# Applied Multivariate: Identifying differences between groups

2017-11-17

```
library(vegan)
library(gridExtra)
library(tidyverse)
```

## Linear discriminant analysis

- Often we have groups that we have defined a priori before doing an analysis and we seek to understand what makes those groups different
- Linear discrimination analysis (LDA), similar to multinomial logistic regression, attempts to find linear combinations of variables that best separate groups when predicting two or more dependent variables, using continuous independent variables.

#### Load the data

We will use the data set morph\_data.csv on github. The data consisted of 8 measurements in all 5 larval stages and the adults of the 6 species of the waterstrider genus Limnoporus (Insecta: Heteroptera: Gerridae). Data was originally sourced from morphometry datasets.

The variables are: Species, stage (1-5 for larvae, 6 for adults), sex (m male, f female, u undetermined), antseg1 - antseg4 (lengths of 1st to 4th antennal segments), midfem, midtib, hindfem, hindtib (lengths of middle and hind femora and tibiae). The values are raw measurements in millimeters.

morph\_data <-read\_csv("https://raw.githubusercontent.com/chrischizinski/SNR\_R\_Group/master/data/morph\_d

```
## Parsed with column specification:
## cols(
##
                    Species = col_character(),
##
                    stage = col_integer(),
##
                    sex = col_character(),
##
                    antseg1 = col_double(),
##
                    antseg2 = col_double(),
##
                    antseg3 = col double(),
##
                    antseg4 = col_double(),
##
                    midfem = col_double(),
##
                    midtib = col_double(),
##
                    hindfem = col_double(),
##
                    hindtib = col_double()
glimpse(morph_data)
## Observations: 541
## Variables: 11
## $ Species <chr> "canali", "canali
## $ stage <int> 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 2, 2, 2, 2, 2, 2, 2, 2, ...
                                                     ## $ sex
```

```
## $ antseg1 <dbl> 0.17, 0.20, 0.18, 0.16, 0.13, 0.18, 0.18, 0.18, 0.20, ...
## $ antseg2 <dbl> 0.15, 0.14, 0.14, 0.13, 0.13, 0.16, 0.12, 0.15, 0.15, ...
## $ antseg3 <dbl> 0.19, 0.18, 0.17, 0.14, 0.16, 0.18, 0.16, 0.16, 0.19, ...
## $ antseg4 <dbl> 0.72, 0.74, 0.62, 0.55, 0.56, 0.52, 0.72, 0.60, 0.67, ...
## $ midfem <dbl> 0.70, 0.76, 0.76, 0.70, 0.72, 0.76, 0.74, 0.76, 0.78, ...
## $ midtib <dbl> 0.80, 0.92, 0.94, 0.92, 0.92, 0.97, 0.91, 0.92, 0.95, ...
## $ hindfem <dbl> 0.43, 0.68, 0.79, 0.74, 0.68, 0.68, 0.70, 0.70, 0.78, ...
## $ hindtib <dbl> 0.72, 0.42, 0.40, 0.42, 0.38, 0.45, 0.42, 0.43, 0.44, ...
```

#### Manipulate the data

## 4 canali

## 5 canali

## 6 canali

1

1

1

-1.33

-1.39

-1.30

For the purpose of this excercise lets limit the data to one species and only the measurements of the antennae.

```
morph data %>%
  select(Species:antseg4, -sex) %>%
  filter(Species == "canali") %>%
  mutate_at(vars(antseg1:antseg4), scale)-> canal_data
head(canal_data)
## # A tibble: 6 x 6
##
     Species stage antseg1 antseg2 antseg3 antseg4
##
     <chr>>
             <int>
                      <dbl>
                              <dbl>
                                      <dbl>
                                               <dbl>
                     -1.31
                              -1.33
                                      -1.38
                                               -1.27
## 1 canali
                 1
                                      -1.42
                                               -1.22
## 2 canali
                 1
                     -1.26
                              -1.36
## 3 canali
                 1
                     -1.30
                              -1.36
                                      -1.45
                                               -1.51
```

-1.68

-1.65

-1.75

Conduct the linear discriminant analysis using MASS::1da()

