Lab 3

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Section 6.3 Worked Example

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

Load libraries

```
require(adegenet)
require(LandGenCourse)
require(pegas)
require(PopGenReport)
require(dplyr)
require(poppr)
require(here)
```

1. Overview

The data set we will use is ralu.loci

2. Import straight from the package after library is loaded

```
## /// 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
##
      Oploidy: 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
##
      Opop: 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
##
      Oploidy: 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
      Opop: 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
                               0.035
                      0.000
## C 136.522 6
                               0.000
## D 83.338 6
                      0.000
                               0.000
## E 226.803 36
                      0.000
                             0.001
## F 0.024 3
                      0.999
                              1.000
## G 12.349 6
                      0.055
                               0.008
## H 76.813 28
                      0.000
                               0.000
# Chi-squared test: p-value
HWE.test <- data.frame(sapply(seppop(Frogs.genind),</pre>
                              function(ls) pegas::hw.test(ls, B=0)[,3]))
HWE.test.chisq <- t(data.matrix(HWE.test))</pre>
{cat("Chi-squared test (p-values):", "\n")
```

```
round(HWE.test.chisq,3)}
## Chi-squared test (p-values):
                                    D
                                          Ε
## 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
              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
              1.000 0.812 1.000 1.000 0.156 1.000 0.477 0.470
## Egg
## Frog
              1.000 0.719 0.070 0.722 0.587 1.000 0.564 0.172
              0.809 0.059 1.000 0.028 0.560 0.717 0.474 0.108
## GentianL
              1.000 0.054 0.885 0.709 0.868 1.000 0.291 0.000
## ParagonL
## 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
# Monte Carlo: p-value
HWE.test <- data.frame(sapply(seppop(Frogs.genind),</pre>
                              function(ls) pegas::hw.test(ls, B=1000)[,4]))
HWE.test.MC <- t(data.matrix(HWE.test))</pre>
{cat("MC permuation test (p-values):", "\n")
round(HWE.test.MC,3)}
## MC permuation test (p-values):
##
                  Α
                        В
                              C
                                    D
                                          E F
                                                  G
                                                         Η
## Airplane
              0.026 1.000 1.000 0.402 0.625 1 0.234 0.005
## Bachelor
              1.000 0.454 1.000 1.000 0.856 1 0.455 0.628
## BarkingFox 1.000 0.207 0.067 1.000 0.703 1 1.000 0.185
## Bob
              1.000 1.000 1.000 1.000 0.012 1 1.000 0.266
## Cache
              1.000 0.398 0.143 1.000 1.000 1 1.000 0.627
              1.000 1.000 1.000 1.000 0.093 1 0.524 0.441
## Egg
## Frog
              1.000 1.000 0.070 1.000 0.461 1 1.000 0.182
## GentianL
              1.000 0.064 1.000 0.056 0.652 1 0.672 0.159
              1.000 0.161 1.000 1.000 1.000 1 0.308 0.065
## ParagonL
## Pothole
              1.000 1.000 1.000 1.000 0.520 1 0.503 1.000
## ShipIsland 1.000 0.609 1.000 0.681 0.116 1 0.549 0.454
## Skyhigh
              1.000 0.341 0.163 0.118 0.137 1 0.073 0.041
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))</pre>
                                 # Type this line again to see results table
Prop.loci.out.of.HWE
          Chisq
## A 0.0000000 0.08333333
## B 0.0000000 0.0000000
## C 0.16666667 0.00000000
## D 0.16666667 0.00000000
## E 0.16666667 0.08333333
## F 0.0000000 0.0000000
## G 0.0000000 0.00000000
## H 0.08333333 0.16666667
```

