Lab 3

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Section 6.3 Worked Example

https://bookdown.org/hhwagner1/LandGenCourse_book/WE_3.html

Load libraries

```
require(adegenet)
```

```
## Loading required package: adegenet

## Loading required package: ade4

##
##    /// adegenet 2.1.10 is loaded ////////////
##
##    > overview: '?adegenet'
##    > tutorials/doc/questions: 'adegenetWeb()'
##    > bug reports/feature requests: adegenetIssues()
```

```
require(LandGenCourse)
```

```
## Loading required package: LandGenCourse
```

```
require(pegas)
```

```
## Loading required package: pegas

## Loading required package: ape

## Registered S3 method overwritten by 'pegas':
##   method      from
##   print.amova ade4

##
## Attaching package: 'pegas'

## The following object is masked from 'package:ape':
##
##     mst

## The following object is masked from 'package:ade4':
##
##     amova
```

```
require(PopGenReport)
```

```
## Loading required package: PopGenReport

## Loading required package: knitr
## Registered S3 method overwritten by 'GGally':
##   method from
##   +.gg   ggplot2

## Registered S3 method overwritten by 'genetics':
##   method      from
##   [.haplotype pegas
```

```r
require(dplyr)
```

```
## Loading required package: dplyr

##
## Attaching package: 'dplyr'

## The following object is masked from 'package:ape':
##
##     where

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```r
require(poppr)
```

```
## Loading required package: poppr

## This is poppr version 2.9.6. To get started, type package?poppr
## OMP parallel support: available
```

1. Overview

The data set we will use is ralu.loci

2. Import straight from the package after library is loaded

```r
data(ralu.loci, package="LandGenCourse")
Frogs <- data.frame(FrogID = paste(substr(ralu.loci$Pop, 1, 3),
                                   row.names(ralu.loci), sep="."), ralu.loci)
Frogs.genind <- adegenet::df2genind(X=Frogs[,c(4:11)], sep=":", ncode=NULL,
                          ind.names= Frogs$FrogID, loc.names=NULL,
                          pop=Frogs$Pop, NA.char="NA", ploidy=2,
                          type="codom", strata=NULL, hierarchy=NULL)
Frogs.genind
```

```
## /// GENIND OBJECT /////////
##
##  // 181 individuals; 8 loci; 39 alleles; size: 55.5 Kb
##
##  // Basic content
##    @tab:  181 x 39 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 3-9)
##    @loc.fac: locus factor for the 39 columns of @tab
##    @all.names: list of allele names for each locus
##    @ploidy: ploidy of each individual  (range: 2-2)
##    @type:  codom
##    @call: adegenet::df2genind(X = Frogs[, c(4:11)], sep = ":", ncode = NULL,
##     ind.names = Frogs$FrogID, loc.names = NULL, pop = Frogs$Pop,
##     NA.char = "NA", ploidy = 2, type = "codom", strata = NULL,
##     hierarchy = NULL)
##
##  // Optional content
##    @pop: population of each individual (group size range: 7-23)
```

Get info on genind object, check that they are polymorphic

```
Frogs.genind
```

```
## /// GENIND OBJECT /////////
##
##  // 181 individuals; 8 loci; 39 alleles; size: 55.5 Kb
##
##  // Basic content
##    @tab:  181 x 39 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 3-9)
##    @loc.fac: locus factor for the 39 columns of @tab
##    @all.names: list of allele names for each locus
##    @ploidy: ploidy of each individual  (range: 2-2)
##    @type:  codom
##    @call: adegenet::df2genind(X = Frogs[, c(4:11)], sep = ":", ncode = NULL,
##     ind.names = Frogs$FrogID, loc.names = NULL, pop = Frogs$Pop,
##     NA.char = "NA", ploidy = 2, type = "codom", strata = NULL,
##     hierarchy = NULL)
##
##  // Optional content
##    @pop: population of each individual (group size range: 7-23)
```

```
summary(Frogs.genind)
```

```
##
## // Number of individuals: 181
## // Group sizes: 21 8 14 13 7 17 9 20 19 13 17 23
## // Number of alleles per locus: 3 4 4 4 9 3 4 8
## // Number of alleles per group: 21 21 20 22 20 19 19 25 18 14 18 26
## // Percentage of missing data: 10.64 %
## // Observed heterozygosity: 0.1 0.4 0.09 0.36 0.68 0.02 0.38 0.68
## // Expected heterozygosity: 0.17 0.47 0.14 0.59 0.78 0.02 0.48 0.74
```

