice one mutation model in: S = Single Step Model, T = Two Phase Model, G = Geometric Model, and provide an additional coefficient (C) for T and G models)
- **MUTAT** (Mutation rate, assumed the same for all loci)
- **NBAR** (Prior value for the effective size)
- **VARP1** (Variance of the prior log-distribution of effective sizes. Ex: VARP1=3 allows for searches with 20- to 40-fold relative variations of effective size)
- **RHOCORN** (Coefficient of correlation between effective sizes in successive intervals)
- **GBAR** (Number of generations since the assumed origin of the population)
- **VARP2** (Variance of the prior log-distribution of time intervals during which the population is assumed of constant size)
- **DMAXPLUS** = DMAX+1 (DMAX is the maximal distance between alleles (number of microsatellite motifs) that is used in the estimation algorithm)
- **Diagonale** (A smoothing parameter to balance the observed covariance structure with a theoretical diagonal variance matrix and avoid numerical instability. Diagonale = 0.5 is a robust choice)
- **NumberBatch** (number of batch (nbatch) for metrop in MCMC)
- **LengthBatch** (length of batch (blen) for metrop in MCMC)
- **SpaceBatch** (space of batch (nspac) for metrop in MCMC)

Additional variables used by NatSizeDist(), LogSizeDist() and NTdist()

- **TMAX** (Length of the period for which the distributions of N_e or $\text{Log}(N_e)$ in the past are generated)
- **NBT** (Number of time intervals. Example: If TMAX=1000 generations, and NBT=100, estimates are calculated every 10th generation until 1000 generations ago)

B. FIRST STEP: THETA FUNCTION

The Theta() function allows you to get a first view of data, and to prepare a parameter file to run VarEff(). The function can be used in two ways:

1) DIRECTLY

Example with data file "InputTest.txt":

```
> Theta(infile='InputTest.txt', parafile="Test",NBLOC=20)
[1] Estimation of effective sizes Ne(T) in the past
[1] from microsatellites data
[1]
[1] This function builds a parameter file 'parafile.R'
[1] allowing you to run VarEff with the command: source('parafile.R')
[1]
[1] *** Data ***
[1] Name of the data file= InputTest.txt
[1] Number of microsatellite markers= 20
[1] Name of the job = Test
```

Then Theta() asks the user to provide values for the other variables of VarEff()

2) ANSWER TO THE QUESTIONS

```
> Theta()
[1] Estimation of effective sizes Ne(T) in the past
[1] from microsatellites data
[1]
[1] This function builds a parameter file 'parafile.R'
[1] allowing you to run VarEff with the command: source('parafile.R')
[1]
[1] *** Data ***
Name of the data file= InputTest.txt
Number of microsatellite markers= 20
Give a name for this job = Test
```

Then Theta() reads the data, shows the distribution of allele distance frequencies in the sample (Fig.1), provides global estimates of theta and write them on a .Theta file,

```
...
[1] Theta (4*Ne*u) is estimated using 3 estimators correlated to
[1] Present, Intermediate and Ancestral population size;
[1] results are written to a .Theta file
[1]
[1] Mean values of estimates Theta_0, Theta_1, Theta_2
[1] 6.746623 14.359955 15.753006
[1] Imbalance indices ln(Theta_1/Theta_0) and ln(Theta_2/Theta_0)
[1] 0.7554014 0.8479892
...
```

asks the user to provide values for the other variables of VarEff(), and writes a .R file to run the VarEff() function (table 1).

