RDA trait prediction tutorial

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This tutorial accompanies the paper "The paradox of adaptive trait clines with non-clinal patterns in the underlying genes"

If the following packages are not installed, be sure to install them first:

```
install.packages("vegan")
install.packages("lfmm")

if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

BiocManager::install("LEA")
```

Load the libraries and set your working directory:

```r
libraries_needed <- c("vegan", "LEA", "lfmm")

for (i in 1:length(libraries_needed)){
  library(libraries_needed[i],character.only = TRUE) #laptop
}
```

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

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

```
## This is vegan 2.6-4
```

```
##
## Attaching package: 'LEA'
```

```
## The following object is masked from 'package:lattice':
##
##     barchart
```

```r
knitr::opts_knit$set(root.dir = "~/Documents/GitHub/MVP-NonClinalAF/tutorial")
#change code to your working directory
```

Load the data

- A matrix of genotypes in 012 format (counts of reference allele)
  - number of rows = number of individuals
  - number of columns = number of SNPs
- A table with information about sampled individuals (each individual in a row)
- A table with information about SNPs (each SNP in a row)

This data was simulated in SLiM and is associated with the complex multivariate simulation presented in Figure 6 in the paper. Briefly, a non-Wright-Fisher model was simulated on a landscape with 6 environmental variables that reflect different aspects of thermal stress and precipitation in British Columbia. The simulation included 6 environmental traits, each of which adapted to a different environmental variable.

The `ind` table includes the xy location for each individual, the 6 exact trait values (note that these won't exactly equal the trait value calculated from the genotype matrix because of MAF filtering), and the 6 environmental values at their xy location.

The `muts` table includes the linkage group `LG`, the position of the mutation on the genetic map `pos_pyslim`, a unique ID `mutname`, the allele frequency based on the 1000 sampled individuals `a_freq_subset`, and whether or not it had effects on one or more phenotypes `causal`.

Note that you will have to change the working directory to where the data is stored on your computer.

```
G <- read.table(unz("Genotypes.txt.zip", "Genotypes.txt"))
dim(G) # 1000 individuals and 26371 loci
```

```
## [1]  1000 26371
```

```
ind <- read.table("Individuals.txt", header=TRUE)
dim(ind) #corresponds to rows in G
```

```
## [1] 1000   15
```

```
head(ind)
```

```
##   ind_index         x        y phenotype1_mat phenotype2_MTWetQ phenotype3_MTDQ
## 1        33 0.4061840 0.233272      0.7379670        -0.1914210        0.142992
## 2        34 0.4256970 0.837158     -0.2740810        -0.4283320       -0.012880
## 3        44 0.6716730 0.581744     -0.6763260         0.0866287       -0.470671
## 4        45 0.0174659 0.329922     -0.2832210        -0.4039420       -0.333718
## 5        46 0.0697436 0.121221      0.0576163        -0.3032010        0.731600
## 6        64 0.1433610 0.233237      0.2656280        -0.2318730        0.214004
##   phenotype4_PDM phenotype5_PwarmQ phenotype6_PWM  env1_mat env2_MTWetQ
## 1      -0.131894         -0.452497     -0.5416510  0.762432  -0.1622380
## 2       0.221058          0.229296      0.0665969 -0.339330  -0.4076670
## 3       0.218813          0.260098     -0.1034650 -0.567733   0.0909278
## 4      -0.200554         -0.345915     -0.4100010 -0.245860  -0.2007590
## 5       0.195679         -0.367110      0.1962960 -0.121500  -0.3063790
## 6      -0.239776         -0.517086     -0.2582660  0.103057  -0.2863170
##    env3_MTDQ  env4_PDM env5_PwarmQ   env6_PWM
## 1  0.2612810 -0.332078   -0.471502 -0.4778210
## 2 -0.0582962  0.288030    0.198810  0.0176800
## 3 -0.4143530  0.234899    0.230255 -0.0535109
## 4 -0.2674550 -0.265260   -0.389828 -0.4589790
## 5  0.7028580  0.211275   -0.352307  0.2127510
## 6  0.1657060 -0.190813   -0.476650 -0.2927740
```

```
muts <-  read.table("SNPs.txt", header=TRUE)
dim(muts) #corresponds to columns in G
```

```
## [1] 26371     5
```

```
head(muts)
```

```
##   LG pos_pyslim mutname a_freq_subset causal
## 1  1          8     1-8        0.0175  FALSE
## 2  1         27    1-27        0.0120  FALSE
## 3  1         34    1-34        0.0370  FALSE
## 4  1         81    1-81        0.0330  FALSE
## 5  1         97    1-97        0.0170  FALSE
## 6  1        153   1-153        0.0225  FALSE
```

```
rownames(G) <- as.character(paste0("i_",ind$ind_index))
colnames(G) <- as.character(muts$mutname)
#G <- as.matrix(G)
head(G[,1:10])
```

```
##      1-8 1-27 1-34 1-81 1-97 1-153 1-154 1-206 1-287 1-304
## i_33   0    0    0    0    0     0     0     0     0     0
## i_34   0    0    0    0    0     0     0     0     0     0
## i_44   0    0    0    0    0     0     0     0     0     0
## i_45   0    0    0    0    0     0     0     0     0     0
## i_46   0    0    0    0    0     0     0     0     1     0
## i_64   2    0    0    0    0     0     0     0     0     0
```