Test for HWE with pegas:

```
round(pegas::hw.test(Frogs.genind, B = 1000), digits = 3)
```

```
##     chi^2 df Pr(chi^2 >) Pr.exact
## A  40.462  3       0.000    0.000
## B  17.135  6       0.009    0.032
## C 136.522  6       0.000    0.000
## D  83.338  6       0.000    0.000
## E 226.803 36       0.000    0.000
## F   0.024  3       0.999    1.000
## G  12.349  6       0.055    0.005
## H  76.813 28       0.000    0.000
```

```r
# Chi-squared test: p-value
HWE.test <- data.frame(sapply(seppop(Frogs.genind),
                              function(ls) pegas::hw.test(ls, B=0)[,3]))
HWE.test.chisq <- t(data.matrix(HWE.test))
{cat("Chi-squared test (p-values):", "\n")
round(HWE.test.chisq,3)}
```

```
## Chi-squared test (p-values):

##                A     B     C     D     E     F     G     H
## Airplane   0.092 0.359 1.000 0.427 0.680 1.000 0.178 0.051
## Bachelor   1.000 0.557 0.576 0.686 0.716 1.000 0.414 0.609
## BarkingFox 0.890 0.136 0.005 0.533 0.739 0.890 0.708 0.157
## Bob        0.764 0.864 0.362 0.764 0.033 1.000 0.860 0.287
## Cache      1.000 0.325 0.046 0.659 0.753 1.000 0.709 0.402
## Egg        1.000 0.812 1.000 1.000 0.156 1.000 0.477 0.470
## Frog       1.000 0.719 0.070 0.722 0.587 1.000 0.564 0.172
## GentianL   0.809 0.059 1.000 0.028 0.560 0.717 0.474 0.108
## ParagonL   1.000 0.054 0.885 0.709 0.868 1.000 0.291 0.000
## Pothole    1.000 1.000 1.000 0.488 0.248 1.000 0.296 0.850
## ShipIsland 0.807 0.497 1.000 0.521 0.006 1.000 0.498 0.403
## Skyhigh    0.915 0.493 0.063 0.001 0.155 1.000 0.126 0.078
```

```r
# Monte Carlo: p-value
HWE.test <- data.frame(sapply(seppop(Frogs.genind),
                              function(ls) pegas::hw.test(ls, B=1000)[,4]))
HWE.test.MC <- t(data.matrix(HWE.test))
{cat("MC permuation test (p-values):", "\n")
round(HWE.test.MC,3)}
```

```
## MC permuation test (p-values):

##                A     B     C     D     E F     G     H
## Airplane   0.015 1.000 1.000 0.405 0.645 1 0.279 0.007
## Bachelor   1.000 0.420 1.000 1.000 0.867 1 0.464 0.586
## BarkingFox 1.000 0.224 0.052 1.000 0.753 1 1.000 0.167
## Bob        1.000 1.000 1.000 1.000 0.018 1 1.000 0.261
## Cache      1.000 0.397 0.165 1.000 1.000 1 1.000 0.608
## Egg        1.000 1.000 1.000 1.000 0.078 1 0.541 0.471
## Frog       1.000 1.000 0.078 1.000 0.464 1 1.000 0.173
## GentianL   1.000 0.070 1.000 0.058 0.700 1 0.645 0.177
## ParagonL   1.000 0.162 1.000 1.000 1.000 1 0.313 0.069
## Pothole    1.000 1.000 1.000 1.000 0.554 1 0.505 1.000
## ShipIsland 1.000 0.625 1.000 0.675 0.137 1 0.558 0.464
## Skyhigh    1.000 0.338 0.166 0.100 0.101 1 0.083 0.036
```

```r
alpha=0.05 # /96
Prop.loci.out.of.HWE <- data.frame(Chisq=apply(HWE.test.chisq<alpha, 2, mean),
           MC=apply(HWE.test.MC<alpha, 2, mean))
Prop.loci.out.of.HWE             # Type this line again to see results table
```

```
##        Chisq         MC
## A 0.00000000 0.08333333
## B 0.00000000 0.00000000
## C 0.16666667 0.00000000
## D 0.16666667 0.00000000
## E 0.16666667 0.08333333
## F 0.00000000 0.00000000
## G 0.00000000 0.00000000
## H 0.08333333 0.16666667
```

```
Prop.pops.out.of.HWE <- data.frame(Chisq=apply(HWE.test.chisq<alpha, 1, mean),
           MC=apply(HWE.test.MC<alpha, 1, mean))
Prop.pops.out.of.HWE
```

```
##            Chisq    MC
## Airplane   0.000 0.250
## Bachelor   0.000 0.000
## BarkingFox 0.125 0.000
## Bob        0.125 0.125
## Cache      0.125 0.000
## Egg        0.000 0.000
## Frog       0.000 0.000
## GentianL   0.125 0.000
## ParagonL   0.125 0.000
## Pothole    0.000 0.000
## ShipIsland 0.125 0.000
## Skyhigh    0.125 0.125
```