-1.39

-1.39

-1.31

-1.55

-1.48

-1.42

```
canal_lda<-MASS::lda(stage~antseg1 + antseg2 + antseg3 + antseg4, data = canal_data)
canal_lda
## Call:
## lda(stage ~ antseg1 + antseg2 + antseg3 + antseg4, data = canal_data)
## Prior probabilities of groups:
                               3
## 0.1111111 0.1111111 0.1111111 0.2222222 0.2222222 0.2222222
##
## Group means:
##
        antseg1
                   antseg2
                              antseg3
                                         antseg4
## 1 -1.3073501 -1.3594034 -1.4443907 -1.4868663
## 2 -1.1332171 -1.1490818 -1.1510214 -1.2348777
## 3 -0.8167818 -0.8640406 -0.8315750 -0.9156920
## 4 -0.3028084 -0.2399942 -0.1731241 -0.0805297
## 5 0.4283631 0.5487119
                           0.6043044
                                      0.6886356
     1.5031198 1.3775452 1.2823132 1.2106121
##
## Coefficients of linear discriminants:
```

```
##
                  LD1
                              LD2
                                         LD3
                                                    LD4
## antseg1 14.5047455 -7.2205800
                                  3.8168030
                                             2.764369
## antseg2 -3.8449898
                       1.2049129 -8.6374675 -2.428811
## antseg3 -1.7878986
                                   4.3236873 -3.984543
                       0.9023135
## antseg4 -0.2461576 5.5171016 0.5203163 3.569601
##
## Proportion of trace:
##
      LD1
             LD2
                    LD3
                            I.D4
## 0.9719 0.0276 0.0003 0.0002
names(canal_lda)
##
    [1] "prior"
                   "counts"
                             "means"
                                       "scaling" "lev"
                                                            "svd"
                                                                       "N"
##
    [8] "call"
                   "terms"
                             "xlevels"
```

### Explanation of the output

#### Prior

From the raw data, the break down in proportions of the number of individuals in each group

```
canal_lda$prior
```

```
## 1 2 3 4 5 6
## 0.1111111 0.1111111 0.2222222 0.222222 0.2222222
```

#### Group means

The mean value for each of the variables for each group

#### canal\_lda\$means

```
## antseg1 antseg2 antseg3 antseg4
## 1 -1.3073501 -1.3594034 -1.4443907 -1.4868663
## 2 -1.1332171 -1.1490818 -1.1510214 -1.2348777
## 3 -0.8167818 -0.8640406 -0.8315750 -0.9156920
## 4 -0.3028084 -0.2399942 -0.1731241 -0.0805297
## 5 0.4283631 0.5487119 0.6043044 0.6886356
## 6 1.5031198 1.3775452 1.2823132 1.2106121
```

#### Coefficients

These are the coffeicients to describe the linear discrimination. For example: LD1 =  $14.5047455 \times \text{Group1} + -3.8449898 \times \text{Group2} + -1.7878986 \times \text{Group3} + \dots$ 

#### canal\_lda\$scaling

```
## LD1 LD2 LD3 LD4

## antseg1 14.5047455 -7.2205800 3.8168030 2.764369

## antseg2 -3.8449898 1.2049129 -8.6374675 -2.428811

## antseg3 -1.7878986 0.9023135 4.3236873 -3.984543

## antseg4 -0.2461576 5.5171016 0.5203163 3.569601
```

You can also standardize the coefficients (loadings), which provides the relative strength of each factor on linear discriminant analysis

```
loadings <-canal_lda$scaling
col.ss <- colSums(loadings^2)
sweep(loadings,2,sqrt(col.ss),"/")

## LD1 LD2 LD3 LD4
## antseg1 0.95969829 -0.78391461 0.36703621 0.4257438
## antseg2 -0.25440157 0.13081342 -0.83060701 -0.3740641
## antseg3 -0.11829530 0.09796121 0.41577985 -0.6136643
## antseg4 -0.01628688 0.59897356 0.05003531 0.5497585</pre>
```