RDA trait prediction funciton

This function predicts an environmental trait through the back-transformation of the RDA "site score" of an individual to a chosen environmental variable (Equation 1 in the manuscript). It makes the prediction for all the individuals that were used to run the RDA.

```
rda_trait_pred <- function(rdaobj, env_row, K){
  #rdaobj is RDA object
  #envi row is the row of the environment in the biplot output
  #K is the number of RDA axes
  scores <- scores(rdaobj, choices=1:K)
  ind.sc <- scores$sites
  pred <- matrix(NA, nrow=nrow(ind.sc), ncol=K)
  for (k in 1:K){
    pred[,k] <- ind.sc[,k]*eigenvals(rdaobj)[k]*summary(rdaobj)$biplot[env_row,k]
  }
  trait_pred <- scale(rowSums(pred))
 return(trait_pred)
}
```

Example of an RDA-predicted environmental trait value

1. First, run the RDA:

Scale the environmental variables to have a mean of 0 and standard deviation of 1

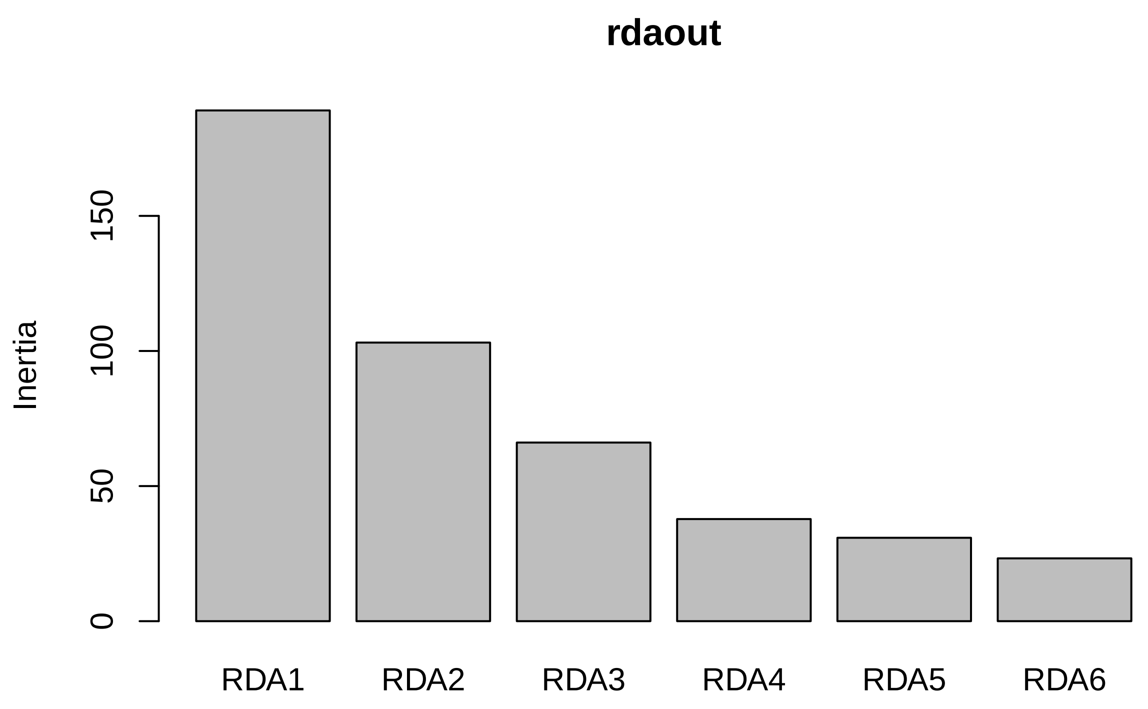
```
ind$env1_mat <- scale(ind$env1_mat)
ind$env2_MTWetQ <- scale(ind$env2_MTWetQ)
ind$env3_MTDQ <- scale(ind$env3_MTDQ)
ind$env4_PDM <- scale(ind$env4_PDM)
ind$env5_PwarmQ <- scale(ind$env5_PwarmQ)
ind$env6_PWM <- scale(ind$env6_PWM)

# Run the RDA
rdaout <- rda(G ~ ind$env1_mat +
                ind$env2_MTWetQ +
                ind$env3_MTDQ +
                ind$env4_PDM +
                ind$env5_PwarmQ +
                ind$env6_PWM
              )
```