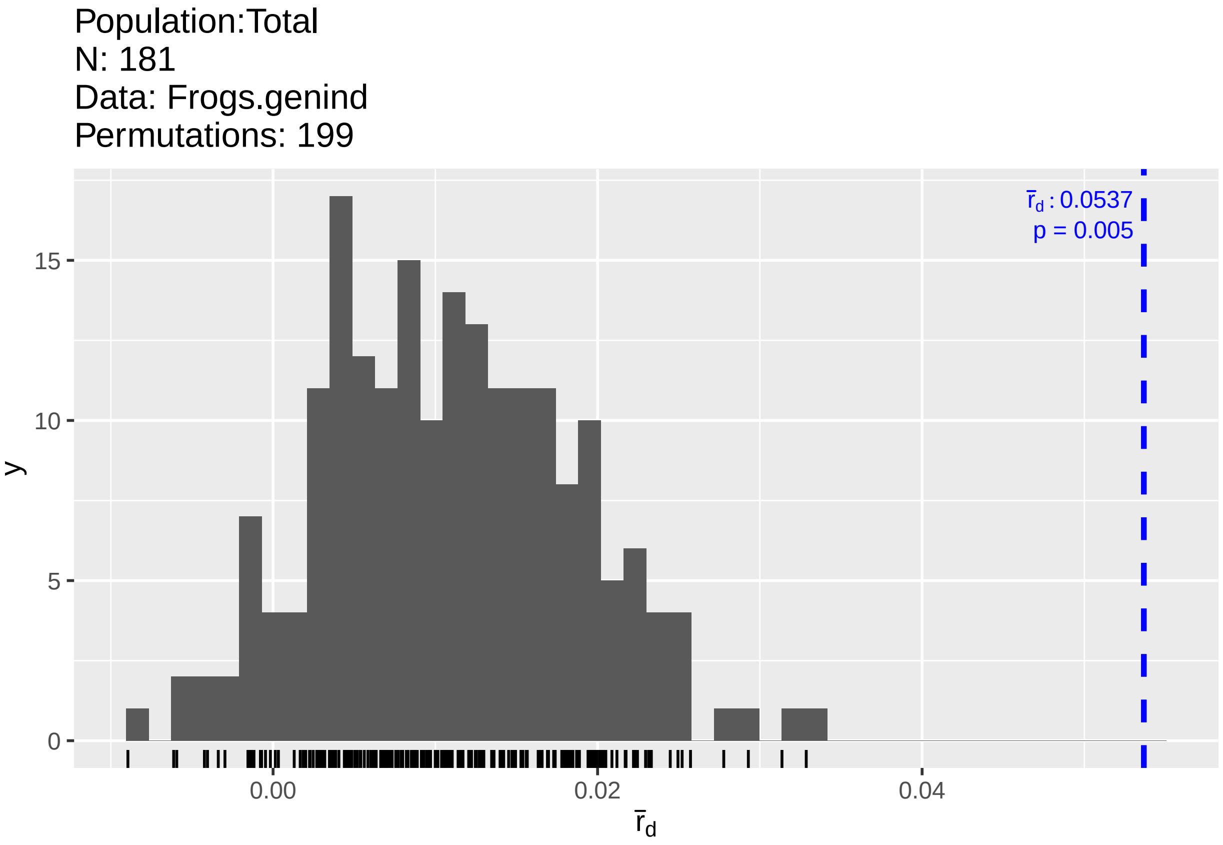
```
Chisq.fdr <- matrix(p.adjust(HWE.test.chisq,method="fdr"),
                    nrow=nrow(HWE.test.chisq))
MC.fdr <- matrix(p.adjust(HWE.test.MC, method="fdr"),
                    nrow=nrow(HWE.test.MC))

Prop.pops.out.of.HWE <- data.frame(Chisq=apply(HWE.test.chisq<alpha, 1, mean),
           MC=apply(HWE.test.MC<alpha, 1, mean),
           Chisq.fdr=apply(Chisq.fdr<alpha, 1, mean),
           MC.fdr=apply(MC.fdr<alpha, 1, mean))
Prop.pops.out.of.HWE
```

```
##            Chisq    MC Chisq.fdr MC.fdr
## Airplane   0.000 0.250     0.000      0
## Bachelor   0.000 0.000     0.000      0
## BarkingFox 0.125 0.000     0.000      0
## Bob        0.125 0.125     0.000      0
## Cache      0.125 0.000     0.000      0
## Egg        0.000 0.000     0.000      0
## Frog       0.000 0.000     0.000      0
## GentianL   0.125 0.000     0.000      0
## ParagonL   0.125 0.000     0.125      0
## Pothole    0.000 0.000     0.000      0
## ShipIsland 0.125 0.000     0.000      0
## Skyhigh    0.125 0.125     0.125      0
```

