Introduction to the R package rich

Jean-Pierre Rossi

CBGP - INRA Montpellier http://www6.montpellier.inra.fr/cbgp
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1 Installation

The current stable version (rich 1.0.1) is available from CRAN. You can also install rich from R by simply typing:

```
install.packages("rich", dep=TRUE)
```

The development version is hosted by R-Forge and you can install it from R by typing:

```
install.packages("rich", repos="http://R-Forge.R-project.org", dep=TRUE)
```

2 What is rich?

Rich is a set of functions designed to perform simple species richness analyses. Readers will find interesting R resources in the package vegan (Oksanen et al. 2016).

3 Basic features

The function rich computes the species richness on the basis of bootstrap estimation.

```
library(rich)
data(ef)

# Bootstrap estimation based on 99 randomizations
o1 <- rich(matrix=ef, nrandom=99)
o1
```

```
## $cr
## [1] 121
##
## $mr
## [1] 10.4
##
## $mrsd
## [1] 4.938239
##
## $bootCR
##          cr.obs  cr.boot cr.bcorr  cr.bias  cr.se  cr.lbn  cr.ubn
## 121 151.6667 151.6667    0.0000     0 4.938239 167.5105
```
The mean species richness, i.e. the average value over the sampling units is given in the slot `$mr` and its standard deviation is given in `$mrsd`. The cumulated richness is given in `$cr`.

The bootstrap estimate of the cumulated richness is stored in `$bootCR`. `$cr.obs` is simply the observed cumulated value whereas the corresponding bootstrapped value is reported in `$cr.boot`.

We can plot the mean and cumulated bootstrap estimates of species richness:

```r
# plot bootstrapped cumulated and mean richness values
library(gplots)
col <- c("lightblue", "lavender")
d <- c(o1$bootCR$cr.boot, o1$bootMR$mr.boot)

cl.l <- c(o1$bootCR$cr.boot + o1$bootCR$cr.se,
o1$bootMR$mr.boot + o1$bootMR$mr.se)
ci.u <- c(o1$bootCR$cr.boot, o1$bootMR$mr.boot)
ci.l ; ci.u

## [1] 98.41708 92.03316

## [1] 90.33333 76.12828

barplot2(d, col = col, ylim=c(0,o1$bootCR$cr.obs),
          plot.ci = TRUE, ci.l = ci.l, ci.u = ci.u, ylab="richness",
          names.arg=c("cumulated\nrichness", "mean\nrichness"))
```
The dispersion of the bootstrap estimates can be used to compute a corrected value (Manly 1997). rich provides these corrected estimates and the outputs can be visualized as follows:

```r
# plot corrected estimates
col <- c("mistyrose", "cornsilk")
d <- c(o1$bootCR$cr.boot, o1$bootCR$cr.bcorr-o1$bootCR$cr.boot)
d2 <- c(o1$bootMR$mr.bcorr, o1$bootMR$mr.boot-o1$bootMR$mr.bcorr)
dd <- cbind(d, d2)
barplot2(dd, col=col, names.arg=c("cumulated richness", "mean richness"))
legend("topright", legend=c("bootstrap estimate", "correction"),
       col="black", pch=c(22,22), bty="n", pt.bg=col)
```
4 Comparing species richness

4.1 Principle

One very common question is to determine if the species richness estimated in two sampling areas are statistically significant. This is for example the question if we sample fauna in plots under conventional agriculture and organic farming. Usually we perform sampling in each site, using a set of sampling units such as traps for insects, soil monoliths for soil fauna, surfers in rivers surveys etc. Each sampling unit brings a set of species forming a list whose length is the species richness.

Imagine a very simple framework where sites A and B are sampled using $n$ sampling units. Each unit brings one estimation of species richness. We thus end up with $n$ local estimates of the richness $S$ for each site.

If we want to compare the richness of site A and B we must be careful. A very common strategy is to perform a student t test which amounts to compare the mean richness of each site. This corresponds to averaging each set of $n$ values and compare the resulting means. This is not a comparison of the richness in site A and B, but rather a comparisons of the density of species richness in each site. Many users go for a student t test because they need a statistical test and that means they need replicates.

