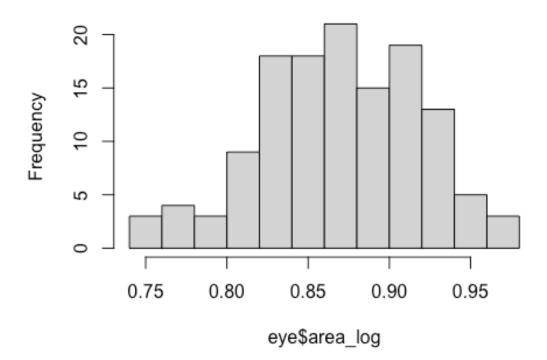
eye_morph_selection_analyses.R

```
#####Full analysis for: Wright et al. Adaptive divergence in Heliconius eyes#
####
#Load packages
library(MASS)
library(lme4)
## Loading required package: Matrix
library(ggplot2)
library(stats)
library(effects)
## Loading required package: carData
## lattice theme set by effectsTheme()
## See ?effectsTheme for details.
library(splines)
library(xlsx)
library(car)
library(lattice)
library(grid)
library(gridExtra)
library(pbkrtest)
library(readx1)
library(dplyr)
##
## Attaching package: 'dplyr'
## The following object is masked from 'package:gridExtra':
##
##
       combine
## The following object is masked from 'package:car':
##
##
       recode
## The following object is masked from 'package:MASS':
##
##
       select
## The following objects are masked from 'package:stats':
##
##
       filter, lag
```

```
## The following objects are masked from 'package:base':
##
##
       intersect, setdiff, setequal, union
#import data
eye <- read.csv("~/Documents/LMU/Eye morphology/selection manuscript/eye morp</pre>
hology cp mp f1.csv")
#is the data properly structured?
str(eye)
## 'data.frame': 131 obs. of 14 variables:
                    : chr "CAM_13031" "CAM_13086" "CAM_13087" "CAM_13118" .
## $ ID
                           "PAN 2007" "PAN 2007" "PAN 2007" "PAN 2007" ...
## $ collection
                   : chr
                           "female" "female" "female" ...
## $ sex
                    : chr
                           "CP" "CP" "CP" "CP" ...
## $ type
                    : chr
                           "stock" "stock" "stock" ...
## $ brood
                    : chr
                           "insectary" "insectary" "insectary" .
## $ wild insectary : chr
. .
## $ raised
                           "Gamboa" "Gamboa" "Gamboa" ...
                    : chr
                           "Shane" "Shane" "Shane" ...
## $ observer
                    : chr
## $ inter eye width: num 1.54 1.58 1.55 1.54 1.26 ...
## $ tibia_length : num NA NA 5.9 6 4.85 ...
## $ L area
                   : num 8.32 7.28 9.12 8.22 6.51 ...
## $ L whole count : int 14322 13569 15142 14059 11818 15035 13497 13746 1
5346 13951 ...
## $ R area
                    : num 8.2 7.12 8.76 8.42 NA ...
## $ R whole count : int 13707 13745 14954 14456 NA 15107 NA NA NA NA ...
eye$type <- as.factor(eye$type)</pre>
eye$sex <- as.factor(eye$sex)</pre>
eye$brood <- as.factor(eye$brood)</pre>
eye$wild_insectary <- as.factor(eye$wild insectary)</pre>
eye$raised <- as.factor(eye$raised)</pre>
eye$observer <- as.factor(eye$observer)</pre>
str(eye) #now correct
## 'data.frame':
                   131 obs. of 14 variables:
                     : chr "CAM 13031" "CAM 13086" "CAM 13087" "CAM 13118" .
## $ ID
                    : chr "PAN 2007" "PAN 2007" "PAN 2007" "PAN 2007" ...
## $ collection
                    : Factor w/ 2 levels "female", "male": 1 1 1 1 2 2 2 1 1
## $ sex
1 ...
## $ type
                    : Factor w/ 4 levels "CP", "CPxMP", "MP", ...: 1 1 1 1 3 1 3
1 1 1 ...
## $ brood
                    : Factor w/ 14 levels "check", "N156", ...: 14 14 14 14 NA
14 NA 14 14 14 ...
## $ wild insectary : Factor w/ 1 level "insectary": 1 1 1 1 NA 1 NA 1 1 1 .
```

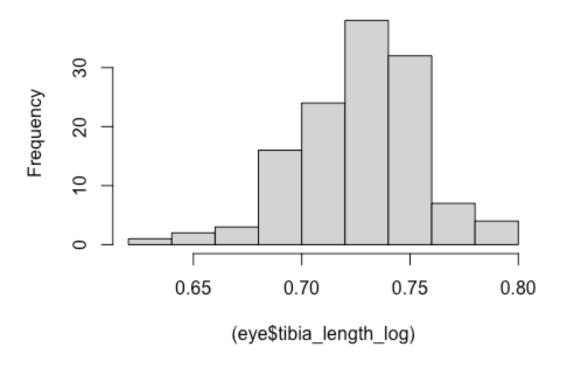
```
$ raised
                     : Factor w/ 1 level "Gamboa": 1 1 1 1 1 1 1 1 1 ...
                     : Factor w/ 2 levels "Lisa", "Shane": 2 2 2 2 1 2 1 1 1 1
## $ observer
    $ inter eye width: num 1.54 1.58 1.55 1.54 1.26 ...
##
## $ tibia_length
                     : num NA NA 5.9 6 4.85 ...
## $ L_area
                     : num 8.32 7.28 9.12 8.22 6.51 ...
## $ L_whole_count : int 14322 13569 15142 14059 11818 15035 13497 13746 1
5346 13951 ...
## $ R area
                     : num 8.2 7.12 8.76 8.42 NA ...
    $ R whole_count
                     : int 13707 13745 14954 14456 NA 15107 NA NA NA NA ...
######new composite variables of facet count & corneal area###
#when left eye was missing or damaged, right is used
eye$whole_count <- ifelse(is.na(eye$L_whole_count), eye$R_whole_count, eye$L_</pre>
whole count)
eye$area <- ifelse(is.na(eye$L_area), eye$R_area, eye$L_area)</pre>
#log-transformed variables
eye$area_log <- (log10(eye$area))</pre>
hist(eye$area_log)
```

Histogram of eye\$area_log



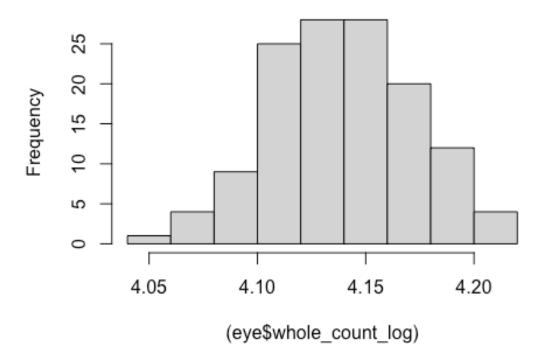
```
eye$tibia_length_log <- (log10(eye$tibia_length))
hist((eye$tibia_length_log))</pre>
```

Histogram of (eye\$tibia_length_log)



```
eye$whole_count_log <- (log10(eye$whole_count))
hist((eye$whole_count_log))</pre>
```

Histogram of (eye\$whole_count_log)



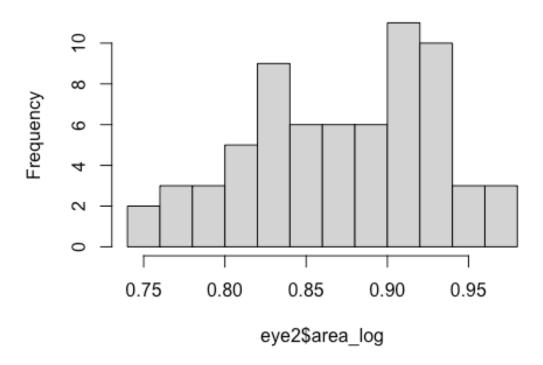
```
#some individuals are missing body size estimate (tibia length)
#remove from the dataset
eye <- eye[!(is.na(eye$tibia_length) | eye$tibia_length==""), ]</pre>
#how many per species & sex
t.first <- eye[match(unique(eye$ID), eye$ID),]</pre>
t.first %>%
  group_by(collection) %>%
  summarize(count = n())
## # A tibble: 3 × 2
##
     collection count
##
     <chr>
                 <int>
## 1 PAN 2007
                    12
## 2 PAN_2015
                    29
## 3 PAN_2017
                    86
#CP
       female
                 19
#CP
       male
                 15
#CPxMP female
                 15
#CPxMP male
                 15
       female
#MP
                  15
       male
                  18
#MP
```

```
#MPxCP female
#MPxCP male
                 15
#dataset without F1 hybrids
eye2 <- eye[-which(eye$type=="CPxMP"
                  eye$type=="MPxCP"),]
#how many per species & sex
t.second <- eye2[match(unique(eye2$ID), eye2$ID),]</pre>
t.second %>%
  group_by(type,sex) %>%
  summarize(count = n())
## `summarise()` has grouped output by 'type'. You can override using the
## `.groups` argument.
## # A tibble: 4 × 3
## # Groups:
              type [2]
   type sex
                 count
     <fct> <fct> <int>
##
## 1 CP
          female
                     19
## 2 CP
          male
                     15
## 3 MP female
                     15
## 4 MP
          male
                     18
      female
#CP
                19
#CP
      male
                15
#MP
      female
                15
#MP
      male
                 18
####Correlations between left and right sides####
#using all processed samples (pure species + F1 hybrids)
cor.test(eye$L whole count,eye$R whole count, method="pearson")
##
## Pearson's product-moment correlation
##
## data: eye$L whole count and eye$R whole count
## t = 43.863, df = 116, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## 0.9586848 0.9798957
## sample estimates:
##
        cor
## 0.971152
```

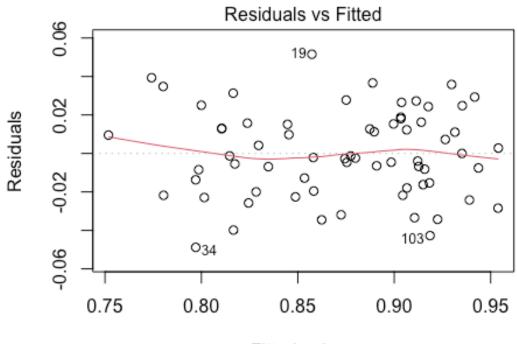
```
cor.test(eye$L area,eye$R area, method="pearson")
##
## Pearson's product-moment correlation
##
## data: eye$L area and eye$R area
## t = 63.881, df = 116, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## 0.9800032 0.9903235
## sample estimates:
##
         cor
## 0.9860831
#import dataset with L & R hind tibia Lengths
legs <- read.csv("~/Documents/LMU/Eye morphology/selection manuscript/legs.cs</pre>
v")
cor.test(legs$L_length,legs$R_length, method="pearson")
##
## Pearson's product-moment correlation
## data: legs$L length and legs$R length
## t = 25.475, df = 101, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## 0.8984434 0.9523152
## sample estimates:
         cor
## 0.9302284
####Correlations between body size, facet count, corneal area####
#without hybrids, onle pure species
cor.test(eye2$tibia_length,eye2$whole_count, method="pearson")
##
## Pearson's product-moment correlation
## data: eye2$tibia length and eye2$whole count
## t = 6.7425, df = 65, p-value = 4.934e-09
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## 0.4744268 0.7640020
## sample estimates:
##
         cor
## 0.6415262
cor.test(eye2$area,eye2$whole count, method="pearson")
##
  Pearson's product-moment correlation
```

```
##
## data: eye2$area and eye2$whole count
## t = 14.361, df = 65, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## 0.7991763 0.9195831
## sample estimates:
         cor
## 0.8719898
cor.test(eye2$tibia length,eye2$area, method="pearson")
##
## Pearson's product-moment correlation
##
## data: eye2$tibia length and eye2$area
## t = 13.167, df = 65, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## 0.7704437 0.9071889
## sample estimates:
##
         cor
## 0.8528228
####WHAT IS THE RELATIONSHIP BETWEEN FACET COUNT AND CORNEAL AREA?###
#pure species only
hist(eye2$area_log)
```

Histogram of eye2\$area_log

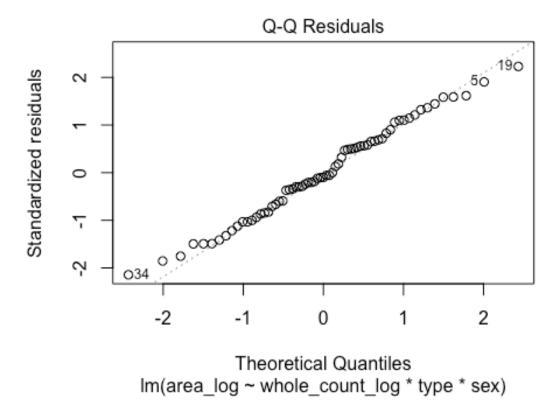


d1 <- lm(area_log~whole_count_log*type*sex, data=eye2)
plot(d1, which=1)</pre>



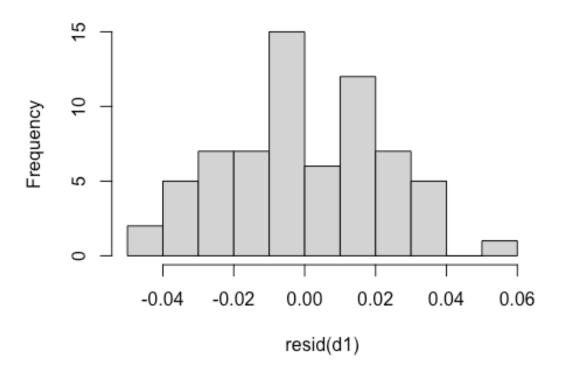
Fitted values Im(area_log ~ whole_count_log * type * sex)

plot(d1, which=2)



hist(resid(d1))

Histogram of resid(d1)

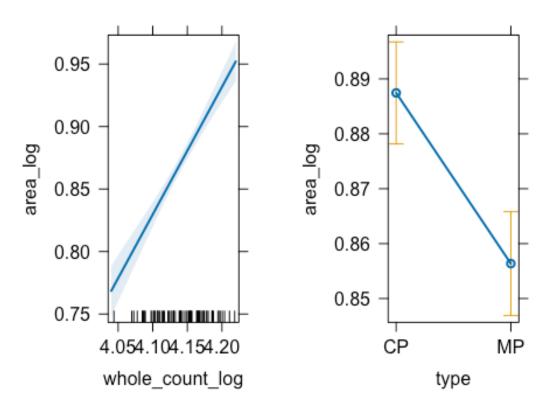


```
drop1(d1, test="Chisq") #3-way interaction 0.098, remove
## Single term deletions
##
## Model:
## area_log ~ whole_count_log * type * sex
                            Df Sum of Sq
                                                      AIC Pr(>Chi)
                                              RSS
                                         0.033481 -493.30
## <none>
## whole_count_log:type:sex 1 0.0013944 0.034875 -492.57 0.09824 .
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
d2 <- update(d1,.~.-whole_count_log:type:sex)</pre>
drop1(d2, test="Chisq") #all 2-way interactions ns
## Single term deletions
##
## Model:
## area_log ~ whole_count_log + type + sex + whole_count_log:type +
       whole_count_log:sex + type:sex
##
                        Df Sum of Sq
                                           RSS
                                                   AIC Pr(>Chi)
## <none>
                                      0.034875 -492.57
## whole_count_log:type 1 0.00089661 0.035772 -492.86
```

```
## whole count log:sex
                         1 0.00023606 0.035111 -494.11
                                                          0.5014
## type:sex
                         1 0.00000165 0.034877 -494.56
                                                          0.9551
d3 <- update(d2,.~.-type:sex)</pre>
drop1(d3, test="Chisq")
## Single term deletions
##
## Model:
## area log ~ whole count log + type + sex + whole count log:type +
       whole count log:sex
##
                        Df Sum of Sq
                                            RSS
                                                    AIC Pr(>Chi)
## <none>
                                      0.034877 -494.56
## whole_count_log:type 1 0.00125965 0.036136 -494.18
                                                          0.1231
## whole_count_log:sex
                       1 0.00041413 0.035291 -495.77
d4 <- update(d3,.~.-whole count log:sex)
drop1(d4, test="Chisq")
## Single term deletions
##
## Model:
## area log ~ whole count log + type + sex + whole count log:type
##
                        Df Sum of Sq
                                           RSS
                                                   AIC Pr(>Chi)
## <none>
                                      0.035291 -495.77
## sex
                         1 0.00072766 0.036019 -496.40
                                                          0.2423
## whole count log:type 1 0.00141206 0.036703 -495.14
                                                        0.1050
d5 <- update(d4,.~.-whole count log:type)
drop1(d5, test="Chisq") #sex ns
## Single term deletions
##
## Model:
## area_log ~ whole_count_log + type + sex
##
                   Df Sum of Sq
                                     RSS
                                             AIC
                                                   Pr(>Chi)
                                0.036703 -495.14
## <none>
## whole_count_log 1
                       0.060948 0.097651 -431.58 5.631e-16 ***
## type
                    1
                       0.007038 0.043741 -485.39 0.0006073 ***
                       0.000860 0.037563 -495.59 0.2128677
## sex
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
d6 <- update(d5,.~.-sex)
drop1(d6, test="Chisq")
## Single term deletions
##
## Model:
## area_log ~ whole_count_log + type
                   Df Sum of Sq
                                     RSS
                                             AIC
                                                   Pr(>Chi)
## <none>
                                0.037563 -495.59
```

```
## whole count log 1 0.070850 0.108413 -426.58 < 2.2e-16 ***
                      0.010692 0.048255 -480.81 4.194e-05 ***
## type
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
plot(allEffects(d6))
```

