Dcifer: Genetic Distance Between Polyclonal Infections

Package `dcifer` calculates genetic distance between polyclonal infections by estimating relatedness from biallelic and multiallelic data [1]. In addition to estimates, the package provides a likelihood function and statistical inference. Functions for reading and reformatting data, performing preparatory steps, and visualizing the results are also included. We will illustrate the analysis process using microhaplotype data from two health facilities in Maputo and Inhambane provinces of Mozambique [2].

```
library(dcifer)
pardef <- par(no.readonly = TRUE)
```

Read and reformat data, estimate COI and population allele frequencies

Dcifer relatedness estimation requires sample data in a specific format and estimates of complexity of infection (COI) and population allele frequencies. Here is an example of some preparation steps using Mozambique dataset. First, read in original data stored in a `.csv` file. The name of that file is the first argument to the function that reads and reformats the data:

```
sfile <- system.file("extdata", "MozParagon.csv", package = "dcifer")
dsmp <- readDat(sfile, svar = "sampleID", lvar = "locus", avar = "allele")
str(dsmp, list.len = 2)
```

```
> List of 52
>  $ 8025874217:List of 87
>   ..$ t1  : Named num [1:5] 1 1 0 0 0
>   .. ..- attr(*, "names")= chr [1:5] "D10--D6--FCR3--V1-S.0" "HB3.0" "W2.0" "t1.0" ...
>   ..$ t10 : Named num [1:4] 1 0 0 0
>   .. ..- attr(*, "names")= chr [1:4] "D10--D6--HB3.0" "U659.0" "t10.0" "t10.2"
>   .. [list output truncated]
>  $ 8025874237:List of 87
>   ..$ t1  : Named num [1:5] 0 0 1 0 0
>   .. ..- attr(*, "names")= chr [1:5] "D10--D6--FCR3--V1-S.0" "HB3.0" "W2.0" "t1.0" ...
>   ..$ t10 : Named num [1:4] 1 0 0 0
>   .. ..- attr(*, "names")= chr [1:4] "D10--D6--HB3.0" "U659.0" "t10.0" "t10.2"
>   .. [list output truncated]
>   [list output truncated]
```

```
# optionally, extract location information
meta <- unique(read.csv(sfile)[c("sampleID", "province")])
meta <- meta[match(names(dsmp), meta$sampleID), ]  # order samples as in dsmp
```

Next, estimate COI for all the samples - here we use naive estimation, first ranking loci of a sample by the number of detected alleles, and then using a locus with a prescribed rank (`lrank`) to determine COI:

```
lrank <- 2
coi   <- getCOI(dsmp, lrank = lrank)
```

Finally, estimate population allele frequencies, adjusting for COI:

```
afreq <- calcAfreq(dsmp, coi, tol = 1e-5)
str(afreq, list.len = 2)
```

```
> List of 87
>  $ t1  : Named num [1:5] 0.4239 0.2808 0.1415 0.1116 0.0422
>   ..- attr(*, "names")= chr [1:5] "D10--D6--FCR3--V1-S.0" "HB3.0" "W2.0" "t1.0" ...
>  $ t10 : Named num [1:4] 0.8539 0.12717 0.00942 0.00951
>   ..- attr(*, "names")= chr [1:4] "D10--D6--HB3.0" "U659.0" "t10.0" "t10.2"
>   [list output truncated]
```

In some situations, population allele frequencies might be estimated from a different (e.g. larger) dataset and are provided separately (in a `.csv` file). In that case, after the frequencies are read in, they need to be checked against the the existing data object to make sure that all the loci and alleles are in the same order. Function `matchAfreq` performs the checking and rearranges sample data to conform to the provided allele frequencies. For that procedure, loci and alleles in both lists (`dsmp` and `afreq`) have to be named; otherwise the names are not required, and the order of loci and alleles is assumed to be the same for sample data and allele frequencies. If `afreq` contains "extra" alleles that are not listed in `dsmp`, these alleles are added to `dsmp`. The opposite situation (alleles listed and present in `dsmp` but not listed in `afreq`) will result in an error.

```
afile  <- system.file("extdata", "MozAfreq.csv", package = "dcifer")
afreq2 <- readAfreq(afile, lvar = "locus", avar = "allele", fvar = "freq")
dsmp2  <- matchAfreq(dsmp, afreq2)
```

Estimate relatedness

As a first step in relatedness estimation, we set $M = 1$ (only one pair of strains between two infections can be related) and test the hypothesis that infections are unrelated ($H_0: r = 0$). This is done with `ibdDat` function. Then we explore significantly related pairs in more detail.