Imagine that 2 sites are sampled with 2 replicates and that the data are as follows:

<table>
<thead>
<tr>
<th></th>
<th>site 1</th>
<th>site 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample 1</td>
<td>species 1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>species 2</td>
<td>0</td>
</tr>
<tr>
<td>sample 2</td>
<td>species 1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>species 2</td>
<td>1</td>
</tr>
</tbody>
</table>

Both sites have a mean richness of 1 species per sample. However the cumulated richness is 2 in site 1 and only 1 in site 2. This very simple example illustrates the importance of considering the cumulated richness in biodiversity surveys. The problem is that we end up comparing only 2 values, one per site, with no replicate. This impairs statistical comparisons by mean of usual tests.

Randomization tests offer a solution that is clearly explained in Manly (1997). The function c2cv (standing for compare 2 cumulated values) implements such comparison of 2 values of species richness using a randomization procedure. Note that the function only handles 2 values comparison and thus does not allow multi-site direct analysis.

4.2 Functions c2cv and c2m

4.2.1 c2cv

c2cv stands for compare 2 cumulated values.
data(efeb)
out <- c2cv(com1=efeb$ef, com2=efeb$eb, nrandom=100, verbose=FALSE)

out$res

##
## cv1 121.00000000
## cv2 22.00000000
## cv1-cv2 99.00000000
## p 0.00990099
## quantile 0.025 -30.00000000
## quantile 0.975 45.00000000
## randomized cv1-cv2 3.34653465
## nrandom 100.00000000

The difference between the richness in site 1 and site 2 is given by \(\text{cv1-cv2}\) and equals 99 species in the example. The corresponding value after randomizations is indicated by \(\text{randomized cv1-cv2}\) and is much smaller. The \(n\) randomized values are used to compute the quantiles at \(p = 0.975\) and \(p = 0.025\) corresponding to a global interval of 95%. We can see that the observed difference is well above the upper quantile value (ca. 31) indicating that the observed difference is much larger than expected under the null hypothesis of “no difference between sites”.

4.2.2 \(\text{c2m}\)

\(\text{c2m}\) compares mean richness of two populations and any type of data can be processed. We illustrate the function using the example of the golden jackals given p. 4 in Manly (1997).

# The example of mandible length of male and female
males<-c(120, 107, 110, 116, 114, 111, 113, 117, 114, 112)
females<-c(110, 111, 107, 108, 110, 105, 107, 106, 111, 111)
out <- c2m(pop1=males, pop2=females, nrandom=99)
out$res

##
## mv1 113.400
## mv2 108.600
## mv1-mv2 4.800
## p 0.020
## quantile 0.025 -3.105
## quantile 0.975 2.905
## randomized mv1-mv2 0.084
## nrandom 99.000
out$res contains the results while out$rand gives the values of the difference between the means to be compared after randomization. We can plot an histogram of these values:

\texttt{hist(out$rand)}

![Histogram of out$rand](image)

It is interesting to add the vertical lines corresponding to the observed value (in red) and the quantile values for probability values of 0.975 and 0.025 (in blue):

\texttt{hist(out$rand)}
\texttt{abline(v=out$res[3,1], col="red")}
\texttt{abline(v=out$res[5,1], col="blue")}
\texttt{abline(v=out$res[6,1], col="blue")}
Let’s see what happens when the populations are very similar. In the following example we simulate normal populations and compare them using `c2m`:

```r
pop1<-rnorm(10)
pop2<-rnorm(10)
out <- c2m(pop1=pop1, pop2=pop2, nrandom=99)
out$res
```

```r
##
## mv1  0.075668974
## mv2  0.029476487
## mv1-mv2  0.046192487
## p       0.430000000
## quantile 0.025 -0.882134199
## quantile 0.975  0.892886766
## randomized mv1-mv2  0.006518599
## nrandom        99.000000000
```

```r
hist(out$rand)
abline(v=out$res[3,1], col="red")
abline(v=out$res[5,1], col="blue")
abline(v=out$res[6,1], col="blue")
```
The observed difference lies between the quantiles.