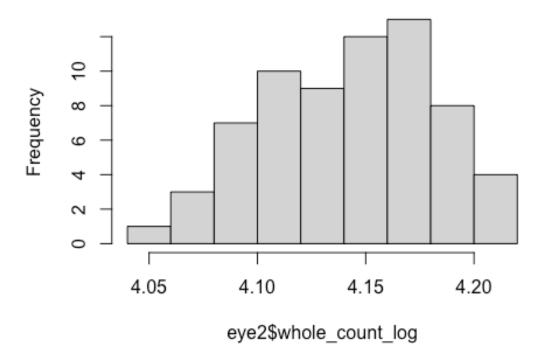
vhole_count_log effect plot type effect plot



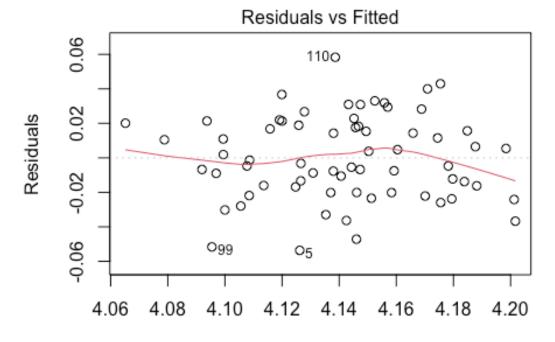
```
Anova(d6, test="Chisq")
## Anova Table (Type II tests)
##
## Response: area_log
                    Sum Sq Df F value
                                         Pr(>F)
## whole_count_log 0.070850 1 120.715 2.269e-16 ***
                  0.010692 1 18.216 6.650e-05 ***
## type
## Residuals
                  0.037563 64
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
#facet count p<0.001
#type p<0.001
Anova(d4, test="Chisq")
```

```
## Anova Table (Type II tests)
##
## Response: area_log
                         Sum Sq Df F value
                                               Pr(>F)
## whole_count_log
                       0.060948 1 107.0742 3.918e-15 ***
## type
                       0.007038 1 12.3644 0.0008246 ***
## sex
                       0.000728 1 1.2784 0.2625574
## whole_count_log:type 0.001412 1
                                     2.4807 0.1203373
## Residuals
                       0.035291 62
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
#facet count:species n.s. p=0.12
####ANALYSES OF FACET COUNT - C & M only####
#full statistical model with only C & M
hist(eye2$whole_count_log)
```

Histogram of eye2\$whole_count_log

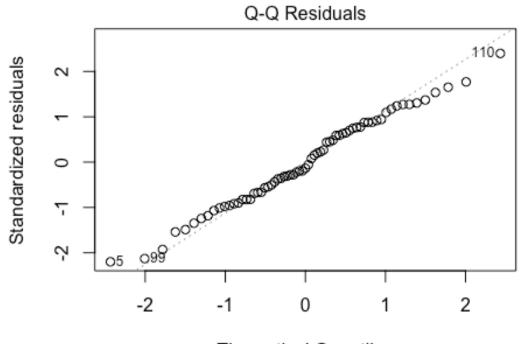


```
m1 <- lm(whole_count_log~type*sex+tibia_length_log, data=eye2)
plot(m1, which=1)</pre>
```



Fitted values
Im(whole_count_log ~ type * sex + tibia_length_log)

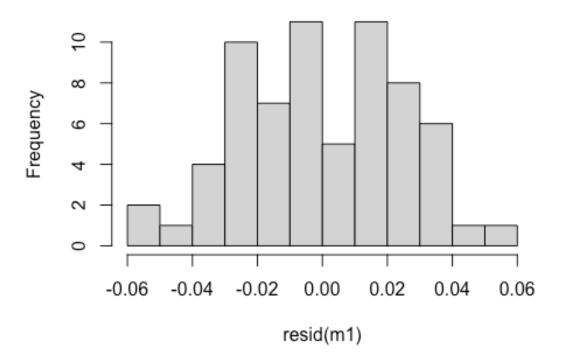
plot(m1, which=2)



Theoretical Quantiles
Im(whole_count_log ~ type * sex + tibia_length_log)

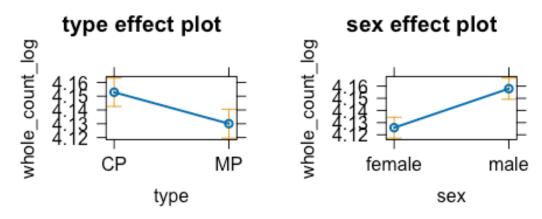
hist(resid(m1))

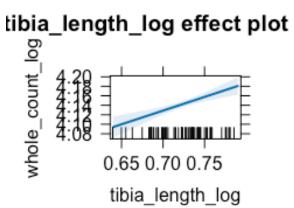
Histogram of resid(m1)



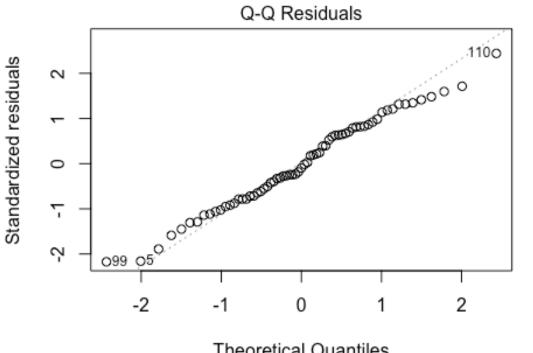
```
drop1(m1, test="Chisq") #type:sex interaction ns
## Single term deletions
##
## Model:
## whole_count_log ~ type * sex + tibia_length_log
                    Df Sum of Sq
                                      RSS
                                              AIC Pr(>Chi)
                                 0.039226 -488.69
## <none>
## tibia length log 1 0.0127784 0.052005 -471.79 1.382e-05 ***
## type:sex
                     1 0.0000828 0.039309 -490.55
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
m2 <- update(m1,.~.-type:sex)</pre>
drop1(m2, test="Chisq") #all three significant
## Single term deletions
##
## Model:
## whole_count_log ~ type + sex + tibia_length_log
##
                    Df Sum of Sq
                                              AIC Pr(>Chi)
                                      RSS
## <none>
                                 0.039309 -490.55
## type
                     1 0.0045653 0.043874 -485.19 0.006663 **
                     1 0.0170625 0.056371 -468.39 8.892e-07 ***
## sex
```

```
## tibia_length_log 1 0.0127559 0.052065 -473.72 1.429e-05 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
plot(allEffects(m2))
```





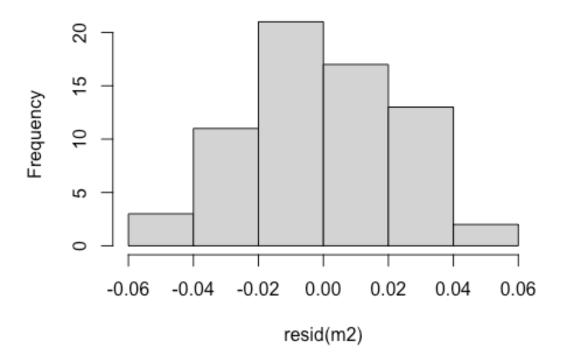
```
plot(m2, which = 2)
```



Theoretical Quantiles
Im(whole_count_log ~ type + sex + tibia_length_log)

hist(resid(m2))

Histogram of resid(m2)



```
Anova(m2, test.statistic="Chisq")
## Anova Table (Type II tests)
##
## Response: whole_count_log
##
                    Sum Sq Df F value
                                       Pr(>F)
## type
                  0.004565 1 7.3168 0.008775 **
                  0.017063 1 27.3460 2.060e-06 ***
## sex
## Residuals
                  0.039309 63
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
#type p=0.0087
#sex p>0.0001
#tibia p>0.0001
Anova(m1, test.statistic="Chisq")
## Anova Table (Type II tests)
## Response: whole_count_log
                    Sum Sq Df F value
                                       Pr(>F)
## type
                  0.004565 1 7.2159 0.009262 **
```

```
## sex
                   0.017063 1 26.9687 2.435e-06 ***
0.000083 1 0.1309 0.718760
## type:sex
                   0.039226 62
## Residuals
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
#type:sex n.s. p=0.78
m2.1 <- update(m2,.~.-type)</pre>
PBmodcomp(m2, m2.1, nsim = 1000) #same result with parametric bootstrapping as
with Anova
## Bootstrap test; time: 2.51 sec; samples: 1000; extremes: 5;
## large : whole count log ~ type + sex + tibia length log
## whole_count_log ~ sex + tibia_length_log
           stat df p.value
##
         7.3617 1 0.006663 **
## LRT
## PBtest 7.3617
                   0.005994 **
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '* 0.05 '.' 0.1 ' ' 1
#####plot of facet count based on MAM####
#using tibia length
#theme settings for plots
require(grid)
pub<- theme_update(</pre>
 panel.grid.major=element_line(colour=NA),
  panel.grid.minor=element_line(colour=NA),
 panel.background = element_rect(colour = NA, fill=NA, linewidth = 0.5),
 panel.border = element rect(linetype = "solid", colour = "black",fill=NA),
 axis.line.x = element line(color="black"),
 axis.line.y = element_line(color="black"),
 axis.title.x=element_text(size=15, face="bold", hjust=0.5, vjust=0.5, angle=0),
 axis.title.y=element_text(size=15, face="bold", hjust=0.5, vjust=1, angle=90),
 axis.text.x=element_text(colour="black",angle=0,size=15),
 axis.text.y=element_text(colour="black",angle=0,size=15),
 axis.ticks=element line(colour="black",linewidth=0.5))
#get estimated marginal means of the final model (m2)
######**species**####
library(tidyverse)
## — Attaching core tidyverse packages —
                                                              – tidyverse 2.
0.0 -
## √ forcats 1.0.0

√ stringr

                                     1.5.0
## ✓ lubridate 1.9.2

√ tibble

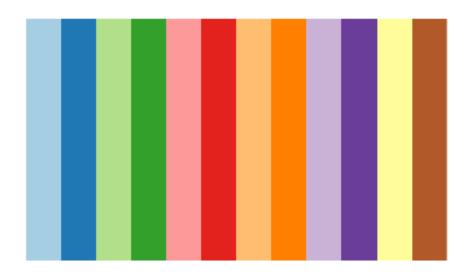
                                     3.2.1
```

```
## √ purrr

√ tidyr

                1.0.1
                                       1.3.0
## ✓ readr
                2.1.4
## — Conflicts -
                                                           tidyverse conflict
s() —
## * dplyr::combine() masks gridExtra::combine()
## * tidyr::expand() masks Matrix::expand()
## * dplyr::filter() masks stats::filter()
## X dplyr::lag()
                       masks stats::lag()
## * tidyr::pack()
                       masks Matrix::pack()
## * dplyr::recode() masks car::recode()
## * dplyr::select() masks MASS::select()
## * purrr::some()
                       masks car::some()
## * tidyr::unpack() masks Matrix::unpack()
## 1 Use the conflicted package (<a href="http://conflicted.r-lib.org/">http://conflicted.r-lib.org/</a>) to force al
l conflicts to become errors
library(emmeans)
library(multcomp)
## Loading required package: mvtnorm
## Loading required package: survival
## Loading required package: TH.data
##
## Attaching package: 'TH.data'
##
## The following object is masked from 'package:MASS':
##
##
       geyser
library(multcompView)
model_means <- emmeans(object = m2, specs = ~ type, type = "response")</pre>
#p-value comparison table
pwpm(model_means, adjust="bonferroni", diffs = F)
                   MP
           CP
## CP [4.153] 0.0088
## MP
              [4.130]
##
## Row and column labels: type
## Upper triangle: P values
## Diagonal: [Estimates] (emmean) type = "response"
#effect size
eff size(model means, sigma = sigma(m2), edf = df.residual(m2))
## contrast estimate
                         SE df lower.CL upper.CL
## CP - MP 0.913 0.347 63 0.219
```

```
##
## Results are averaged over the levels of: sex
## sigma used for effect sizes: 0.02498
## Confidence level used: 0.95
#add letters to each mean to indicate difference
model_means_cld <- cld(object = model_means,</pre>
                       reversed=T,
                       adjust="bonferroni",
                       Letters = letters,
                       alpha = 0.05)
#set marginal means at a dataframe
facet <- as.data.frame(summary(model_means_cld))</pre>
#species
#color selection for consistenty with other papers
library(RColorBrewer)
display.brewer.pal(12, 'Paired')
```



Paired (qualitative)

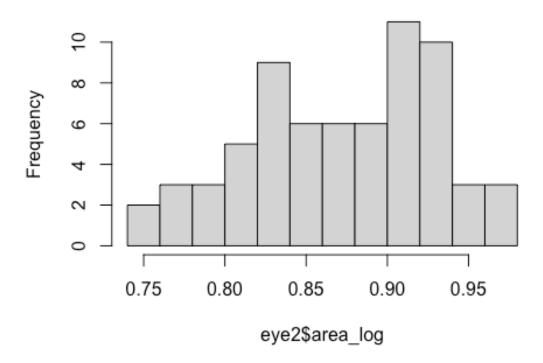
```
brewer.pal(12, 'Paired')
```

```
## [1] "#A6CEE3" "#1F78B4" "#B2DF8A" "#33A02C" "#FB9A99" "#E31A1C" "#FDBF6F"
## [8] "#FF7F00" "#CAB2D6" "#6A3D9A" "#FFFF99" "#B15928"
facet$type <- factor(facet$type, c("CP", "MP"))</pre>
species <- ggplot(facet, aes(type,emmean))+</pre>
  geom_errorbar(data=facet,mapping=aes(x=type, ymin=upper.CL, ymax=lower.CL),
color="grey45",
                width=0, linewidth=1, position = position_dodge(0.4))+
  geom_point(data=facet, aes(type,emmean, color=type), size=4,
             position = position dodge(0.4))+
  geom_text(aes(label = .group, y = emmean), position = position_dodge(0.4),
            vjust=0.3, hjust=-0.2, size=4.5) +
  labs(x="",y="e.m.m. log10 (facet count)")+
  scale_y_continuous(breaks=seq(4.11, 4.17, 0.02), limits=c(4.10, 4.174))+
  scale_x_discrete(labels=c("", "", ""))+
  scale_color_manual(values = c("#1F78B4","#FDBF6F"))+
  theme_update(axis.title.y=element_text(size=14,face="bold",hjust=0.5,vjust=
2.5, angle=90))+
  theme(plot.margin = margin(0.4,0.5,0.3,0.75, "cm"))+
  theme(legend.position="none", legend.key=element blank(),legend.title=eleme
nt blank(),
        legend.text=element text(size=10))+
  theme(axis.text.x=element_blank())+
  theme(text = element text(family = "Times New Roman"))+
  theme(axis.title.y = element_text(vjust = 5))+
  ggtitle("species effect") + theme(plot.title = element_text(hjust = 0.5, si
ze=16, face="bold"))
#####**sex**####
model_means2 <- emmeans(object = m2, specs = ~ sex, type = "response")</pre>
#p-value comparison table
pwpm(model means2, adjust="bonferroni", diffs = F)
           female
                     male
## female [4.126] <.0001
## male
                  [4.158]
##
## Row and column labels: sex
## Upper triangle: P values
## Diagonal: [Estimates] (emmean) type = "response"
#effect size
eff_size(model_means2, sigma = sigma(m2), edf = df.residual(m2))
## contrast
                              SE df lower.CL upper.CL
                  estimate
## female - male
                    -1.29 0.271 63
                                       -1.83
                                               -0.744
##
```