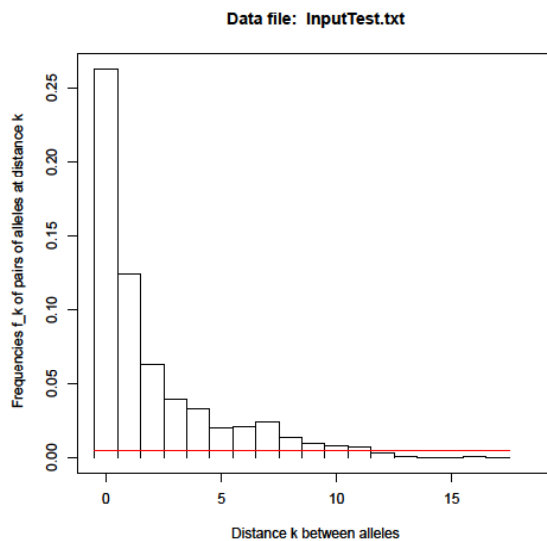


Figure 1: Distribution of the frequencies of allele distances

```
VarEff(parafile = 'Test',
infile = 'InputTest.txt',
NBLOC = 20,
JMAX = 4,
MODEL = 'S',
MUTAT = 0.01,
NBAR = 1000,
VARP1 = 3,
RHOCORN = 0,
GBAR = 5000,
VARP2 = 3,
DMAXPLUS = 13,
Diagonale = 0.5,
NumberBatch = 10000,
LengthBatch = 1,
SpaceBatch = 1)
```

Table 1: The Test.R file generated by Theta()

C. SECOND STEP: VAREFF FUNCTION

The VarEff function can be used in two ways:

1) DIRECTLY

Enter the command providing values to all the variables

```
> VarEff(parafile = 'Test', infile = 'InputTest.txt', NBLOC = 20,
JMAX = 4, MODEL = 'S', MUTAT = 0.01, NBAR = 1000, VARP1 = 3,
RHOCORN = 0, GBAR = 5000, VARP2 = 3, DMAXPLUS = 13, Diagonale = 0.5,
NumberBatch = 10000, LengthBatch = 1, SpaceBatch = 1)
```

Or, when Theta() has been previously run, launch with:

```
> source("Test.R")
```

2) ANSWER THE QUESTIONS

Call the function VarEff(), then answer the questions (red) thanks to the explanation (blue) (Table 2). In that case, it begins like Theta(), providing the global theta estimates, showing the distribution of allele distances (and keeping the plot to file), giving guidelines to choose parameters, and building a .R file containing all the chosen parameters.

At the end of the calculations, VarEff() returns global theta values (as Theta() does) and summaries of adjustment criteria of data to model and the distribution of posterior probabilities, which are added to the .Theta file.

```
...
[1] Mean values of estimates Theta_0, Theta_1, Theta_2
[1] 6.746623 14.359955 15.753006
[1] Imbalance indices ln(Theta_1/Theta_0) and ln(Theta_2/Theta_0)
```

```

[1] 0.7554014 0.8479892
[1] Mean and standard deviation of Quadratic deviations from data
[1] 10.163939 2.622715
[1] Mean and standard deviation of log prior probabilities
[1] -17.995083 1.517846

```

The main result of VarEff() is the .Batch file, which reports a list of demographic evolutions described by step functions. Each line includes:

```

Column 1: the number i of the simulated state (from 1 to Numberbatch)
Column 2: quadratic deviation of data from the i-th simulated state
Column 3: natural logarithm of the prior probability the i-th state
Columns 4 to JMAX+4: the JMAX+1 population sizes in the i-th state
Columns JMAX+5 to 2 JMAX+4: times of size changes in the i-th state
Columns 2 JMAX + 5: value of the C parameter of the mutation model

```

Results are kept in the .Batch files in reduced scales: theta's for population sizes, products of generation numbers times mutation rate for times of size changes. The additional C parameter is set to 0 for the Single Step Mutation Model, positive for geometrical model or negative for the Two Phase Model.

Table 2: Questions and Answers when launching VarEff()

```

> VarEff()
[1] Estimation of effective sizes Ne(T) in the past
[1] from microsatellites data
[1] Note that this R code makes use of the mcmc library
[1]
[1] *** Data ***
Name of the data file= InputTest.txt
Number of microsatellite markers= 20
Give a name for this job = Test
[1] *** Demographic model ***
[1] Choose the number of transitions between periods with constant Ne
Value of JMAX = 4
[1] *** Mutation model ***
[1] Fix the mutation model (S, T or G)
[1] (S = Single Step Model, T = Two Phase Model, G = Geometric Model)
[1] and the prior value of its C parameter, 0 < C < 1
[1] e.g. answer ' T 0.15 ' for the Two Phase Model with a proportion
15% of two motifs steps
Mutation model and C coefficient? S
[1] Give the mutation rate MUTAT
MUTAT = 0.01
[1] *** Priors about population sizes ***
[1]
[1] Prior expected value of Effective Population Size Ne:
Prior Ne = 1000
Variance of log(Ne)= 3
[1] Prior correlation between successive population sizes:
correlation= 0
[1] *** Priors about time and time intervals ***
[1]
[1] Prior time since the Origin (number of generations)
Origin time (generations) = 1000
[1] Prior about time intervals:
Variance of log(time interval)= 3