In some cases, the sets of values to be compared can overlap. This is what happen in our second example below. We have recorded the maximum temperature at sites where either *Tomicus destruens* or *T. piniperda*, two closely related species of bark beetles, have been recorded. The species are sympatric in 4 sites which leads to an overlap *i.e.* values common to both species between the distributions to be compared. The values common to both populations are passed to the function `c2m` by the argument `pop3`.

```r
data(Tomicus)
out <- c2m(pop1=Tomicus$destruens, pop2=Tomicus$piniperda, pop3=Tomicus$both, nrandom=99)
out$res
```

```r
##
## mv1 19.2432432
## mv2 14.2083333
## mv1-mv2 5.0349099
## p 0.0100000
## quantile 0.025 -2.2321509
## quantile 0.975 1.9470439
## randomized mv1-mv2 -0.2614189
## nrandom 99.0000000
```
The value of $mv_1 - mv_2$ lies outside the envelope defined by the quantiles which indicates a difference in species tolerance to temperature.

c2m can be used to make comparisons between two objects generated by the function `rich`:

```r
data(ef)
o1 <- rich(matrix=ef, nrandom=99, verbose=TRUE)
data(ea)
o2 <- rich(matrix=ea, nrandom=99, verbose=TRUE)
out <- c2m(pop1=o1$sumrow, pop2=o2$sumrow, nrandom=999, verbose=TRUE)
hist(out$rand)
abline(v=out$res[3,1], col="red")
abline(v=out$res[5,1], col="blue")
abline(v=out$res[6,1], col="blue")```
5 Rarefaction curves

5.1 rarc

rich allows to compute the rarefaction curve which corresponds to the changes in the species richness with sampling intensity. User selects the set of values of sampling size for which the estimate of species richness is needed, the number of randomizations to be performed and the values of the upper and lower bounds. rarc returns a data frame ($out$) with the bootstrap estimates of species richness, the corresponding statistical envelop and the average number of individuals for the sample size to be investigated.

data(ef)
t <- rarc(ef,samplesize=c(5,10,15,20,25), nrandom=30, p1=0.975, p2=0.025)
head(t)

## $out
## mean.richness lb.richness ub.richness mean.nb.individuals samples
## 1 35.03333 21.625 50.00 397.200 168.8724
## 2 51.90000 38.000 64.65 783.900 246.5735
## 3 66.20000 48.525 82.55 1193.733 363.1112
## 4 74.86667 56.425 91.10 1630.233 454.8685
## 5 84.96667 69.725 99.65 1999.900 438.0184
t can be used to plot the changes in richness according to sample size:

```r
plot(t$out[,6],t$out[,1], type="b", ylim=range(c(t$out[,2],t$out[,3])),
     xlab="number of sampling units", ylab="richness")
points(t$out[,6] , t$out[,2], type="l", col="red")
points(t$out[,6] , t$out[,3], type="l", col="blue")
```

Note that the function uses bootstrap which means sampling with replacement. The consequence is that the richness estimated for a sample size equal to the size of the dataset is not equal to the observed richness, it is lower. For example the species richness of the dataset `ef` is 121 and the number of sampling units is 30:

```r
data(ef)
r <- rich(ef)
r$cr
```

```r
## [1] 121
```
If we perform rarefaction curve for (say) samples of \{10, 20, 30\} we get:

```r
data(ef)
t <- rarc(ef, samplesize=c(10, 20, 30), nrandom=30, p1=0.975, p2=0.025)
t
```

```r
# $out
#   mean.richness lb.richness ub.richness mean.nb.individuals samples
# 1    52.83333     38.700     68.100         837.100   345.9349
# 2    76.40000     60.175     92.825        1670.200   474.6547
# 3    88.53333     73.525    103.000        2406.133   510.2116
# sample
# 1   10
# 2   20
# 3   30
```

For \(n = 30\) the bootstrap estimate of the richness is ca. 90 while the observed value is 121. If we plot the curve and add the observed value the difference is clear:

```r
plot(t$out[,6], t$out[,1], type="b", ylim=range(c(t$out[,2], t$out[,3])),
    xlab="number of sampling units", ylab="richness")
points(t$out[,6] , t$out[,2], type="l", col="red")
points(t$out[,6] , t$out[,3], type="l", col="blue")
abline(h=r$cr, lty="dashed")
```
The interest of such estimates by bootstrap is that the variance of the estimate is meaningful whatever the sampling size which is not the case of rarefaction curves based on resampling with replacement. When replacement is not allowed, the variance decreases with increasing sampling size and becomes null for the maximum sampling size.

When the argument `save` is set to `TRUE`, `rarc` returns an additional list (`$bootstrapped.val`) which corresponds to the raw bootstrapped values used to compute the quantiles. It may be useful for users who want to compute standard errors for example. The example below shows how to compute the standard errors.

