RADseq Usnea

December 5, 2018

```
In [2]: ### Copying data files
        #system("cp /home/fgrewe/Usnea/Usnea_RAD1234/6-pyrad7/outfiles/c90d6m4p3.vcf .")
        #system("cp /home/fgrewe/Usnea/Usnea_RAD1234/9-DAPC/Usnea.pop .")
        system("~/bin/vcftools --vcf c90d6m4p3.vcf --max-missing 0.5 --maf 0.05 --out Usnea.vcft
```

1. ” 2. ’VCFtools - 0.1.15’ 3. ’(C) Adam Auton and Anthony Marcketta 2009’ 4. ” 5. ’Parameters as interpreted:’ 6. ’\t–vcf c90d6m4p3.vcf’ 7. ’\t–maf 0.05’ 8. ’\t–max-missing 0.5’ 9. ’\t–out Usnea.vcftools.vcf’ 10. ’\t–recode’ 11. ” 12. ’Eighth Header entry should be INFO: INFO ’ 13. ’After filtering, kept 101 out of 101 Individuals’ 14. ’Outputting VCF file...’ 15. ’After filtering, kept 4146 out of a possible 18069 Sites’ 16. ’Run Time = 0.00 seconds’

```
In [3]: ### Required packages
        library(vcfR)
        library(adegenet)
        library(hierfstat)
        library(qqman)
        library(mmod)
```

```
   *****       ***   vcfR   ***       *****
   This is vcfR 1.6.0
     browseVignettes('vcfR') # Documentation
     citation('vcfR') # Citation
   *****       *****      *****       *****

Loading required package: ade4

   /// adegenet 2.1.0 is loaded ////////////

   > overview: '?adegenet'
   > tutorials/doc/questions: 'adegenetWeb()'
   > bug reports/feature requests: adegenetIssues()



Attaching package: hierfstat

The following object is masked from package:adegenet:
```

```
    read.fstat


For example usage please run: vignette('qqman')

Citation appreciated but not required:
Turner, S.D. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. bio
```

```
In [4]: ### Loading vcf file into R genind object
        vcf <- read.vcfR("Usnea.vcftools.vcf.recode.vcf")
        data.genlight <- vcfR2genlight(vcf, n.cores = 10)
        pop.file <- read.table('Usnea.pop', header=F)
        pop(data.genlight) <- pop.file[,2]
        data.genind <- df2genind(as.data.frame(data.genlight), pop=pop(data.genlight), ploidy=1,
        data.genind
```

```
Scanning file to determine attributes.
File attributes:
  meta lines: 11
  header line: 12
  variant count: 4146
  column count: 110
Meta line 11 read in.
All meta lines processed.
gt matrix initialized.
Character matrix gt created.
  Character matrix gt rows: 4146
  Character matrix gt cols: 110
  skip: 0
  nrows: 4146
  row_num: 0
Processed variant: 4146
All variants processed


Warning message in vcfR2genlight(vcf, n.cores = 10):
Found 14 loci with more than two alleles.
Objects of class genlight only support loci with two alleles.
14 loci will be omitted from the genlight object.