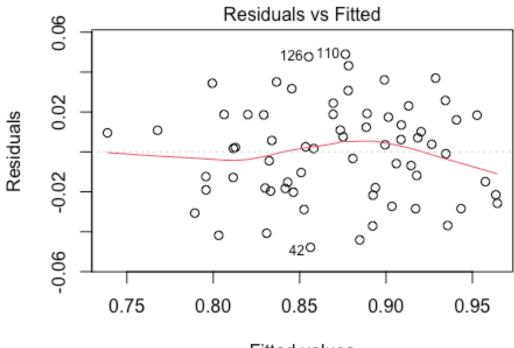
```
## Results are averaged over the levels of: type
## sigma used for effect sizes: 0.02498
## Confidence level used: 0.95
#add Letters to each mean to indicate difference
model means cld2 <- cld(object = model means2,
                        reversed = T,
                        adjust="bonferroni",
                        Letters = letters,
                        alpha = 0.05)
#set marginal means at a dataframe
facet2 <- as.data.frame(summary(model means cld2))</pre>
#plot marginal means
facet2$sex <- factor(facet2$sex, c("male", "female"))</pre>
#sex
sex <- ggplot(facet2, aes(sex,emmean))+</pre>
  geom errorbar(data=facet2,mapping=aes(x=sex, ymin=upper.CL, ymax=lower.CL),
color="grey45",
                width=0, linewidth=1)+
  geom point(data=facet2, aes(sex,emmean), size=4)+
  geom_text(aes(label = .group, y = emmean),
            vjust=0.3, hjust=-0.3, size=4.5) +
  labs(x="",y="")+
  scale_y_continuous(breaks=seq(4.11, 4.17, 0.02), limits=c(4.10, 4.174),
                     position="right")+
  scale_x_discrete(labels = c("", ""))+
  theme_update(axis.title.y=element_text(size=14,face="bold",hjust=0.5,vjust=
2.5, angle=90))+
  theme(legend.position=c(0.88,0.89), legend.key=element blank(),legend.title
=element blank(),
        legend.text=element text(size=10))+
  theme(plot.margin = margin(0.4,1.05,0.3,0.85, "cm"))+
  theme(axis.text.x=element_blank())+
  theme(text = element_text(family = "Times New Roman"))+
  ggtitle("sex effect") + theme(plot.title = element text(hjust = 0.5, size=1
6, face="bold"))
######**species:sex**####
model_means3 <- emmeans(object = m2, specs = ~ type:sex, type = "response")</pre>
#p-value comparison table
pwpm(model_means3, adjust="bonferroni", diffs = F)
             CP female MP female CP male MP male
##
## CP female [4.137] 0.0527 <.0001 1.0000
```

```
## MP female
                         [4.114] <.0001 <.0001
## CP male
                                  [4.169] 0.0527
## MP male
                                          [4.146]
##
## Row and column labels: type:sex
## Upper triangle: P values
                             adjust = "bonferroni"
## Diagonal: [Estimates] (emmean) type = "response"
#add Letters to each mean to indicate difference
model_means_cld3 <- cld(object = model_means3,</pre>
                        adjust = "bonferroni",
                        reversed=T,
                        Letters = letters,
                        alpha = 0.05)
#set marginal means at a dataframe
facet3 <- as.data.frame(summary(model means cld3))</pre>
#plot marginal means
facet3$sex <- factor(facet3$sex, c("male", "female"))</pre>
facet3$type <- factor(facet3$type, c("CP", "MP"))</pre>
#species:sex plot
combo1<-ggplot(facet3, aes(type,emmean))+</pre>
  geom_errorbar(data=facet3,mapping=aes(x=type, ymin=upper.CL, ymax=lower.CL,
color=sex),
                width=0, linewidth=1, position = position dodge(width=0.5))+
  geom point(data=facet3, aes(type,emmean, color=sex), size=3,
             position = position_dodge2(width=0.5))+
  geom text(aes(label = .group, y = emmean),
            vjust=0.3, hjust=-0.3, size=4.5, position = position_dodge2(width=
(0.5)) +
  labs(x="",y="e.m.m. log10 (facet count)", color="")+
  scale y continuous(breaks=seq(4.09, 4.21, 0.03), limits=c(4.08, 4.21))+
  scale_x_discrete(labels=c("H. cydno", "H. melp"))+
  scale color manual(values=c("black", "grey65"))+
  theme(legend.position="", legend.key=element_blank(),legend.title=element_b
lank(),
        legend.text=element text(size=10))+
  theme(plot.margin = margin(1,0.5,0.5,0.5, "cm"))+
  theme(legend.text=element_text(size=11))+
  theme(legend.key=element blank())+
  theme(axis.text.x=element text(face = "bold"))+
  theme(text = element_text(family = "Times New Roman"))
####ANALYSES OF CORNEAL AREA - C & M ONLY####
hist(eye2$area log)
```

Histogram of eye2\$area_log

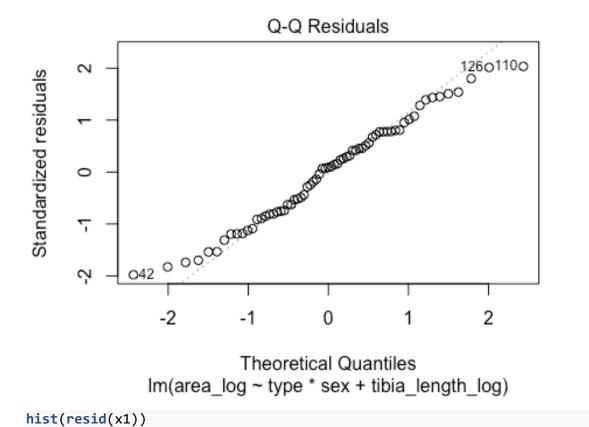


```
x1 <- lm(area_log~type*sex+tibia_length_log, data=eye2)
plot(x1,which=1)</pre>
```

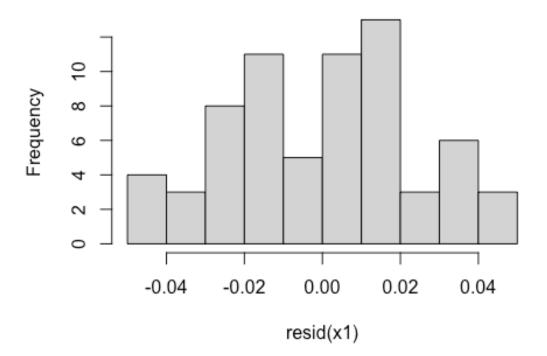


Fitted values
Im(area_log ~ type * sex + tibia_length_log)

plot(x1,which=2)



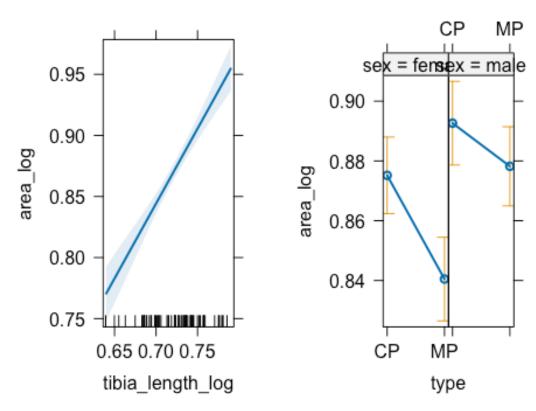
Histogram of resid(x1)



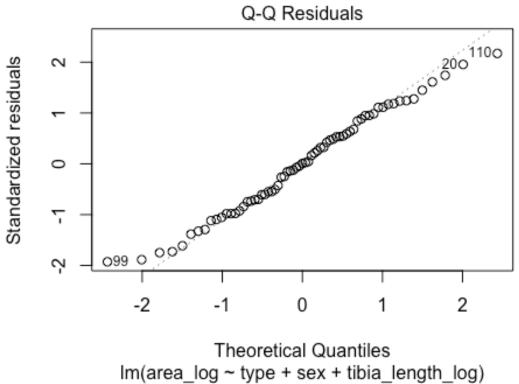
```
drop1(x1, test="Chisq") #interaction weakly trending (0.08)
## Single term deletions
##
## Model:
## area_log ~ type * sex + tibia_length_log
                   Df Sum of Sq
                                     RSS
                                             AIC Pr(>Chi)
                                0.038397 -490.12
## <none>
## tibia length log 1 0.057777 0.096174 -430.60 4.388e-15 ***
## type:sex
                    1 0.001702 0.040100 -489.21
                                                   0.08824 .
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
Anova(x1, test="Chisq") #interaction n.s. p=0.102, remove
## Anova Table (Type II tests)
## Response: area log
                     Sum Sq Df F value
##
                                          Pr(>F)
## type
                   0.005391 1 8.7050 0.004473 **
                   0.012487 1 20.1622 3.159e-05 ***
## tibia_length_log 0.057777 1 93.2916 5.608e-14 ***
## type:sex
                   0.001702 1
                                2.7485 0.102394
## Residuals
                   0.038397 62
```

```
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
plot(allEffects(x1))
```

tibia_length_log effect plot type*sex effect plot

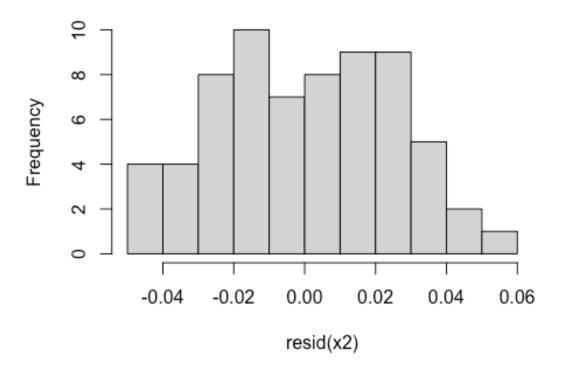


```
x2 <- update(x1,.~.-type:sex)</pre>
drop1(x2, test="Chisq")
## Single term deletions
##
## Model:
## area log ~ type + sex + tibia length log
                                                    Pr(>Chi)
##
                    Df Sum of Sq
                                       RSS
                                               AIC
## <none>
                                  0.040100 -489.21
                        0.005391 0.045491 -482.76
## type
                                                   0.003647 **
                        0.012487 0.052586 -473.05 2.028e-05 ***
## sex
## tibia_length_log 1
                        0.057551 0.097651 -431.58 1.144e-14 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
plot(x2, which=2)
```



hist(resid(x2))

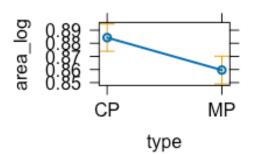
Histogram of resid(x2)

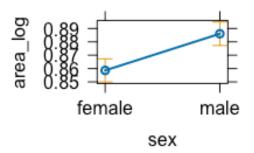


```
Anova(x2, test="Chisq")
## Anova Table (Type II tests)
##
## Response: area_log
                     Sum Sq Df F value
##
                                          Pr(>F)
                   0.005391 1 8.4699 0.004985 **
## type
                   0.012487 1 19.6177 3.844e-05 ***
## sex
## tibia_length_log 0.057551 1 90.4172 8.610e-14 ***
## Residuals
                   0.040100 63
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
#type p=0.004
#sex p>0.001
#tibia p>0.001
plot(allEffects(x2))
```

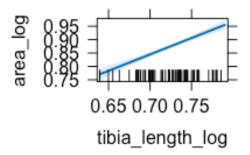
type effect plot

sex effect plot





tibia_length_log effect plot



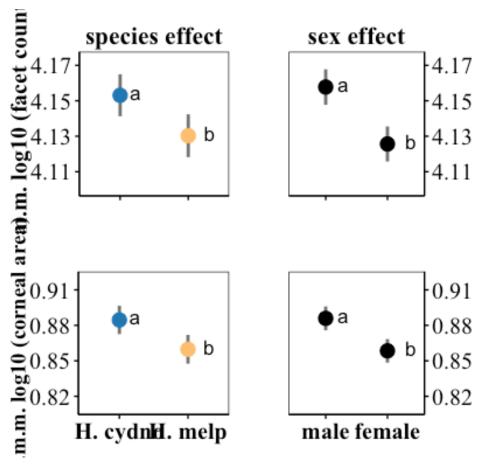
```
#####plot of corneal area based on MAM####
\#get\ estimated\ marginal\ means\ of\ the\ final\ model\ (x2)
#####**species**####
model_means_x <- emmeans(object = x2, specs = ~ type, type = "response")</pre>
#p-value comparison table
pwpm(model_means_x, adjust="bonferroni", diffs = F)
           CP
## CP [0.885] 0.0050
## MP
              [0.860]
##
## Row and column labels: type
## Upper triangle: P values
## Diagonal: [Estimates] (emmean) type = "response"
#effect size
eff_size(model_means_x, sigma = sigma(x2), edf = df.residual(x2))
```

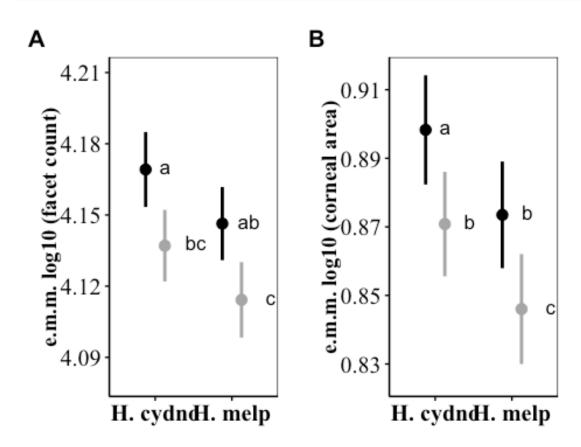
```
## contrast estimate SE df lower.CL upper.CL
## CP - MP
                0.983 0.349 63
                                  0.286
                                             1.68
## Results are averaged over the levels of: sex
## sigma used for effect sizes: 0.02523
## Confidence level used: 0.95
#add Letters to each mean to indicate difference
model_means_cld_x <- cld(object = model_means_x,</pre>
                         reversed=T,
                         adjust="bonferroni",
                         Letters = letters,
                         alpha = 0.05)
#set marginal means at a dataframe
area <- as.data.frame(summary(model means cld x))</pre>
#species
area$type <- factor(area$type, c("CP", "MP"))</pre>
species2 <- ggplot(area, aes(type,emmean))+</pre>
  geom errorbar(data=area,mapping=aes(x=type, ymin=upper.CL, ymax=lower.CL),
color="grey45",
                width=0, linewidth=1, position = position_dodge(0.4))+
  geom_point(data=area, aes(type,emmean,color=type), size=4,
             position = position dodge(0.4))+
  geom_text(aes(label = .group, y = emmean), position = position_dodge(0.4),
            vjust=0.3, hjust=-0.2, size=4.5) +
  labs(x="",y="e.m.m. log10 (corneal area)")+
  scale_y_continuous(breaks=seq(0.82, 0.92, 0.03), limits=c(0.81, 0.92))+
  scale_x_discrete(labels=c("H. cydno", "H. melp"))+
  scale color manual(values = c("#1F78B4","#FDBF6F"))+
  theme update(axis.title.y=element text(size=14,face="bold",hjust=0.5,vjust=
2.5, angle=90)+
  theme(legend.position="none", legend.key=element_blank(),legend.title=eleme
nt blank(),
        legend.text=element text(size=10))+
  theme(plot.margin = margin(0.3,0.5,0,0.75, "cm"))+
  theme(axis.text.x=element text(face = "bold"))+
  theme(text = element text(family = "Times New Roman"))+
  theme(axis.title.y = element_text(vjust = 5))+
  ggtitle("") + theme(plot.title = element_text(hjust = 0.5, size=16, face="bo
ld"))
#####**sex**####
model_means2_x <- emmeans(object = x2, specs = ~ sex, type = "response")</pre>
```

```
#p-value comparison table
pwpm(model means2 x, adjust="bonferroni", diffs = F)
##
           female
                     male
## female [0.858] <.0001
## male
                  [0.886]
##
## Row and column labels: sex
## Upper triangle: P values
## Diagonal: [Estimates] (emmean) type = "response"
#effect size
eff_size(model_means2_x, sigma = sigma(x2), edf = df.residual(x2))
## contrast
                  estimate
                              SE df lower.CL upper.CL
## female - male
                     -1.09 0.264 63
                                       -1.62
##
## Results are averaged over the levels of: type
## sigma used for effect sizes: 0.02523
## Confidence level used: 0.95
#add letters to each mean to indicate difference
model means cld2 x <- cld(object = model means2 x,
                          reversed = T,
                          adjust="bonferroni",
                          Letters = letters,
                          alpha = 0.05)
#set marginal means at a dataframe
area2 <- as.data.frame(summary(model means cld2 x))</pre>
#plot marginal means
area2$sex <- factor(area2$sex, c("male", "female"))</pre>
#sex
sex2<- ggplot(area2, aes(sex,emmean))+</pre>
  geom_errorbar(data=area2,mapping=aes(x=sex, ymin=upper.CL, ymax=lower.CL),
color="grey45",
                width=0, linewidth=1)+
  geom point(data=area2, aes(sex,emmean), size=4)+
  geom_text(aes(label = .group, y = emmean),
            vjust=0.3, hjust=-0.3, size=4.5) +
  labs(x="",y="")+
  scale_y_continuous(breaks=seq(0.82, 0.92, 0.03), limits=c(0.81, 0.92),
                     position="right")+
  scale_x_discrete(labels = c("male", "female"))+
  theme_update(axis.title.y=element_text(size=14,face="bold",hjust=0.5,vjust=
2.5, angle=90))+
  theme(legend.position=c(0.88,0.89), legend.key=element_blank(),legend.title
=element blank(),
```

```
legend.text=element text(size=10))+
  theme(plot.margin = margin(0.3,1.05,0,0.85, "cm"))+
  theme(axis.text.x=element_text(face = "bold"))+
  theme(text = element text(family = "Times New Roman"))+
  ggtitle("") + theme(plot.title = element_text(hjust = 0.5, size=16,face="bo
ld"))
######**species:sex**####
model_means3_x <- emmeans(object = x2, specs = ~ type:sex, type = "response")</pre>
#p-value comparison table
pwpm(model means3 x, adjust="bonferroni", diffs = F)
             CP female MP female CP male MP male
##
                          0.0299 0.0002 1.0000
## CP female
              [0.871]
## MP female
                         [0.846] <.0001 0.0002
## CP male
                                  [0.898] 0.0299
## MP male
                                          [0.873]
##
## Row and column labels: type:sex
## Upper triangle: P values
                             adjust = "bonferroni"
## Diagonal: [Estimates] (emmean) type = "response"
#add Letters to each mean to indicate difference
model means cld3 x \leftarrow cld(object = model means3 x,
                          adjust = "bonferroni",
                          reversed = T,
                          Letters = letters,
                          alpha = 0.05)
#set marginal means at a dataframe
area3 <- as.data.frame(summary(model_means_cld3_x))</pre>
#plot marginal means
area3$sex <- factor(area3$sex, c("male", "female"))</pre>
#species:sex plot
combo2<-ggplot(area3, aes(type,emmean))+</pre>
  geom_errorbar(data=area3,mapping=aes(x=type, ymin=upper.CL, ymax=lower.CL,
color=sex),
                width=0, linewidth=1, position = position_dodge(width=0.5))+
  geom_point(data=area3, aes(type,emmean, color=sex), size=3,
             position = position dodge2(width=0.5))+
  geom text(aes(label = .group, y = emmean),
            vjust=0.3, hjust=-0.3, size=4.5, position = position_dodge2(width=
(0.5)) +
  labs(x="",y="e.m.m. log10 (corneal area)", color="")+
  scale_y_continuous(breaks=seq(0.83, 0.91, 0.02), limits=c(0.825, 0.915),
```

```
position="left")+
  scale_x_discrete(labels=c("H. cydno", "H. melp"))+
  scale_color_manual(values=c("black", "grey65"))+
  theme(legend.position="", legend.key=element_blank(),legend.title=element_b
lank(),
        legend.text=element_text(size=10))+
  theme(plot.margin = margin(1,0.5,0.5,0.5, "cm"))+
  theme(legend.text=element_text(size=11))+
  theme(legend.key=element_blank())+
  theme(axis.text.x=element text(face = "bold"))+
  theme(text = element_text(family = "Times New Roman"))
####MASTER PLOT OF ALL 4 EMMS - C & M ONLY####
library(ggpubr)
#####**species & sex separate**####
ggarrange(species, sex, species2, sex2,
                       ncol = 2, nrow = 2, widths = c(1, 1.05),
                       font.label = list(size = 15))
```

