euroMix tutorial, version 1.1

Thore Egeland and Guro Dørum

January 8, 2014

This document gives an introduction to the R software package euroMix developed for analysing mixtures while accounting for related contributors and linkage. The package contains functions for simulating mixtures with relatives for both linked and unlinked markers, and for computing likelihood ratios conditioned on pedigrees. euroMix relies heavily on the R package paramlink (Egeland et al., 2013). Simulation of mixtures with relatives for linked markers uses MERLIN, while the R version of Familias is used to compute likelihood ratios that also accommodate theta-correction, mutation models and silent allele frequencies. Previous literature and methods have assumed the relationships between typed contributors to be same for the competing hypotheses. This restriction does not apply for our approach. The calculations are generalised to allow for general, possibly inbred, pedigrees. There is also a function pvalue.machine with accompanying wrapper functions that calculate tail probabilities for LRs. Planned extensions include handling of continuous data and artefacts, and to accommodate drop-in and drop-out in the computation of likelihood ratios.

euroMix is developed in close cooperation with the R package forensim (Haned, 2011) and this document is inspired by the simillar documentation for forensim. It should however be noted that forensim is a much larger program and project compared to euroMix. euroMix is designed for users familliar with R and is not intended to be user friendly for other users.

euroMix version 1.1 is available from the R-Forge repository (http://r-forge.r-project.org), from which versions for all platforms (Windows, Mac OS X, Linux) can be downloaded. In the following sections we describe how to install euroMix and illustrate with examples how to use the main functions. Some very specialised functions considered to be of little relevance to users are not covered in this document, but the documentation is available online.

1 Installation

euroMix requires that the freely available R software is installed. See http://www.cran.r-project.org/ on how to install R. euroMix is available from the R-Forge repository, and Windows and Linux users can install euroMix by typing the command

```
> install.packages("euroMix", repos="http://R-Forge.R-project.org")
```

in the R command window. Mac OS users install euroMix with the command

```
> install.packages("euroMix", repos="http://R-Forge.R-project.org",
+                  type="source")
```

After installation the package is loaded with the command

```
> library(euroMix)
```

The R packages paramlink, Familias and forensim are automatically installed together with euroMix. Some functions in euroMix also relies on MERLIN, which must be installed manually. MERLIN can be downloaded from http://www.sph.umich.edu/csg/abecasis/merlin/index.html. It is important to make sure that MERLIN is correctly pointed to in the PATH environment as explained in the documentation of the merlin function in paramlink.

2 Compute the likelihood for mixtures

We will show with an example how to compute the likelihood ratio for a set of hypotheses where the evidence is a DNA mixture. The prosecution and defense hypotheses are

- H_P: The victim and the suspect contributed to the mixture.
- H_D: The victim and the father of the suspect contributed to the mixture.

We start by defining the alleles, their frequencies and the observed alleles in the mixture.

```
> alleles <- 1:4 # Defines alleles labelled 1, 2, 3 and 4
> afreq <- c(0.044, 0.166, 0.11, 0.68) # Assigns allele frequencies
> R <- 1:3 # Assigns mixture evidence alleles as 1/2/3
```

Next, we need to creat pedigrees that account for the relationship between the contributors to the mixture. The pedigrees are created with functions from paramlink. We will briefly present some of the functions in paramlink here, more detailed information can be found in the paramlink documentation (http://cran.r-project.org/web/packages/paramlink/paramlink.pdf). The female victim is unrelated to the other contributors and is therefore created as a pedigree with a single member using the function singleton. The relationship between the male suspect and his father is accounted for by creating a nuclear family with the function nuclearPed. It is important that the victim is assigned an index that does not coincide with the indices of the individuals in the nuclear family, in this case she is given the index 4.

```
> x <- singleton(4, sex=2) # Victim pedigree, singleton female called 4
> y <- nuclearPed(1) # Suspect pedigree
```

The pedigree functions return a linkdat object, which is essentially a list of information linked to the pedigree. See the paramlink documentation for more information about linkdat objects.

