Redundancy analysis Outlier SNPs 6 pops

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2023-03-22

Seascape Redundancy Analysis

This code follows that documented by Tom Jenkins.

Prepare genetic data for redundancy analysis.

Notes before execution:

1. Make sure all required R packages are installed.
2. Set working directory to the location of this R script.

```
# Load packages
library(adegenet)
```

```
## Loading required package: ade4
```

```
##
##    /// adegenet 2.1.6 is loaded ////////////
##
##    > overview: '?adegenet'
##    > tutorials/doc/questions: 'adegenetWeb()'
##    > bug reports/feature requests: adegenetIssues()
```

```
library(poppr)
```

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

```
## This is poppr version 2.8.6.99.18. To get started, type package?poppr
## OMP parallel support: available
##
## This version of poppr is under development.
## If you find any bugs, please report them at https://github.com/grunwaldlab/poppr/issues
```

```
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
```

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

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

```
library(reshape2)
library(ggplot2)
library(vcfR)
```

```
##
##    *****       ***   vcfR   ***       *****
##    This is vcfR 1.12.0
##      browseVignettes('vcfR') # Documentation
##      citation('vcfR') # Citation
##    *****       *****      *****       *****
```

VCF file and strata file (environmental and population info) are saved in PATH: /home/azyck/NB_capture_both/NB_ddhaplo/

```
# Preparinng the data
my_vcf_out6_nolfmm <- read.vcfR("6pops_outlierloci_nolfmm.recode.vcf")
```

```
## Scanning file to determine attributes.
## File attributes:
##   meta lines: 77
##   header_line: 78
##   variant count: 78
##   column count: 69
## Meta line 77 read in.
## All meta lines processed.
## gt matrix initialized.
## Character matrix gt created.
##   Character matrix gt rows: 78
##   Character matrix gt cols: 69
##   skip: 0
##   nrows: 78
##   row_num: 0
## Processed variant: 78
## All variants processed
```

```
strata_out6_nolfmm <- read.table("strata_6pops", header=TRUE)
```

```
rad_out6_nolfmm.filt <- vcfR2genind(my_vcf_out6_nolfmm, strata = strata_out6_nolfmm, pop = c(rep("BAR",
```

```
rad_out6_nolfmm.filt
```

```
## /// GENIND OBJECT /////////
##
##  // 60 individuals; 78 loci; 156 alleles; size: 112.2 Kb
##
##  // Basic content
##    @tab:  60 x 156 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 2-2)
##    @loc.fac: locus factor for the 156 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 = t(x), sep = sep, pop = ..2, strata = ..1)
##
```

```
##  // Optional content
##    @pop: population of each individual (group size range: 10-10)
##    @strata: a data frame with 9 columns ( Individual, Population, Latitude., Longitude., SewageEfflu
```

```
# Explore data -no LFMM
rad_out6_nolfmm.filt
```

```
## /// GENIND OBJECT /////////
##
##  // 60 individuals; 78 loci; 156 alleles; size: 112.2 Kb
##
##  // Basic content
##    @tab:  60 x 156 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 2-2)
##    @loc.fac: locus factor for the 156 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 = t(x), sep = sep, pop = ..2, strata = ..1)
##
##  // Optional content
##    @pop: population of each individual (group size range: 10-10)
##    @strata: a data frame with 9 columns ( Individual, Population, Latitude., Longitude., SewageEfflu
```

```
nLoc(rad_out6_nolfmm.filt) # number of loci
```

```
## [1] 78
```

```
nPop(rad_out6_nolfmm.filt) # number of sites
```

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

```
nInd(rad_out6_nolfmm.filt) # number of individuals
```

```
## [1] 60
```

```
summary(rad_out6_nolfmm.filt$pop) # sample size
```

```
## BAR BIS  GB KIC MCD PVD
##  10  10  10  10  10  10
```

```
# Calculate allele frequencies for each site - no LFMM
allele_freqs_out6_nolfmm = data.frame(rraf(rad_out6_nolfmm.filt, by_pop = TRUE, correction = FALSE), ch
```

```
# Keep only the first of the two alleles for each SNP (since p=1-q). - no LFMM
allele_freqs_out6_nolfmm = allele_freqs_out6_nolfmm[, seq(1, dim(allele_freqs_out6_nolfmm)[2], 2)]
```

```
# Export allele frequencies - no LFMM
write.csv(allele_freqs_out6_nolfmm, file = "all_allele_freqs_out6_nolfmm.csv", row.names = TRUE)
```

#————# # # Calculate minor allele frequencies # #————#

```
# Separate genind object by site - no LFMM
site_list_out6_nolfmm = seppop(rad_out6_nolfmm.filt)
names(site_list_out6_nolfmm)
```

```
## [1] "BAR" "BIS" "GB"  "KIC" "MCD" "PVD"
```

```
# Calculate the minor allele frequency for each site - no LFMM
maf_list_out6_nolfmm = lapply(site_list_out6_nolfmm, FUN = minorAllele)
```

```
# Convert list to dataframe - no LFMM
maf_out6_nolfmm = as.data.frame(maf_list_out6_nolfmm) %>% t() %>% as.data.frame()
```

```
# Export minor allele frequencies - no LFMM
write.csv(maf_out6_nolfmm, file = "minor_allele_freqs_out6_nolfmm.csv", row.names = TRUE)
```

Prepare environmental data for redundancy analysis.