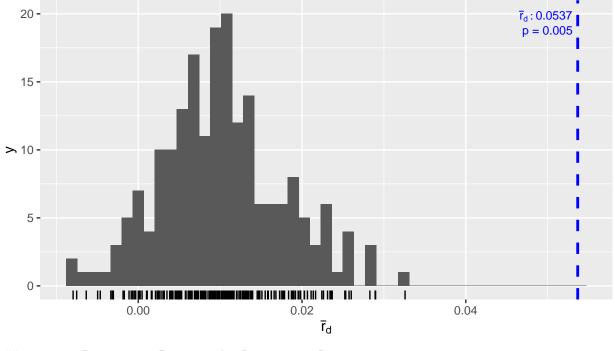
```
Prop.pops.out.of.HWE <- data.frame(Chisq=apply(HWE.test.chisq<alpha, 1, mean),
           MC=apply(HWE.test.MC<alpha, 1, mean))</pre>
Prop.pops.out.of.HWE
##
              Chisq
## Airplane
              0.000 0.250
## Bachelor
              0.000 0.000
## BarkingFox 0.125 0.000
## Bob
              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"),</pre>
                    nrow=nrow(HWE.test.chisq))
MC.fdr <- matrix(p.adjust(HWE.test.MC, method="fdr"),</pre>
                    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),</pre>
           Chisq.fdr=apply(Chisq.fdr<alpha, 1, mean),</pre>
           MC.fdr=apply(MC.fdr<alpha, 1, mean))</pre>
Prop.pops.out.of.HWE
##
                       MC Chisq.fdr MC.fdr
              Chisq
              0.000 0.250
                               0.000
## Airplane
## Bachelor
              0.000 0.000
                               0.000
## BarkingFox 0.125 0.000
                               0.000
                                          0
                               0.000
## Bob
              0.125 0.125
## Cache
              0.125 0.000
                               0.000
                                          0
              0.000 0.000
                               0.000
## Egg
## Frog
              0.000 0.000
                               0.000
## GentianL
              0.125 0.000
                               0.000
                                          0
## ParagonL
                                          0
              0.125 0.000
                               0.125
## Pothole
              0.000 0.000
                               0.000
                                          0
## ShipIsland 0.125 0.000
                               0.000
                                          0
## Skyhigh
              0.125 0.125
                               0.125
```