Optionally, sort samples by clinic or geographic location:

```
provinces <- c("Maputo", "Inhambane")
nsite     <- table(meta$province)[provinces]
ord       <- order(factor(meta$province, levels = provinces))
dsmp <- dsmp[ord]
coi  <- coi[ ord]
```

```
dres <- ibdDat(dsmp, coi, afreq, pval = TRUE, confint = TRUE, rnull = 0,
              alpha = 0.05, nr = 1e3)
```

Look at the results for a single pair of samples:

```
dres[9, 5, ]
```

```
>   estimate    p_value   CI_lower   CI_upper
> 0.31100000 0.06203213 0.00000000 0.68000000
```

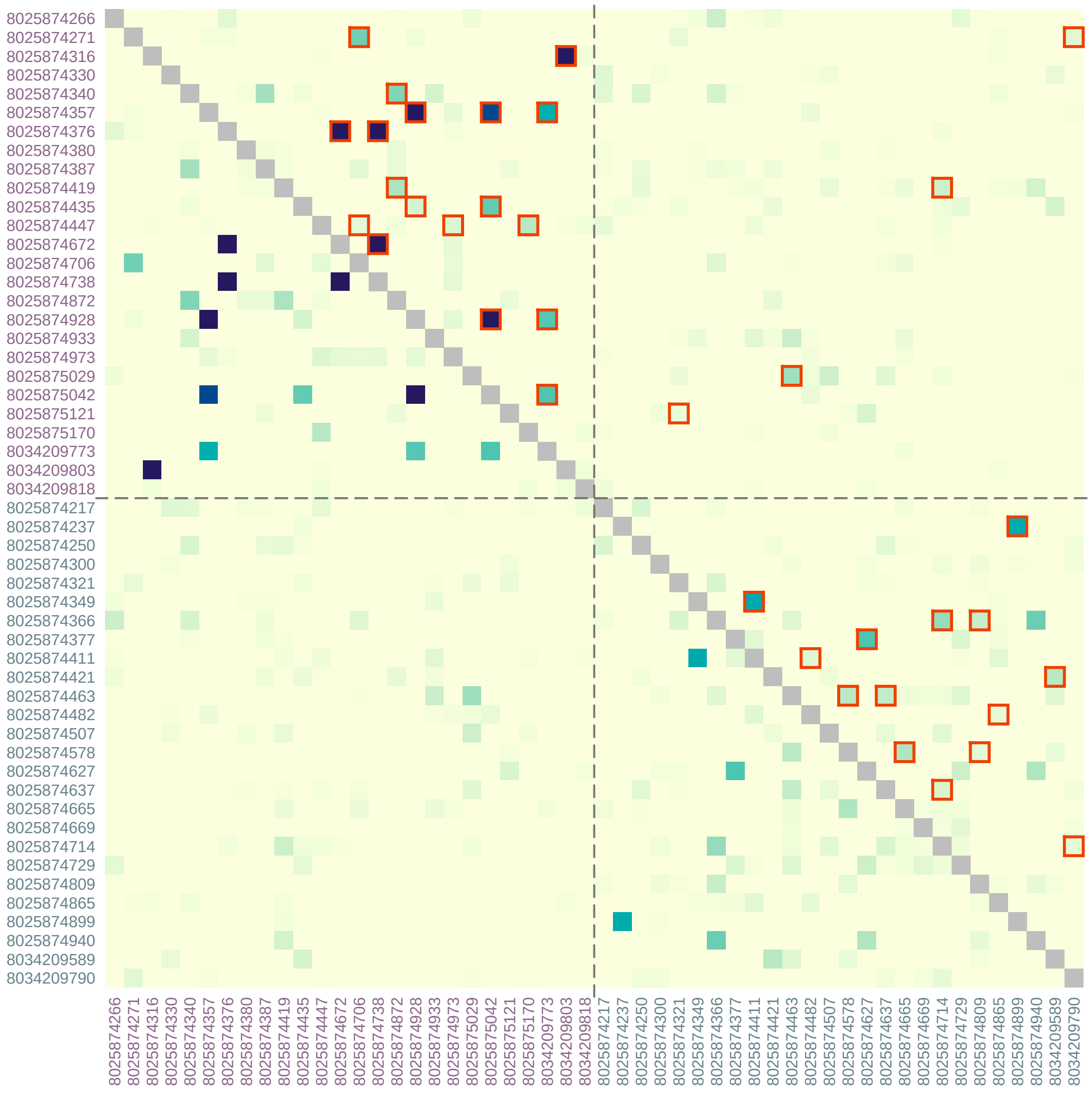
Visualize the results

When pairwise relatedness is calculated within a single dataset, `ibdDat` returns triangular matrices. For plotting, we can make them symmetric. Then significantly related pairs can be outlined in either or both triangles.