Environmental variables:

- Sewage Effluent (PW stats)
- Mean temperature (deg C)
- Mean Salinity (psu)
- Mean pH
- Mean Dissolved Oxygen (mg/L)

Environmental data for each population is saved in a `strata_pop6` file that can be accessed here

```
# All environmental data was previously saved in strata file
strata_pop6 <- read.table("strata_6pops", header=TRUE)
strata_pop6
```

```
##    Individual Population Latitude. Longitude. SewageEffluent Temperature
## 1       BAR_1        BAR    41.741    -71.305      17.881340       22.08
## 2      BAR_10        BAR    41.741    -71.305      17.881340       22.08
## 3       BAR_2        BAR    41.741    -71.305      17.881340       22.08
## 4       BAR_3        BAR    41.741    -71.305      17.881340       22.08
## 5       BAR_4        BAR    41.741    -71.305      17.881340       22.08
## 6       BAR_5        BAR    41.741    -71.305      17.881340       22.08
## 7       BAR_6        BAR    41.741    -71.305      17.881340       22.08
## 8       BAR_7        BAR    41.741    -71.305      17.881340       22.08
## 9       BAR_8        BAR    41.741    -71.305      17.881340       22.08
## 10      BAR_9        BAR    41.741    -71.305      17.881340       22.08
## 11      BIS_1        BIS    41.545    -71.431       8.824636       21.39
## 12     BIS_10        BIS    41.545    -71.431       8.824636       21.39
## 13      BIS_2        BIS    41.545    -71.431       8.824636       21.39
## 14      BIS_3        BIS    41.545    -71.431       8.824636       21.39
## 15      BIS_4        BIS    41.545    -71.431       8.824636       21.39
## 16      BIS_5        BIS    41.545    -71.431       8.824636       21.39
## 17      BIS_6        BIS    41.545    -71.431       8.824636       21.39
## 18      BIS_7        BIS    41.545    -71.431       8.824636       21.39
## 19      BIS_8        BIS    41.545    -71.431       8.824636       21.39
## 20      BIS_9        BIS    41.545    -71.431       8.824636       21.39
## 21       GB_1         GB    41.654    -71.445      14.596049       22.27
## 22      GB_10         GB    41.654    -71.445      14.596049       22.27
## 23       GB_2         GB    41.654    -71.445      14.596049       22.27
## 24       GB_3         GB    41.654    -71.445      14.596049       22.27
## 25       GB_4         GB    41.654    -71.445      14.596049       22.27
## 26       GB_5         GB    41.654    -71.445      14.596049       22.27
## 27       GB_6         GB    41.654    -71.445      14.596049       22.27
## 28       GB_7         GB    41.654    -71.445      14.596049       22.27
## 29       GB_8         GB    41.654    -71.445      14.596049       22.27
## 30       GB_9         GB    41.654    -71.445      14.596049       22.27
## 31      KIC_1        KIC    41.698    -71.247      56.312594       21.50
## 32     KIC_10        KIC    41.698    -71.247      56.312594       21.50
```

```
## 33      KIC_2        KIC    41.698    -71.247      56.312594       21.50
## 34      KIC_3        KIC    41.698    -71.247      56.312594       21.50
## 35      KIC_4        KIC    41.698    -71.247      56.312594       21.50
## 36      KIC_5        KIC    41.698    -71.247      56.312594       21.50
## 37      KIC_6        KIC    41.698    -71.247      56.312594       21.50
## 38      KIC_7        KIC    41.698    -71.247      56.312594       21.50
## 39      KIC_8        KIC    41.698    -71.247      56.312594       21.50
## 40      KIC_9        KIC    41.698    -71.247      56.312594       21.50
## 41      MCD_1        MCD    41.547    -71.203      12.111228       22.24
## 42     MCD_10        MCD    41.547    -71.203      12.111228       22.24
## 43      MCD_2        MCD    41.547    -71.203      12.111228       22.24
## 44      MCD_3        MCD    41.547    -71.203      12.111228       22.24
## 45      MCD_4        MCD    41.547    -71.203      12.111228       22.24
## 46      MCD_5        MCD    41.547    -71.203      12.111228       22.24
## 47      MCD_6        MCD    41.547    -71.203      12.111228       22.24
## 48      MCD_7        MCD    41.547    -71.203      12.111228       22.24
## 49      MCD_8        MCD    41.547    -71.203      12.111228       22.24
## 50      MCD_9        MCD    41.547    -71.203      12.111228       22.24
## 51      PVD_1        PVD    41.816    -71.391      59.860038       15.80
## 52     PVD_10        PVD    41.816    -71.391      59.860038       15.80
## 53      PVD_2        PVD    41.816    -71.391      59.860038       15.80
## 54      PVD_3        PVD    41.816    -71.391      59.860038       15.80
## 55      PVD_4        PVD    41.816    -71.391      59.860038       15.80
## 56      PVD_5        PVD    41.816    -71.391      59.860038       15.80
## 57      PVD_6        PVD    41.816    -71.391      59.860038       15.80
## 58      PVD_7        PVD    41.816    -71.391      59.860038       15.80
## 59      PVD_8        PVD    41.816    -71.391      59.860038       15.80
## 60      PVD_9        PVD    41.816    -71.391      59.860038       15.80
##    Salinity   pH  DO.
## 1     29.08 7.69 5.37
## 2     29.08 7.69 5.37
## 3     29.08 7.69 5.37
## 4     29.08 7.69 5.37
## 5     29.08 7.69 5.37
## 6     29.08 7.69 5.37
## 7     29.08 7.69 5.37
## 8     29.08 7.69 5.37
## 9     29.08 7.69 5.37
## 10    29.08 7.69 5.37
## 11    27.32 7.94 7.05
## 12    27.32 7.94 7.05
## 13    27.32 7.94 7.05
## 14    27.32 7.94 7.05
## 15    27.32 7.94 7.05
## 16    27.32 7.94 7.05
## 17    27.32 7.94 7.05
## 18    27.32 7.94 7.05
## 19    27.32 7.94 7.05
## 20    27.32 7.94 7.05
## 21    18.82 7.67 4.57
## 22    18.82 7.67 4.57
## 23    18.82 7.67 4.57
## 24    18.82 7.67 4.57
## 25    18.82 7.67 4.57
```

```
## 26    18.82 7.67 4.57
## 27    18.82 7.67 4.57
## 28    18.82 7.67 4.57
## 29    18.82 7.67 4.57
## 30    18.82 7.67 4.57
## 31    28.31 7.84 6.07
## 32    28.31 7.84 6.07
## 33    28.31 7.84 6.07
## 34    28.31 7.84 6.07
## 35    28.31 7.84 6.07
## 36    28.31 7.84 6.07
## 37    28.31 7.84 6.07
## 38    28.31 7.84 6.07
## 39    28.31 7.84 6.07
## 40    28.31 7.84 6.07
## 41    20.68 7.69 8.76
## 42    20.68 7.69 8.76
## 43    20.68 7.69 8.76
## 44    20.68 7.69 8.76
## 45    20.68 7.69 8.76
## 46    20.68 7.69 8.76
## 47    20.68 7.69 8.76
## 48    20.68 7.69 8.76
## 49    20.68 7.69 8.76
## 50    20.68 7.69 8.76
## 51    18.82 7.68 4.90
## 52    18.82 7.68 4.90
## 53    18.82 7.68 4.90
## 54    18.82 7.68 4.90
## 55    18.82 7.68 4.90
## 56    18.82 7.68 4.90
## 57    18.82 7.68 4.90
## 58    18.82 7.68 4.90
## 59    18.82 7.68 4.90
## 60    18.82 7.68 4.90
```

```
# Export data as a csv file
write.csv(strata_pop6, file="environmental_data.csv", row.names = FALSE)
```