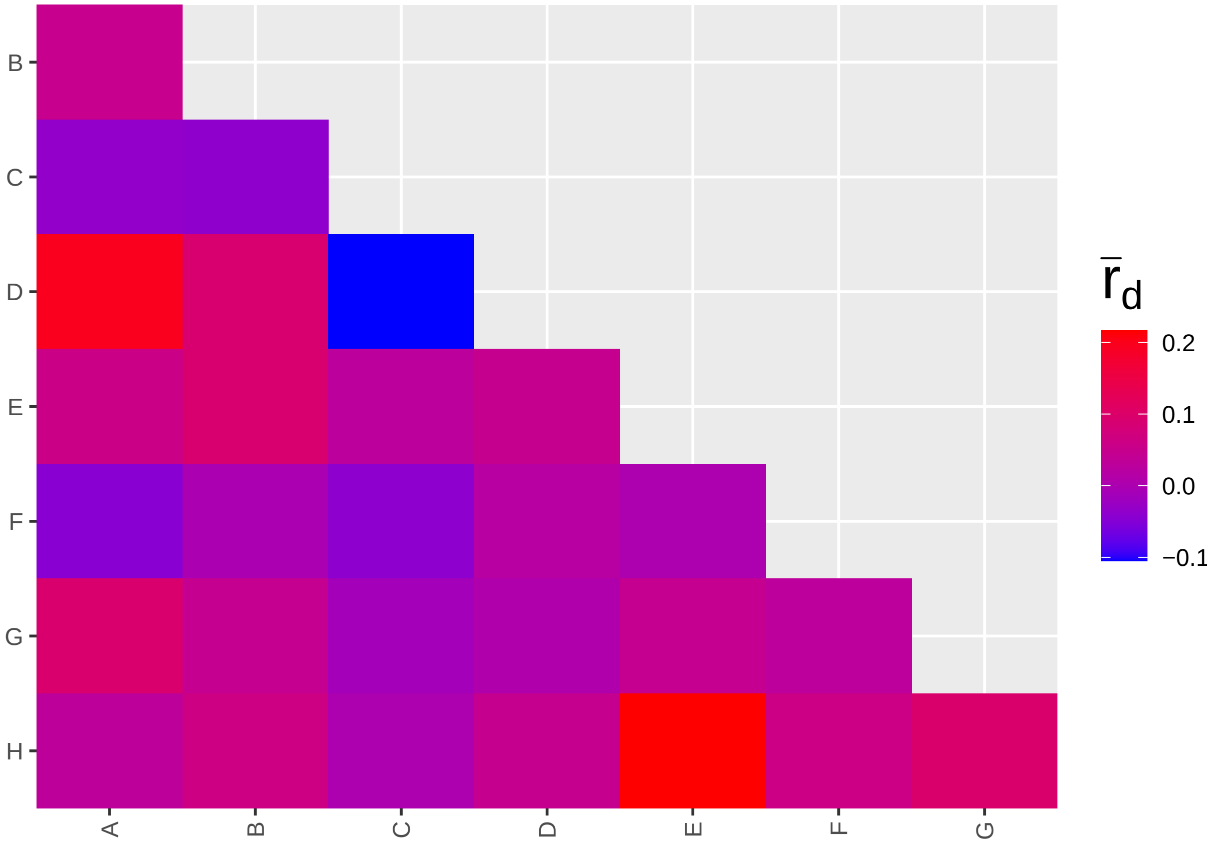
Linkage Disequilibrium

```
poppr::ia(Frogs.genind, sample=199)
```



```
##          Ia        p.Ia       rbarD        p.rD 
## 0.33744318 0.00500000 0.05366542 0.00500000
```

```
LD.pair <- poppr::pair.ia(Frogs.genind)
```



```
LD.pair
```

```
##          Ia   rbarD
## A:B  0.0485  0.0492
## A:C -0.0314 -0.0335
## A:D  0.1886  0.1966
## A:E  0.0560  0.0569
## A:F -0.0272 -0.0452
## A:G  0.0931  0.0935
## A:H  0.0294  0.0304
## B:C -0.0329 -0.0375
## B:D  0.0903  0.0911
## B:E  0.0910  0.0910
## B:F -0.0013 -0.0025
## B:G  0.0451  0.0452
## B:H  0.0621  0.0623
## C:D -0.0859 -0.1049
## C:E  0.0247  0.0284
## C:F -0.0311 -0.0397
## C:G -0.0107 -0.0118
## C:H  0.0012  0.0015
## D:E  0.0455  0.0458
## D:F  0.0094  0.0199
## D:G  0.0069  0.0070
## D:H  0.0461  0.0462
## E:F  0.0013  0.0025
## E:G  0.0453  0.0454
## E:H  0.2153  0.2159
## F:G  0.0167  0.0299
## F:H  0.0296  0.0606
## G:H  0.0942  0.0953
```

Null Alleles

```
# Null alleles: depends on method! See help file.
Null.alleles <- PopGenReport::null.all(Frogs.genind)
```