2. Next, check the biplot output and decide how many RDA axes to use in the prediction.

```
# Check the biplot output
rdaout$CCA$biplot
```

```
##                       RDA1        RDA2       RDA3        RDA4        RDA5
## ind$env1_mat    -0.5004451  0.01863416 -0.5476147  0.54418964 -0.37298079
## ind$env2_MTWetQ  0.4523477  0.19158505 -0.8296969 -0.11392353  0.03132509
## ind$env3_MTDQ   -0.7128197 -0.22757653  0.1200761  0.48309488  0.33816827
## ind$env4_PDM    -0.3843437  0.04904761  0.8297919 -0.24744529  0.09707124
## ind$env5_PwarmQ  0.6524152  0.56221376  0.4505148  0.02427266 -0.04630498
## ind$env6_PWM     0.3209795 -0.05176393  0.7346369  0.19495732 -0.08780176
##                       RDA6
## ind$env1_mat    -0.1186113
## ind$env2_MTWetQ -0.2373183
## ind$env3_MTDQ   -0.2791780
## ind$env4_PDM    -0.3011107
## ind$env5_PwarmQ -0.2292885
## ind$env6_PWM    -0.5557731
```

```
# Decide how many RDA axes to use in calculation
  a<- screeplot(rdaout)
```



```r
str(a)
```

```
## List of 4
##  $ x   : num [1:6] 0.7 1.9 3.1 4.3 5.5 6.7
##  $ y   : num [1:6] 189 103.1 66.1 37.8 30.9 ...
##  $ xlab: NULL
##  $ ylab: NULL
```