I also prepared spatial data for the redundancy analysis which is documented here.

Allele frequency, environmental, and spatial csv files are saved to your working directory and must be imported into the Rscript to run the redundancy analysis. Working directory `/home/azyck/NB_capture_both/NB_ddhaplo/PopSeaGenA`

Redundancy Analysis

```
# Load packages
library(psych)
```

```
##
## Attaching package: 'psych'
```

```
## The following objects are masked from 'package:ggplot2':
##
```

```
##     %+%, alpha
```

```
library(dplyr)
library(adespatial)
```

```
## Registered S3 methods overwritten by 'adegraphics':
##   method         from
##   biplot.dudi    ade4
##   kplot.foucart  ade4
##   kplot.mcoa     ade4
##   kplot.mfa      ade4
##   kplot.pta      ade4
##   kplot.sepan    ade4
##   kplot.statis   ade4
##   scatter.coa    ade4
##   scatter.dudi   ade4
##   scatter.nipals ade4
##   scatter.pco    ade4
##   score.acm      ade4
##   score.mix      ade4
##   score.pca      ade4
##   screeplot.dudi ade4
```

```
## Registered S3 method overwritten by 'spdep':
##   method   from
##   plot.mst ape
```

```
## Registered S3 methods overwritten by 'adespatial':
##   method             from
##   plot.multispati    adegraphics
##   print.multispati   ade4
##   summary.multispati ade4
```

```
##
## Attaching package: 'adespatial'
```

```
## The following objects are masked from 'package:adegenet':
##
##     chooseCN, global.rtest, local.rtest
```

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

```
library(vegan)
```

```
## Loading required package: permute
```

```
## Loading required package: lattice
```

```
## This is vegan 2.5-7
```

```
# Import genetic data - no LFMM
allele_freqs_out6_nolfmm = read.csv("all_allele_freqs_out6_nolfmm.csv", row.names = 1, check.names = FA
```