Display the results with sample ID's written on the margins. Label colors correspond to locations (health facilities):

```
par(mar = c(3, 3, 1, 1))
nsmp  <- length(dsmp)
atsep <- cumsum(nsite)[-length(nsite)]

# create symmetric matrix
```

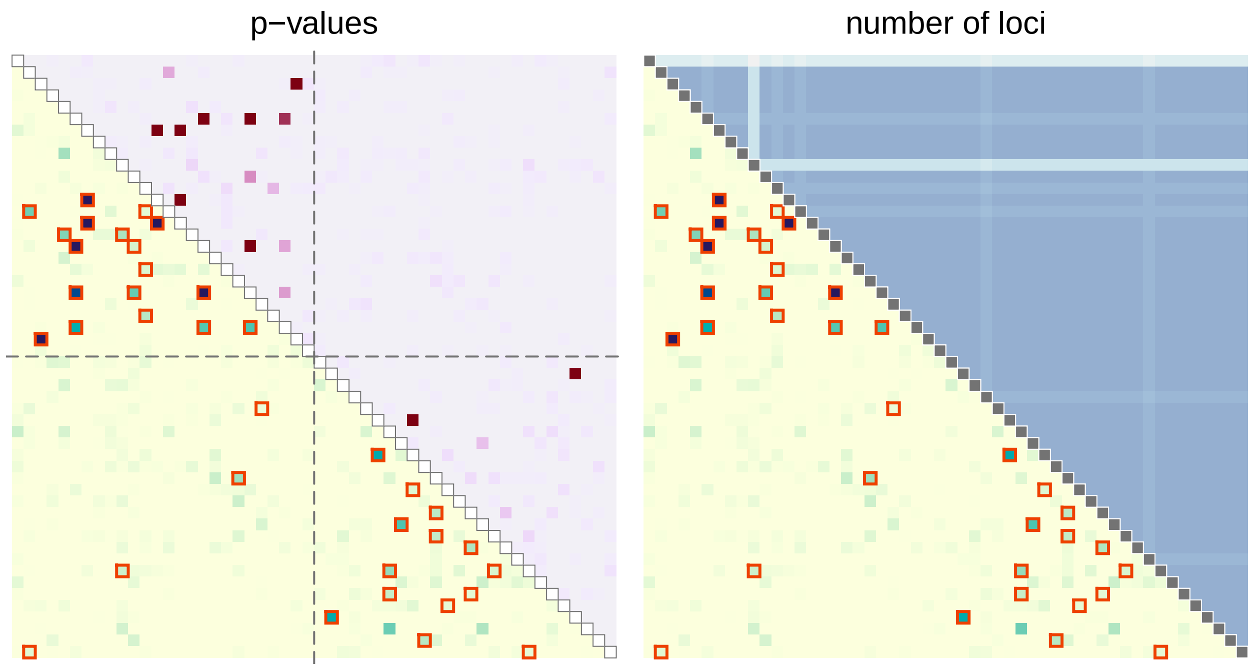
```r
dmat <- dres[, , "estimate"]
dmat[upper.tri(dmat)] <- t(dmat)[upper.tri(t(dmat))]

# determine significant, indices for upper triangle
alpha <- 0.05                              # significance level
isig <- which(dres[, , "p_value"] <= alpha, arr.ind = TRUE)
col_id <- rep(c("plum4", "lightblue4"), nsite)
plotRel(dmat, isig = isig[, 2:1], draw_diag = TRUE, alpha = alpha, idlab = TRUE,
        col_id = col_id)
abline(v = atsep, h = atsep, col = "gray45", lty = 5)
```