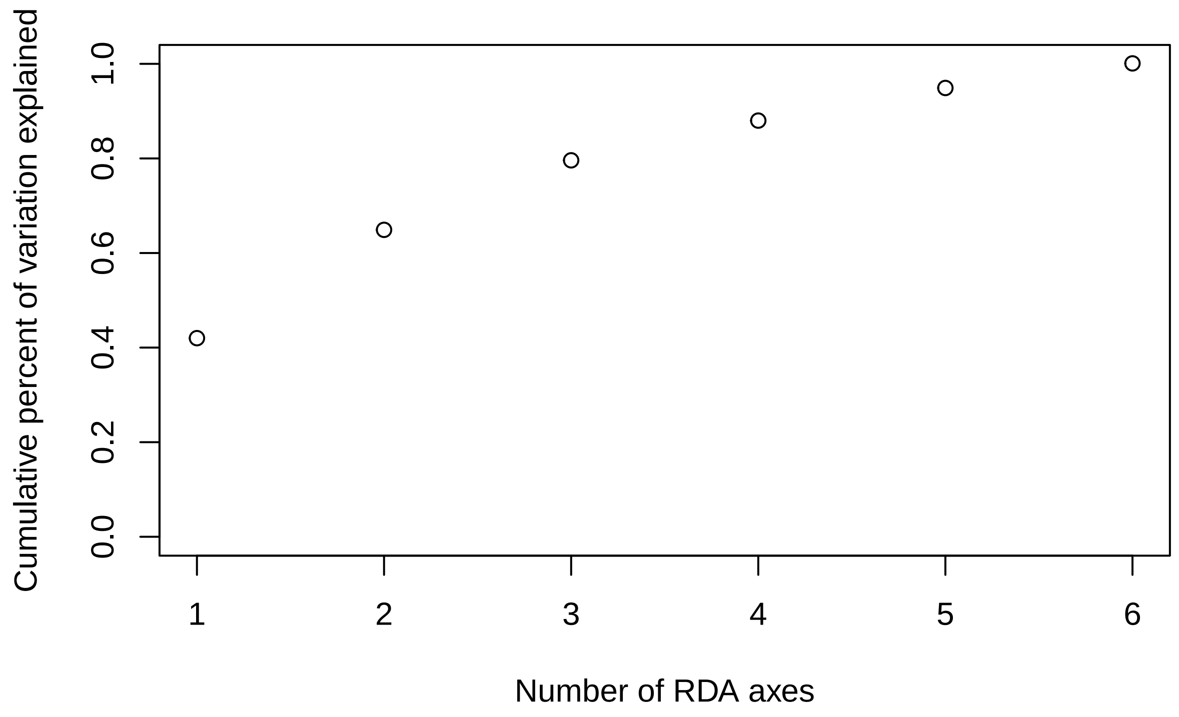
```r
a$y # save this it's the eigenvalues
```

```
## [1] 189.02481 103.10740  66.10749  37.79033  30.86247  23.24983
```

```r
prop_var <- round(a$y[1:6]/sum(a$y),3)
cumsum(prop_var)
```

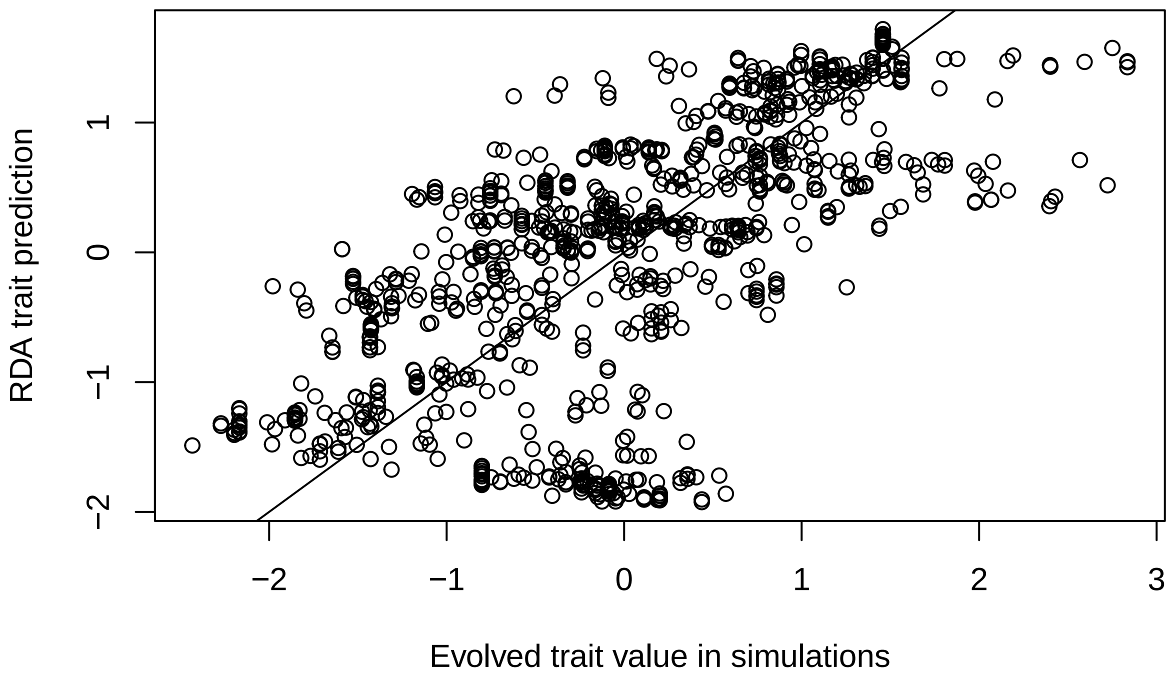
```
## [1] 0.420 0.649 0.796 0.880 0.949 1.001
```

```r
plot(cumsum(prop_var), xlab="Number of RDA axes",
     ylab="Cumulative percent of variation explained", ylim=c(0,1))
```