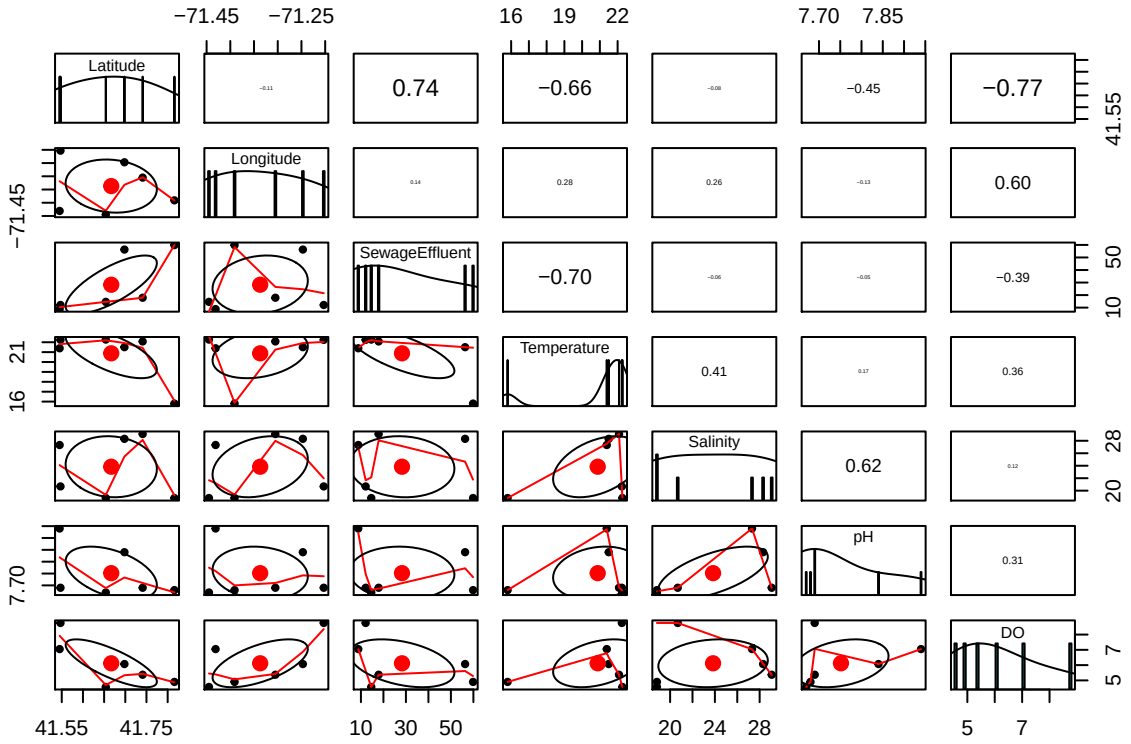
```
# Import environmental data
env.raw_out6 = read.csv("environmental_data_6pops.csv", row.names = 1)
```

```
# Import spatial data
dbmem.raw_out6 = read.csv("dbmems_6pops.csv")
```