Linkage Disequilibrium

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

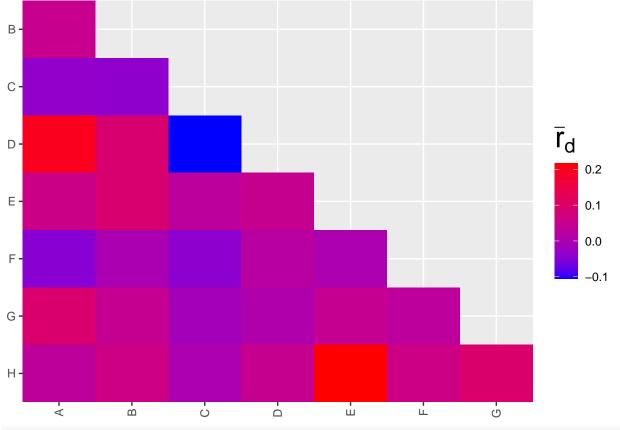
Population:Total N: 181

Data: Frogs.genind Permutations: 199



rbarDp.Ia ## 0.33744318 0.00500000 0.05366542 0.00500000

LD.pair <- poppr::pair.ia(Frogs.genind)</pre>



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
```

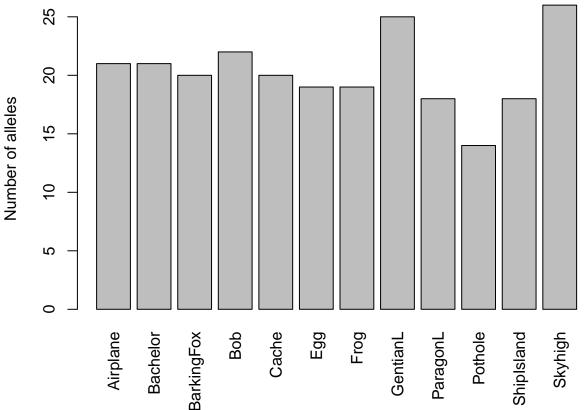
```
## E:H 0.2153 0.2159
## F:G 0.0167 0.0299
## F:H 0.0296 0.0606
## G:H 0.0942 0.0953
```

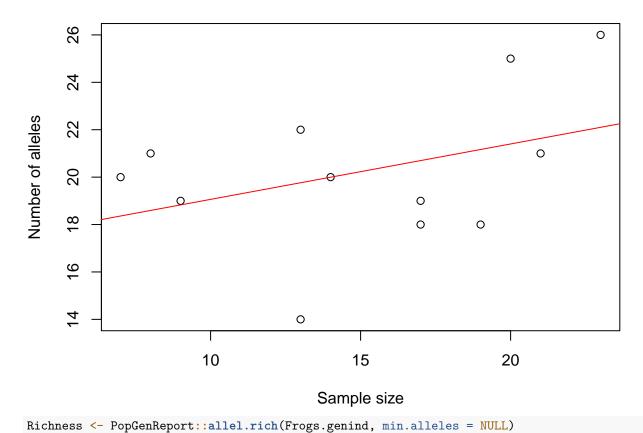
Null Alleles

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

Genetic Diversity

allelic richness



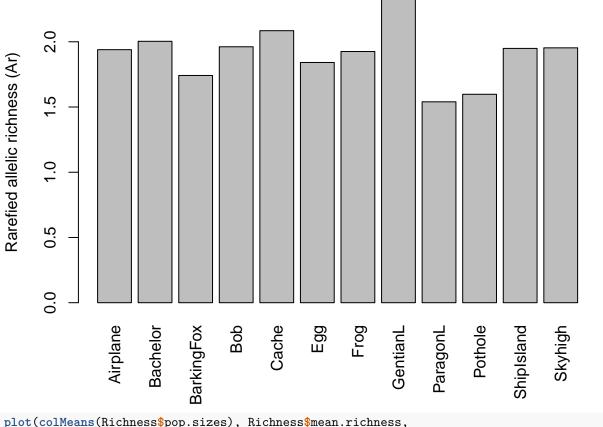


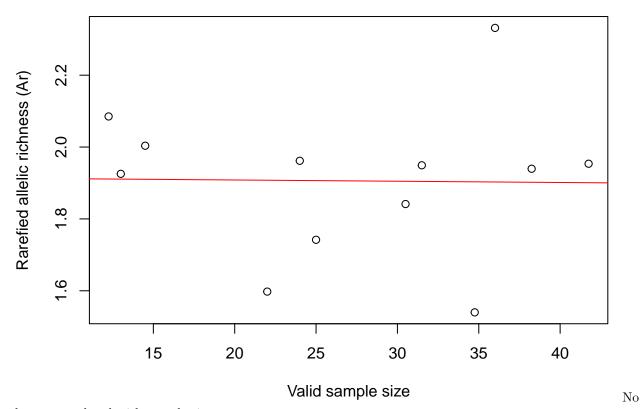
```
Richness$alleles.sampled

## [1] 6

par(mar=c(5.5, 4.5.1.1))
```

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





longer correlated with sample size.

6.4 Exercise

Task: Drop offspring (seeds, OffID==1) from dataset pulsatilla_genotypes.csv, check for HWE by site and locus and calculate Hexp for each site.

Drop offspring

Using adegenet and base R