3. In this case, the first 3 RDA axes explain 80% of the variance. Note that choosing too many axes may result in overfitting. Here is an example of a trait prediction for MAT using the first 3 RDA axes:

```
# Make the trait prediction for MAT (1st row in biplot output)
K = 3 # use 3 RDA axes to make the trait prediciton

MATtraitPredict <- rda_trait_pred(rdaout, 1, K)

# Since this is a simulation, we can compare the prediction to the true value
# Similarly, an empirical study could compare an empirically measured trait value
# to the RDA-predicted trait value to test how well landscape genomic data
# can predict functional traits
plot(scale(ind$phenotype1_mat), MATtraitPredict, xlab="Evolved trait value in simulations",
     ylab="RDA trait prediction")
abline(0,1)
```



```
#Correlation between the prediction and the true value:
cor(ind$phenotype1_mat, MATtraitPredict)
```

```
##           [,1]
## [1,] 0.6461756
```

Compare to other functions in RDA

Note that the `predict` function and it's variations in the R package `vegan` do not make the same kind of predictions as `rda_trait_pred`. Here are the types of outputs produced by the function `predict` and it's variations:

```
# This option in the `predict` function outputs the scores for each locus in RDA space
loci_scores_predict <- predict(rdaout, type="sp", newdata=G, scaling=2)
str(loci_scores_predict)
```

```
##  num [1:26371, 1:6] -0.00957 -0.01685 -0.00659 0.08155 0.03396 ...
##  - attr(*, "dimnames")=List of 2
##   ..$ : chr [1:26371] "1-8" "1-27" "1-34" "1-81" ...
##   ..$ : chr [1:6] "RDA1" "RDA2" "RDA3" "RDA4" ...
```

```
# This option in the `predict` function outputs the fitted values from the multiple regression
# performed on each locus within each individual
fitted_values_predict <- predict(rdaout, newdata=G, type="response")
str(fitted_values_predict)
```

```
##  num [1:1000, 1:26371] 0.1031 0.0345 -0.0436 0.1821 0.1753 ...
##  - attr(*, "dimnames")=List of 2
##   ..$ : chr [1:1000] "i_33" "i_34" "i_44" "i_45" ...
##   ..$ : chr [1:26371] "1-8" "1-27" "1-34" "1-81" ...
```

```
  # As a side note, it outputs the same thing as the `fitted` funciton
  fitted_values_predict2 <- fitted(rdaout)
  str(fitted_values_predict2)
```

```
##  num [1:1000, 1:26371] 0.1031 0.0345 -0.0436 0.1821 0.1753 ...
##  - attr(*, "METHOD")= chr "PCA"
##  - attr(*, "dimnames")=List of 2
##   ..$ : chr [1:1000] "i_33" "i_34" "i_44" "i_45" ...
##   ..$ : chr [1:26371] "1-8" "1-27" "1-34" "1-81" ...
```

```
# This option  in the `predict` function outputs the individual scores in RDA space
# based on a linear combination of the predictor variables
X <- data.frame(ind$env1_mat ,
                ind$env2_MTWetQ ,
                ind$env3_MTDQ ,
                ind$env4_PDM ,
                ind$env5_PwarmQ ,
                ind$env6_PWM)
ind_scores_predict <- predict(rdaout, type="lc", new=X, scal=2)
str(ind_scores_predict)
```

```
##  num [1:1000, 1:6] -2.034 -0.19 0.543 0.253 -0.508 ...
##  - attr(*, "dimnames")=List of 2
##   ..$ : chr [1:1000] "1" "2" "3" "4" ...
##   ..$ : chr [1:6] "RDA1" "RDA2" "RDA3" "RDA4" ...
```

The `predict` function and it's variations make predictions in RDA space, and therefore do not output the same kind of predictions as `RDA_trait_predict` and Equation 1 in the paper.

Understanding how the RDA is built on multiple regressions

Prior to ordination in the RDA, each locus is used in a multiple regression model with the environmental variables to produce fitted values for that locus across individuals.