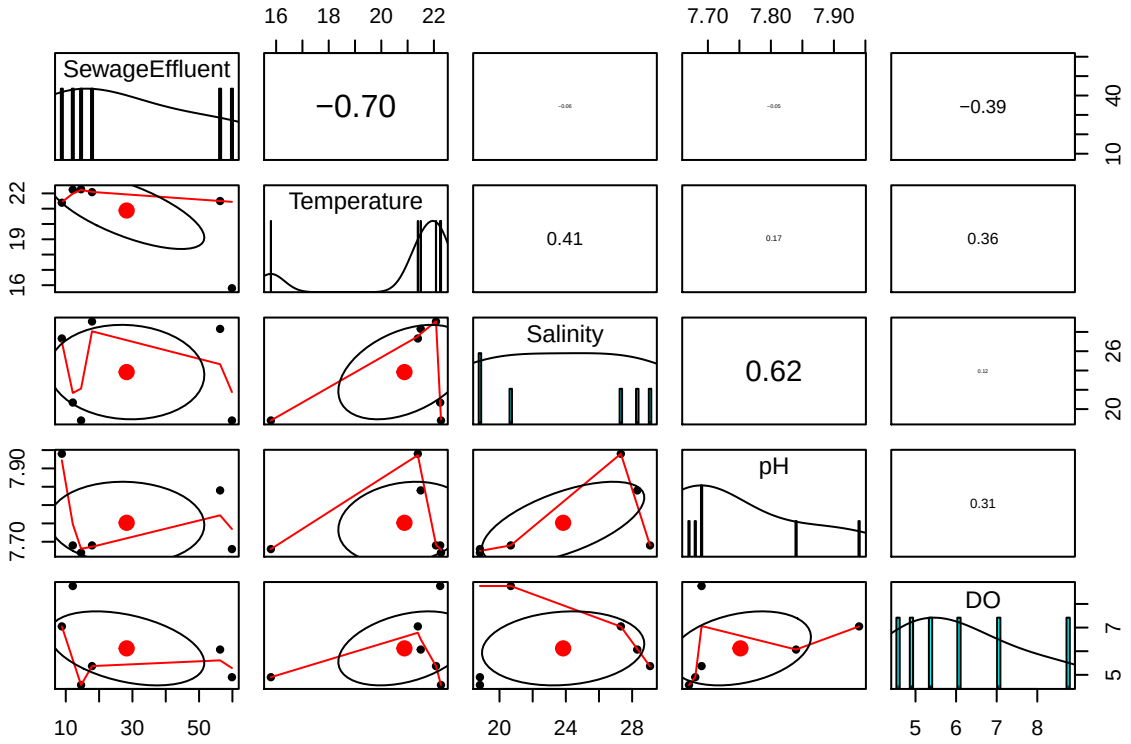
```
# Set seed
set.seed(123)
```

#————————# # # Multicollinearity checks # #————————#

```
# Plot and run correlation test on environmental variables
pairs.panels(env.raw_out6, scale = TRUE)
```




```
# Remove correlated variables
env.data_out6 = subset(env.raw_out6, select = -c(Latitude,Longitude))
pairs.panels(env.data_out6, scale = TRUE)
```




```
#standardize the environmental data
# Scale and center variables
env.z_out6 <- decostand(env.data_out6, method = "standardize")
# Variables are now centered around a mean of 0
round(apply(env.z_out6, 2, mean), 1)
```

```
## SewageEffluent    Temperature       Salinity             pH             DO
##              0              0              0              0              0
```

```
# and scaled to have a standard deviation of 1
apply(env.z_out6, 2, sd)
```

```
## SewageEffluent    Temperature       Salinity             pH             DO
##              1              1              1              1              1
```

```
# Combine all environmental variables and dbmems - no LFMM
env.full_out6_nolfmm = cbind(env.z_out6, dbmem.raw_out6)
str(env.full_out6_nolfmm)
```

```
## 'data.frame':    6 obs. of  7 variables:
##  $ SewageEffluent: num  -0.445 -0.834 -0.586 1.203 -0.693 ...
##  $ Temperature   : num  0.477 0.203 0.552 0.246 0.54 ...
##  $ Salinity      : num  1.07 0.711 -1.025 0.913 -0.645 ...
##  $ pH            : num  -0.551 1.682 -0.729 0.789 -0.551 ...
##  $ DO            : num  -0.4784 0.5932 -0.9886 -0.0319 1.6839 ...
##  $ MEM1          : num  -0.0648 1.2691 1.237 -1.211 -1.1771 ...
##  $ MEM2          : num  1.147 -0.976 -0.255 -0.338 -1.104 ...
```

#————# # # Identify significant variables # #————#

```
# Use forward selection to identify significant environmental variables with ordiR2step
# first we neeed to create a null model and then a full model
## Null model
RDA0_out6_nolfmm <- rda(allele_freqs_out6_nolfmm ~ 1,  env.full_out6_nolfmm)

## Full model
RDAfull_out6_nolfmm <- rda(allele_freqs_out6_nolfmm ~ SewageEffluent + Temperature + Salinity + pH + DO
```

```
adjR2.RDAfull_out6_nolfmm <- RsquareAdj(RDAfull_out6_nolfmm)$adj.r.squared
```

```
# Running ordiR2step to identify significant environmental variables in the model
mod <- ordiR2step(RDA0_out6_nolfmm, scope = formula(RDAfull_out6_nolfmm), Pin = 0.1, permutations = 100
```

```
## Step: R2.adj= 0
## Call: allele_freqs_out6_nolfmm ~ 1
##
##                   R2.adjusted
## + pH               0.19959263
## + SewageEffluent   0.02627450
## + Temperature      0.02319929
## <none>             0.00000000
## + DO              -0.10171167
## + Salinity        -0.13897256
```

```
## 'nperm' >= set of all permutations: complete enumeration.
```

```
## Set of permutations < 'minperm'. Generating entire set.
```

```
##      Df      AIC      F Pr(>F)
## + pH  1 -0.23182 2.2468 0.1444
```

Only pH is identified, but the p-value is 0.1444. I couldn't run ordiR2step with R2scope as true. This parameter uses adjusted R2 as the stopping criterion - only models with lower adjusted R2 than the scope are accepted.

#————————# # # Redundancy analysis # #————————#

```
# Perform RDA with all variables - no LFMM
rda1_out6_nolfmm = rda(allele_freqs_out6_nolfmm ~ pH, data = env.full_out6_nolfmm, scale = TRUE)
rda1_out6_nolfmm
```

```
## Call: rda(formula = allele_freqs_out6_nolfmm ~ pH, data =
## env.full_out6_nolfmm, scale = TRUE)
##
##               Inertia Proportion Rank
## Total         78.0000     1.0000
## Constrained   23.0552     0.2956    1
## Unconstrained 54.9448     0.7044    4
## Inertia is correlations
##
## Eigenvalues for constrained axes:
##   RDA1
## 23.055
##
## Eigenvalues for unconstrained axes:
##    PC1    PC2    PC3    PC4
## 27.252 14.073  8.449  5.171
```

```
# Model summaries - no LFMM
RsquareAdj(rda1_out6_nolfmm) # adjusted Rsquared
```

```
## $r.squared
## [1] 0.2955793
##
## $adj.r.squared
## [1] 0.1194741
```

```
vif.cca(rda1_out6_nolfmm) # variance inflation factor (<10 OK)
```

```
## pH
##  1
```

```
anova.cca(rda1_out6_nolfmm, permutations = 1000) # full model
```

```
## 'nperm' >= set of all permutations: complete enumeration.
```

```
## Set of permutations < 'minperm'. Generating entire set.
```

```
## Permutation test for rda under reduced model
## Permutation: free
## Number of permutations: 719
##
## Model: rda(formula = allele_freqs_out6_nolfmm ~ pH, data = env.full_out6_nolfmm, scale = TRUE)
##          Df Variance      F Pr(>F)
## Model     1   23.055 1.6784 0.1806
## Residual  4   54.945
```

```
anova.cca(rda1_out6_nolfmm, permutations = 1000, by="margin") # per variable
```