/// GENIND OBJECT /////////

 // 101 individuals; 4,132 loci; 8,264 alleles; size: 5.1 Mb

 // Basic content
   @tab:  101 x 8264 matrix of allele counts
```

```
   @loc.n.all: number of alleles per locus (range: 2-2)
   @loc.fac: locus factor for the 8264 columns of @tab
   @all.names: list of allele names for each locus
   @ploidy: ploidy of each individual  (range: 1-1)
   @type:  codom
   @call: df2genind(X = as.data.frame(data.genlight), ind.names = indNames(data.genlight),
    loc.names = locNames(data.genlight), pop = pop(data.genlight),
    ploidy = 1)

 // Optional content
   @pop: population of each individual (group size range: 45-56)
```

In [5]:
```
### Pairwise Fst
pwD <- pairwise_D(data.genind)
pwGstN <- pairwise_Gst_Nei(data.genind)
pwGstH <- pairwise_Gst_Hedrick(data.genind)
pwGstN
pwGstH
pwD
```

```
Warning message in HsHt(g):
Need at least two population to calculate differentiationWarning message in HsHt(g):
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Need at least two population to calculate differentiation

          11
21 0.6975744


          11
21 0.9285895


          11
21 0.5991588
```

In [6]:
```
obj_seplocus <- seploc(data.genind);
#Calculation of Gst Nei
obj_pwGst <- lapply(obj_seplocus, pairwise_Gst_Nei);
obj_pairwiseGstnum <- sapply(obj_pwGst, as.numeric);
obj_pairwiseGstnum[obj_pairwiseGstnum<0] <- 0;
#Calculation of Gst Hedrick
obj_pwGstH <- lapply(obj_seplocus, pairwise_Gst_Hedrick);
obj_pairwiseGstHnum <- sapply(obj_pwGstH, as.numeric);
obj_pairwiseGstHnum[obj_pairwiseGstHnum<0] <- 0;
#Calculation of D
obj_pwD <- lapply(obj_seplocus, pairwise_D);
obj_pairwiseDnum <- sapply(obj_pwD, as.numeric);
obj_pairwiseDnum[obj_pairwiseDnum<0] <- 0;
```

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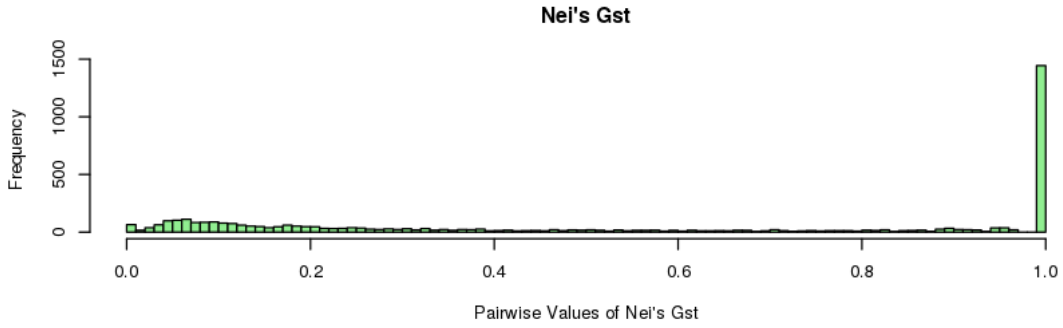
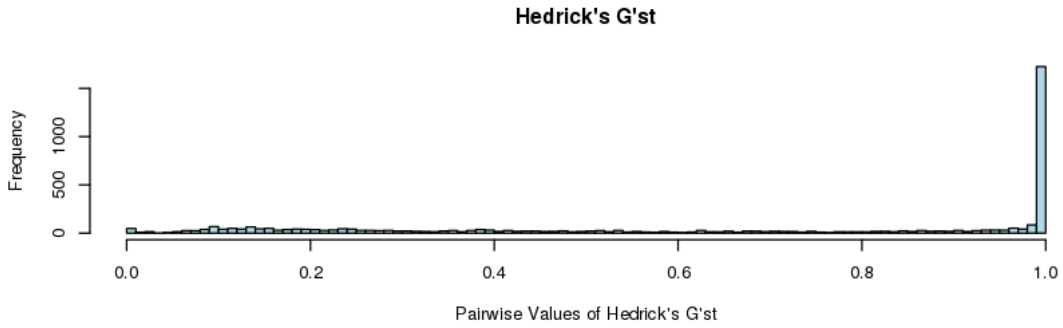
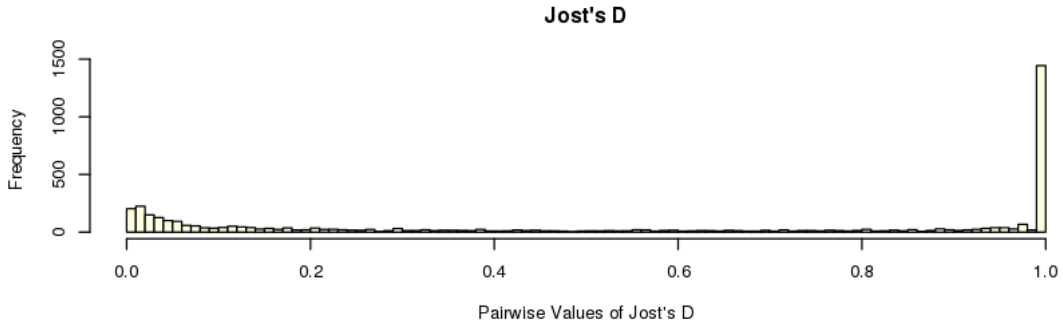
Need at least two population to calculate differentiationWarning message in HsHt(g):
Need at least two population to calculate differentiation
```

In [7]:
```
#Priting 3 figures arranged in 3 rows and 1 column
par(mfrow=c(3,1))
hist(obj_pairwiseGstnum, breaks=100, main="Nei's Gst", xlab="Pairwise Values of Nei's Gs
hist(obj_pairwiseGstHnum, breaks=100, main="Hedrick's G'st", xlab="Pairwise Values of He