SNP Genotype ~ *Env1* + *Env2* + *Env3* etc.

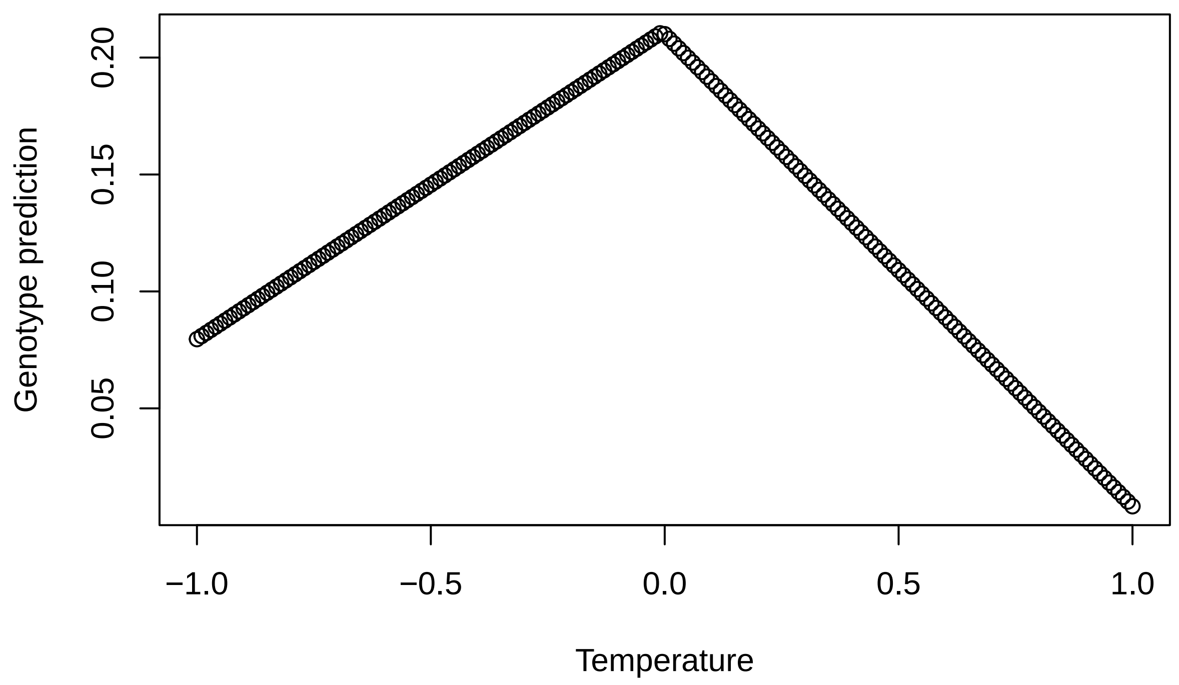
For example for the first SNP in the data:

```
 # multiple regression of 1st locus

mod <- lm(G[,1] ~ ind$env1_mat + ind$env2_MTWetQ + ind$env3_MTDQ +  ind$env4_PDM +
                ind$env5_PwarmQ +   ind$env6_PWM)
 coef(summary(mod))
```

```
##                     Estimate  Std. Error    t value     Pr(>|t|)
## (Intercept)      0.035000000 0.007404071  4.7271290 2.605782e-06
## ind$env1_mat    -0.035729076 0.011338501 -3.1511287 1.675100e-03
## ind$env2_MTWetQ -0.062538545 0.012165353 -5.1407094 3.295576e-07
## ind$env3_MTDQ    0.007471565 0.013224098  0.5649962 5.722040e-01
## ind$env4_PDM    -0.088670803 0.014835808 -5.9768099 3.169042e-09
## ind$env5_PwarmQ -0.069588746 0.014273932 -4.8752331 1.264746e-06
## ind$env6_PWM     0.047203246 0.013582893  3.4751982 5.325440e-04
```