For these symmetric distance measures, one of the triangles can be used to display other relevant information, such as p-values, geographic distance, or a number of non-missing loci between two samples. For that, use `add = TRUE`.

```r
par(mfrow = c(1, 2), mar = c(1, 0, 1, 0.2))
plotRel(dres, draw_diag = TRUE, alpha = alpha)
mtext("p-values", 3, 0.2)
# p-values for upper triangle
```

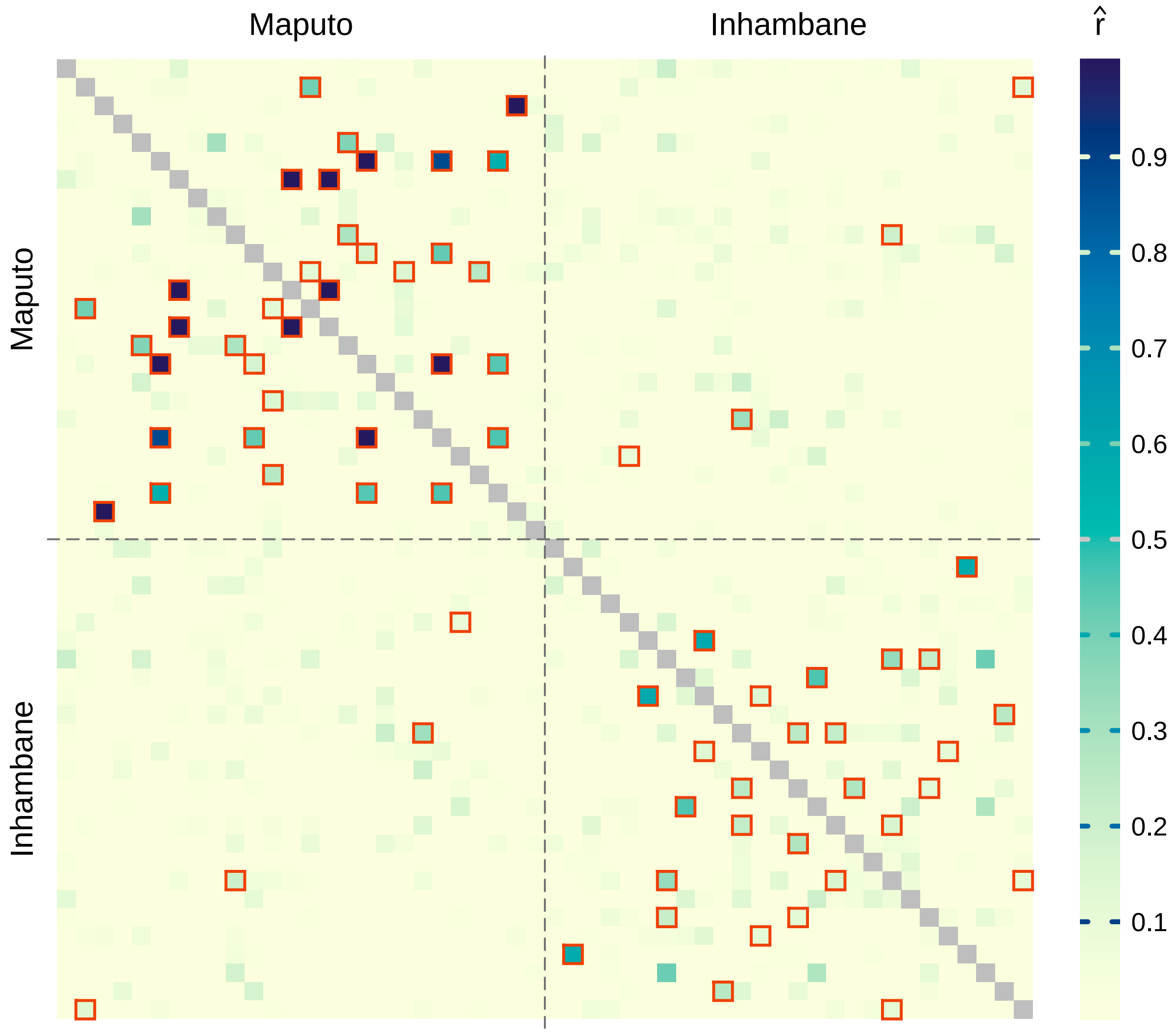
```
pmat <- matrix(NA, length(dsmp), length(dsmp))
pmat[upper.tri(pmat)] <- t(log(dres[, , "p_value"]))[upper.tri(pmat)]
pmat[pmat == -Inf] <- min(pmat[is.finite(pmat)])#*1.2
plotRel(pmat, rlim = NULL, draw_diag = TRUE, col = hcl.colors(101, "Red-Purple"),
        sig = FALSE, add = TRUE, col_diag = "white", border_diag = "gray45")
abline(v = atsep, h = atsep, col = "gray45", lty = 5)

# number of non-missing loci for upper triangle
dmiss <- lapply(dsmp, function(lst) sapply(lst, function(v) all(!v)))
nmat <- matrix(NA, nsmp, nsmp)
for (jsmp in 2:nsmp) {
  for (ismp in 1:(jsmp - 1)) {
    nmat[ismp, jsmp] <- sum(!dmiss[[ismp]] & !dmiss[[jsmp]])
  }
}
nrng <- range(nmat, na.rm = TRUE)  #
par(mar = c(1, 0.2, 1, 0))
plotRel(dres, draw_diag = TRUE, alpha = alpha)
mtext("number of loci", 3, 0.2)
coln <- hcl.colors(diff(nrng)*2.4, "Purple-Blue", rev = TRUE)[1:(diff(nrng) + 1)]
plotRel(nmat, rlim = NA, col = coln, add = TRUE,
        draw_diag = TRUE, col_diag = "gray45", border_diag = "white")
```



```
par(pardef)
```