#### Variation expained

The amount of variation explained by the each of the linear discriminant axis

```
prop = canal_lda$svd^2/sum(canal_lda$svd^2)
prop
```

```
## [1] 0.9719389713 0.0275790703 0.0003069345 0.0001750239
```

#### Graphing the results

```
# extracting the data
lda_pred <- predict(canal_lda)</pre>
lda_scores<-data.frame(stage = lda_pred$class, lda_pred$x)</pre>
#developing hulls for each stage group
hull1<- lda_scores[lda_scores$stage ==1,][chull(lda_scores[lda_scores$stage ==1,c("LD1", "LD2")]),]
hull2<- lda_scores[lda_scores$stage ==2,][chull(lda_scores[lda_scores$stage ==2,c("LD1", "LD2")]),]
hull3<- lda_scores[lda_scores$stage ==3,][chull(lda_scores[lda_scores$stage ==3,c("LD1", "LD2")]),]
hull4<- lda_scores[lda_scores$stage ==4,][chull(lda_scores[lda_scores$stage ==4,c("LD1", "LD2")]),]
hull5<- lda_scores[lda_scores$stage ==5,][chull(lda_scores[lda_scores$stage ==5,c("LD1", "LD2")]),]
hull6<- lda scores[lda scores$stage ==6,][chull(lda scores[lda scores$stage ==6,c("LD1", "LD2")]),]
all_hulls <- rbind(hull1, hull2, hull3, hull4, hull5, hull6)
#plot using gglot2
ggplot(data = lda_scores) +
  geom_point(aes(x = LD1, y = LD2, color = stage), size = 2) +
  geom_polygon(data = all_hulls,aes(x = LD1, y = LD2, color = stage, fill = stage), alpha = 0.25) +
  coord_equal() +
  theme_classic()
```

#### Assessing the accuracy of the predicitions

```
ct <- table(canal_data$stage, lda_pred$class)
ct</pre>
```

##

```
##
          2
             3
                  5
##
          1
             0
                   0
    1
                0
       0 10
##
    2
             0
##
    3 0 0 10 0 0 0
##
          0 0 20
       0 0 0 0 20 0
##
       0
          0 0 0 0 20
# percent correct for each stage
diag(prop.table(ct, 1))
        2
          3 4
## 0.9 1.0 1.0 1.0 1.0 1.0
# total percent correct
sum(diag(prop.table(ct)))
## [1] 0.9888889
```

MANOVA and MANCOVA

Two common multivariate regression models are the multivariate analysis of variance (MANOVA; an extension of the univariate ANOVA) when all predictor variables are categorical, and the multivariate analysis of covariance (MANCOVA; extension of univariate ANCOVA) when predictor variables are categorical and continuous.