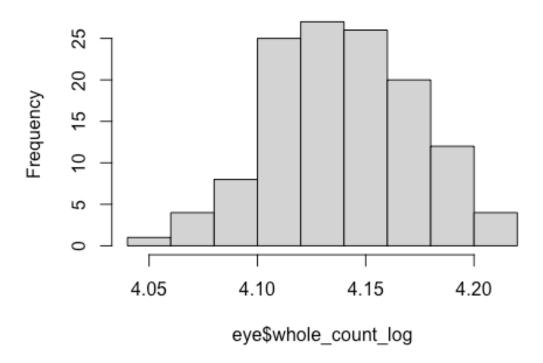




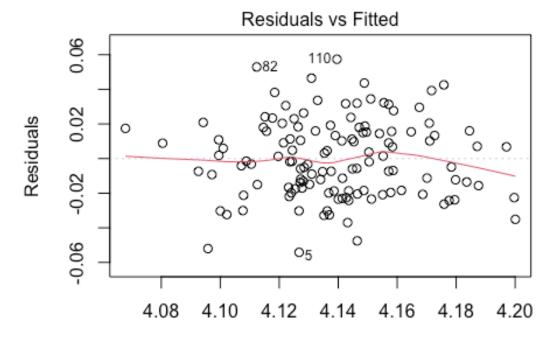
```
####ANALYSES OF FACET COUNT - C, M & F1 HYBRIDS####

#full statistical model
hist(eye$whole_count_log)
```

Histogram of eye\$whole_count_log

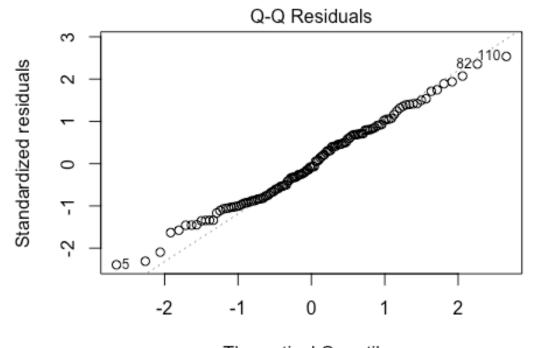


```
w1 <- lm(whole_count_log~type*sex+tibia_length_log, data=eye)
plot(w1, which=1)</pre>
```



Fitted values
Im(whole_count_log ~ type * sex + tibia_length_log)

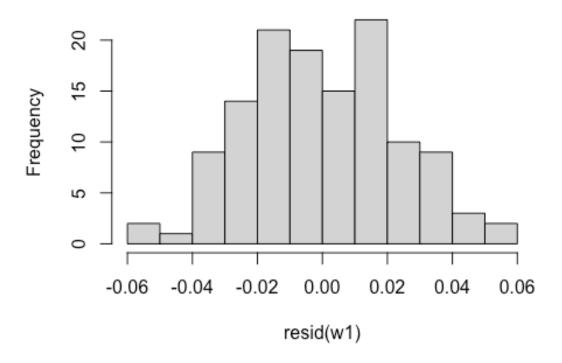
plot(w1, which=2)



Theoretical Quantiles
Im(whole_count_log ~ type * sex + tibia_length_log)

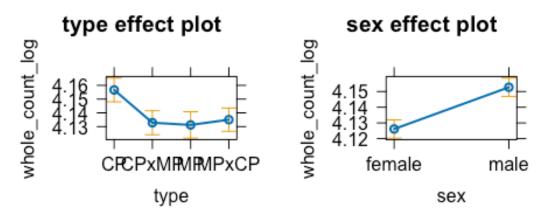
hist(resid(w1))

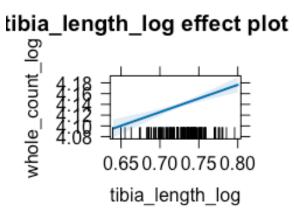
Histogram of resid(w1)



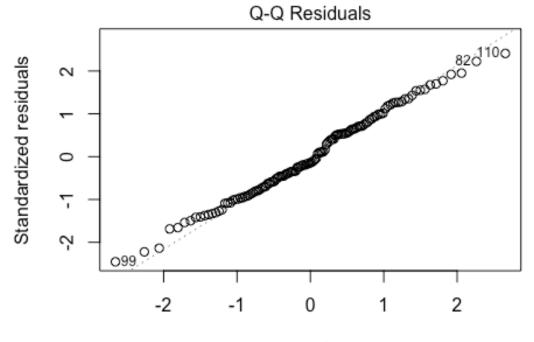
```
drop1(w1, test="Chisq")
## Single term deletions
##
## Model:
## whole_count_log ~ type * sex + tibia_length_log
                    Df Sum of Sq
                                      RSS
                                              AIC Pr(>Chi)
## <none>
                                 0.064274 -945.78
## tibia length log 1 0.0177991 0.082073 -916.73 2.52e-08 ***
## type:sex
                     3 0.0022994 0.066573 -947.31
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
w2 <- update(w1,.~.-type:sex) #type:sex interaction ns
drop1(w2, test="Chisq") #all three significant
## Single term deletions
##
## Model:
## whole_count_log ~ type + sex + tibia_length_log
##
                    Df Sum of Sq
                                              AIC Pr(>Chi)
                                      RSS
## <none>
                                 0.066573 -947.31
## type
                        0.011743 0.078316 -932.68 0.0001255 ***
                        0.022228 0.088801 -912.72 1.459e-09 ***
## sex
```

```
## tibia_length_log 1 0.016372 0.082945 -921.39 1.261e-07 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
plot(allEffects(w2))
```





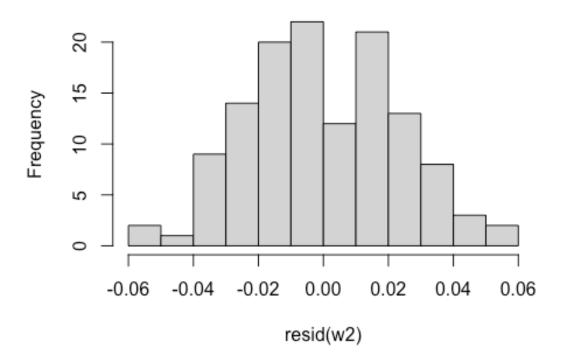
```
plot(w2, which = 2)
```



Theoretical Quantiles
Im(whole_count_log ~ type + sex + tibia_length_log)

hist(resid(w2))

Histogram of resid(w2)



```
Anova(w2, test.statistic="Chisq")
## Anova Table (Type II tests)
##
## Response: whole_count_log
                      Sum Sq Df F value
##
                                            Pr(>F)
                               3 7.1147 0.0001924 ***
## type
                    0.011743
                    0.022228
                               1 40.4010 3.807e-09 ***
## sex
## tibia_length_log 0.016372
                               1 29.7570 2.639e-07 ***
## Residuals
                    0.066573 121
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
#type p>0.001
#sex p>0.001
#tibia p>0.001
Anova(w1, test.statistic="Chisq")
## Anova Table (Type II tests)
## Response: whole_count_log
##
                      Sum Sq Df F value
                                           Pr(>F)
                               3 7.1865 0.0001794 ***
## type
                    0.011743
```

```
## sex
                   0.022228
                              1 40.8088 3.465e-09 ***
## tibia length log 0.017799
                              1 32.6774 8.336e-08 ***
                   0.002299
## type:sex
                              3 1.4072 0.2441719
## Residuals
                   0.064274 118
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
#type:sex p=0.244
#posthoc comparisons
#by species type
library(multcomp)
posthoc <- glht(w2, linfct=mcp(type="Tukey"))</pre>
summary(posthoc,adjusted(type="bonferroni"))
##
##
    Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
##
##
## Fit: lm(formula = whole_count_log ~ type + sex + tibia_length_log,
      data = eye)
##
## Linear Hypotheses:
                      Estimate Std. Error t value Pr(>|t|)
##
## CPxMP - CP == 0
                     ## MP - CP == 0
                     -0.025446
                                 0.007153 -3.558 0.003213 **
                                 0.006118 -3.535 0.003471 **
## MPxCP - CP == 0
                     -0.021629
## MP - CPxMP == 0
                                 0.006941 -0.232 1.000000
                     -0.001608
## MPxCP - CPxMP == 0 0.002208
                                 0.006148 0.359 1.000000
## MPxCP - MP == 0
                      0.003817
                                 0.006453
                                           0.591 1.000000
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- bonferroni method)
#####plot of facet count based on MAM####
######**species**####
model_means_w2 <- emmeans(object = w2, specs = ~ type, type = "response")</pre>
#p-value comparison table
pwpm(model means w2, adjust="bonferroni", diffs = F)
             CP
                  CPxMP
##
                             MP
                                  MPxCP
## CP
         [4.157] 0.0006 0.0032
                                0.0035
## CPxMP
                [4.133]
                        1.0000
                                 1.0000
## MP
                        [4.131]
                                 1.0000
## MPxCP
                                [4.135]
```

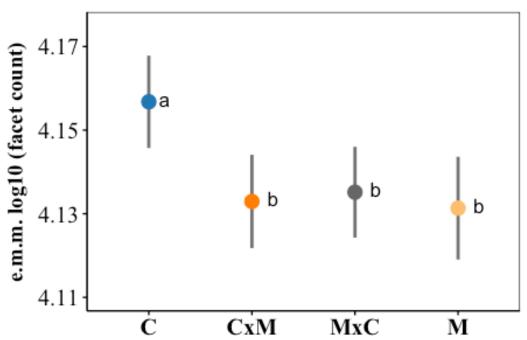
```
##
## Row and column labels: type
## Upper triangle: P values
                             adjust = "bonferroni"
## Diagonal: [Estimates] (emmean) type = "response"
#effect size
eff_size(model_means_w2, sigma = sigma(w2), edf = df.residual(w2))
## contrast
                 estimate
                             SE df lower.CL upper.CL
## CP - CPxMP
                                       0.501
                   1.0163 0.260 121
                                                1.532
## CP - MP
                   1.0848 0.313 121
                                       0.466
                                                1.704
## CP - MPxCP
                   0.9221 0.267 121
                                       0.393
                                                1.452
## CPxMP - MP
                   0.0686 0.296 121
                                      -0.517
                                                0.655
## CPxMP - MPxCP -0.0942 0.262 121 -0.613
                                                0.425
## MP - MPxCP
                 -0.1627 0.275 121
                                      -0.708
                                                0.382
##
## Results are averaged over the levels of: sex
## sigma used for effect sizes: 0.02346
## Confidence level used: 0.95
#add Letters to each mean to indicate difference
model_means_cld_w2 <- cld(object = model_means_w2,</pre>
                         reversed=T,
                         adjust="bonferroni",
                         Letters = letters,
                         alpha = 0.05)
#set marginal means at a dataframe
facet_w2 <- as.data.frame(summary(model_means_cld_w2))</pre>
#species
#color selection for consistenty with other papers
library(RColorBrewer)
display.brewer.pal(12, 'Paired')
```



Paired (qualitative)