```

```

[1] *** Smoothing the Covariance matrix ***
[1]
[1] Calculation of an approximate likelihood assumes the theoretical
[1] variance-covariance matrix V of observations is known.
[1] It is in fact replaced by a combination of the sample estimate Vs
[1] and of the diagonal matrix Vd made of theoretical variances:
[1] V = (1-D) * Vs + D * Vd
[1] D must be > 0 and less than 1
[1] D = 1 means that only theoretical variances are considered
[1] and correlations are ignored
[1] D = 0 means that only observed data are considered, a choice
[1] that may raise numerical instability
[1] For more detail read the help and article
[1] A medium choice, D = 0.5 , is generally robust
D = 0.5
[1] *** Length of the MCMC chain (look at the metrop specifications) ***
[1] Values recommended: nbatch = 10000, blen=10, nspac=10
[1] Enter the three values you wish to use
nbatch = 10000
blen = 1
nspac = 1
Read 240 items
[1] Effective Number of markers = 20
[1] Plot of the (mean) distribution of distances between alleles
[1] Choose the range 0:DMAX of allele distances to be analysed
[1] e.g. so that f_k < 0.005 for k > DMAX (under the red line)
Enter DMAX = 12
[1] Mean values of estimates Theta_0, Theta_1, Theta_2
[1] 6.746623 14.359955 15.753006
[1] Imbalance indices ln(Theta_1/Theta_0) and ln(Theta_2/Theta_0)
[1] 0.75540135 0.84798912
[1] Mean and standard deviation of Quadratic deviations from data
[1] 9.979541 2.662895
[1] Mean and standard deviation of log prior probabilities
[1] -18.563440 1.760705

```

D. THIRD STEP: OUTPUTS FROM RESULTS FILES

To obtain the distribution of **(1)** effective size (N_e) or **(2)** $\text{Log}(N_e)$ at a number of generations in the past, run the function **(1)** NatSizeDist() or **(2)** LogSizeDist()

Both functions work the same. They use the job.Batch file created by VarEff(). Results can be shown in natural census values of population sizes, or in reduced theta scale if mutation rate is set to 0 in these functions.

1) DIRECTLY

Example:

```
NatSizeDist(NameBATCH= Test.Batch, MUTAT=0.01, TMAX=1000, NBT=20)
```

In this example the estimates of N_e will be calculated every 50-th generation from 0 to 1000 generations ago.

2) ANSWER THE QUESTIONS

Call the function `NatSizeDist()`, then answer the questions (same answers, using the reduced scales):

```
> NatSizeDist()
[1] VarEff - View past effective sizes Ne(T)
[1]           and save posterior distributions
Name of the batch file= Test.Batch
[1] Mutation rate: Enter 0 to use reduced scales Theta's and G*μ
mutation rate = 0
[1] Enter the length of the period to be analysed
[1] give time in the reduced time scale G*μ
Length of the period = 10
Number of time intervals= 20
[1] Results are expressed in reduced scales Theta's and G*μ
[1] Plot of mean results
```

Results include:

> a plot (Fig.2),

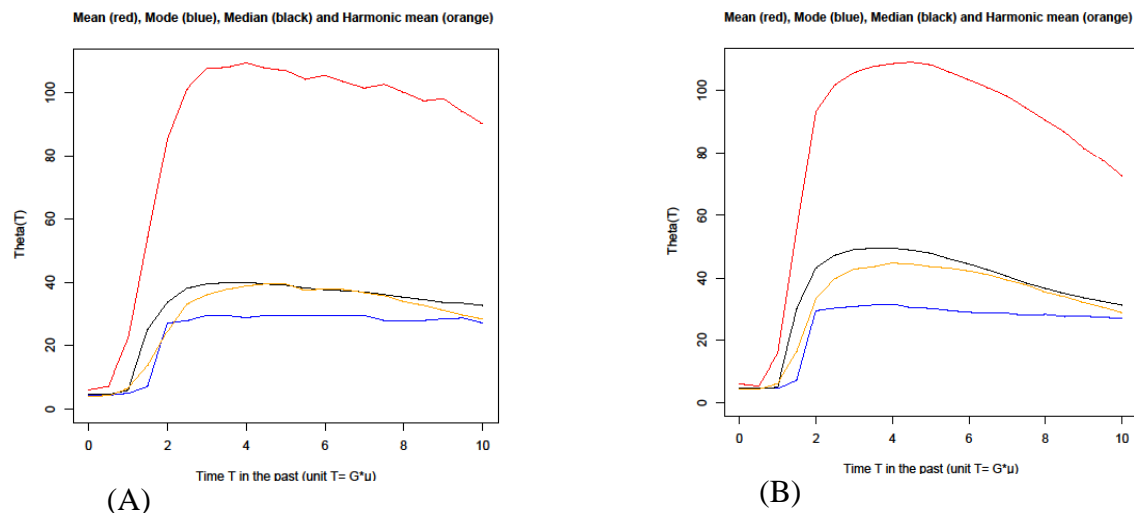


Figure 2: Estimates of N_e in the past (reduced scale).

(A) using a short MCMC chain as in Table 1; (B) using a long chain (10000, 10, 10)

> a file `job.Nstat` (or `job.Lstat`) giving statistics at the different times:

1. Generations (if `MUTAT` is not 0) or the corresponding reduced time
2. Arithmetic means of N_e or $\text{Log}(N_e)$
3. Harmonic means of N_e (not provided for $\text{Log}(N_e)$)
4. Mode
5. Median
6. Quantile 5%
7. Quantile 95%

> and a big file, `job.Ndist` (or `job.Ldist`) containing the detailed densities of the posterior distributions of N_e (or $\text{Log}(N_e)$), with 512 points. Such files are used by the `plotNdistrib()` function (see below).

D. THIRD STEP: PLOTS OF POSTERIOR DENSITIES

1) TWO DIMENSIONAL SUMMARY: function NTdist():

The function NTdist() uses the job.Batch file and makes a figure to summarise the posterior distribution of N_e or $\text{Log}(N_e)$ in a certain period (TMAX) given by the user.

The graph is plotted and saved as a text and as a .pdf file

Example:

Directly using the function as:

```
> NTdist(NameBATCH= Test.Batch, , MUTAT=0.01, TMAX=1000)
```

In this example the summary will be from 0 to 1000 generations ago.

By answering the questions (example giving a “true” mutation rate)

```
> NTdist()  
[1] VarEff - View joint distribution of T and Ne(T)  
[1] and save this joint posterior distributions  
Name of the batch table= Test.Batch  
[1] Mutation rate: Enter 0 to use reduced scales Theta's and G*μ  
mutation rate = 0.01  
[1] Enter the length of the period to be analysed  
[1] give time in number of generations  
Length of the period = 1000  
[1] Assumed mutation rate= 0.01  
[1] Plotting the 2D ( g ,log10( Ne(g) ) ) distribution  
[1] Time range: from 0 to 1000 (generations)  
[1] Minimum and Maximum values of log10( Ne ) in the plot:  
[1] Minimum = 1.20233626012957  
[1] Maximum = 4.08949542800206  
[1] Coverage = 97.2457 %
```

The result is shown as a plot (Fig. 3)