Although multiple regression is a linear combination of multiple variables, it is able to model complex multivariate responses that appear to be non-monotonic in any one dimension. For example, let's look at a the relationship between explanatory variable temperature and the response variable genotype, across decreasing and increasing values of the other explanatory variables:

```
otherenv <- c(seq(1,0,length.out=100), seq(0,1,length.out=101))
newdata=data.frame(ind.env1_mat = seq(-1,1, by=0.01),
                   ind.env2_MTWetQ = otherenv,
                   ind.env3_MTDQ = otherenv,
                   ind.env4_PDM = otherenv,
                   ind.env5_PwarmQ =otherenv,
                   ind.env6_PWM = otherenv)
pred <- t(newdata)*(coef(summary(mod))[2:7,1]) + coef(summary(mod))[1,1]
plot(seq(-1,1, by=0.01), colSums(pred), xlab="Temperature", ylab="Genotype prediction")
```



Thus, there is flexibility with the RDA to capture the way environmental variables may influence the patterns at one locus in a different way than at another locus, which may not correlate with the relationship between the environment and population structure.

It may be interesting for some studies to understand how each locus is shaped by the environment - in other words, what are the slopes associated with the environmental variables in the multiple regression model for each locus?

Unfortunately there is not a way to output these slopes in the R package **vegan**, but we can reproduce the first step of the RDA to get the regression coefficients: (vegan source code at https://github.com/cran/vegan/blob/master/R/simpleRDA2.R)

```
X <- data.frame(ind$env1_mat ,
                ind$env2_MTWetQ ,
                ind$env3_MTDQ ,
                ind$env4_PDM ,
                ind$env5_PwarmQ ,
                ind$env6_PWM)
```

```r
# Perform qr decomposition to do the regression for all SNPs at the same time
 Q <- qr(X, tol=1e-6)
# str(Q) run this line if you want to understand the structure of Q

# Get the matrix of regression coefficients
 Qr.coeff <- qr.coef(Q, G)
 # This matrix has each SNP in a column and the regression coefficients
 # for that SNP corresponds to each environmental variable.
 # This is the step that is not performed in the `vegan` package -
 # the package skips directly to predicting the fitted values,
 # on which the ordination is performed.

# Here is an example of regression coefficients for the first 10 SNPs:
 head(Qr.coeff[,1:10])
```

```
##                         [,1]         [,2]         [,3]          [,4]
## ind.env1_mat    -0.035729076  0.007247705 -0.001047802 -6.104895e-02
## ind.env2_MTWetQ -0.062538545  0.039624398  0.101004048  9.680467e-05
## ind.env3_MTDQ    0.007471565 -0.005698865  0.039132724  7.995858e-02
## ind.env4_PDM    -0.088670803  0.017639011  0.027586476 -1.237919e-01
## ind.env5_PwarmQ -0.069588746 -0.043489428  0.005967464  1.262363e-01
## ind.env6_PWM     0.047203246 -0.012944110 -0.061687810 -6.657131e-03
##                         [,5]        [,6]        [,7]         [,8]        [,9]
## ind.env1_mat    -0.063099759  0.02064869  0.02539415  0.002292975 -0.07305243
## ind.env2_MTWetQ  0.023736921 -0.03019428  0.01360083  0.112457789 -0.02354021
## ind.env3_MTDQ   -0.019240464 -0.02199593 -0.06875513  0.043179895  0.10418350
## ind.env4_PDM    -0.003946225 -0.11767281  0.05887330  0.032681938 -0.09642371
## ind.env5_PwarmQ -0.032384913  0.05976386 -0.07535789  0.008011003 -0.03393128
## ind.env6_PWM     0.018107020  0.06710304  0.06788231 -0.068827598  0.02928042
##                       [,10]
## ind.env1_mat     0.03694418
## ind.env2_MTWetQ -0.04964375
## ind.env3_MTDQ   -0.05660635
## ind.env4_PDM     0.01149236
## ind.env5_PwarmQ -0.01743073
## ind.env6_PWM    -0.01118472
```

```r
# Note that the regression coefficients for the first SNP from this
# approach is exactly the same as from our model above:
 Qr.coeff[,1]
```

```
##    ind.env1_mat ind.env2_MTWetQ   ind.env3_MTDQ    ind.env4_PDM ind.env5_PwarmQ
##    -0.035729076    -0.062538545     0.007471565    -0.088670803    -0.069588746
##    ind.env6_PWM
##     0.047203246
```

```r
 coef(summary(mod))[,1]
```

```
##     (Intercept)    ind$env1_mat ind$env2_MTWetQ   ind$env3_MTDQ    ind$env4_PDM
##     0.035000000    -0.035729076    -0.062538545     0.007471565    -0.088670803
## ind$env5_PwarmQ    ind$env6_PWM
##    -0.069588746     0.047203246
```