The victim is genotyped to 1/2 and the suspect is genotyped to 3/3, while the suspect's father is unknown. The known genotypes of the victim (4) and suspect (3) are put into a list. Each element in the list is given as (i, g_i) where i denotes the index of the individual and g_i denotes its genotype, i.e. (4,1,2) means that individual 4 has genotype 1/2.

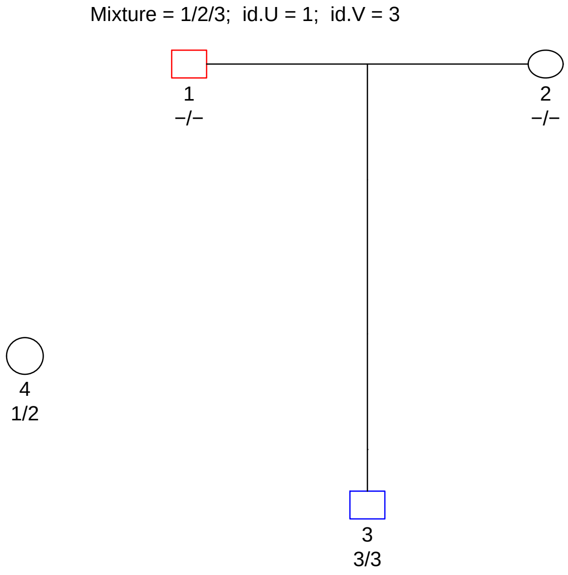
```
> known <- list(c(3,3,3), c(4,1,2)) #Known genotypes of 3 and 4
```

The likelihood of the mixture evidence is computed separately under the defense hypothesis and the prosecution hypothesis with the function paraMix. Under H_p there are no unknown contributors and this is signified by the argument id.U=NULL.

```
> lp <- paraMix(list(x,y), R, id.U=NULL,alleles=alleles, afreq=afreq,
+                known=known)$likelihood
```

Under H_d the father (1) is specified as an unknown contributor by setting id.U=1, while the suspect is specified as a known (genotyped) non-contributor by setting id.V=3. Note that we can choose to plot the pedigree (plot=TRUE) with the hypothesised contributors (in this case the suspect and father) marked.

```
> ld <- paraMix(list(x,y), R, id.U=1, id.V=3, alleles=alleles,
+                 afreq=afreq, known=known,plot=TRUE)$likelihood
```



Finally, the likelihood ratio is computed as the ratio between the two likelihoods.

```
> lp/ld # Calculates required LR as 3.125
```

```
[1] 3.125
```

For likelihood ratio computations with theta correction, mutations and silent alleles, the function `famMix`, based on `Familias`, can be used. `Familias` first needs to loaded it into `R`. We will consider the same example as above, but with theta correction. Unlike `paraMix`, `famMix` can not handle singletons, i.e. pedigrees with only one individual, and unrelated individuals must therefore be specified as part of the pedigree.

```
> library(Familias)
> theta <- 0.01
> x <- nuclearPed(1)
> x <- addOffspring(x,father=1,noff=1)
```

In `famMix`, the known genotypes of 3 and 4 are specified as a marker object created with the function `marker` in `paramlink`. The likelihood is computed separately under H_p and H_d. The marker object with the known genotypes is specified in `famMix` through the argument partialmarker, while id.U and id.V are specified as for `paraMix`.

```
> m1 <- marker(x, 4, c(1,2), 3, c(3,3), alleles=alleles, afreq=afreq)
> lp <- famMix(x, R, id.U=NULL, partialmarker=m1,
+ theta=theta)$likelihood
> ld <- famMix(x, R, id.U=1, id.V=3, partialmarker=m1,
+ theta=theta)$likelihood
```

```
> lp/ld
```

```
[1] 2.886771
```

3 Compute a p-value corresponding to LR

The function pvalue.machine computes a p-value corresponding to a given likelihood ratio. The p-value is the probability of observing a likelihood ratio under H_d that is at least as large as the one observed.

We will consider an example with nine markers where we allow for drop-in and drop-out in the evidence. The hypotheses to be considered are

- H_P: The victim and the suspect contributed to the mixture.
- H_D: The victim and an unrelated unknown contributed to the mixture.