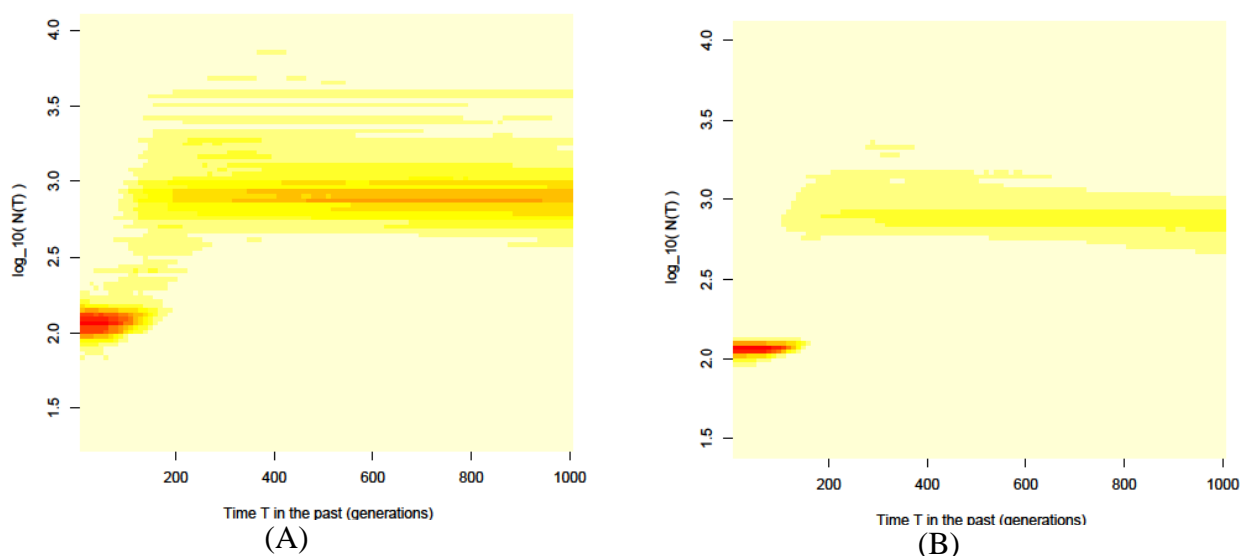


Figure 3. Global plot of the posterior distribution of N_e in the past (A) using a short MCMC chain as in Table 1; (B) using a long chain (10000, 10, 10)

2) PLOTS OF POSTERIOR DISTRIBUTIONS

The function `plotNdistrib()` makes use of files `.Ndist` or `.Ldist` previously built by `NatSizeDist()` or `LogSizeDist()`. It allows the user to exhibit the density of the posterior distribution of N_e (or of $\text{Log}(N_e)$) at several times in the past.

Compared to `NTdist()` that gives a rough but global view of these densities, `plotNdistrib()` gives a precise view of these densities at a small number of times. The global 2D plot given by `NTdis()` may help choosing the times when `plotNdistrib()` is used.

The function is interactive, allowing the user to check several plots. The last plot is saved to a `.pdf` file (Fig.4)..

Call the function as `plotNdistrib("job.Ndist")`, or as `plotNdistrib()` and answer the questions:

NOTE: the example refers to the same previous example, using extractions from `LogSizeDist()`, to provide densities of $\text{Log}(\text{Theta})$.

In Fig. 4-A, posterior densities are quite erratic, because the MCMC chain used in the example was very short: MCMC parameters were (10000,1,1) whereas getting good results generally requires 100 times longer runs, with parameters set at (10000,10,10) for example, as in Fig. 4-B.

```
> plotNdistrib()
[1] *** Plot posterior distributions of N(T) ***
    Name of the 'parafilename.Ndist' file = test.Ldist
[1] 1           0
[1] 2           50
[1] 3          100
[1] 4          150
[1] 5          200
[1] 6          250
[1] 7          300
[1] 8          350
[1] 9          400
[1] 10         450
[1] 11         500
[1] 12         550
[1] 13         600
[1] 14         650
[1] 15         700
[1] 16         750
[1] 17         800
[1] 18         850
[1] 19         900
[1] 20         950
[1] 21        1000
[1] Choose the number of times (suggestion: <=5) you wish to plot the
distribution
    number of times (stop on 0): 4
    case # 1 , instant number = : 1
    case # 2 , instant number = : 3
    case # 3 , instant number = : 4
    case # 4 , instant number = : 5
```

```

[1] Choosing the range of Ne values
[1] note that a log_10 scale of sizes is used here
[1] maximal Ne value is 4.287239. Give an upper value :
    enter maximum Ne value= 4.5
[1] minimal Ne value is 1.351914. Give a lower value :
    enter minimum Ne value= 1.3
[1] Choose the number of times (suggestion: <=5) you wish to plot the
distribution
    number of times (stop on 0): 0