hist(obj_pairwiseDnum, breaks=100, main="Jost's D", xlab="Pairwise Values of Jost's D",
```

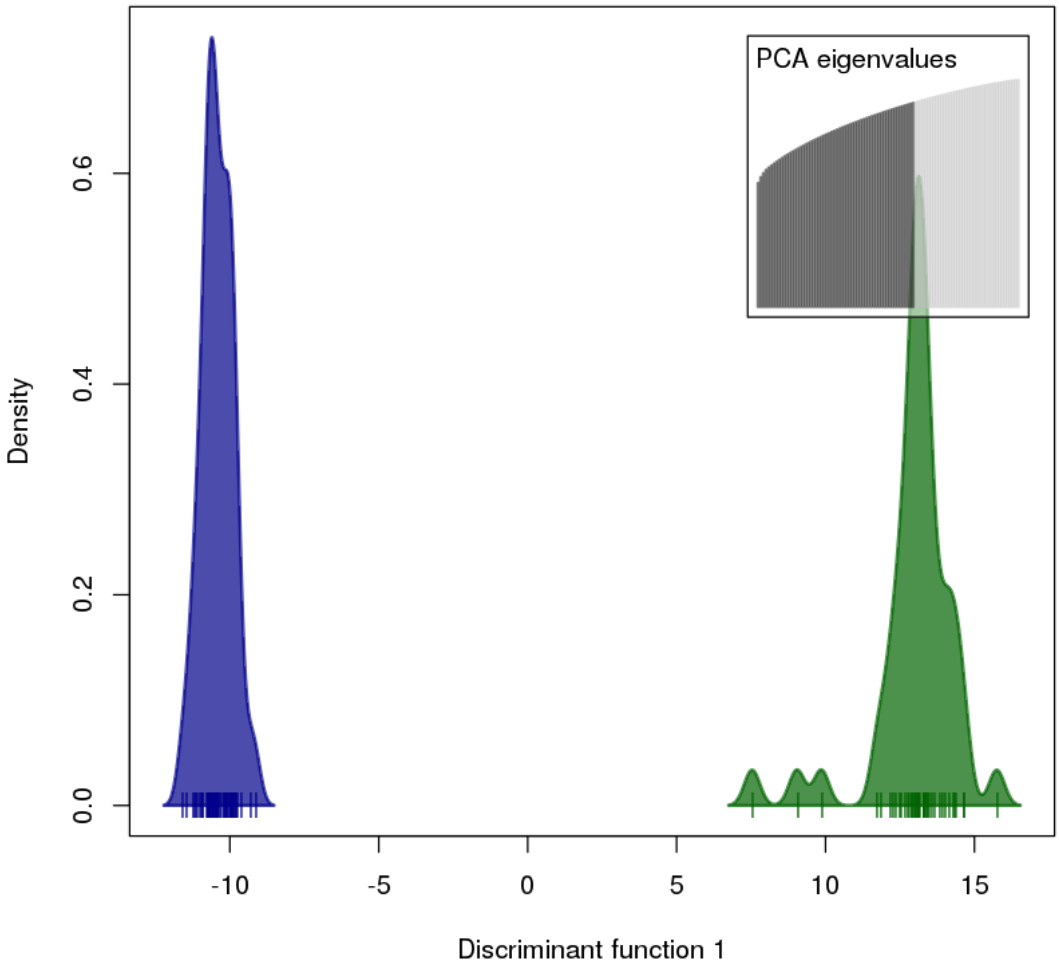


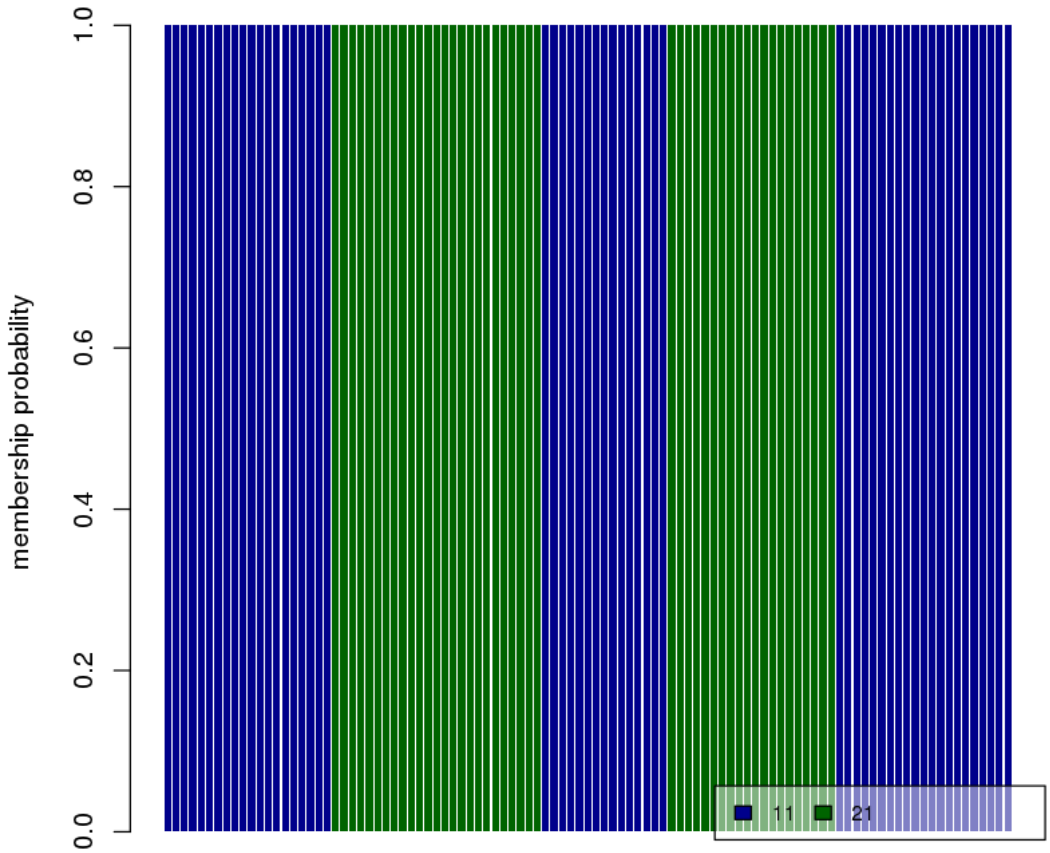




In [8]:
```
### DAPC
dapc1 <- dapc(data.genind, n.pca = 60, n.da = 2)
mycol <- c('darkblue','darkgreen')
```

In [9]:
```
#print DAPC
scatter(dapc1, col = mycol, scree.pca = TRUE, posi.pca = "topright")
compoplot(dapc1, posi='bottomright', ncol = 1, col = mycol, cleg = 0.8, cex.names = 0.4)
```


PCA eigenvalues
Density
0.0
0.2
0.4
0.6
-10
-5
0
5
10
15
Discriminant function 1



In [10]:
```
# 11 = Usena antarctica
# 21 = Usena aurantiaco-atra
```

In [11]:
```
### save all in pdf
pdf("usnea_popgen.pdf")
par(mfrow=c(3,1))
hist(obj_pairwiseGstnum, breaks=100, main="Nei's Gst", xlab="Pairwise Values of Nei's G
hist(obj_pairwiseGstHnum, breaks=100, main="Hedrick's G'st", xlab="Pairwise Values of H
hist(obj_pairwiseDnum, breaks=100, main="Jost's D", xlab="Pairwise Values of Jost's D",
par(mfrow=c(2,1))
scatter(dapc1, col = mycol, scree.pca = TRUE, posi.pca = "topright")
compoplot(dapc1, posi='bottomright', ncol = 1, col = mycol, cleg = 0.8, cex.names = 0.4
dev.off()
```

png: 2