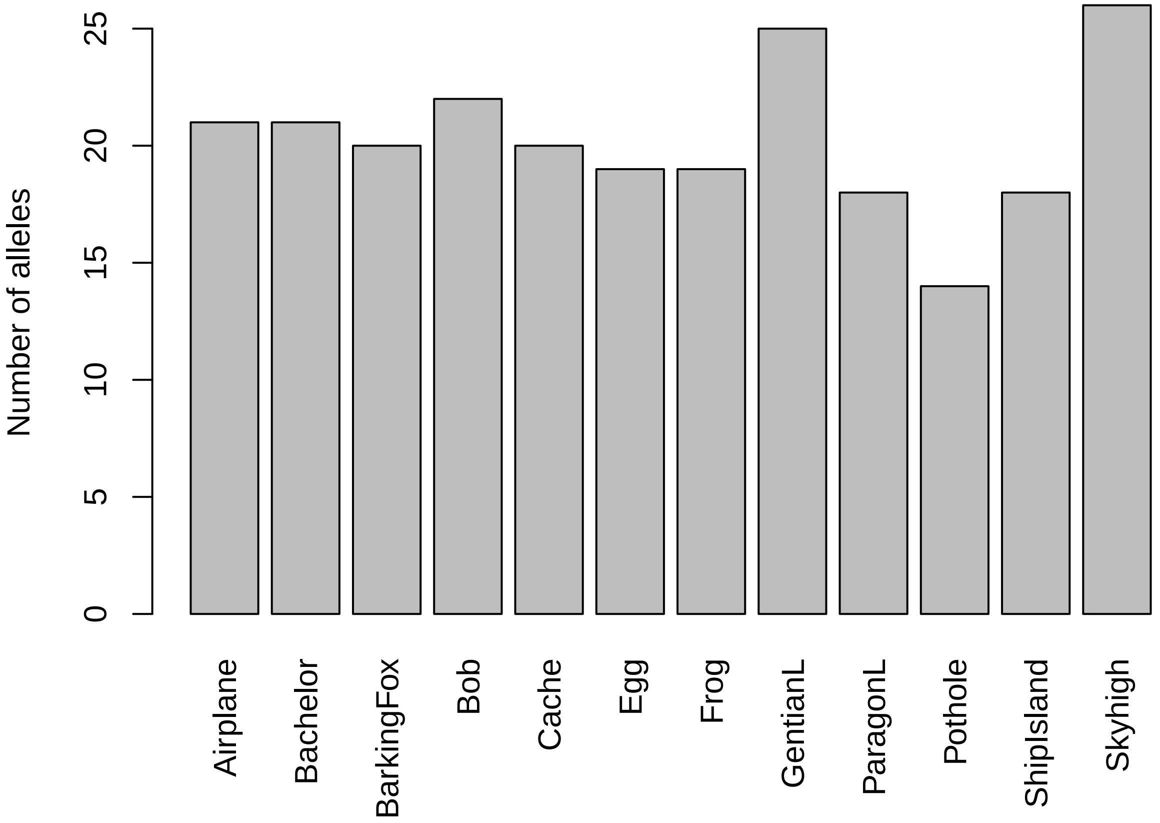
Genetic Diversity

allelic richness

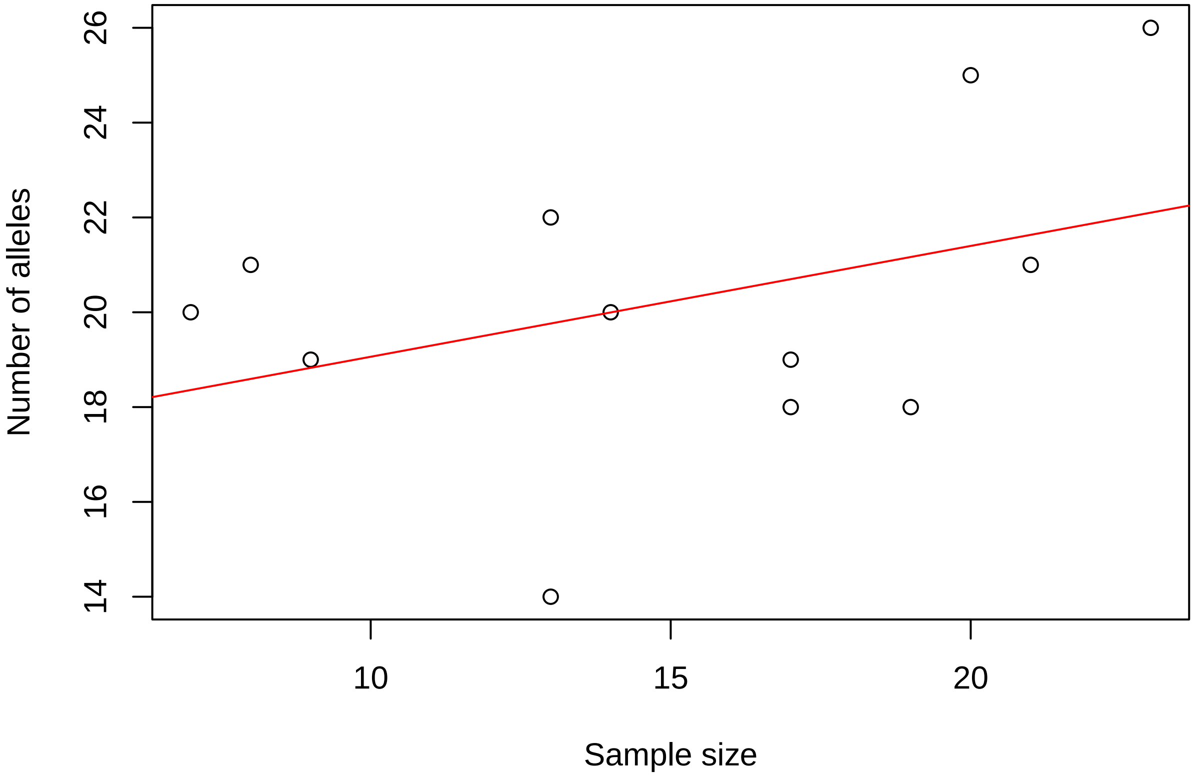
```
Sum <- adegenet::summary(Frogs.genind)
names(Sum)
```

```
## [1] "n"          "n.by.pop"   "loc.n.all" "pop.n.all" "NA.perc"    "Hobs"
## [7] "Hexp"
```

```
par(mar=c(5.5, 4.5,1,1))
barplot(Sum$pop.n.all, las=3,
       xlab = "", ylab = "Number of alleles")
```