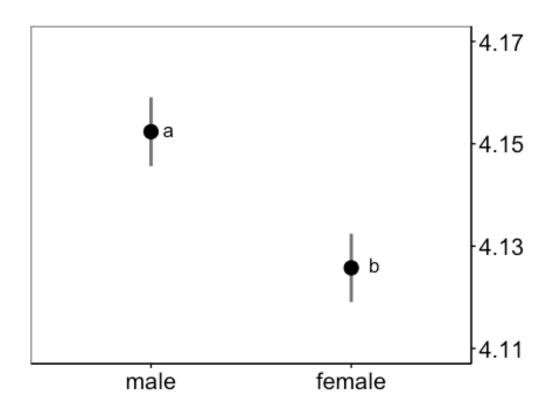
```
brewer.pal(12, 'Paired')
## [1] "#A6CEE3" "#1F78B4" "#B2DF8A" "#33A02C" "#FB9A99" "#E31A1C" "#FDBF6F"
  [8] "#FF7F00" "#CAB2D6" "#6A3D9A" "#FFFF99" "#B15928"
facet_w2$type <- factor(facet_w2$type, c("CP", "CPxMP", "MPxCP", "MP"))</pre>
ggplot(facet_w2, aes(type,emmean))+
  geom_errorbar(data=facet_w2,mapping=aes(x=type, ymin=upper.CL, ymax=lower.C
L), color="grey45",
                width=0, linewidth=1, position = position_dodge(0.4))+
  geom_point(data=facet_w2, aes(type,emmean, color=type), size=4,
             position = position dodge(0.4))+
  geom_text(aes(label = .group, y = emmean), position = position_dodge(0.4),
            vjust=0.3, hjust=-0.2, size=4.5) +
  labs(x="",y="e.m.m. log10 (facet count)")+
  scale_y_continuous(breaks=seq(4.11, 4.17, 0.02), limits=c(4.11, 4.175))+
  scale_x_discrete(labels=c("C", "CxM" , "MxC" , "M"))+
  scale_color_manual(values = c("#1F78B4", "#FF7F00", "grey40", "#FDBF6F"))+
  theme_update(axis.title.y=element_text(size=14,face="bold",hjust=0.5,vjust=
2.5, angle=90))+
  theme(plot.margin = margin(1,0.5,0.5,0.75, "cm"))+
 #theme_update(axis.text.x=element_text(vjust=0.5, angle=45))+
```

facet count



```
#####**sex**###
model_means_w2.1 <- emmeans(object = w2, specs = ~ sex, type = "response")</pre>
#p-value comparison table
pwpm(model_means_w2.1, adjust="bonferroni", diffs = F)
##
           female
                     male
## female [4.126]
                  <.0001
## male
                  [4.152]
##
## Row and column labels: sex
## Upper triangle: P values
## Diagonal: [Estimates] (emmean) type = "response"
#effect size
eff_size(model_means_w2.1, sigma = sigma(w2), edf = df.residual(w2))
```

```
## contrast
                  estimate SE df lower.CL upper.CL
## female - male
                     -1.13 0.193 121
                                        -1.52
                                                -0.753
##
## Results are averaged over the levels of: type
## sigma used for effect sizes: 0.02346
## Confidence level used: 0.95
#add Letters to each mean to indicate difference
model means cld w2.1 <- cld(object = model means w2.1,
                            reversed = T,
                            adjust="bonferroni",
                            Letters = letters,
                            alpha = 0.05)
#set marginal means at a dataframe
facet w2.1 <- as.data.frame(summary(model means cld w2.1))</pre>
#plot marginal means
facet w2.1$sex <- factor(facet w2.1$sex, c("male", "female"))</pre>
#sex
ggplot(facet_w2.1, aes(sex,emmean))+
  geom_errorbar(data=facet_w2.1, mapping=aes(x=sex, ymin=upper.CL, ymax=lower.
CL), color="grey45",
                width=0, linewidth=1)+
  geom_point(data=facet_w2.1, aes(sex,emmean), size=4)+
  geom_text(aes(label = .group, y = emmean),
            vjust=0.3, hjust=-0.3, size=4.5) +
  labs(x="",y="")+
  scale y continuous(breaks=seq(4.11, 4.17, 0.02), limits=c(4.11, 4.17),
                     position="right")+
  scale_x_discrete(labels = c("male", "female"))+
  theme_update(axis.title.y=element_text(size=14,face="bold",hjust=0.5,vjust=
2.5, angle=90)+
  theme(legend.position=c(0.88,0.89), legend.key=element_blank(),legend.title
=element blank(),
        legend.text=element text(size=10))+
 theme(plot.margin = margin(1,0.25,0.25,0.7, "cm"))
```



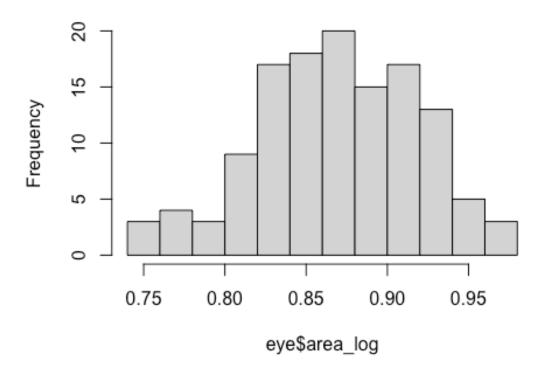
```
#####**species:sex**###
model_means_w2.2 <- emmeans(object = w2, specs = ~ type:sex, type = "response")</pre>
")
#p-value comparison table
pwpm(model_means_w2.2, adjust="bonferroni", diffs = F)
##
                CP female CPxMP female MP female MPxCP female CP male CPxMP m
ale
## CP female
                  [4.143]
                                0.0027
                                           0.0150
                                                        0.0162 <.0001
                                                                            1.0
000
## CPxMP female
                                [4.120]
                                          1.0000
                                                                            <.0
                                                        1.0000 <.0001
001
## MP female
                                          [4.118]
                                                        1.0000 <.0001
                                                                            0.0
268
## MPxCP female
                                                       [4.122] <.0001
                                                                            0.0
403
## CP male
                                                                [4.170]
                                                                            0.0
027
                                                                           [4.1
## CPxMP male
46]
## MP male
## MPxCP male
```

```
##
                MP male MPxCP male
## CP female
                 1.0000
                            1.0000
## CPxMP female 0.0534
                            0.0045
## MP female
                            0.0052
                 <.0001
## MPxCP female 0.0805
                            <.0001
## CP male
                 0.0150
                            0.0162
## CPxMP male
                 1.0000
                            1,0000
## MP male
                [4.145]
                            1.0000
## MPxCP male
                            [4.148]
##
## Row and column labels: type:sex
## Upper triangle: P values adjust = "bonferroni"
## Diagonal: [Estimates] (emmean) type = "response"
#add Letters to each mean to indicate difference
model_means_cld_w2.2 <- cld(object = model_means_w2.2 ,</pre>
                             adjust = "bonferroni",
                             reversed=T.
                             Letters = letters,
                             alpha = 0.05
#set marginal means at a dataframe
facet_w2.2 <- as.data.frame(summary(model_means_cld_w2.2 ))</pre>
#plot marginal means
facet_w2.2 $sex <- factor(facet_w2.2 $sex, c("male", "female"))</pre>
facet_w2.2 $type <- factor(facet_w2.2 $type, c("CP", "CPXMP", "MPXCP", "MP"))</pre>
#species:sex plot
combo3 <- ggplot(facet_w2.2 , aes(type,emmean))+</pre>
  geom errorbar(data=facet w2.2 ,mapping=aes(x=type, ymin=upper.CL, ymax=lowe
r.CL, color=sex),
                width=0, linewidth=1, position = position_dodge(width=0.5))+
  geom point(data=facet w2.2 , aes(type,emmean, color=sex), size=3,
             position = position_dodge2(width=0.5))+
  geom_text(aes(label = .group, y = emmean),
            vjust=0.3, hjust=-0.3, size=4.5, position = position dodge2(width=
(0.5)) +
  labs(x="",y="e.m.m. log10 (facet count)", color="")+
  scale y continuous(breaks=seq(4.09, 4.19, 0.02), limits=c(4.09, 4.19),
                     position="left")+
  scale_x_discrete(labels=c("C", "CxM", "MxC", "M"))+
  scale_color_manual(values = c("black", "grey"))+
  theme(legend.position="none", legend.key=element blank(),legend.title=eleme
nt blank(),
        legend.text=element text(size=10))+
  theme(plot.margin = margin(1,0.9,0.5,0.5, "cm"))+
  theme(legend.text=element_text(size=11))+
  theme(legend.key=element blank())+
```

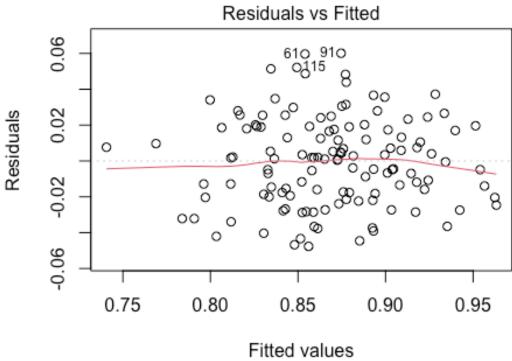
```
theme(axis.text.x=element_text(face = "bold"))+
theme(text = element_text(family = "Times New Roman"))

####ANALYSES OF CORNEAL AREA - C, M, & F1 HYBRIDS###
hist(eye$area_log)
```

Histogram of eye\$area_log

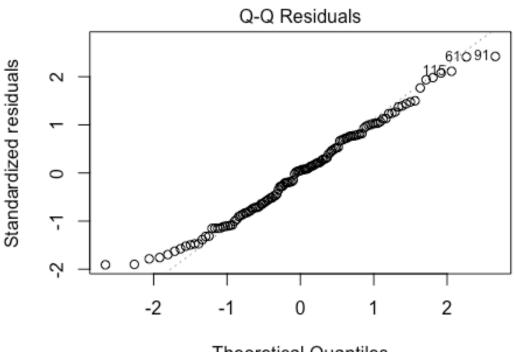


```
k1 <- lm(area_log~type*sex+tibia_length_log, data=eye)
plot(k1,which=1)</pre>
```



Im(area_log ~ type * sex + tibia_length_log)

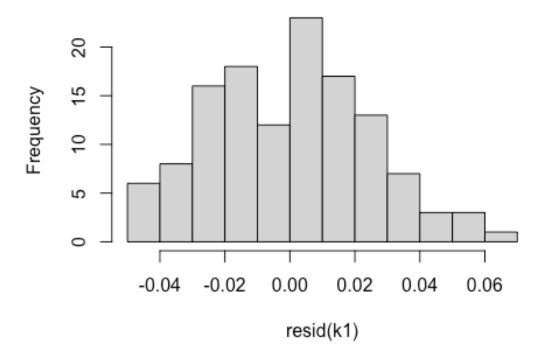
plot(k1, which=2)



Theoretical Quantiles
Im(area_log ~ type * sex + tibia_length_log)

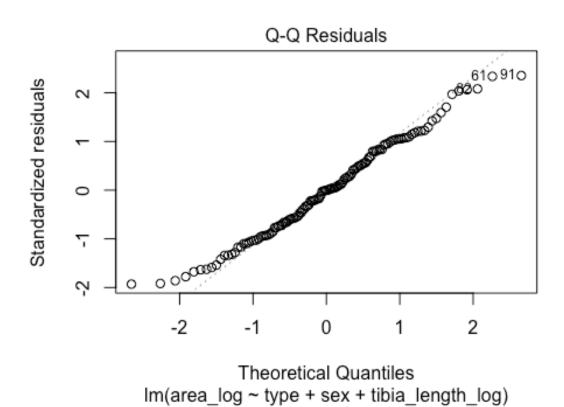
hist(resid(k1))

Histogram of resid(k1)



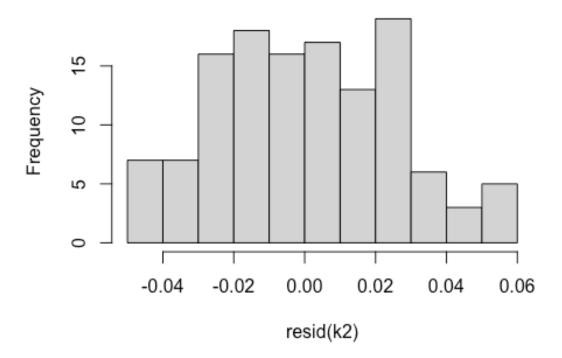
```
drop1(k1, test="Chisq") #type:sex n.s.
## Single term deletions
##
## Model:
## area_log ~ type * sex + tibia_length_log
                    Df Sum of Sq
                                      RSS
                                              AIC Pr(>Chi)
                                 0.077995 -921.20
## <none>
                                                    <2e-16 ***
## tibia length log 1 0.089577 0.167573 -826.08
## type:sex
                     3 0.001785 0.079780 -924.33
                                                    0.4115
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
k2 <- update(k1,.~.-type:sex)</pre>
drop1(k2, test="Chisq") #all effects significant
## Single term deletions
##
## Model:
## area_log ~ type + sex + tibia_length_log
                    Df Sum of Sq
                                             AIC
                                                  Pr(>Chi)
##
                                     RSS
                                 0.07978 -924.33
## <none>
## type
                        0.020439 0.10022 -901.36 2.276e-06 ***
                        0.020961 0.10074 -896.70 5.238e-08 ***
## sex
```

```
## tibia_length_log 1 0.090602 0.17038 -829.96 < 2.2e-16 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
plot(k2,which=2)</pre>
```



hist(resid(k2))

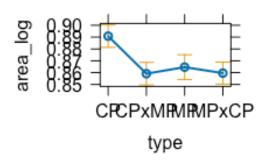
Histogram of resid(k2)

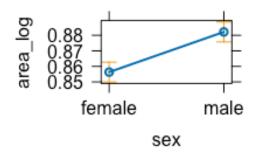


```
Anova(k2, test="Chisq")
## Anova Table (Type II tests)
##
## Response: area_log
                     Sum Sq Df F value
##
                                           Pr(>F)
                   0.020439
                              3 10.333 4.180e-06 ***
## type
                              1 31.791 1.144e-07 ***
                   0.020961
## sex
## tibia_length_log 0.090602
                              1 137.413 < 2.2e-16 ***
## Residuals
                   0.079780 121
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
#type p>0.001
#sex p>0.001
#tibia p>0.001
plot(allEffects(k2))
```

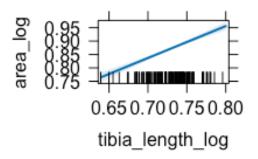
type effect plot

sex effect plot





tibia_length_log effect plot

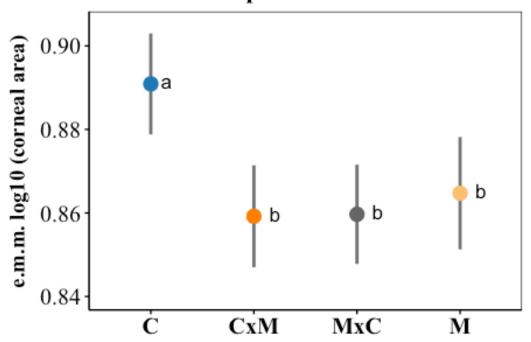


```
#species comparison
posthoc_size2<-glht(k2, linfct=mcp(type="Tukey"))</pre>
summary(posthoc_size2, adjusted(type="bonferroni"))
##
##
     Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
##
##
## Fit: lm(formula = area_log ~ type + sex + tibia_length_log, data = eye)
## Linear Hypotheses:
##
                       Estimate Std. Error t value Pr(>|t|)
                                  0.006475 -4.897 1.83e-05 ***
## CPxMP - CP == 0
                      -0.031704
## MP - CP == 0
                      -0.026166
                                  0.007830 -3.342 0.00665 **
## MPxCP - CP == 0
                                  0.006698 -4.661 4.89e-05 ***
                      -0.031222
## MP - CPxMP == 0
                       0.005538
                                  0.007599
                                             0.729
                                                    1.00000
## MPxCP - CPxMP == 0 0.000482
                                  0.006730
                                             0.072
                                                    1.00000
## MPxCP - MP == 0
                                  0.007064 -0.716 1.00000
                      -0.005056
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- bonferroni method)
```

```
Anova(k1, test="Chisq")
## Anova Table (Type II tests)
## Response: area log
##
                     Sum Sq Df F value
                                            Pr(>F)
## type
                   0.020439
                              3 10.3073 4.456e-06 ***
                             1 31.7124 1.231e-07 ***
## sex
                   0.020961
                              1 135.5228 < 2.2e-16 ***
## tibia_length_log 0.089577
## type:sex
                   0.001785
                              3
                                  0.9002
                                            0.4434
## Residuals
                   0.077995 118
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
#type:sex p=0.44
#####plot of corneal area based on MAM####
#####**species**####
model means k2 <- emmeans(object = k2, specs = ~ type, type = "response")
#p-value comparison table
pwpm(model_means_k2, adjust="bonferroni", diffs = F)
##
             CP
                  CPxMP
                             MP
                                  MPxCP
## CP
         [0.891] <.0001 0.0066 <.0001
## CPxMP
                 [0.859] 1.0000 1.0000
## MP
                         [0.865] 1.0000
## MPxCP
                                [0.860]
##
## Row and column labels: type
## Upper triangle: P values adjust = "bonferroni"
## Diagonal: [Estimates] (emmean) type = "response"
#effect size
eff_size(model_means_k2, sigma = sigma(k2), edf = df.residual(k2))
## contrast
                  estimate
                             SE df lower.CL upper.CL
## CP - CPxMP
                   1.2347 0.264 121
                                       0.711
                                                1.758
## CP - MP
                   1.0190 0.312 121
                                       0.402
                                                1.636
## CP - MPxCP
                   1.2159 0.272 121
                                       0.677
                                                1.755
## CPxMP - MP
                   -0.2157 0.296 121
                                      -0.802
                                                0.371
## CPxMP - MPxCP -0.0188 0.262 121
                                      -0.538
                                                0.500
## MP - MPxCP
                   0.1969 0.275 121
                                      -0.348
                                                0.742
##
## Results are averaged over the levels of: sex
## sigma used for effect sizes: 0.02568
## Confidence level used: 0.95
```