We start by loading data integrated in euroMix, including an allele frequency database (db2), a mixture and genotype profiles for a victim and a suspect (all found in the data object 'ex9m').

```
> data(db2)
> data(ex9m)
> M <- nrow(R)
> markers <- rownames(R)
```

We choose here to compute likelihood ratios with the LR function in forensim, but in general any LR model can be used. LR can handle drop-in and drop-out but not related contributors, but in this example there are no related contributors. We use a drop-in probability of 0.47 and a drop-out probability of 0.05. A loop goes through all markers and computes the LR for each marker. Finally, the total LR is found as the product of the LRs from each marker.

```
> lr <- A <- numeric()
> for(i in 1:M){
+   r <- as.numeric(R[i,!is.na(R[i,])])
+   v <- as.numeric(V[i,])
+   s <- as.numeric(S[i,])
+   afreq <- db2[db2[,1]==markers[i],3]
+   A[i] <- choose(length(afreq)+1,2)
+   names(afreq) <- db2[db2[,1]==markers[i],2]
+   lr[i] <- LR(r, Tp=c(v,s), Td=v, Vp=NULL, Vd=s,
+             xp=0, xd=1, theta=0, prDHet=rep(0.47,4),
+               prDHom=rep(0.47,4)^2, prC=0.05, freq=afreq)$LR
+ }
> LR.suspect <- prod(lr)
```

Next, we need to compute the LRs for all genotypes that may occur under H_d, that is, the LRs we get when we replace the suspect's profile with all genotype profiles that an unrelated unknown may have. At the same time we find the probability for each genotype of occuring. One loop goes through the markers and a second loop goes through the genotypes for each marker. The results are a matrix of LRs where the likelihood ratios are sorted in descending order within each row, and a matrix of corresponding probabilities.

```
> LR.table <- P.table <- matrix(0,M,max(A))
> for(i in 1:M){
+   afreq <- db2[db2$Marker==markers[i],]$Frequency
+   names(afreq) <- db2[db2$Marker==markers[i],]$Allele
+   #All possible genotypes for the given marker
+   gtAll <- as.matrix(expand.grid(names(afreq),names(afreq)))
+   gtAll <- gtAll[gtAll[,1]<=gtAll[,2],]
+   #Compute Likelihood ratios and corresponding
+   #genotype probabilities for each genotype
+   lrAll <- pAll <- numeric()
+   for(j in 1:nrow(gtAll)) {
+     rm <- gtAll[j,]
+     r <- as.numeric(R[i,!is.na(R[i,])])
+     v <- as.numeric(V[i,])
+     s <- as.numeric(S[i,])
+     lrAll[j] <- LR(r, Tp=c(v,rm), Td=v, Vp=NULL, Vd=rm, xp=0,
+                    xd=1, theta=0, prDHet=rep(0.47,4),
+                    prDHom=rep(0.47,4)^2, prC=0.05, freq=afreq)$LR
+     pAll[j] <- ifelse(rm[1]==rm[2], prod(afreq[as.character(rm)]),
+                       2*prod(afreq[as.character(rm)]) )
+   }
+   LR.table[i,1:length(lrAll)] <- lrAll
+   P.table[i,1:length(lrAll)] <- pAll
+ }
```

Finally, the p-value is computed with pvalue.machine by inserting the observed LR, the matrix of LRs and the matrix of probabilities.

```
> pvalue.machine(LR.suspect, LR.table, P.table)
```

```
[1] 1.403838e-09
```

The function pvalue.machine is the most generic method for computing p-values. It is independent of LR model since the user provides the function with precalculated likelihood ratios. Alternatively, one of the functions LRpvalue or LRp can be used. Both functions take as input mixture data in form of sample and reference profiles and allele frequencies and compute all the necessary likelihood ratios with the LR function in forensim. The user must provide values for drop-out and drop-in probabilities (the drop-out probability can be estimated with e.g. forensim's LRmix). The difference between the two functions is that LRp takes as input data frames, while LRpvalue reads data directly from CSV files. The CSV files must be of the same format as for LRmix (see the LRmix tutorial for details), however they must only contain data for autosomal markers. We first illustrate how LRp can be used for the example above.

```
> LRp(sampleData=R,victimData=V,suspectData=S,db=db2,hp=c('V','S'),
+     hd=c('V','U'),prD=0.47,prC=0.05 )

$LR
[1] 67429230

$pvalue
[1] 1.403838e-09
```