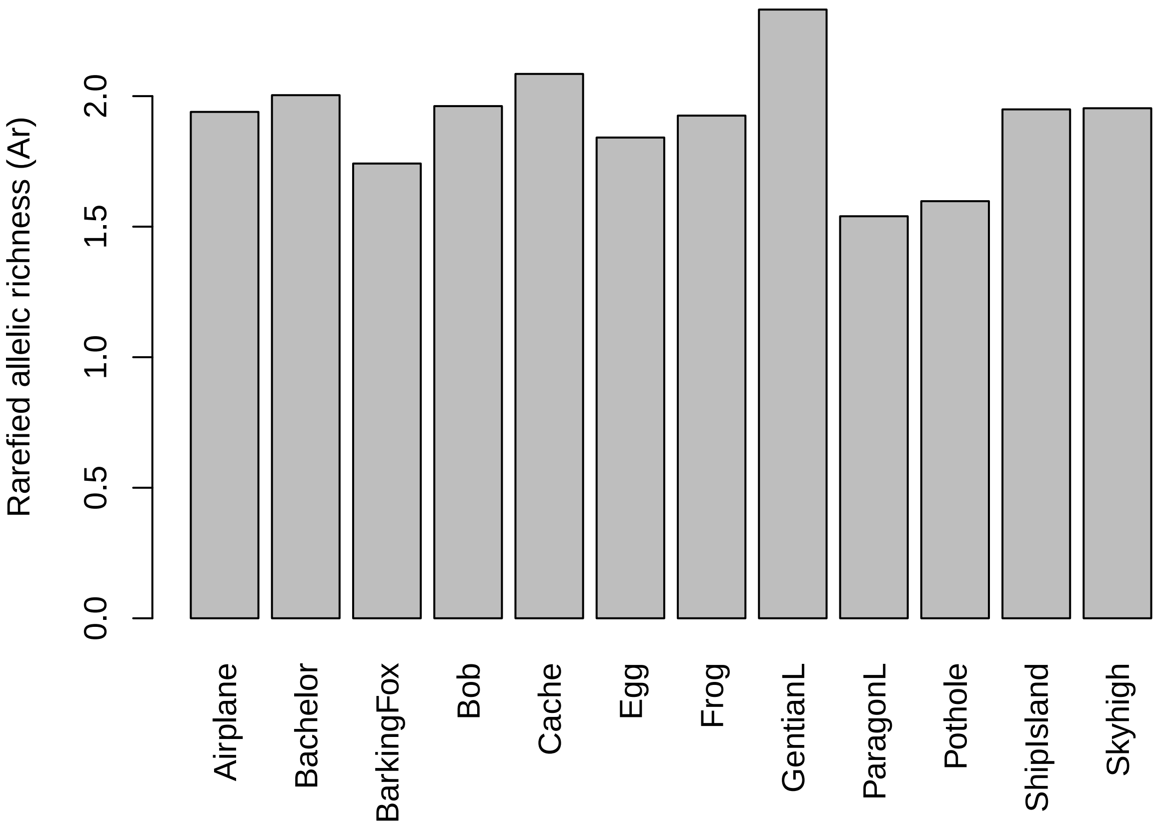
```
plot(Sum$n.by.pop, Sum$pop.n.all,
       xlab = "Sample size", ylab = "Number of alleles")
abline(lm(Sum$pop.n.all ~ Sum$n.by.pop), col = "red")
```