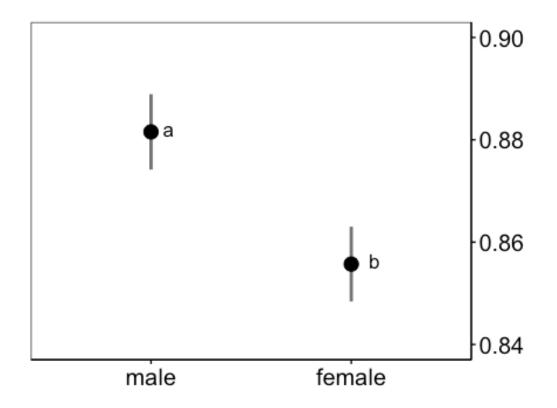
```
#add letters to each mean to indicate difference
model means cld k2 <- cld(object = model means k2,
                          reversed=T,
                          adjust="bonferroni",
                          Letters = letters,
                          alpha = 0.05)
#set marginal means at a dataframe
area k2 <- as.data.frame(summary(model means cld k2))</pre>
area_k2$type <- factor(area_k2$type, c("CP", "CPxMP", "MPxCP", "MP"))</pre>
#species
ggplot(area k2, aes(type,emmean))+
  geom_errorbar(data=area_k2,mapping=aes(x=type, ymin=upper.CL, ymax=lower.CL
), color="grey45",
                width=0, linewidth=1, position = position_dodge(0.4))+
  geom_point(data=area_k2, aes(type,emmean,color=type), size=4,
             position = position dodge(0.4)+
  geom_text(aes(label = .group, y = emmean), position = position_dodge(0.4),
            vjust=0.3, hjust=-0.2, size=4.5) +
  labs(x="",y="e.m.m. log10 (corneal area)")+
  scale_y_continuous(breaks=seq(0.82, 0.92, 0.02), limits=c(0.84, 0.905))+
  scale_x_discrete(labels=c("C", "CxM", "MxC","M"))+
  scale color manual(values = c("#1F78B4","#FF7F00","grey40","#FDBF6F"))+
  theme update(axis.title.y=element text(size=14,face="bold",hjust=0.5,vjust=
2.5, angle=90))+
  theme(legend.position="none", legend.key=element blank(),legend.title=eleme
nt blank(),
        legend.text=element_text(size=10))+
  theme(plot.margin = margin(1,0.5,0.5,0.75, "cm"))+
  theme(axis.text.x=element text(face = "bold"))+
  theme(text = element_text(family = "Times New Roman"))+
  ggtitle("species effect") + theme(plot.title = element_text(hjust = 0.5, si
ze=16,face="bold"))
```

species effect



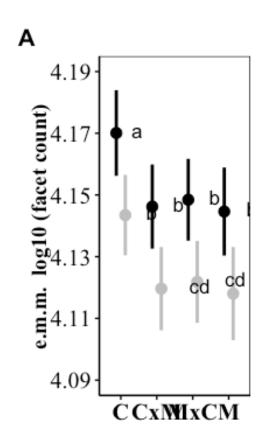
```
#####**sex**###
model_means2_k2.1 <- emmeans(object = k2, specs = ~ sex, type = "response")</pre>
#p-value comparison table
pwpm(model_means2_k2.1, adjust="bonferroni", diffs = F)
           female
                     male
## female [0.856] <.0001
## male
                  [0.882]
##
## Row and column labels: sex
## Upper triangle: P values
## Diagonal: [Estimates] (emmean) type = "response"
#effect size
eff_size(model_means2_k2.1, sigma = sigma(k2), edf = df.residual(k2))
##
   contrast
                  estimate
                             SE df lower.CL upper.CL
                     -1.01 0.19 121
   female - male
##
                                       -1.38
##
## Results are averaged over the levels of: type
## sigma used for effect sizes: 0.02568
## Confidence level used: 0.95
```

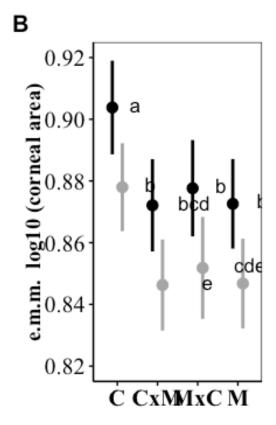
```
#add Letters to each mean to indicate difference
model means cld2 k2.1 <- cld(object = model means2 k2.1,
                             reversed = T,
                             adjust="bonferroni",
                             Letters = letters,
                             alpha = 0.05)
#set marginal means at a dataframe
area k2.1 <- as.data.frame(summary(model means cld2 k2.1))</pre>
#plot marginal means
area k2.1$sex <- factor(area k2.1$sex, c("male", "female"))</pre>
ggplot(area_k2.1, aes(sex,emmean))+
  geom_errorbar(data=area_k2.1,mapping=aes(x=sex, ymin=upper.CL, ymax=lower.C
L), color="grey45",
                width=0, linewidth=1)+
  geom point(data=area k2.1, aes(sex,emmean), size=4)+
  geom_text(aes(label = .group, y = emmean),
            vjust=0.3, hjust=-0.3, size=4.5) +
  labs(x="",y="")+
  scale_y_continuous(breaks=seq(0.84, 0.90, 0.02), limits=c(0.84, 0.90),
   position="right")+
  scale x discrete(labels = c("male", "female"))+
  theme update(axis.title.y=element text(size=14,face="bold",hjust=0.5,vjust=
2.5, angle=90))+
  theme(legend.position=c(0.88,0.89), legend.key=element blank(),legend.title
=element blank(),
        legend.text=element_text(size=10))+
 theme(plot.margin = margin(1,0.25,0.25,0.7, "cm"))
```



```
######**species:sex**####
model_means3_k2.2 <- emmeans(object = k2, specs = ~ type:sex, type = "respons")</pre>
e")
#p-value comparison table
pwpm(model_means3_k2.2, adjust="bonferroni", diffs = F)
##
                CP female CPxMP female MP female MPxCP female CP male CPxMP m
ale
## CP female
                  [0.878]
                                 <.0001
                                           0.0310
                                                         0.0002 <.0001
                                                                            1.0
000
## CPxMP female
                                [0.846]
                                           1.0000
                                                         1.0000 <.0001
                                                                            <.0
001
## MP female
                                          [0.852]
                                                         1.0000 <.0001
                                                                            0.7
813
## MPxCP female
                                                        [0.847] <.0001
                                                                            0.0
681
## CP male
                                                                [0.904]
                                                                            <.0
001
                                                                           [0.8
## CPxMP male
72]
## MP male
## MPxCP male
```

```
##
                MP male MPxCP male
## CP female
                 1.0000
                            1.0000
## CPxMP female 0.0113
                            0.0418
## MP female
                 <.0001
                            0.4942
## MPxCP female 0.0072
                            <.0001
## CP male
                 0.0310
                            0.0002
## CPxMP male
                 1.0000
                            1.0000
## MP male
                [0.878]
                            1.0000
## MPxCP male
                           [0.873]
##
## Row and column labels: type:sex
## Upper triangle: P values
                              adjust = "bonferroni"
## Diagonal: [Estimates] (emmean) type = "response"
#add letters to each mean to indicate difference
model_means_cld3_k2.2 <- cld(object = model_means3_k2.2,</pre>
                             adjust = "bonferroni",
                             reversed = T,
                             Letters = letters,
                             alpha = 0.05)
#set marginal means at a dataframe
area_k2.2 <- as.data.frame(summary(model_means_cld3_k2.2))</pre>
#plot marginal means
area k2.2$sex <- factor(area k2.2$sex, c("male", "female"))
#species:sex plot
combo4<-ggplot(area_k2.2, aes(type,emmean))+</pre>
  geom_errorbar(data=area_k2.2,mapping=aes(x=type, ymin=upper.CL, ymax=lower.
CL, color=sex),
                width=0, linewidth=1, position = position dodge(width=0.5))+
  geom_point(data=area_k2.2, aes(type,emmean, color=sex), size=3,
             position = position dodge2(width=0.5))+
  geom_text(aes(label = .group, y = emmean),
            vjust=0.3, hjust=-0.3, size=4.5, position = position_dodge2(width=
(0.5)) +
  labs(x="",y="e.m.m. log10 (corneal area)", color="")+
  scale y continuous(breaks=seq(0.82, 0.92, 0.02), limits=c(0.82, 0.92))+
  scale_x_discrete(labels=c("C", "CxM", "MxC", "M"))+
  scale_color_manual(values=c("black", "grey65"))+
  theme(legend.position="", legend.key=element_blank(),legend.title=element_b
lank(),
        legend.text=element text(size=10))+
  theme(plot.margin = margin(1,0.5,0.5,0.5, "cm"))+
  theme(legend.text=element text(size=11))+
  theme(legend.key=element blank())+
  theme(axis.text.x=element_text(face = "bold"))+
  theme(text = element text(family = "Times New Roman"))
```

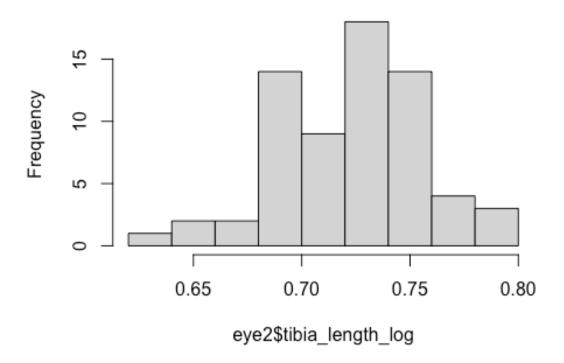




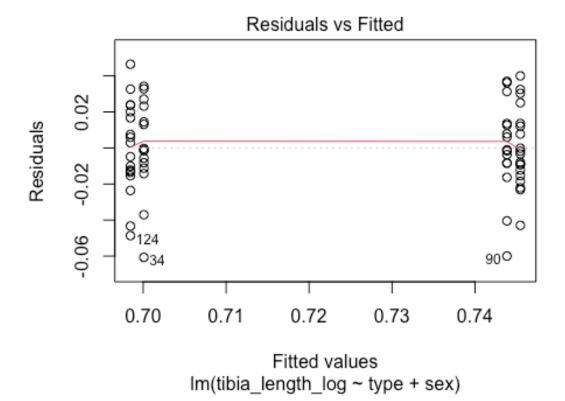
####DO SPECIES DIFFER IN BODY SIZE?####

```
#pure species
hist(eye2$tibia_length_log)
```

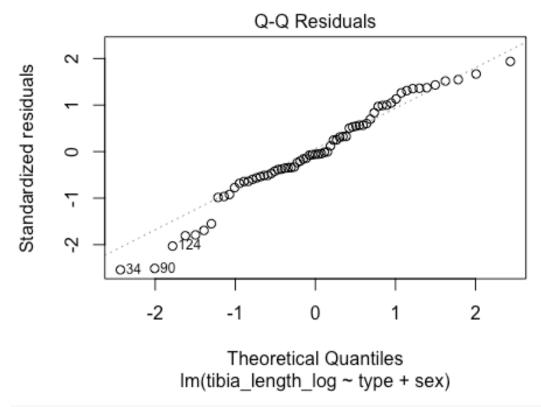
Histogram of eye2\$tibia_length_log



```
size <- lm(tibia_length_log~type+sex, data=eye2)
plot(size, which=1)</pre>
```

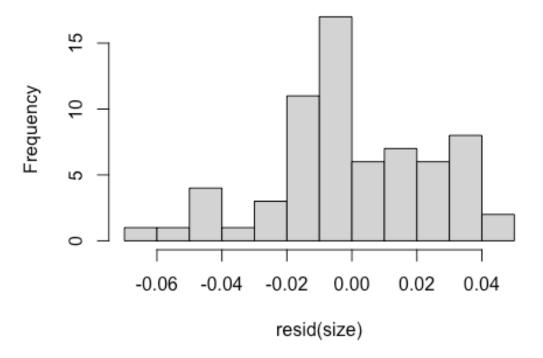


plot(size, which=2)



hist(resid(size))

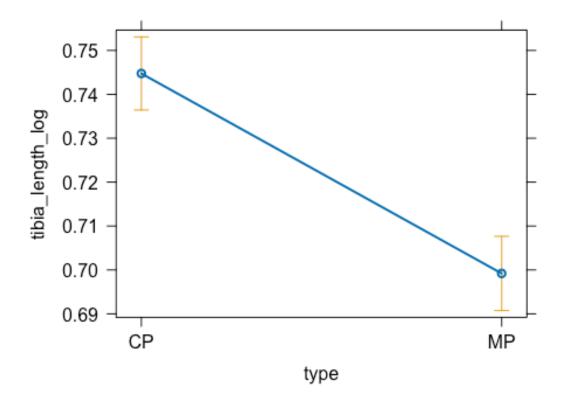
Histogram of resid(size)



```
drop1(size, test="Chisq") #sex n.s.
## Single term deletions
##
## Model:
## tibia_length_log ~ type + sex
          Df Sum of Sq
                                    AIC Pr(>Chi)
                            RSS
                       0.038392 -494.13
## <none>
           1 0.034117 0.072509 -453.53 6.707e-11 ***
## type
## sex
           1 0.000043 0.038435 -496.05
                                           0.7833
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
size2 <- update(size,.~.-sex)</pre>
drop1(size2, test="Chisq")
## Single term deletions
##
## Model:
## tibia_length_log ~ type
                                    AIC Pr(>Chi)
          Df Sum of Sq
                            RSS
##
## <none>
                       0.038435 -496.05
## type 1 0.034749 0.073184 -454.90 5.075e-11 ***
```

```
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
Anova(size2, test="Chisq")
## Anova Table (Type II tests)
##
## Response: tibia_length_log
## Sum Sq Df F value Pr(>F)
## type 0.034749 1 58.765 1.145e-10 ***
## Residuals 0.038435 65
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
#species p<0.001
plot(allEffects(size2))</pre>
```

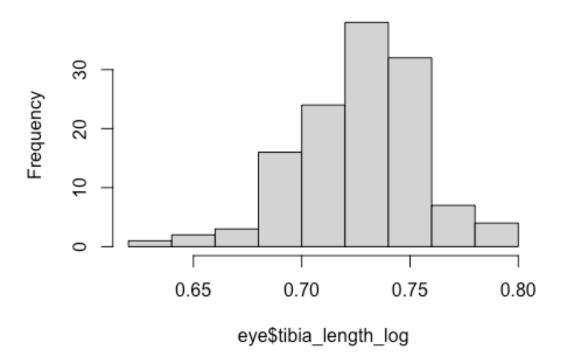
type effect plot



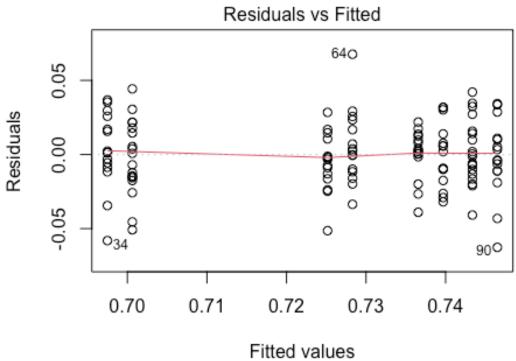
```
Anova(size, test="Chisq")
## Anova Table (Type II tests)
##
## Response: tibia_length_log
## Sum Sq Df F value Pr(>F)
## type 0.034117 1 56.8730 2.075e-10 ***
```

```
## sex    0.000043 1 0.0723 0.7889
## Residuals 0.038392 64
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
#sex p=0.788
#with F1 hybrids
hist(eye$tibia_length_log)
```

Histogram of eye\$tibia_length_log

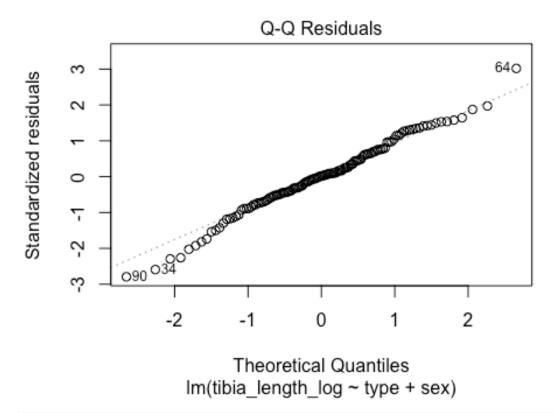


size3 <- lm(tibia_length_log~type+sex, data=eye)
plot(size3, which=1)</pre>



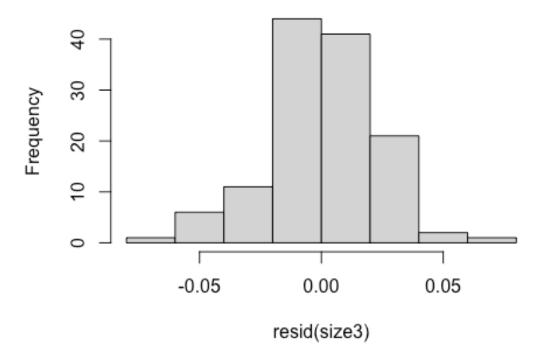
Im(tibia_length_log ~ type + sex)

plot(size3, which=2)



hist(resid(size3))