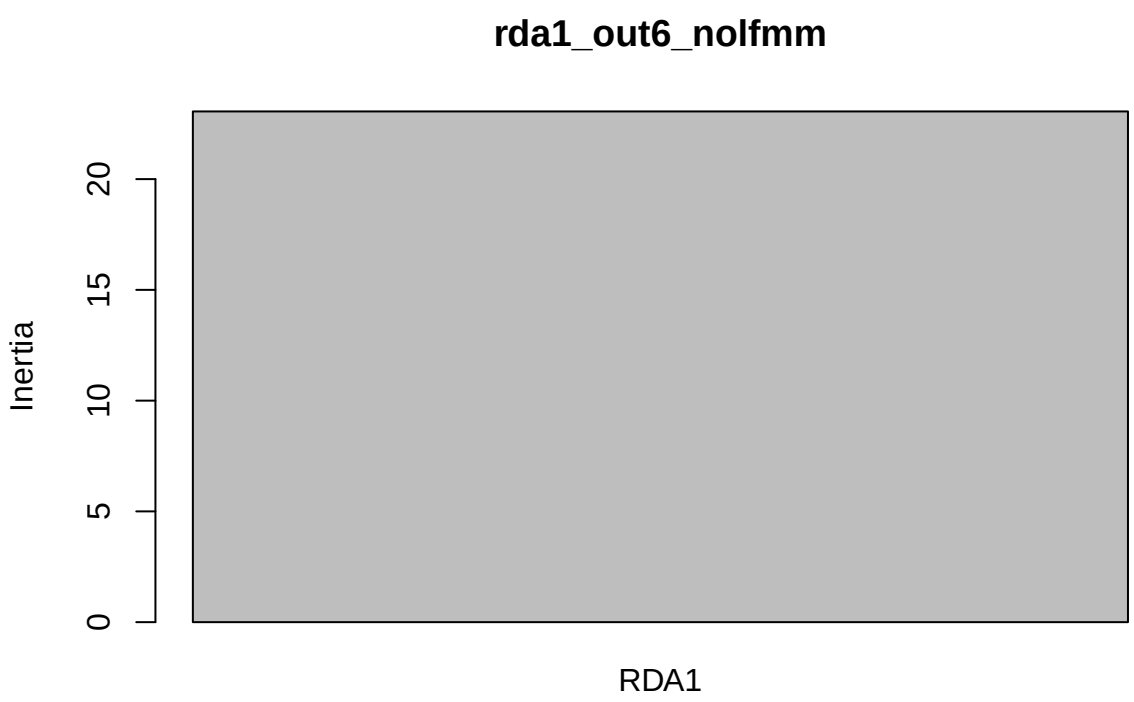
```
## 'nperm' >= set of all permutations: complete enumeration.
## Set of permutations < 'minperm'. Generating entire set.
```

```
## Permutation test for rda under NA model
## Marginal effects of terms
## Permutation: free
## Number of permutations: 719
##
## Model: rda(formula = allele_freqs_out6_nolfmm ~ pH, data = env.full_out6_nolfmm, scale = TRUE)
##          Df Variance      F Pr(>F)
## pH        1   23.055 1.6784 0.1806
## Residual  4   54.945
```

```
# Variance explained by each canonical axis - no LFMM
summary(eigenvals(rda1_out6_nolfmm, model = "constrained"))
```

```
## Importance of components:
##                         RDA1
## Eigenvalue            23.06
## Proportion Explained   1.00
## Cumulative Proportion  1.00
```

```
screeplot(rda1_out6_nolfmm)
```




```
# Create a dataframe to correctly colour regions - no LFMM
col_dframe_out6_nolfmm = data.frame("site" = rownames(allele_freqs_out6_nolfmm))
```

```
# Function to add regional labels to dataframe
addregion_6pops = function(x){
  # If pop label is present function will output the region
  if(x=="BAR") y = " Barrington "
  if(x=="BIS") y = " Bissel Cove "
  if(x=="GB") y = " Greenwich Bay "
  if(x=="KIC") y = " Kickemuit "
  if(x=="MCD") y = " Donovan Marsh "
  if(x=="PVD") y = " Providence "
  return(y)
}
```

```
# Add regional labels - no LFMM
col_dframe_out6_nolfmm$region = sapply(col_dframe_out6_nolfmm$site, addregion_6pops)
```

```
# Add factor levels
region_order_6pops = c(" Barrington "," Bissel Cove "," Greenwich Bay "," Kickemuit "," Donovan Marsh "
col_dframe_out6_nolfmm$region = factor(col_dframe_out6_nolfmm$region, levels = region_order_6pops)
```

```
# Create colour scheme
# blue=#000088, green=#7FC97F, orange=#FF7F00, red=#E31A1C, purple=#9A32CD, pink=#FF1493, yellow=#FFD70
cols_6pops = c("#7FC97F","#00008B","#FF7F00","#9A32CD","#FF1493","#00FFFF")
```

```
# Visualise results of RDA
png("rda_out6_nolfmm_pH.png", width = 8, height = 7, units = "in", res = 600)
```

```
plot(rda1_out6_nolfmm, type="n", scaling = 3)
# SITES
points(rda1_out6_nolfmm, display="sites", pch=21, scaling=3, cex=1.5, col="black",
       bg=cols_6pops[col_dframe_out6_nolfmm$region]) # sites
text(rda1_out6_nolfmm, display="sites", scaling = 3, col="black", font=2, pos=4)
# PREDICTORS
text(rda1_out6_nolfmm, display="bp", scaling=3, col="red1", cex=1, lwd=2)
# SNPS
# text(rda1, display="species", scaling = 3, col="blue", cex=0.7, pos=4) # SNPs
# LEGEND
#legend("bottomright", legend=levels(col_dframe_out6$region), bty="n", col="black",
      #pch=21, cex=1.2, pt.bg=cols_6pops)
# OTHER LABELS
adj.R2 = round(RsquareAdj(rda1_out6_nolfmm)$adj.r.squared, 3)
mtext(bquote(italic("R")^"2"~"= "~.(adj.R2)), side = 3, adj = 0.5)
dev.off()
```

```
## pdf
##   2
```