```r
# Computing the standard deviation instead of the quantiles.
# We set the save argument to TRUE

# We set the save argument to TRUE

# Computing the standard for the third sampling size

# computing the standard error for all sample sizes:
```
stdev <- rep(NA, times=length(samplesize))
for (i in 1:length(samplesize)) {
    stdev[i] <- sd(t$bootstrapped.val[[i]][,1])/
        sqrt(length(t$bootstrapped.val[[i]][,1]))
}

We plot the results:

r <- range(t$out$mean.richness-stdev, t$out$mean.richness+2*stdev)
r

## [1] 28.98770 95.13287

plot(t$out$mean.nb.individuals, t$out$mean.richness, pch=19, ylim=r, xlab="nb individuals", ylab="Mean richness (bootstrap) +/- SD")
arrows(t$out$mean.nb.individuals, t$out$mean.richness-stdev, 
    t$out$mean.nb.individuals, t$out$mean.richness+stdev, 
    length=0.05, angle=90, code=3)

5.2 raref

raref computes the rarefaction curve and interpolates the species richness corresponding to a given density of individuals (not a number of samples!).
data(ef)
    r <- raref(ef, dens=1100, nrandom=50)
head(r$rar)

## nbsp ind sample
## 1  1.00  91.40  1
## 2  17.94 168.06  2
## 3 24.10 235.86  3
## 4 27.96 289.56  4
## 5 32.54 362.68  5
## 6 39.28 494.86  6

r$Sinterp

## [1] 1100.00000 62.93212

We plot the curve and the interpolated value:

plot(r$rar$ind, r$rar$nbsp, type="b")
abline(v=r$Sinterp[1], lty="dashed"); abline(h=r$Sinterp[2], lty="dashed")
points(r$Sinterp[1], r$Sinterp[2], pch=3, col="red")
5.3 raref2

raref2 computes another estimation of the species richness by thinning the data matrix so that the overall corresponding density is comprised in a fixed interval.

```r
# Example code snippet

data(ef)
r2 <- raref2(matrix=ef, dens=1100, tolerance=0.01, nrandom=50)
r2$mean.boot

# Output
## [1] 71.06
```

We can add this second estimate to the rarefaction curve derived from the outputs of `raref`:

```r
plot(r$rar$ind, r$rar$nbsp, type="p", cex=0.5)
points(r$Sinterp[1], r$Sinterp[2], pch=8, col="blue")
points(1100, r2$mean.boot, pch=3, col="red")
legend(x="bottomright", legend=c("raref", "raref2"), bty="n",
       pch=c(8,3), col=c("blue", "red"))
```

5.4 shared

shared computes the richness of each group of sample depicting a community, the number of species shared by pairs of communities and the total number of species for each pairs of community. Two or more communities can be compared:
```
sp1<-c(1,2,3,4,5)
sp2<-c(0,0,0,0,0)
sp3<-c(1,1,0,0,0)
sp4<-c(0,0,0,0,0)
site1<-cbind(sp1, sp2, sp3, sp4)
colnames(site1)<-c("sp1", "sp2", "sp3", "sp4")
site1

## sp1 sp2 sp3 sp4
## [1,] 1 0 1 0
## [2,] 2 0 1 0
## [3,] 3 0 0 0
## [4,] 4 0 0 0
## [5,] 5 0 0 0
```

```
sp1<-c(1,2,3)
sp2<-c(1,0,0)
sp3<-c(0,0,0)
sp4<-c(0,0,0)
site2<-cbind(sp1, sp2, sp3, sp4)
colnames(site2)<-c("sp1", "sp2", "sp3", "sp4")
site2

## sp1 sp2 sp3 sp4
## [1,] 1 1 0 0
## [2,] 2 0 0 0
## [3,] 3 0 0 0
```

```
sp1<-c(1,2,3,4)
sp2<-c(1,0,0,0)
sp3<-c(1,0,0,0)
sp4<-c(1,0,0,0)
site3<-cbind(sp1, sp2, sp3, sp4)
colnames(site3)<-c("sp1", "sp2", "sp3", "sp4")
site3

## sp1 sp2 sp3 sp4
## [1,] 1 1 1 1
## [2,] 2 0 0 0
## [3,] 3 0 0 0
## [4,] 4 0 0 0
```
# we create a list containing the sites to be compared:
data<-list(site1,site2, site3)
names(data)<-c("site1","site2","site3")

shared(data)

## site1 site2 site3
## site1 2 1 2
## site2 3 2 2
## site3 4 4 4

shared returns a matrix whose diagonal is the richness of each community.

data(efeb)
shared(efeb)

## ef eb
## ef 121 9
## eb 134 22

References