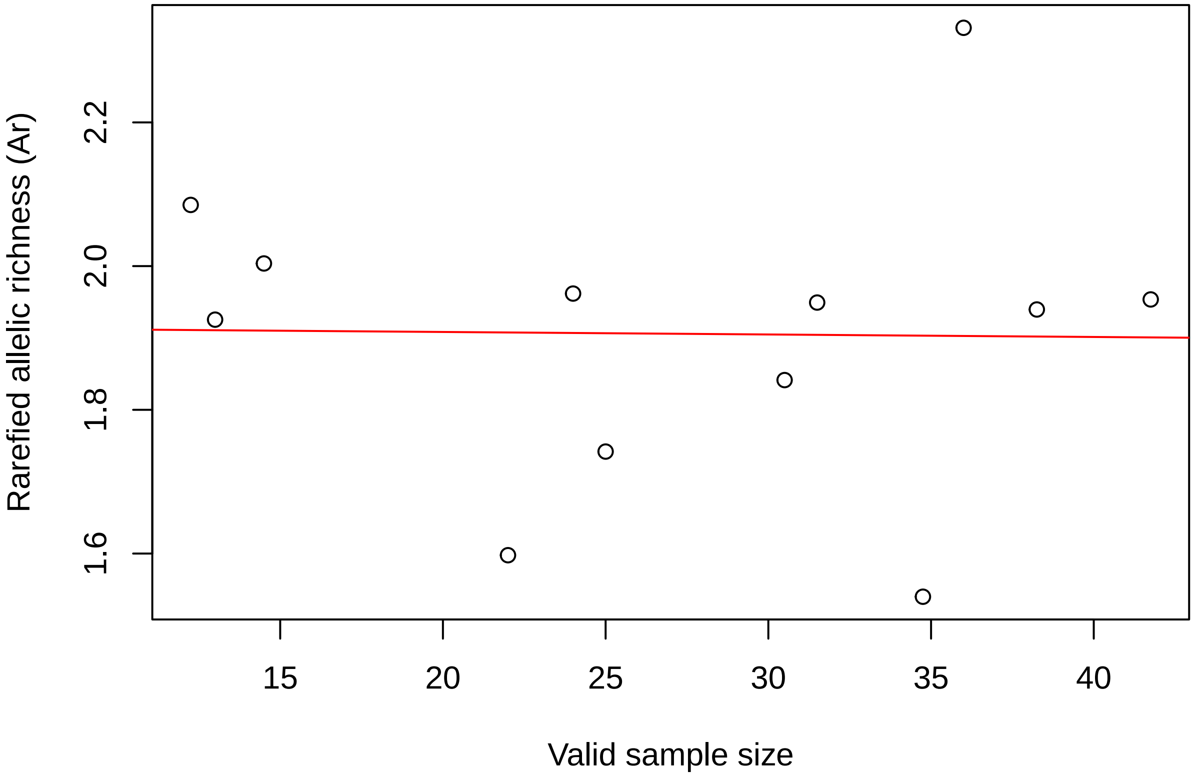
```
Richness <- PopGenReport::allel.rich(Frogs.genind, min.alleles = NULL)
Richness$alleles.sampled
```

```
## [1] 6
```

```
par(mar=c(5.5, 4.5,1,1))
barplot(Richness$mean.richness, las=3, ylab="Rarefied allelic richness (Ar)")
```



```
plot(colMeans(Richness$pop.sizes), Richness$mean.richness,
     xlab="Valid sample size",
     ylab="Rarefied allelic richness (Ar)")
abline(lm(Richness$mean.richness ~ colMeans(Richness$pop.sizes)), col="red")
```



6.4 Exercise

```
Flowers <- read.csv("./downloads/pulsatilla_genotypes.csv", header=TRUE)
as_tibble(Flowers)
```

```
## # A tibble: 536 x 19
##       ID OffID Population        X        Y loc1_a loc1_b loc2_a loc2_b loc3_a
##    <int> <int> <chr>           <dbl>    <dbl>  <int>  <int>  <int>  <int>  <int>
##  1    62     0 A21         4426941. 5427173.    340    340    422    422    413
##  2    64     0 A21         4426933. 5427178.    334    334    424    424    417
##  3    65     0 A21         4426936. 5427173.    338    340    417    422    417
##  4    66     0 A21         4426937. 5427174.    340    344    422    422    411
##  5    68     0 A21         4426934. 5427171.    336    342    417    422    423
##  6    69     0 A21         4426933. 5427166.    336    346    422    422    417
##  7    75     0 A21         4426925. 5427175.    340    340    422    422    415
##  8    76     0 A21         4426925. 5427173.    338    340    417    422    413
##  9    77     0 A21         4426922. 5427174.    344    352    422    422    415
## 10    78     0 A21         4426922. 5427174.    342    352    417    424    425
## # i 526 more rows
## # i 9 more variables: loc3_b <int>, loc4_a <int>, loc4_b <int>, loc5_a <int>,
## #   loc5_b <int>, loc6_a <int>, loc6_b <int>, loc7_a <int>, loc7_b <int>
```