For reference, add a colorbar legend to the plot. It can be placed beside the main plot:

```
layout(matrix(1:2, 1), width = c(7, 1))
par(mar = c(1, 1, 2, 1))#, mgp = c(0, 0, 0.5))
plotRel(dmat, draw_diag = TRUE, isig = rbind(isig, isig[, 2:1]))
atclin <- cumsum(nsite) - nsite/2
abline(v = atsep, h = atsep, col = "gray45", lty = 5)
mtext(provinces, side = 3, at = atclin, line = 0.2)
mtext(provinces, side = 2, at = atclin, line = 0.2)
par(mar = c(1, 0, 2, 3))
plotColorbar()
```

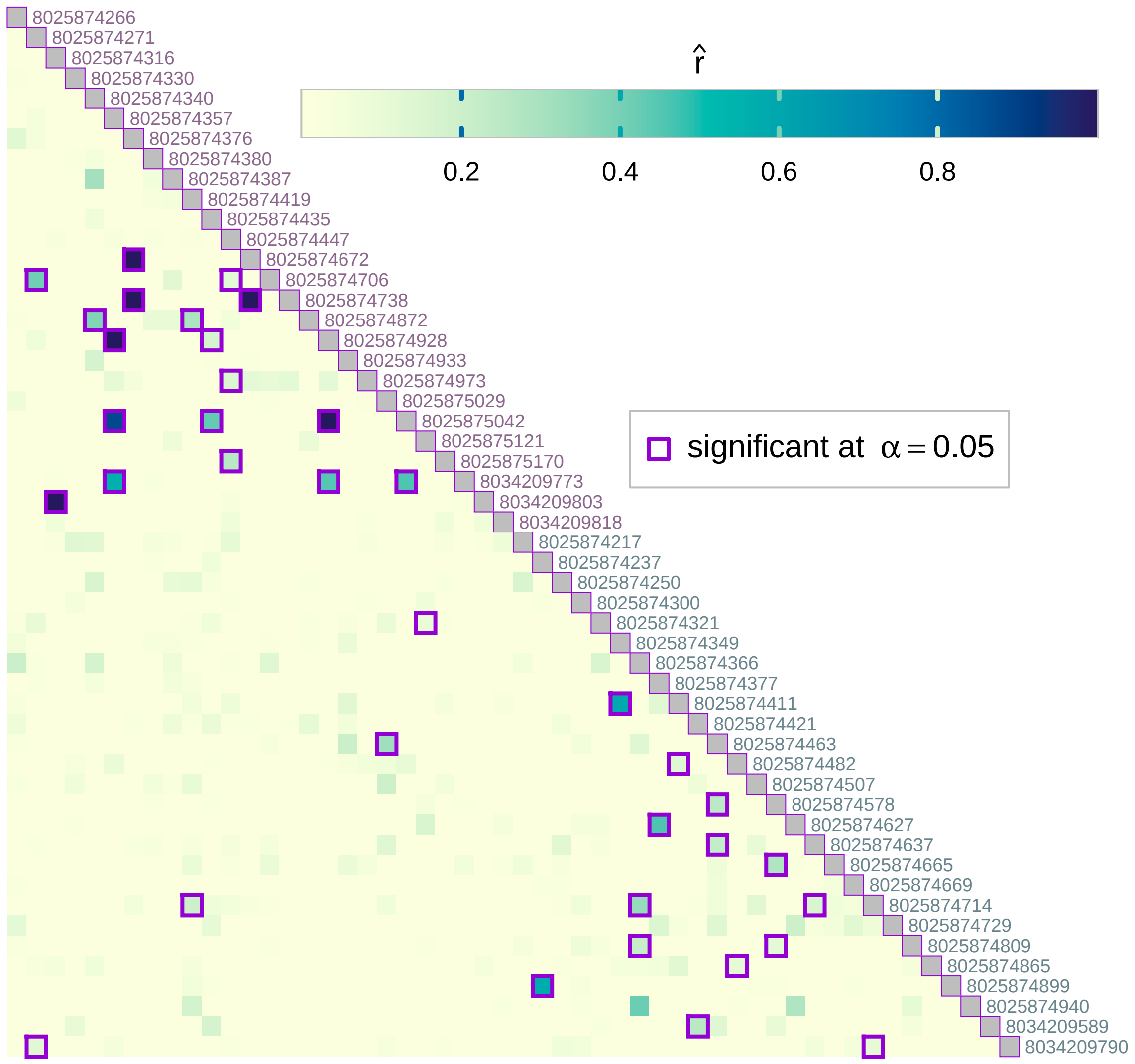


```
par(pardef)
```

The colorbar can also be located in the empty space left by the triangular matrix. In the example below, we specify horizontal colorbar and provide custom tick mark locations:

```
# horizontal colorbar (needed?)
par(mar = c(1, 1, 1, 3))
border_sig = "darkviolet"
plotRel(dres, draw_diag = TRUE, border_diag = border_sig, alpha = alpha,
        border_sig = border_sig, lwd_sig = 2)
legend(32, 20, pch = 0, col = border_sig, pt.lwd = 2, pt.cex = 1.4,
       box.col = "gray", legend = expression("significant at" ~~ alpha == 0.05))
text(1:nsmp + 0.3, 1:nsmp - 0.5, labels = names(dsmp), col = col_id, adj = 0,
     cex = 0.6, xpd = TRUE)
par(fig = c(0.25, 1, 0.81, 0.92), mar = c(1, 1, 1, 1), new = TRUE)
plotColorbar(at = c(0.2, 0.4, 0.6, 0.8), horiz = TRUE)
ncol <- 301
```

```r
lines(c(0, ncol, ncol, 0, 0), c(0, 0, 1, 1, 0), col = "gray")
```



```r
par(pardef)
```