```
library(adegenet)
# 1. CSV file "./downloads/pulsatilla_genotypes.csv" --> data frame
# with base R function read.csv()
Flr <- read.csv("./downloads/pulsatilla_genotypes.csv", header=TRUE)
# 2. Select only adults with base R indexing of data frame
# rows where OffID==0, all columns
Flr <- Flr[Flr$0ffID==0,]</pre>
# 3. Combine columns with base R function paste()
Flr <- data.frame(Flr[,1:5],loc1 = paste(Flr[,6], Flr[,7], sep=":"),</pre>
                            loc2 = paste(Flr[,8], Flr[,9], sep=":"),
                            loc3 = paste(Flr[,10], Flr[,11], sep=":"),
                            loc4 = paste(Flr[,12], Flr[,13], sep=":"),
                            loc5 = paste(Flr[,14], Flr[,15], sep=":"),
                            loc6 = paste(Flr[,16], Flr[,17], sep=":"),
                            loc7 = paste(Flr[,18], Flr[,19], sep=":"))
# 4. Create genind object with "adegenet" function df2genind()
# using NA.char = "NA"
Flr.genind <- df2genind(X=Flr[,c(6:12)], sep=":", ncode=NULL, ind.names= Flr$ID, loc.names=names(Flr[,c
# 5. Check genind object
Flr.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
##
      Oploidy: ploidy of each individual (range: 2-2)
##
      @type: codom
      @call: df2genind(X = Flr[, c(6:12)], sep = ":", ncode = NULL, ind.names = Flr$ID,
##
       loc.names = names(Flr[, c(6:12)]), pop = Flr$Population,
##
##
       NA.char = "NA", ploidy = 2, type = "codom", strata = NULL,
       hierarchy = NULL)
##
##
   // Optional content
##
##
      Opop: population of each individual (group size range: 14-56)
```

```
## // 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
```

Count the number of individuals in each pop

```
table(Flr$Population)

##

## A03 A21 A25 A26 A41 A45 G05a

## 42 21 56 21 14 22 45
```

With Gstudio this time

```
library(gstudio)
## Warning: replacing previous import 'dplyr::union' by 'raster::union' when
## loading 'gstudio'
## Warning: replacing previous import 'dplyr::intersect' by 'raster::intersect'
## when loading 'gstudio'
## Warning: replacing previous import 'dplyr::select' by 'raster::select' when
## loading 'gstudio'
## Registered S3 method overwritten by 'gstudio':
##
     method
                 from
##
     print.locus genetics
##
## Attaching package: 'gstudio'
## The following object is masked from 'package:pegas':
##
##
       Fst
## The following objects are masked from 'package:adegenet':
##
       alleles, ploidy
library(adegenet)
# 1. CSV file "./downloads/pulsatilla_qenotypes.csv" --> data frame
# with "gstudio" function read_population()
g.Flr <- read_population("./downloads/pulsatilla_genotypes.csv",</pre>
                       type = "column",locus.columns = c(6:19))
# 2. Select only adults with base R indexing of data frame
# rows where OffID==0, all columns
```

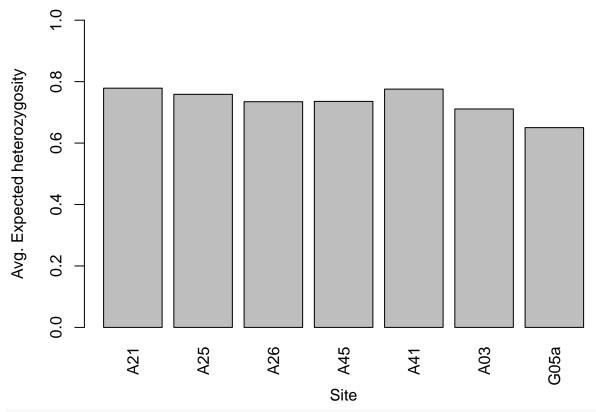
```
g.Flr <- g.Flr[g.Flr$0ffID==0,]
# 3. Nothing to do here
# 4. Create genind object with "adegenet" function df2genind()
# using NA.char = ""
g.Flr.genind <- df2genind(X=g.Flr[,c(6:12)], sep=":", ncode=NULL, ind.names=g.Flr$ID, loc.names=NULL, p
# 5. Check genind object
g.Flr.genind
## /// GENIND OBJECT ///////
##
   // 221 individuals; 7 loci; 105 alleles; size: 129.8 Kb
##
##
   // Basic content
##
      Otab: 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
##
##
      Oploidy: ploidy of each individual (range: 2-2)
##
      @type: codom
     @call: df2genind(X = g.Flr[, c(6:12)], sep = ":", ncode = NULL, ind.names = g.Flr$ID,
##
##
      loc.names = NULL, pop = g.Flr$Population, NA.char = "", ploidy = 2,
      type = "codom", strata = NULL, hierarchy = NULL)
##
##
##
   // Optional content
##
      Opop: population of each individual (group size range: 14-56)
summary(g.Flr.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
Check for HWE by site and locus and calculate Hexp for each site
Test for HWE with pegas by site
# Chi-squared test: p-value
HWE.test <- data.frame(sapply(seppop(g.Flr.genind),</pre>
                              function(ls) pegas::hw.test(ls, B=0)[,3]))
HWE.test.chisq <- t(data.matrix(HWE.test))</pre>
{cat("Chi-squared test (p-values):", "\n")
round(HWE.test.chisq,3)}
## Chi-squared test (p-values):
        loc1_a loc2_a loc3_a loc4_a loc5_a loc6_a loc7_a
        0.296  0.730  0.555  0.457  0.068  0.858  0.530
## A21
## A25
        0.069 0.992 0.404 0.076 0.018 0.930 0.587
```