Filter offspring (seeds) from the dataset

```
Flowers <- filter(Flowers, OffID==0)
as_tibble(Flowers)
```

```
## # A tibble: 221 x 19
##       ID OffID Population        X        Y loc1_a loc1_b loc2_a loc2_b loc3_a
##    <int> <int> <chr>         <dbl>    <dbl>  <int>  <int>  <int>  <int>  <int>
##  1    62     0 A21        4426941. 5427173.    340    340    422    422    413
##  2    64     0 A21        4426933. 5427178.    334    334    424    424    417
##  3    65     0 A21        4426936. 5427173.    338    340    417    422    417
##  4    66     0 A21        4426937. 5427174.    340    344    422    422    411
##  5    68     0 A21        4426934. 5427171.    336    342    417    422    423
##  6    69     0 A21        4426933. 5427166.    336    346    422    422    417
##  7    75     0 A21        4426925. 5427175.    340    340    422    422    415
##  8    76     0 A21        4426925. 5427173.    338    340    417    422    413
##  9    77     0 A21        4426922. 5427174.    344    352    422    422    415
## 10    78     0 A21        4426922. 5427174.    342    352    417    424    425
## # i 211 more rows
## # i 9 more variables: loc3_b <int>, loc4_a <int>, loc4_b <int>, loc5_a <int>,
## #   loc5_b <int>, loc6_a <int>, loc6_b <int>, loc7_a <int>, loc7_b <int>
```

Count the number of individuals in each pop

```
Adults.by.site<-split(Flowers, Flowers$Population, T)
sapply(Adults.by.site, count)
```

```
## $A03.n
## [1] 42
##
## $A21.n
## [1] 21
##
## $A25.n
## [1] 56
##
## $A26.n
## [1] 21
##
## $A41.n
## [1] 14
##
## $A45.n
## [1] 22
##
## $G05a.n
## [1] 45
```

Dataframe with the first 5 columns, then paste loc1_a:loc1_b, etc.

```
Flowers <- data.frame(Flowers[,1:5],loc1 = paste(Flowers$loc1_a, Flowers$loc1_b, sep=":"), loc2 = paste
as_tibble(Flowers)
```

```
## # A tibble: 221 x 12
##       ID OffID Population        X        Y loc1   loc2  loc3  loc4  loc5  loc6
##    <int> <int> <chr>         <dbl>    <dbl> <chr>  <chr> <chr> <chr> <chr> <chr>
##  1    62     0 A21        4426941. 5427173. 340:3~ 422:~ 413:~ 446:~ 121:~ 155:~
##  2    64     0 A21        4426933. 5427178. 334:3~ 424:~ 417:~ 444:~ 122:~ 155:~
##  3    65     0 A21        4426936. 5427173. 338:3~ 417:~ 417:~ 446:~ 135:~ 153:~
##  4    66     0 A21        4426937. 5427174. 340:3~ 422:~ 411:~ 446:~ 122:~ 157:~
##  5    68     0 A21        4426934. 5427171. 336:3~ 417:~ 423:~ 448:~ 119:~ 155:~
##  6    69     0 A21        4426933. 5427166. 336:3~ 422:~ 417:~ 444:~ 122:~ 155:~
##  7    75     0 A21        4426925. 5427175. 340:3~ 422:~ 415:~ 442:~ 121:~ 152:~
##  8    76     0 A21        4426925. 5427173. 338:3~ 417:~ 413:~ 446:~ 126:~ 155:~
##  9    77     0 A21        4426922. 5427174. 344:3~ 422:~ 415:~ 446:~ 121:~ 155:~
## 10    78     0 A21        4426922. 5427174. 342:3~ 417:~ 425:~ 446:~ 121:~ 157:~
## # i 211 more rows
## # i 1 more variable: loc7 <chr>
```

Create ‘genind’ object:

```
Flowers.genind <- df2genind(X=Flowers[,c(6:12)], sep=":", ncode=NULL, ind.names= Flowers$ID, loc.names=
```

Get info on genind object:

```
Flowers.genind
```

```
## /// GENIND OBJECT /////////
##
##  // 221 individuals; 7 loci; 105 alleles; size: 130.8 Kb
##
##  // Basic content
##    @tab:  221 x 105 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 8-25)
##    @loc.fac: locus factor for the 105 columns of @tab
##    @all.names: list of allele names for each locus
##    @ploidy: ploidy of each individual  (range: 2-2)
##    @type:  codom
##    @call: df2genind(X = Flowers[, c(6:12)], sep = ":", ncode = NULL, ind.names = Flowers$ID,
##     loc.names = names(Flowers[, c(6:12)]), pop = Flowers$Population,
##     NA.char = "NA", ploidy = 2, type = "codom", strata = NULL,
##     hierarchy = NULL)
##
##  // Optional content
##    @pop: population of each individual (group size range: 14-56)
```

```
summary(Flowers.genind)
```

```
##
## // Number of individuals: 221
## // Group sizes: 21 56 21 22 14 42 45
## // Number of alleles per locus: 18 8 25 8 19 14 13
## // Number of alleles per group: 63 68 54 50 51 73 53
## // Percentage of missing data: 0.9 %
## // Observed heterozygosity: 0.74 0.54 0.89 0.71 0.74 0.68 0.74
## // Expected heterozygosity: 0.83 0.57 0.89 0.74 0.81 0.76 0.83
```