#————————# # # Partial redundancy analysis # #————————#

```
# Perform RDA while controlling for geographical location - no LFMM
pRDA_out6_nolfmm = rda(allele_freqs_out6_nolfmm ~ pH + Condition(MEM1+MEM2),
           data = env.full_out6_nolfmm, scale = TRUE)
pRDA_out6_nolfmm
```

```
## Call: rda(formula = allele_freqs_out6_nolfmm ~ pH + Condition(MEM1 +
## MEM2), data = env.full_out6_nolfmm, scale = TRUE)
##
##               Inertia Proportion Rank
## Total         78.0000     1.0000
## Conditional   18.0473     0.2314    2
## Constrained   25.7432     0.3300    1
## Unconstrained 34.2095     0.4386    2
## Inertia is correlations
##
## Eigenvalues for constrained axes:
##   RDA1
## 25.743
##
## Eigenvalues for unconstrained axes:
##    PC1    PC2
## 21.153 13.057
```

```
RsquareAdj(pRDA_out6_nolfmm) # adjusted Rsquared
```

```
## $r.squared
## [1] 0.3300411
##
## $adj.r.squared
## [1] 0.1845826
```

```
vif.cca(pRDA_out6_nolfmm) # variance inflation factor (<10 OK)
```

```
##     MEM1     MEM2       pH
## 1.045845 1.343501 1.389347
```

```
anova.cca(pRDA_out6_nolfmm, permutations = 1000) # full model
```

```
## 'nperm' >= set of all permutations: complete enumeration.
```

```
## Set of permutations < 'minperm'. Generating entire set.
```

```
## Permutation test for rda under reduced model
## Permutation: free
## Number of permutations: 719
##
## Model: rda(formula = allele_freqs_out6_nolfmm ~ pH + Condition(MEM1 + MEM2), data = env.full_out6_nol
##          Df Variance     F Pr(>F)
## Model     1   25.743 1.505 0.3611
## Residual  2   34.209
```

```
anova.cca(pRDA_out6_nolfmm, permutations = 1000, by = "margin") # per variable
```

```
## 'nperm' >= set of all permutations: complete enumeration.
## Set of permutations < 'minperm'. Generating entire set.
```

```
## Permutation test for rda under NA model
## Marginal effects of terms
## Permutation: free
## Number of permutations: 719
##
## Model: rda(formula = allele_freqs_out6_nolfmm ~ pH + Condition(MEM1 + MEM2), data = env.full_out6_nol
##          Df Variance     F Pr(>F)
## pH        1   25.743 1.505 0.3611
## Residual  2   34.209
```