#### Load the data

```
bryc data <- read.table("http://ecology.msu.montana.edu/labdsv/R/labs/lab1/bryceveg.R")</pre>
bryc_data %>% rownames_to_column("site") -> bryc_data
bryc_site_data <- read.table("http://ecology.msu.montana.edu/labdsv/R/labs/lab2/brycesite.R")</pre>
bryc_site_data %>%
 rownames_to_column("site") %>%
  full_join(bryc_data, by = "site") -> bryc_site_data
glimpse(bryc_site_data)
## Observations: 160
## Variables: 182
              <chr> "bcnp__1", "bcnp__2", "bcnp__3", "bcnp__4", "bcnp__5"...
## $ site
## $ plotcode <int> 50001, 50002, 50003, 50004, 50005, 50006, 50007, 5000...
## $ annrad
              <int> 241, 222, 231, 254, 232, 216, 288, 279, 187, 279, 271...
## $ asp
              <int> 30, 50, 50, 360, 300, 330, 150, 220, 360, 125, 125, 2...
              <dbl> 1.00, 0.96, 0.96, 0.93, 0.48, 0.76, 0.25, 0.00, 0.93,...
## $ av
              <chr> "deep", "shallow", "shallow", "shallow", "shallow", "...
## $ depth
## $ east
              <dbl> 388220.9, 388477.2, 388384.0, 388307.5, 389026.3, 389...
## $ elev
              <int> 8720, 8360, 8560, 8660, 8480, 8560, 8560, 8680, 8240,...
## $ grorad
              <int> 162, 156, 159, 166, 159, 155, 169, 169, 146, 167, 168...
## $ north
              <int> 4144784, 4147573, 4147347, 4146971, 4146846, 4146853,...
## $ pos
              <chr> "ridge", "mid_slope", "mid_slope", "ridge", "up_slope...
```

```
<chr> "pc", "pc", "pc", "pc", "pc", "pc", "pc", "pc", "pc", "bp",...
## $ quad
## $ slope
   <int> 9, 2, 2, 0, 2, 2, 3, 2, 3, 3, 2, 6, 3, 4, 3, 4, 2, 1,...
## $ junost
   ## $ ameuta
   ## $ arcpat
   <dbl> 1.0, 0.5, 1.0, 1.0, 4.0, 1.0, 4.0, 2.0, 0.0, 5.0, 2.0...
## $ arttri
   ## $ atrcan
   ## $ berfre
## $ ceamar
   <dbl> 0.5, 0.0, 0.5, 0.5, 0.5, 1.0, 1.0, 0.0, 0.0, 0.5, 0.5...
## $ cerled
   ## $ cermon
   ## $ chrdep
## $ chrnau
   ## $ chrpar
   ## $ chrvis
   ## $ eurlan
   <dbl> 0.0, 2.0, 1.0, 0.0, 1.0, 0.5, 0.0, 0.5, 0.5, 0.0, 0.0...
## $ juncom
## $ pacmyr
   ## $ pruvir
## $ purtri
   ## $ quegam
   ## $ rhutri
   ## $ ribcer
   ## $ roswoo
## $ samcoe
   ## $ shearg
   ## $ sherot
   ## $ symore
   ## $ arcuva
   ## $ artarb
   ## $ artfri
   ## $ artpyg
   ## $ atrcon
   <dbl> 1.0, 0.0, 0.5, 1.0, 0.5, 1.0, 0.5, 0.0, 1.0, 0.5, 1.0...
## $ berrep
## $ ericor
   ## $ gutsar
   ## $ tetcan
   ## $ agrcri
   ## $ agrdas
## $ agrscr
   ## $ agrsmi
   ## $ bougra
   ## $ broano
   ## $ brocil
   ## $ broine
   ## $ carrss
   <dbl> 0.5, 0.5, 0.5, 0.5, 1.0, 0.5, 0.5, 0.5, 0.5, 0.5, 0.5...
## $ elysal
   ## $ fesovi
   ## $ hiljam
   ## $ junbal
   ## $ koenit
   ## $ muhmon
   ## $ muhric
   ## $ oryhym
```

```
## $ orymic
  ## $ phlpra
  ## $ poacom
  ## $ poafen
  ## $ poanev
  ## $ poapra
  ## $ sithys
  ## $ sticom
  ## $ stilet
  ## $ stipin
  ## $ achmil
  ## $ agogla
  ## $ anemul
  ## $ antros
  ## $ apoand
  ## $ arahol
## $ arapen
  ## $ arefen
  ## $ artcar
  ## $ artlud
  ## $ astagr
  ## $ astchi
  ## $ astcon
  ## $ asthum
  ## $ astken
  ## $ astmeg
  ## $ astmis
  ## $ astten
  ## $ balsag
  ## $ calnut
  ## $ caschr
  ## $ caslin
  ## $ chadou
  ## $ cirneo
  ## $ compal
  ## $ corkin
  ## $ creint
  ## $ crycon
  ## $ cympur
  ## $ dessop
## $ drasub
  ## $ echtri
  ## $ eriala
  ## $ erican
  ## $ erieat
## $ erifla
  ## $ eripan
  ## $ eripum
  ## $ erirac
  ## $ erisub
  ## $ eriumb
  ## $ eupfen
  ## $ euplur
```

```
## $ euprob
  ## $ fraves
  ## $ genaff
  ## $ gerfre
  <dbl> 0.5, 0.5, 0.5, 0.0, 0.0, 0.5, 0.0, 0.5, 0.0, 0.5, 0.0.
## $ gerric
  ## $ gilcon
  ## $ haparm
  ## $ heddru
  ## $ hymaca
  ## $ hymfil
  ## $ hymric
  ## $ ipoagg
## $ irimis
  ## $ ivesab
  ## $ leppun
  ## $ lesint
  ## $ leueri
  ## $ ligpor
  ## $ linkin
## $ linlew
  ## $ litinc
  ## $ litmul
  ## $ lotuta
## $ lupkin
  ## $ lupser
  ## $ lyggra
  ## $ lygspi
## $ macgri
  ## $ molpar
  ## $ oenbra
  ## $ oencae
  ## $ oencor
  ## $ oenfla
  ## $ oenlav
## $ opueri
  ## $ orofas
  ## $ pedcan
  ## $ pedsim
  ## $ pencae
  ## $ pencom
  ## $ penlei
## $ penuta
  ## $ phllon
  ## $ phycha
  ## $ potcon
  ## $ potcri
  ## $ potgra
  ## $ pteand
  ## $ pyrvir
  ## $ salibe
  ## $ sclwhi
  ## $ senmul
  ## $ sphcoc
  ## $ stapin
```

```
## $ steten
  ## $ strcor
  ## $ swerad
  ## $ taroff
## $ thafen
  ## $ towmin
  ## $ tradub
  ## $ valacu
## $ vicame
```