```

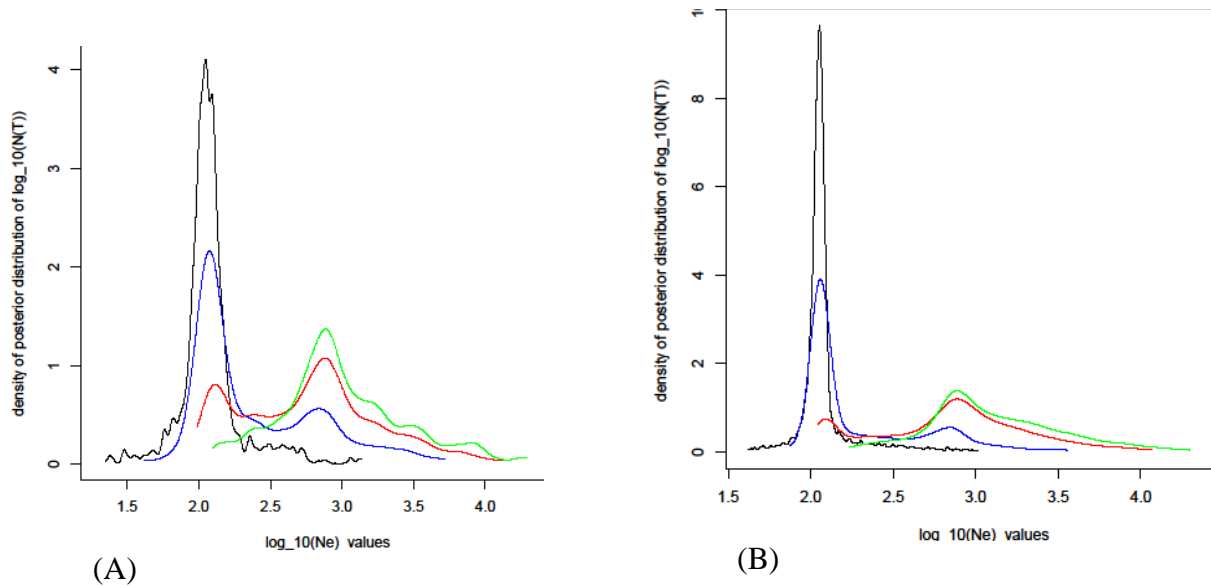


Figure 4. Posterior distributions of $Log(Ne)$, at several times in the past.
 Black: $T=0$ (current time), Blue: $T=50$; Red: $T=150$; Green: $T=200$.
 (A) using a short MCMC chain as in Table 1; (B) using a long chain (10000, 10, 10)

E. RECOMMENDATIONS

-Preliminary test:

With little batch 1000 * 10 * 10 and a DMAX=minimum distances of alleles (upper red lines of figure 1), make different trials

1. JMAX (exemple: JMAX=2, JMAX=3, JMAX=4)
2. NBAR (start with the value between Theta0-Theta1 and a large VARP1 then increase. Choose NBAR such that the results revealed narrow quantiles (5%-95%, last column in .NSTAT file)
3. GBAR (start with a large value and with a large VARP2, then increase or decrease GBAR to check the best values, then use smaller VARP2 values)
4. Increase DMAX to the maximum, to check robustness (note that calculation time increases with DMAX, and large DMAX may lead to numerical problems)

-Final result with:

- Batch for MCMC is often (NumberBatch = 10000, LengthBatch = 10, SpaceBatch = 10)
- RHOCORN is often equal at 0
- Diagonale is often set to 0.5

TEST FILE “InputTest.txt”

```
9
4 12 2 0 14 21 15 3 9
12
3 30 10 17 15 0 1 0 0 0 0 4
18
2 0 0 0 0 0 0 0 0 0 0 1 29 16 1 3 24 4
5
8 3 43 16 10
9
13 17 17 1 0 1 16 9 6
5
1 3 16 58 2
5
9 19 46 5 1
11
1 8 3 7 35 13 0 0 0 12 1
17
1 0 0 0 0 0 0 0 0 1 15 5 53 0 2 0 3
9
13 38 10 0 3 5 0 6 5
14
1 21 0 0 1 24 2 0 0 0 0 7 21 3
11
7 1 0 0 0 1 3 12 11 34 11
13
7 33 9 1 0 0 0 5 4 1 12 5 3
11
2 9 0 0 6 0 0 18 32 11 2
10
18 8 1 8 6 1 17 20 0 1
10
8 17 8 5 2 3 4 2 21 10
15
5 11 19 9 3 0 0 0 0 0 9 8 2 9 5
14
1 2 6 0 0 0 2 11 5 25 13 1 13 1
8
3 35 26 6 7 0 1 2
14
1 4 0 0 0 38 18 7 9 1 0 0 1 1
```