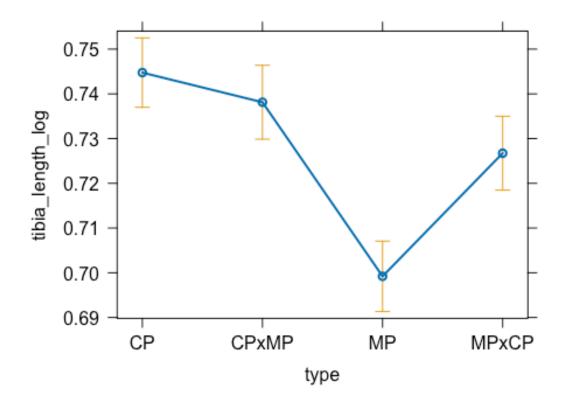
Histogram of resid(size3)



```
drop1(size3, test="Chisq") #sex n.s.
## Single term deletions
##
## Model:
## tibia_length_log ~ type + sex
          Df Sum of Sq
                                    AIC Pr(>Chi)
                            RSS
                       0.063930 -954.46
## <none>
           3 0.040227 0.104158 -898.47 2.208e-13 ***
## type
## sex
           1 0.000310 0.064241 -955.84
                                            0.433
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
size4 <- update(size3,.~.-sex)</pre>
drop1(size4, test="Chisq")
## Single term deletions
##
## Model:
## tibia_length_log ~ type
          Df Sum of Sq
                                    AIC Pr(>Chi)
                            RSS
##
## <none>
                       0.064241 -955.84
## type 3 0.039932 0.104173 -900.45 2.961e-13 ***
```

```
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Anova(size4, test="Chisq")
## Anova Table (Type II tests)
## Response: tibia_length_log
              Sum Sq Df F value
                                    Pr(>F)
##
                     3 25.486 6.842e-13 ***
            0.039932
## type
## Residuals 0.064241 123
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
#type p>0.001
plot(allEffects(size4))
```

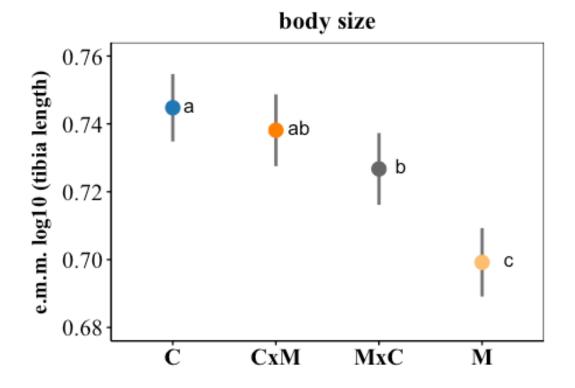
type effect plot



```
Anova(size3, test="Chisq")
## Anova Table (Type II tests)
##
## Response: tibia_length_log
## Sum Sq Df F value Pr(>F)
## type 0.040227 3 25.5890 6.541e-13 ***
```

```
## sex
            0.000310 1 0.5919
                                   0.4432
## Residuals 0.063930 122
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
\#sex p=0.44
#species comparison
posthoc_size<-glht(size4, linfct=mcp(type="Tukey"))</pre>
summary(posthoc_size, adjusted(type="bonferroni"))
##
##
    Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
##
##
## Fit: lm(formula = tibia length log ~ type, data = eye)
## Linear Hypotheses:
                      Estimate Std. Error t value Pr(>|t|)
##
                     -0.006628 0.005725 -1.158
## CPxMP - CP == 0
                                                   1.0000
## MP - CP == 0
                     -0.045552
                                0.005585 -8.157 2.03e-12 ***
## MPxCP - CP == 0
                     -0.018022 0.005725 -3.148
                                                   0.0124 *
## MP - CPxMP == 0
                     ## MPxCP - CPxMP == 0 -0.011394
                                0.005901 -1.931
                                                   0.3348
## MPxCP - MP == 0
                                0.005765 4.775 3.00e-05 ***
                      0.027531
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- bonferroni method)
######**Body size emm plot**#####
model_means_size <- emmeans(object = size4, specs = ~ type, type = "response"</pre>
#p-value comparison table
pwpm(model_means_size, adjust="bonferroni", diffs = F)
                             MP
##
             CP
                  CPxMP
                                 MPxCP
## CP
        [0.745] 1.0000 <.0001 0.0124
## CPxMP
                [0.738]
                       <.0001 0.3348
## MP
                        [0.699] <.0001
## MPxCP
                                [0.727]
## Row and column labels: type
## Upper triangle: P values adjust = "bonferroni"
## Diagonal: [Estimates] (emmean) type = "response"
#effect size
eff_size(model_means_size, sigma = sigma(size4), edf = df.residual(size4))
```

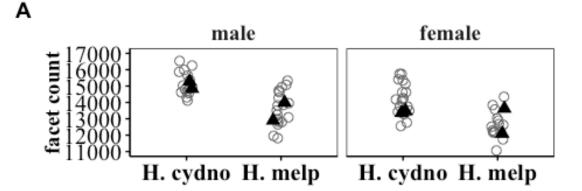
```
## contrast
                  estimate SE df lower.CL upper.CL
## CP - CPxMP
                     0.290 0.251 123 -0.2071
                                                 0.787
## CP - MP
                     1.993 0.275 123
                                       1.4480
                                                 2.538
## CP - MPxCP
                     0.789 0.255 123
                                       0.2829
                                                 1.294
                     1.703 0.275 123 1.1596
## CPxMP - MP
                                                 2.247
## CPxMP - MPxCP
                     0.499 0.260 123 -0.0164
                                                 1.013
## MP - MPxCP
                    -1.205 0.264 123 -1.7266 -0.683
##
## sigma used for effect sizes: 0.02285
## Confidence level used: 0.95
#add Letters to each mean to indicate difference
model_means_cld_size <- cld(object = model_means_size,</pre>
                            reversed=T,
                            adjust="bonferroni",
                            Letters = letters,
                            alpha = 0.05
#set marginal means at a dataframe
sizes <- as.data.frame(summary(model means cld size))</pre>
#size
sizes$type <- factor(sizes$type, c("CP", "CPXMP", "MPXCP", "MP"))</pre>
ggplot(sizes, aes(type,emmean))+
  geom errorbar(data=sizes,mapping=aes(x=type, ymin=upper.CL, ymax=lower.CL),
color="grey45",
                width=0, linewidth=1, position = position dodge(0.4))+
  geom_point(data=sizes, aes(type,emmean,color=type), size=4,
             position = position dodge(0.4))+
  geom_text(aes(label = .group, y = emmean), position = position_dodge(0.4),
            vjust=0.3, hjust=-0.2, size=4.5) +
  labs(x="",y="e.m.m. log10 (tibia length)")+
  scale_y_continuous(breaks=seq(0.68, 0.76, 0.02), limits=c(0.68, 0.76))+
  scale_x_discrete(labels=c("C", "CxM", "MxC", "M"))+
  scale color manual(values = c("#1F78B4","#FF7F00", "grey40", "#FDBF6F"))+
  theme update(axis.title.y=element text(size=14,face="bold",hjust=0.5,vjust=
2.5, angle=90))+
  theme(legend.position="none", legend.key=element blank(),legend.title=eleme
nt blank(),
        legend.text=element_text(size=10))+
  theme(plot.margin = margin(1,0.5,0.5,0.75, "cm"))+
  theme(axis.text.x=element text(face = "bold"))+
  theme(text = element_text(family = "Times New Roman"))+
  ggtitle("body size") + theme(plot.title = element text(hjust = 0.5, size=16
,face="bold"))
```

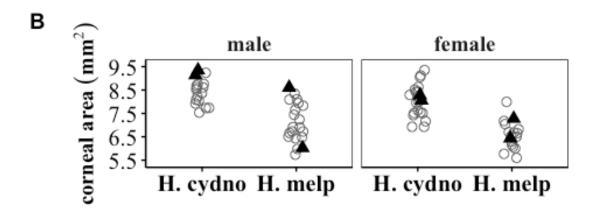


```
####FDR P-VALUE ADJUSTMENTS FROM ALL LMS####
pvalues <- c(0.008775,
              2.06E-06,
              2.77E-05,
              7.19E-01,
              0.004985,
              3.84E-05,
              8.61E-14,
              0.102394,
             0.0001924,
              3.81E-09,
              2.64E-07,
              0.2441719,
              4.18E-06,
              1.14E-07,
              2.20E-16,
             0.4434,
              1.15E-10,
              7.89E-01,
              6.54E-13,
             0.4432,
              2.27E-16,
```

```
0.1203373
adjusted.p <- p.adjust(pvalues, method = "fdr", n = length(pvalues))</pre>
adjusted.p
## [1] 1.287000e-02 5.035556e-06 5.540000e-05 7.532381e-01 7.833571e-03
## [6] 7.040000e-05 6.314000e-13 1.407917e-01 3.256000e-04 1.397000e-08
## [11] 7.260000e-07 2.984323e-01 9.196000e-06 3.582857e-07 2.497000e-15
## [16] 4.877400e-01 5.060000e-10 7.890000e-01 3.597000e-12 4.877400e-01
## [21] 2.497000e-15 1.557306e-01
####COMPARISON TO PREVIOUS PAPER - SEYMOURE ET AL. (2015)####
#means per species & sex from this study
#pure species only
eye2 %>%
  group by(type:sex) %>%
  summarise_at(vars(area), list(name = mean))
## # A tibble: 4 × 2
##
     `type:sex` name
##
     <fct>
                <dbl>
## 1 CP:female
                 8.03
## 2 CP:male
                 8.32
## 3 MP:female
                 6.54
## 4 MP:male
                 7.10
#import data extracted from Seymoure et al. (2015)
method comp <- read.csv("~/Documents/LMU/Eye morphology/selection manuscript/</pre>
method comp.csv")
eye2$sex<- factor(eye2$sex, c("male", "female"))</pre>
method comp$sex<- factor(method comp$sex, c("male", "female"))</pre>
facet_comp <- ggplot(eye2, aes(type,whole_count))+ facet_grid(~sex)+</pre>
  geom_point(data=eye2, aes(type,whole_count), size=2.5,shape=1,color="grey40
             position = position jitter(0.085))+
  geom_point(data=method_comp, aes(type,whole_count), size=3, shape=17,
             position = position_jitter(0.085))+
  labs(x="",y="facet count")+
  scale y continuous(breaks=seq(11000, 17000, 1000), limits=c(11000, 17000))+
  scale_x_discrete(labels=c("H. cydno","H. melp"))+
  theme update(axis.title.y=element text(size=14,face="bold",hjust=0.5,vjust=
2.5, angle=90))+
  theme(legend.position="none", legend.key=element blank(),legend.title=eleme
nt blank(),
        legend.text=element text(size=10))+
  theme(plot.margin = margin(0.4,0.4,0.4,0.75, "cm"))+
 theme(axis.text.x=element_text(face = "bold"))+
```

```
theme(strip.background = element rect(fill = "transparent"))+
  theme(strip.text = element text(size = 14, face = "bold"))+
  theme(text = element text(family = "Times New Roman"))
area comp <- ggplot(eye2, aes(type, area))+ facet grid(~sex)+
  geom point(data=eye2, aes(type,area), size=2.5,shape=1,color="grey40",
             position = position jitter(0.085))+
  geom_point(data=method_comp, aes(type, area), size=3, shape=17,
             position = position_jitter(0.085))+
  labs(x="",y=bquote(bold('corneal area'~(mm^2))))+
  scale y continuous(breaks=seq(5.5, 9.5, 1.00), limits=c(5.4, 9.6))+
  scale_x_discrete(labels=c("H. cydno","H. melp"))+
  theme update(axis.title.y=element text(size=14,face="bold",hjust=0.5,vjust=
2.5.angle=90)+
  theme(legend.position="none", legend.key=element_blank(),legend.title=eleme
nt blank(),
        legend.text=element text(size=10))+
  theme(plot.margin = margin(0.4,0.4,0.4,1.08, "cm"))+
  theme(axis.text.x=element text(face = "bold"))+
  theme(strip.background = element_rect(fill = "transparent"))+
  theme(strip.text = element text(size = 14, face = "bold"))+
  theme(text = element_text(family = "Times New Roman"))
#combine plots
library(ggpubr)
ggarrange(facet_comp, area_comp,
          labels = c("A", "B"),
          vjust=1.23, hjust=-0.25,
          ncol = 1, nrow = 2, widths = c(1, 1)
```





```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
raw 0.33$level <- c(0.33,0.33)
raw_0.67 <- Pst(eye2.1, ci=1, csh=0.67, va=c("whole_count", "area"), boot=100</pre>
0,
                PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
raw_0.67$level <- c(0.67,0.67)
raw_1 <- Pst(eye2.1, ci=1, csh=1.0, va=c("whole count", "area"), boot=1000,
             PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
```

```
raw 1$level <- c(1,1)
raw_1.33 <- Pst(eye2.1, ci=1, csh=1.33, va=c("whole_count", "area"), boot=100
0,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
raw_1.33$level <- c(1.33,1.33)
raw_1.5 <- Pst(eye2.1, ci=1, csh=1.5, va=c("whole count", "area"), boot=1000,
               Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
raw 1.5$level <- c(1.5,1.5)
raw_2 <- Pst(eye2.1, ci=1, csh=2, va=c("whole_count", "area"), boot=1000,
             PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
raw_2$level <- c(2,2)
raw_3 <- Pst(eye2.1, ci=1, csh=3, va=c("whole_count", "area"), boot=1000,</pre>
             PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## [1] "Populations sizes are:"
## CP MP
## 34 33
raw 3$level \leftarrow c(3,3)
raw_4 <- Pst(eye2.1, ci=1, csh=4, va=c("whole_count", "area"), boot=1000,</pre>
             PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
raw 4$level \leftarrow c(4,4)
raw values <- rbind(raw 0.33, raw 0.67, raw 1, raw 1.33, raw 1.5, raw 2, raw
3, raw_4)
#####2) log-transformed measurements####
```

```
log 0.33 <- Pst(eye2.1, ci=1, csh=0.33, va=c("whole count log", "area log"),
boot=1000,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
\log 0.33$level <- c(0.33,0.33)
log_0.67 <- Pst(eye2.1, ci=1, csh=0.67, va=c("whole_count_log", "area_log"),</pre>
boot=1000,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
log 0.67$level <- c(0.67,0.67)
log_1 <- Pst(eye2.1, ci=1, csh=1, va=c("whole_count_log", "area_log"), boot=1</pre>
000,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
log_1$level <- c(1,1)
log_1.33 <- Pst(eye2.1, ci=1, csh=1.33, va=c("whole_count_log", "area_log"),</pre>
boot=1000,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## [1] "Populations sizes are:"
## CP MP
## 34 33
log_1.33$level <- c(1.33,1.33)
log_1.5 <- Pst(eye2.1, ci=1, csh=1.5, va=c("whole_count_log", "area_log"), bo</pre>
ot=1000,
               Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
log_1.5$level <- c(1.5,1.5)
log_2 <- Pst(eye2.1, ci=1, csh=2, va=c("whole_count_log", "area_log"), boot=1</pre>
000,
             Pw = c("CP", "MP"), pe = 0.95)
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
log_2$level <- c(2,2)
log_3 <- Pst(eye2.1, ci=1, csh=3, va=c("whole_count_log", "area_log"), boot=1</pre>
000,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
log_3$level <- c(3,3)
log_4 <- Pst(eye2.1, ci=1, csh=4, va=c("whole_count_log", "area_log"), boot=1</pre>
000,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
log 4$level <- c(4,4)
log values <- rbind(log 0.33, log 0.67, log 1, log 1.33, log 1.5, log 2, log
3, log 4)
#####3) allometric-scaled####
library(allomr)
#maybe different per species & sex? need to do separate?
######CP males####
#subset the data
eye2.1.cp.m <- eye2.1[which(eye2.1$type=="CP"),]
eye2.1.cp.m <- eye2.1.cp.m[which(eye2.1.cp.m$sex=="male"),]</pre>
#apply allometric scaling
count_al_cpm <- as.data.frame(allomr(eye2.1.cp.m$tibia_length, eye2.1.cp.m$wh</pre>
ole count))
## Warning in (function (..., row.names = NULL, check.rows = FALSE, check.nam
es =
## TRUE, : row names were found from a short variable and have been discarded
area al cpm <- as.data.frame(allomr(eye2.1.cp.m$tibia length, eye2.1.cp.m$are
a))
## Warning in (function (..., row.names = NULL, check.rows = FALSE, check.nam
es =
## TRUE, : row names were found from a short variable and have been discarded
#add scaled values back to dataset
eye2.1.cp.m2 <- cbind(eye2.1.cp.m, count_al_cpm$Yx)</pre>
colnames(eye2.1.cp.m2)[colnames(eye2.1.cp.m2) == "count_al_cpm$Yx"] <- "whole</pre>
count al"
```

```
eye2.1.cp.m2 <- cbind(eye2.1.cp.m2, area_al_cpm$Yx)
colnames(eye2.1.cp.m2)[colnames(eye2.1.cp.m2) == "area al cpm$Yx"] <- "area a</pre>
1"
######P males####
#subset the data
eye2.1.mp.m <- eye2.1[which(eye2.1$type=="MP"),]
eye2.1.mp.m <- eye2.1.mp.m[which(eye2.1.mp.m$sex=="male"),]
#apply allometric scaling
count al mpm <- as.data.frame(allomr(eye2.1.mp.m$tibia length, eye2.1.mp.m$wh
ole count))
## Warning in (function (..., row.names = NULL, check.rows = FALSE, check.nam
es =
## TRUE, : row names were found from a short variable and have been discarded
area al mpm <- as.data.frame(allomr(eye2.1.mp.m$tibia length, eye2.1.mp.m$are
a))
## Warning in (function (..., row.names = NULL, check.rows = FALSE, check.nam
es =
## TRUE, : row names were found from a short variable and have been discarded
#add scaled values back to dataset
eye2.1.mp.m2 <- cbind(eye2.1.mp.m, count_al_mpm$Yx)</pre>
colnames(eye2.1.mp.m2)[colnames(eye2.1.mp.m2) == "count al mpm$Yx"] <- "whole</pre>
_count al"
eye2.1.mp.m2 <- cbind(eye2.1.mp.m2, area al mpm$Yx)
colnames(eye2.1.mp.m2)[colnames(eye2.1.mp.m2) == "area_al_mpm$Yx"] <- "area_a</pre>
1"
######CP females####
#subset the data
eye2.1.cp.f <- eye2.1[which(eye2.1$type=="CP"),]
eye2.1.cp.f <- eye2.1.cp.f[which(eye2.1.cp.f$sex=="female"),]
#apply allometic scaling
count al cpf <- as.data.frame(allomr(eye2.1.cp.f$tibia length, eye2.1.cp.f$wh
ole count))
## Warning in (function (..., row.names = NULL, check.rows = FALSE, check.nam
## TRUE, : row names were found from a short variable and have been discarded
```

```
area al cpf <- as.data.frame(allomr(eye2.1.cp.f$tibia_length, eye2.1.cp.f$are
a))
## Warning in (function (..., row.names = NULL, check.rows = FALSE, check.nam
## TRUE, : row names were found from a short variable and have been discarded
#add scaled values back to dataset
eye2.1.cp.f2 <- cbind(eye2.1.cp.f, count_al_cpf$Yx)</pre>
colnames(eye2.1.cp.f2)[colnames(eye2.1.cp.f2) == "count al cpf$Yx"] <- "whole</pre>
count al"
eye2.1.cp.f2 <- cbind(eye2.1.cp.f2, area al cpf$Yx)
colnames(eye2.1.cp.f2)[colnames(eye2.1.cp.f2) == "area al cpf$Yx"] <- "area a</pre>
######P females####
#subset the data
eye2.1.mp.f <- eye2.1[which(eye2.1$type=="MP"),]
eye2.1.mp.f <- eye2.1.mp.f[which(eye2.1.mp.f$sex=="female"),]</pre>
#apply allometric scaling
count al mpf <- as.data.frame(allomr(eye2.1.mp.f$tibia length, eye2.1.mp.f$wh
ole_count))
## Warning in (function (..., row.names = NULL, check.rows = FALSE, check.nam
## TRUE, : row names were found from a short variable and have been discarded
area_al_mpf <- as.data.frame(allomr(eye2.1.mp.f$tibia_length, eye2.1.mp.f$are
a))
## Warning in (function (..., row.names = NULL, check.rows = FALSE, check.nam
## TRUE, : row names were found from a short variable and have been discarded
#add scaled values back to dataset
eye2.1.mp.f2 <- cbind(eye2.1.mp.f, count al mpf$Yx)
colnames(eye2.1.mp.f2)[colnames(eye2.1.mp.f2) == "count_al_mpf$Yx"] <- "whole</pre>
count al"
eye2.1.mp.f2 <- cbind(eye2.1.mp.f2, area_al_mpf$Yx)</pre>
colnames(eye2.1.mp.f2)[colnames(eye2.1.mp.f2) == "area al mpf$Yx"] <- "area a</pre>
1"
#combine datasets for analyses
```

```
eye2.2 <- (rbind(eye2.1.cp.m2, eye2.1.mp.m2, eye2.1.cp.f2, eye2.1.mp.f2))
#and now with allomertic-scaled measurements
all_0.33 <- Pst(eye2.2, ci=1, csh=0.33, va=c("whole_count_al", "area_al"), bo
ot=1000.
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
all_0.33$level <- c(0.33,0.33)
all 0.67 <- Pst(eye2.2, ci=1, csh=0.67, va=c("whole count al", "area al"), bo
ot=1000,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
all 0.67$level <- c(0.67,0.67)
all_1 <- Pst(eye2.2, ci=1, csh=1, va=c("whole_count_al", "area_al"), boot=100
0,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
all_1$level <- c(1.00, 1.00)
all_1.33 <- Pst(eye2.2, ci=1, csh=1.33, va=c("whole count_al", "area_al"), bo
ot=1000,
                PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

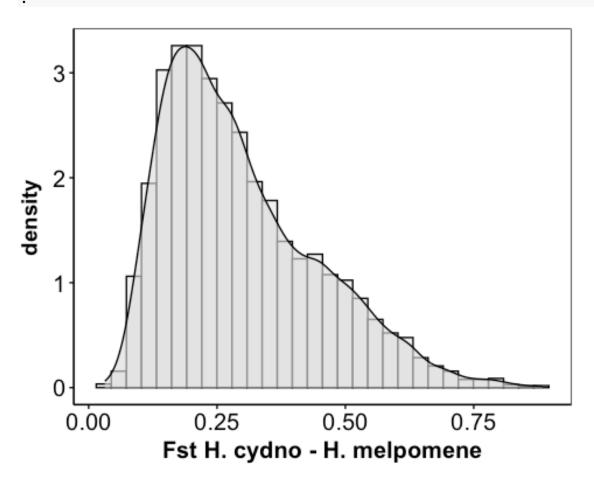
```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
all_1.33$level <- c(1.33,1.33)
all_1.5 <- Pst(eye2.2, ci=1, csh=1.5, va=c("whole_count_al", "area_al"), boot
=1000,
               Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
```