#### Using MANOVA and MANCOVA

#### **MANOVA**

-Independent variable is a categorical variable - Interpret like ANOVA - degfault test statistic is "Pillai" but "Wilks", "Hotelling-Lawley", "Roy" are also available. See ?summary.manova. Wilks' statistic is most popular in the literature, but the default Pillai—Bartlett statistic is recommended by Hand and Taylor (1987)

#### As a **reminder**:

-R provides Type I sequential SS, not the default Type III marginal SS reported by SAS and SPSS. In a nonorthogonal design with more than one term on the right hand side of the equation order will matter (i.e., A+B and B+A will produce different results)! - Type I sequential SS: tests the main effect of factor A, followed by the main effect of factor B after the main effect of A, followed by the interaction effect AB after the main effects. - Type II: tests for each main effect after the other main effect. No significant interaction is assumed (in other words, you should test for interaction first (SS(AB | A, B)) and only if AB is not significant, continue with the analysis for main effects). - Type III: tests for the presence of a main effect after the other main effect and interaction. This approach is therefore valid in the presence of significant interactions. If the interactions are not significant, type II gives a more powerful test.

- NOTE: when data is balanced, the factors are orthogonal, and types I, II and III all give the same results.
- If you wish to find ways to produce Type II or III SS then you will need to find a package to help. For example, regr0 has a drop1() function for mlm models. Install via install.packages("regr0", repos="http://R-Forge.R-project.org")

```
man_out <- manova(cbind(junost,ameuta,arcpat,arttri,atrcan) ~ pos,data = bryc_site_data)
summary(man_out)</pre>
```

```
## Df Pillai approx F num Df den Df Pr(>F)
## pos    4 0.18304   1.477   20   616 0.08253 .
## Residuals 155
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

#### **MANCOVA**

• When you have a categorical and continuous independent variables

```
## Residuals 154
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

#### ANOSIM

Analysis of similarities (ANOSIM) provides a way to test statistically whether there is a significant difference between two or more groups of sampling units.