```
## A26
         0.000 0.576 0.998 0.508 0.846 0.180 0.354
## A45
        0.983 0.828 0.442 0.178 0.359 0.120 0.244
         0.586  0.733  0.179  0.038  0.468  0.956  0.717
## A41
## A03
         1.000 0.193 0.040 0.047 0.000 0.970 0.490
## G05a 0.436 0.672 0.121 0.954 0.394 0.973 0.104
# Monte Carlo: p-value
HWE.test <- data.frame(sapply(seppop(g.Flr.genind),</pre>
                              function(ls) pegas::hw.test(ls, B=1000)[,4]))
HWE.test.MC <- t(data.matrix(HWE.test))</pre>
{cat("MC permuation test (p-values):", "\n")
round(HWE.test.MC,3)}
## MC permuation test (p-values):
        loc1_a loc2_a loc3_a loc4_a loc5_a loc6_a loc7_a
         0.243  0.647  0.959  0.332  0.116  0.719  0.418
## A21
## A25
        0.205 1.000 0.364 0.101 0.599 0.570 0.317
## A26
        0.089 0.728 0.956 0.369 0.975 0.254 0.145
         0.781 0.503 0.506 0.048 0.253 0.007 0.661
## A45
## A41
         0.352  0.809  0.061  0.020  0.630  0.925  0.816
## A03
        0.867 0.027 0.258 0.063 0.005 0.667 0.393
## G05a 0.100 0.541 0.011 0.968 0.132 0.381 0.150
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))</pre>
Prop.loci.out.of.HWE
                                 # Type this line again to see results table
##
              Chisq
## loc1_a 0.1428571 0.0000000
## loc2_a 0.0000000 0.1428571
## loc3_a 0.1428571 0.1428571
## loc4_a 0.2857143 0.2857143
## loc5_a 0.2857143 0.1428571
## loc6_a 0.0000000 0.1428571
## loc7_a 0.0000000 0.0000000
Prop.pops.out.of.HWE <- data.frame(Chisq=apply(HWE.test.chisq<alpha, 1, mean),
           MC=apply(HWE.test.MC<alpha, 1, mean))</pre>
Prop.pops.out.of.HWE
##
            Chisa
## A21 0.0000000 0.0000000
## A25 0.1428571 0.0000000
## A26 0.1428571 0.0000000
## A45 0.0000000 0.2857143
## A41 0.1428571 0.1428571
## A03 0.4285714 0.2857143
## G05a 0.0000000 0.1428571
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"),</pre>
                    nrow=nrow(HWE.test.MC))
Prop.pops.out.of.HWE <- data.frame(Chisq=apply(HWE.test.chisq<alpha, 1, mean),
```

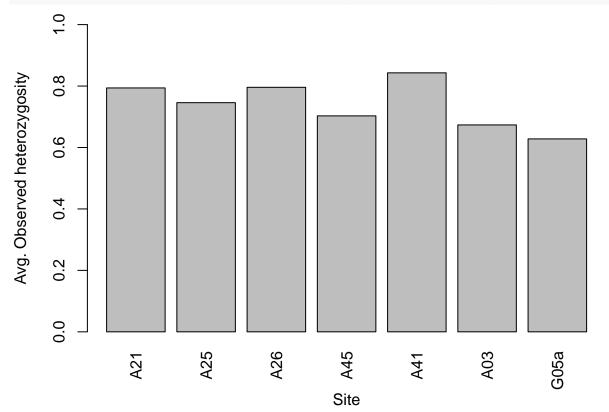
```
MC=apply(HWE.test.MC<alpha, 1, mean),</pre>
           Chisq.fdr=apply(Chisq.fdr<alpha, 1, mean),
           MC.fdr=apply(MC.fdr<alpha, 1, mean))</pre>
Prop.pops.out.of.HWE
##
            Chisq
                         MC Chisq.fdr MC.fdr
## A21 0.0000000 0.0000000 0.0000000
## A25 0.1428571 0.0000000 0.0000000
                                            0
## A26 0.1428571 0.0000000 0.1428571
                                            0
## A45 0.0000000 0.2857143 0.0000000
                                            0
## A41 0.1428571 0.1428571 0.0000000
                                            0
## A03 0.4285714 0.2857143 0.1428571
                                            0
## G05a 0.0000000 0.1428571 0.0000000
                                            0
Hexp for each site
Hobs <- t(sapply(seppop(g.Flr.genind), function(ls) summary(ls)$Hobs))</pre>
Hexp <- t(sapply(seppop(g.Flr.genind), function(ls) summary(ls)$Hexp))</pre>
{cat("Expected heterozygosity (Hexp):", "\n")
round(Hexp, 2)}
## Expected heterozygosity (Hexp):
```