Further analysis of related samples

Examine pairs that are determined to be significantly related at the significance level α more closely by allowing multiple pairs of strains to be related between two infections. Using **ibdEstM**, we also estimate the number of positively related pairs M' of strains and compare results yielded by a constrained model assuming $r_1 = \ldots = r_M$ (faster method) and without the constraint. In addition, we look at the estimates $\hat{r}_{total}$ of overall relatedness.

```r
# First, create a grid of r values to evaluate over
revals <- mapply(generateReval, 1:5, nr = c(1e3, 1e2, 32, 16, 12))
```

```r
sig1 <- sig2 <- vector("list", nrow(isig))
for (i in 1:nrow(isig)) {
  sig1[[i]] <- ibdEstM(dsmp[isig[i, ]], coi[isig[i, ]], afreq, Mmax = 5,
                       equalr = FALSE, reval = revals)
}
```

```
for (i in 1:nrow(isig)) {
  sig2[[i]] <- ibdEstM(dsmp[isig[i, ]], coi[isig[i, ]], afreq, equalr = TRUE)
}
M1      <- sapply(sig1, function(r) sum(r > 0))
M2      <- sapply(sig2, length)
rtotal1 <- sapply(sig1, sum)
rtotal2 <- sapply(sig2, sum)

cor(M1, M2)
```

```
> [1] 0.9486851
```

```
cor(rtotal1, rtotal2)
```

```
> [1] 0.9998965
```

Create a list of significant pairs:

```
samples <- names(dsmp)
sig <- as.data.frame(isig, row.names = FALSE)
sig[c("id1", "id2")]         <- list(samples[isig[, 1]], samples[isig[, 2]])
sig[c("M1", "M2")]           <- list(M1, M2)
sig[c("rtotal1", "rtotal2")] <- list(round(rtotal1, 3), round(rtotal2, 3))
head(sig)
```

```
>   row col        id1        id2 M1 M2 rtotal1 rtotal2
> 1  14   2 8025874706 8025874271  1  1   0.410   0.412
> 2  52   2 8034209790 8025874271  1  1   0.133   0.133
> 3  25   3 8034209803 8025874316  1  1   1.000   1.000
> 4  16   5 8025874872 8025874340  2  2   0.520   0.516
> 5  17   6 8025874928 8025874357  1  1   1.000   1.000
> 6  21   6 8025875042 8025874357  1  1   0.881   0.881
```

For full control, we can use the function `ibdPair` for any two samples and explore the outputs:

```
i <- 18
pair <- dsmp[isig[i, ]]
coii <-  coi[isig[i, ]]

res1 <- ibdPair(pair, coii, afreq, M = M1[i], pval = TRUE, equalr = FALSE,
                reval = revals[[M1[i]]])
res2 <- ibdPair(pair, coii, afreq, M = M2[i], pval = TRUE, equalr = TRUE,
                confreg = TRUE, llik = TRUE, reval = revals[[1]])

res1$rhat                             # estimate with equalr = FALSE
```

```
> [1] 0.31 1.00
```