The R-statistic in ANOSIM is a ratio between within-group and between-group dissimilarities

```
# create the y variable
y<-cbind(bryc_site_data$arcpat,bryc_site_data$artarb,bryc_site_data$carrss,bryc_site_data$purtri,bryc_s
# convert to an x variable
x <- as.factor(bryc_site_data$pos)</pre>
# analysis
anosim_out <- anosim(y, x, distance = "euclidean", permutations = 999)
summary(anosim_out)
##
## Call:
## anosim(dat = y, grouping = x, permutations = 999, distance = "euclidean")
## Dissimilarity: euclidean
## ANOSIM statistic R: 0.0162
         Significance: 0.23
##
##
## Permutation: free
## Number of permutations: 999
##
## Upper quantiles of permutations (null model):
             95% 97.5%
      90%
                           99%
## 0.0306 0.0391 0.0484 0.0597
##
## Dissimilarity ranks between and within classes:
##
              0%
                     25%
                             50%
                                       75%
                                              100%
## Between
           198 2978.50 6359.50 9536.000 12720.0 9823
## bottom
            198 4155.00 6932.50 9591.125 12485.0 190
## low_slope 198 4070.00 6876.75 10634.500 12716.5
## mid_slope 198 3220.75 7323.50 10180.000 12704.0 1431
## ridge
             198 2978.50 6196.50 8470.000 11827.5
## up_slope 198 1628.50 3863.50 6932.500 11978.0
perms<-permustats(anosim_out)</pre>
summary(perms)
##
##
                   SES
                                               upper Pr(perm)
     statistic
                          mean lower median
## R.
        0.0162 0.6818 0.0014
                                     -0.0009
                                              0.0391
                                                          0.23
## (Interval (Upper - Lower) = 0.95)
```

## anosim\_out\$perm

##	TRUE		TRUE	TRUE	TRUE
##	0.012078652347	-0.	.032816017834	0.017721471214	0.011803572877
##	TRUE		TRUE	TRUE	TRUE
##	0.004734578709	-0	.004219384521	-0.006707258341	0.016295295913
##	TRUE		TRUE	TRUE	TRUE
##	0.002899227968	-0	.001738925337	0.001169614851	0.067735367506
##	TRUE		TRUE	TRUE	TRUE
##	0.036388642310	-0	.013310536081	-0.023454355064	-0.015490790372
##	TRUE		TRUE	TRUE	TRUE
##	-0.025731632147	0.	.009431240868	0.004536000006	-0.007285529643
##	TRUE		TRUE	TRUE	TRUE
##	-0.014542560378	0.	.003889942771	-0.025675091157	-0.005015596915
##	TRUE		TRUE	TRUE	TRUE
##	-0.020152312078	0.	.031368477137	0.030313525585	0.001268183823
##	TRUE		TRUE	TRUE	TRUE
##	0.035143299782	0.	.047068704611		
##	TRUE		TRUE	TRUE	TRUE
##	0.011635285246	0.	.014903382553		-0.035794909210
##	TRUE		TRUE	TRUE	TRUE
##	-0.032275276537	0.	.007655453196	-0.020202422365	0.024910645734
##	TRUE		TRUE	TRUE	TRUE
##	0.049297839273	-0	.012026047088	-0.029110773286	0.013193518371
##	TRUE		TRUE	TRUE	TRUE
##	0.041795176769	-0		0.020022302.00	-0.019900144185
##	TRUE	_	TRUE	TRUE	TRUE
##	-0.012362095244	-0	.008055948943	-0.016157018229	-0.002089662202
##	TRUE	^	TRUE	TRUE	TRUE
##	0.030722033356	0.	.003735746461	0.003236787163	0.028683641075
##	TRUE	^	TRUE	TRUE	TRUE
##	-0.020587807717 TRUE	0.	.030652771522	-0.018545585127 TRUE	0.009550296724 TRUE
##	-0.018332633980	^	TRUE .028345730475		-0.018479661637
##	TRUE	0.	TRUE	0.010714240396 TRUE	TRIJE.
##	0.002430559741	٥	.025239314394	-0.001905842490	0.024376440561
##	TRUE	0	TRUE	TRUE.	TRUE
##	-0.023718822116	-0	.003969114212		-0.008669466119
##	TRUE	0	TRUE	TRUE	TRUE
##	0.012239525342	0	.002360911362	0.070208025510	0.007586402205
##	TRUE		TRUE	TRUE	TRUE
	-0.000979786122	0	.022236668072		
##	TRUE		TRUE	TRUE	TRUE
		-0		-0.003416776566	
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```

R value is supposed to vary between 0 and 1 (not between -1 and +1) but you can obtained negative values but they are always close to 0. R value close to 1 indicates high separation between levels of your factor (e.g. control vs treatment samples), while R value close to 0 indicate no separation between levels of your factor.