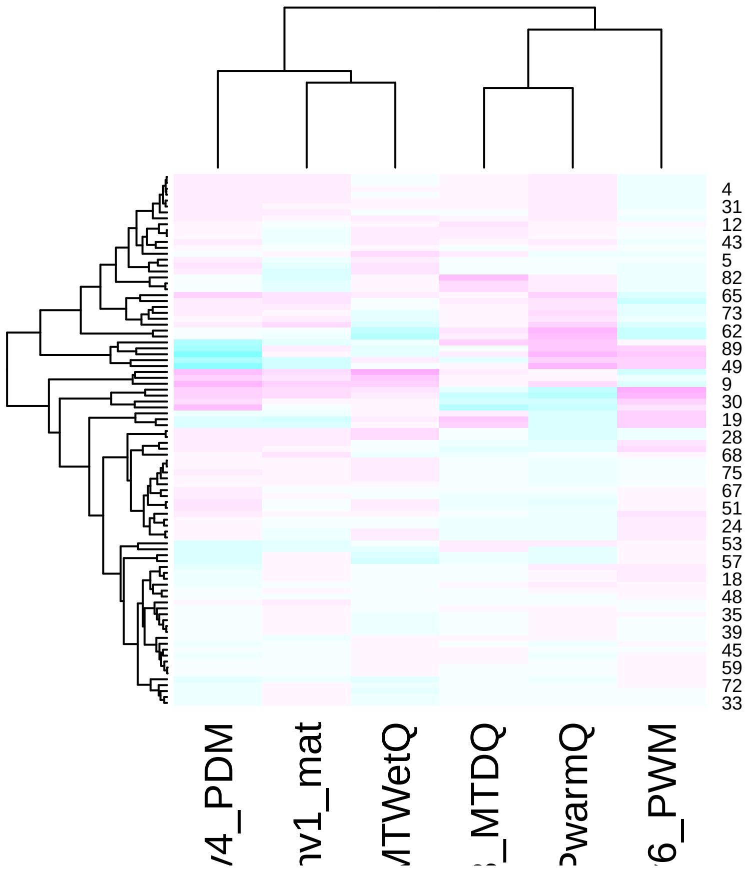
We can visualize the regression coefficients with a heatmap. In this case, we know the causal loci in the simulations, so we will just visualize those loci.

This visualization illustrates how there are unique ways in which environments are combined in the model to predict the pattern at each SNP.

```
# look at the range of slopes
summary(as.numeric(Qr.coeff[,which(muts$causal)]))
```

```
##      Min.   1st Qu.    Median      Mean   3rd Qu.      Max.
## -0.657086 -0.054464  0.001058  0.001041  0.056986  0.447236
```

```
brks <- seq(-0.7, 0.7, by=0.05) #set the color scale

heatmap(t(Qr.coeff[,which(muts$causal)]),
        scale="none", col = cm.colors(length(brks)-1), breaks=brks)
```



Here is the information about the session when the tutorial was built:

```
sessionInfo()
```

```
## R version 4.2.2 (2022-10-31)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur ... 10.16
##
```

```
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] lfmm_1.1        LEA_3.10.1      vegan_2.6-4     lattice_0.20-45
## [5] permute_0.9-7
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.9       knitr_1.41       cluster_2.1.4    magrittr_2.0.3
##  [5] splines_4.2.2    MASS_7.3-58.1    rlang_1.0.6      foreach_1.5.2
##  [9] fastmap_1.1.0    highr_0.10       stringr_1.5.0    tools_4.2.2
## [13] parallel_4.2.2   grid_4.2.2       nlme_3.1-161     mgcv_1.8-41
## [17] xfun_0.36        cli_3.6.0        iterators_1.0.14 htmltools_0.5.4
## [21] yaml_2.3.6       digest_0.6.31    lifecycle_1.0.3  Matrix_1.5-3
## [25] codetools_0.2-18 vctrs_0.5.1      glue_1.6.2       evaluate_0.19
## [29] rmarkdown_2.19   stringi_1.7.8    compiler_4.2.2
```