The data frames R, V, S and db2 (from the previously loaded data object ex9m) contain the sample profile, victim profile, suspect profile and allele frequencies, respectively. The H_p hypothesis is specified with the argument hp=c('V','S') which indicates that the victim (V) and suspect (S) are the contributors to the mixture. H_d is specified with the argument hd=c('V','U') denoting the victim and an unknown as the contributors. The following section demonstrates the use of LRpvalue. The data object 'testdata' loaded here contain the same data as 'ex9m', but the data frames have slightly different formats. The data frames is read to temporary CSV files that LRpvalue can read.

```
> #Load data and read into temporary CSV files
> data(testdata)
> samplefile <- tempfile()
> write.table(sample, samplefile, sep=",", row.names=FALSE)
> victimfile <- tempfile()
> write.table(victim, victimfile, sep=",", row.names=FALSE)
> suspectfile <- tempfile()
> write.table(suspect, suspectfile, sep=",", row.names=FALSE)
> freqfile <- tempfile()
> write.table(freqs, freqfile, sep=",", row.names=FALSE)
> #LRpvalue takes CSV files as input
> LRpvalue(samplefile, victimfile, suspectfile, freqfile,
+          hp=c("V","S"), hd=c("V","U"), prD=0.47, prC=0.05)

$LR
[1] 67429230

$pvalue
[1] 1.403838e-09

> unlink(c(samplefile, victimfile, suspectfile, freqfile))
```

4 Simulate mixtures and generate all possible genotypes

The function simMixParamlink can be used to simulate DNA mixtures. Given a linkdat object that contains marker data for the individuals of interest, it generates a mixture of the genotypes. We create a linkdat object with one of the pedigree functions and an empty marker object. Next, genotypes for individual 1 and 3 are simulated with the markerSim function in paramlink and added to the linkdat object. A mixture of the genotypes of individual 1 and 3 is generated by running simMixParamlink on the linkdat object. In this case the mixture consists of only the allele 4.

```
> alleles <- 1:4 # Defines alleles labelled 1, 2, 3 and 4
> afreq <- c(0.044, 0.166, 0.11, 0.68) # Assigns allele frequencies
> x <- nuclearPed(2) #Create pedigree
> m1 <- marker(x,alleles=alleles,afreq=afreq) #Create empty marker
> y <- markerSim(x,N=1,available=c(1,3),partialmarker=m1,
+                verbose=FALSE,loop=7,seed=2) #Simulate genotypes
>                                                #for individuals 1 and 3
> simMixParamlink(y,alleles)
```

```
[[1]]
[1] 4
```

The function generate creates all genotypes that an unknown contributor may have. It takes as input a mixture, the alleles of the known contributors and the number of unknown contributors. The result is a matrix where each row represent an uknown contributor and columns represent the alleles, columns one and two is the first genotype, comlumns three and four the second genotype, and so on.

```
> R <- 1:3 #Alleles in the mixture
> K <- 1:2 #Alleles of known contributors
> n <- 1 #Number of unknown contributors
> generate(R=R,K=K,x=n)
```

```
     [,1] [,2] [,3] [,4] [,5] [,6]
[1,]    3    3    1    3    2    3
```

References

[1] H. Haned. Forensim: An open-source initiative for the evaluation of statistical methods in forensic genetics. *Forensic Science International: Genetics*, 5(4):265 – 268, 2011.

[2] T. Egeland, N. Pinto and M.D. Vigeland. A general approach to power calculation for relationship testing. *Forensic Science International: Genetics*, doi: http://dx.doi.org/10.1016/j.fsigen.2013.05.001 2013.