```
##
        loc1_a loc2_a loc3_a loc4_a loc5_a loc6_a loc7_a
## A21
          0.82
                 0.59
                         0.92
                                0.74
                                       0.81
                                               0.73
                                                      0.83
## A25
          0.87
                 0.59
                         0.87
                                0.70
                                       0.64
                                               0.77
                                                      0.86
## A26
          0.56
                 0.65
                         0.78
                                0.81
                                       0.76
                                               0.80
                                                      0.78
## A45
          0.78
                 0.56
                         0.86
                                0.67
                                       0.73
                                               0.74
                                                      0.80
          0.76
                         0.77
                                       0.82
                                               0.77
                                                      0.83
## A41
                 0.68
                                0.81
## A03
          0.67
                 0.60
                         0.89
                                0.58
                                       0.85
                                               0.67
                                                      0.72
## G05a
          0.85
                 0.33
                                0.75
                                       0.82
                                               0.50
                         0.83
                                                      0.48
```

```
par(mar=c(5.5, 4.5, 1, 1))
Hobs.pop <- apply(Hobs, MARGIN = 1, FUN = mean)
Hexp.pop <- apply(Hexp, 1, mean)
barplot(Hexp.pop, ylim=c(0,1), las=3, ylab="Avg. Expected heterozygosity", xlab = "Site")</pre>
```



barplot(Hobs.pop, ylim=c(0,1), las=3, ylab="Avg. Observed heterozygosity", xlab = "Site")



Data frame of summary statistics

```
Sum <- summary(g.Flr.genind)</pre>
H.pop <- data.frame(Pop = names(Hobs.pop),</pre>
                     n = Sum[2] n.by.pop,
                                Hobs = Hobs.pop,
                                Hexp = Hexp.pop)
{\tt H.pop}
##
                      Hobs
                                 Hexp
         Pop n
         A21 21 0.7938776 0.7787476
## A21
## A25
         A25 56 0.7459098 0.7587271
## A26
         A26 21 0.7959184 0.7346939
## A45
        A45 22 0.7029375 0.7356625
## A41
         A41 14 0.8430141 0.7756482
## A03
        A03 42 0.6734694 0.7110058
## G05a G05a 45 0.6279942 0.6503175
Save "H.pop" for later in output file (eval = FALSE so that it doesn't do this every time I knit)
saveRDS(H.pop, file = "./output/H.pop.rds")
```

Question: Which site had the lowest expected heterozygosity?

Site G05a had the lowest average expected heterozygosity, at 0.6503175.