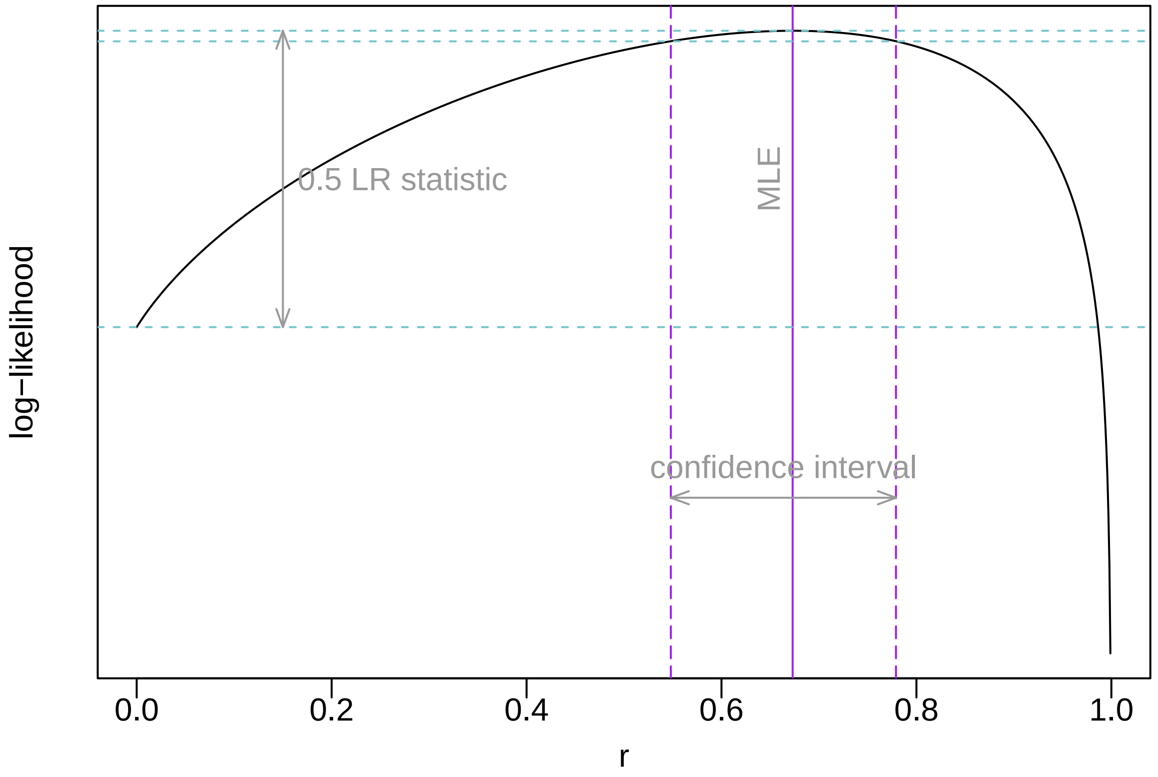
```
rep(res2$rhat, M2[i])                 # estimate with equalr = TRUE
```

```
> [1] 0.673 0.673
```

When `llik = TRUE`, output includes log-likelihood (for a single parameter if we use `equalr = TRUE`), which provides the basis for statistical inference:

```
CI     <- range(res2$confreg)
llikCI <- max(res2$llik) - qchisq(1 - alpha, df = 1)/2
llrng  <- range(res2$llik[is.finite(res2$llik)])
yCI <- llrng + diff(llrng)*0.25
```

```r
yLR <- (res2$llik[1] + max(res2$llik))/2
cols <- c("purple", "cadetblue3", "gray60")

par(mar = c(3, 2.5, 2, 0.1), mgp = c(1.5, 0.3, 0))
plot(revals[[1]], res2$llik, type = "l", xlab = "r", ylab = "log-likelihood",
     yaxt = "n")#, tck = -0.01)
abline(v = res2$rhat, lty = 1, col = cols[1])
abline(h = c(max(res2$llik), llikCI, res2$llik[[1]]), lty = 2, col = cols[2])
abline(v = CI, col = cols[1], lty = 5)
arrows(CI[1], yCI, CI[2], yCI, angle = 20, length = 0.1, code = 3,
       col = cols[3])
arrows(0.15, res2$llik[1], 0.15, max(res2$llik), angle = 20, length = 0.1,
       code = 3, col = cols[3])
text(0.165, yLR, adj = 0, "0.5 LR statistic", col = cols[3])
text(mean(CI), yCI + 0.05*diff(llrng), "confidence interval", col = cols[3])
text(res2$rhat - 0.025, yLR, "MLE", col = cols[3], srt = 90)
```



```r
par(pardef)
```

1. Gerlovina I, Gerlovin B, Rodriguez-Barraquer I, Greenhouse B. Dcifer: An ibd-based method to calculate genetic distance between polyclonal infections. bioRxiv. 2022;

2. Tessema SK, Hathaway NJ, Teyssier NB, Murphy M, Chen A, Aydemir O, et al. Sensitive, highly multiplexed sequencing of microhaplotypes from the plasmodium falciparum heterozygome. The Journal of infectious diseases. 2022;225(7):1227–37.