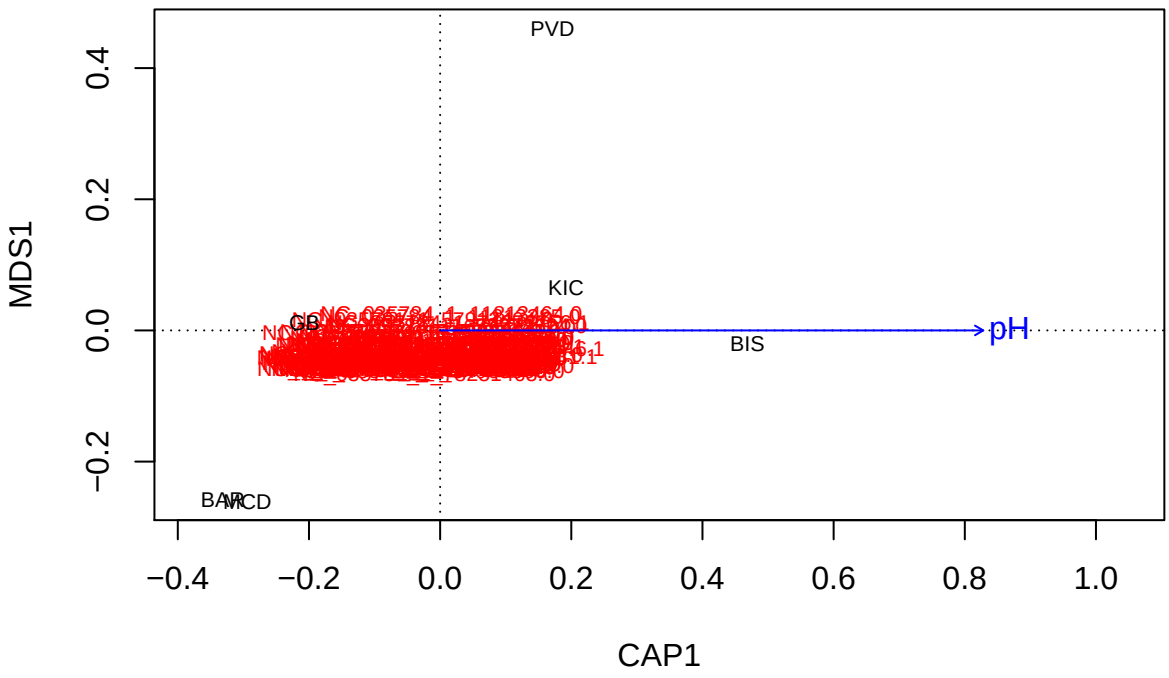
Trying out a distance based RDA (dbRDA)

```
rankindex(env.full_out6_nolfmm, allele_freqs_out6_nolfmm, indices = c("euc", "man", "gow","bra", "kul")
```

```
##         euc         man         gow         bra         kul
## -0.15714286 -0.06785714 -0.05000000 -0.07142857 -0.04285714
```

```
dbRDA_out6_noflmm = capscale(allele_freqs_out6_nolfmm ~ pH, env.full_out6_nolfmm, dist="kul")
```

```
plot(dbRDA_out6_noflmm) # use base plot, might be done with ggplot2
```




```
anova(dbRDA_out6_noflmm) # is the model significant?
```

```
## 'nperm' >= set of all permutations: complete enumeration.
```

```
## Set of permutations < 'minperm'. Generating entire set.
```

```
## Permutation test for capscale under reduced model
## Permutation: free
## Number of permutations: 719
##
## Model: capscale(formula = allele_freqs_out6_nolfmm ~ pH, data = env.full_out6_nolfmm, distance = "ku
##          Df  SumOfSqs      F Pr(>F)
## Model     1 0.0080349 1.9309 0.1583
## Residual  4 0.0166448
```

```
anova(dbRDA_out6_noflmm) # overall test of the significant of the analysis
```

```
## 'nperm' >= set of all permutations: complete enumeration.
```

```
## Set of permutations < 'minperm'. Generating entire set.
```

```
## Permutation test for capscale under reduced model
## Permutation: free
## Number of permutations: 719
##
## Model: capscale(formula = allele_freqs_out6_nolfmm ~ pH, data = env.full_out6_nolfmm, distance = "ku
##          Df  SumOfSqs      F Pr(>F)
## Model     1 0.0080349 1.9309 0.1583
## Residual  4 0.0166448
```

```
anova(dbRDA_out6_noflmm, by="axis", perm.max=500) # test axes for significance
```

```
## 'nperm' >= set of all permutations: complete enumeration.
## Set of permutations < 'minperm'. Generating entire set.
```

```
## Permutation test for capscale under reduced model
## Forward tests for axes
## Permutation: free
## Number of permutations: 719
##
## Model: capscale(formula = allele_freqs_out6_nolfmm ~ pH, data = env.full_out6_nolfmm, distance = "ku
##          Df  SumOfSqs      F Pr(>F)
## CAP1      1 0.0080349 1.9309 0.1583
## Residual  4 0.0166448
```

```
anova(dbRDA_out6_noflmm, by="terms", permu=200) # test for sign. environ. variables
```

```
## 'nperm' >= set of all permutations: complete enumeration.
## Set of permutations < 'minperm'. Generating entire set.
```

```
## Permutation test for capscale under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 719
##
## Model: capscale(formula = allele_freqs_out6_nolfmm ~ pH, data = env.full_out6_nolfmm, distance = "ku
##          Df  SumOfSqs      F Pr(>F)
## pH        1 0.0080349 1.9309 0.1583
## Residual  4 0.0166448
```