```
all 1.5$level <- c(1.50, 1.50)
all_2 <- Pst(eye2.2, ci=1, csh=2, va=c("whole_count_al", "area_al"), boot=100
0,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
all_2$level <- c(2.00, 2.00)
all_3 <- Pst(eye2.2, ci=1, csh=3, va=c("whole_count_al", "area_al"), boot=100
0,
             PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
all 3$level <- c(3.00, 3.00)
all_4 <- Pst(eye2.2, ci=1, csh=4, va=c("whole_count_al", "area_al"), boot=100
0,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 34 33
all_4$level <- c(4.00, 4.00)
all values <- rbind(all 0.33, all 0.67, all 1, all 1.33, all 1.5, all 2, all
3, all_4)
#####combine pst values into one data frame####
pst_values <- rbind(raw_values, log_values, all_values)</pre>
####Fst values from Martin et al. 2013####
fst <- read.csv("~/Documents/LMU/Eye morphology/selection manuscript/fst mart</pre>
in2013.csv")
ggplot(fst,aes(chi_rosFst)) +
  geom_histogram(aes(y = ..density..),
                 colour = 1, fill = "grey95") +
  geom_density(fill="grey85", alpha=0.6)+
  labs(x= "Fst H. cydno - H. melpomene", y="density")
## Warning: The dot-dot notation (`..density..`) was deprecated in ggplot2 3.
## U Please use `after_stat(density)` instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
## `stat bin()` using `bins = 30`. Pick better value with `binwidth`.
```

```
## Warning: Removed 4308 rows containing non-finite values (`stat_bin()`).
## Warning: Removed 4308 rows containing non-finite values (`stat_density()`)
```



```
#####***Plotted with Pst values**###

#with only facet count
pst_values1 <- pst_values[which(pst_values$Quant_Varia=="whole_count_al"),]

quantile(fst$chi_rosFst, 0.95, na.rm=T)

## 95%
## 0.58481

pst_values1$Pst_Values

## [1] 0.8788755 0.9364345 0.9564986 0.9669353 0.9705724 0.9777657 0.9850665
## [8] 0.9887579

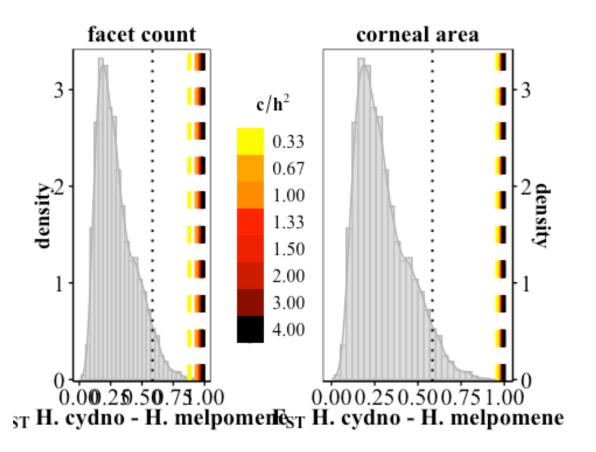
x.expression <- expression(bold(F[ST] ~ "H. cydno -" ~ "H. melpomene"))

count<- ggplot(fst,aes(chi_rosFst)) +
    geom_histogram(aes(y = ..density..),</pre>
```

```
colour = "grey70", fill = "grey90") +
  geom density(colour="grey70",fill="lightgrey", alpha=0.4)+
  geom_vline(aes(xintercept=0.8788755, color="0.33"), size=1.3, linetype="das
hed", key glyph = "rect")+
  geom vline(aes(xintercept=0.9364345, color="0.67"), size=1.3, linetype="das
hed")+
  geom vline(aes(xintercept=0.9564986, color="1.00"), size=1.3, linetype="das
  geom vline(aes(xintercept=0.9669353, color="1.33"), size=1.3, linetype="das
hed")+
  geom_vline(aes(xintercept=0.9705724, color="1.50"), size=1.3, linetype="das
  geom vline(aes(xintercept=0.9777657, color="2.00"), size=1.3, linetype="das
hed")+
  geom_vline(aes(xintercept=0.9850665, color="3.00"), size=1.3, linetype="das
  geom_vline(aes(xintercept=0.9887579, color="4.00"), size=1.3, linetype="das
hed")+
  scale color manual(
    name = expression(bold(c/h^2)),
    values = c("0.33" = "yellow", "0.67" = "orange", "1.00" = "darkorange", "
geom vline(xintercept=0.584, linetype="dotted", color="black", size=0.75)+
  labs(x= x.expression, y="density")+
  scale x continuous(limits = c(0,1.0))+
  scale y continuous(limits = c(0,3.4), expand = c(0,0.02))+
  theme(legend.key=element_blank(), legend.title=element_text(size=12),
        legend.title.align=0.5,legend.text=element_text(size=12))+
  theme(plot.margin = margin(0.5,0,0.5,0.5, "cm"))+
  ggtitle("facet count") + theme(plot.title = element_text(hjust = 0.5, size=
15, face="bold"))+
  theme(text = element text(family = "Times New Roman"))
## Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.
## ## Please use `linewidth` instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
#with only area
pst values2 <- pst values[which(pst values$Quant Varia=="area al"),]</pre>
quantile(fst$chi_rosFst, 0.95, na.rm=T)
##
      95%
## 0.58481
pst_values2$Pst_Values
```

```
## [1] 0.9608007 0.9803010 0.9867153 0.9899785 0.9911042 0.9933132 0.9955322
## [8] 0.9966454
area <- ggplot(fst,aes(chi_rosFst)) +</pre>
  geom_histogram(aes(y = ..density..),
                 colour = "grey70", fill = "grey90") +
  geom density(colour="grey70",fill="lightgrey", alpha=0.4)+
  geom vline(aes(xintercept=0.9608007, color="0.33"), size=1.3, linetype="das
hed",key_glyph = "rect")+
  geom_vline(aes(xintercept=0.9803010, color="0.67"), size=1.3, linetype="das
hed")+
  geom vline(aes(xintercept=0.9867153, color="1.00"), size=1.3, linetype="das
hed")+
  geom vline(aes(xintercept=0.9899785, color="1.33"), size=1.3, linetype="das
hed")+
  geom_vline(aes(xintercept=0.9911042, color="1.50"), size=1.3, linetype="das
hed")+
  geom vline(aes(xintercept=0.9933132, color="2.00"), size=1.3, linetype="das
hed")+
  geom vline(aes(xintercept=0.9955322, color="3.00"), size=1.3, linetype="das
hed")+
  geom_vline(aes(xintercept=0.9966454, color="4.00"), size=1.3, linetype="das
hed")+
  scale color manual(
    name = expression(bold(c/h^2)),
    values = c("0.33" = "yellow", "0.67" = "orange", "1.00" = "darkorange", "
1.33" = "red", "1.50" = "red2",
               "2.00" = "red3", "3.00" = "red4", "4.00" = "black"))+
  geom_vline(xintercept=0.584, linetype="dotted", color="black", size=0.75)+
  labs(x= x.expression, y="density")+
  scale_x_continuous(limits = c(0,1.0))+
  scale y continuous(limits = c(0,3.4), expand = c(0,0.02), position = "right
")+
  theme(legend.key=element blank(), legend.title=element text(size=12),
        legend.title.align=0.5,legend.text=element text(size=12))+
  theme(legend.position='none')+
  theme(plot.margin = margin(0.5,0.5,0.5,0.1, "cm"))+
  ggtitle("corneal area") + theme(plot.title = element_text(hjust = 0.5, size
=15, face="bold"))+
  theme(text = element text(family = "Times New Roman"))
#combine plots
library(ggpubr)
ggarrange(count, area,
          \#labels = c("A", "B"),
          #vjust=1.23, hjust=0.8,
          ncol = 2, nrow = 1, widths = c(1.2, 1))
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
## Warning: Removed 4308 rows containing non-finite values (`stat_bin()`).
## Warning: Removed 4308 rows containing non-finite values (`stat_density()`)
.
## Warning: Removed 2 rows containing missing values (`geom_bar()`).
```



######calculate p-values for each Pst###

#use only cydno vs. melp fst values
fst2 <- as.data.frame(fst\$chi_rosFst)

#remove NAs from the Fst values
fst2.1 <- complete.cases(fst2)
fst3 <- fst2[fst2.1,]

#calculate the proportion of the Fst distribution above each Pst value
proportions <- lapply(pst_values\$Pst_Values, function(x) sum(fst3 > x) / leng
th(fst3))

```
#combine the x values and proportions into a data frame
p.values <- data.frame(x = pst values$Pst Values, p value = unlist(proportion</pre>
s))
pst values <- cbind(pst values, p.values)</pre>
#double-check that Pst values are aligned in new dataset
pst values$Pst Values - pst values$x #all zeros, everything is correct
0 0 0
## [39] 0 0 0 0 0 0 0 0 0
#export to make a table
write.csv(pst values,file='~/Documents/LMU/Eye morphology/selection manuscrip
t/pst table.csv')
####SELECTION TESTS PER SEX SEPARATELY####
library("Pstat")
eye3m <- eye2.1[which(eye2.1$sex=="male"),]</pre>
#####1) raw measurements male####
raw 0.33 m <- Pst(eye3m, ci=1, csh=0.33, va=c("whole count", "area"), boot=10
00,
              PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
raw_0.33_m$level <- c(0.33,0.33)
raw_0.67_m <- Pst(eye3m, ci=1, csh=0.67, va=c("whole_count", "area"), boot=10
00,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## [1] "Populations sizes are:"
## CP MP
## 15 18
raw_0.67_m$level <- c(0.67,0.67)
raw_1_m <- Pst(eye3m, ci=1, csh=1.0, va=c("whole_count", "area"), boot=1000,</pre>
             PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
raw 1 m$level \leftarrow c(1,1)
raw_1.33 m <- Pst(eye3m, ci=1, csh=1.33, va=c("whole_count", "area"), boot=10
00,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
raw 1.33 m$level <- c(1.33,1.33)
raw_1.5_m <- Pst(eye3m, ci=1, csh=1.5, va=c("whole count", "area"), boot=1000</pre>
               Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
raw_1.5_m$level <- c(1.5,1.5)
raw_2 m <- Pst(eye3m, ci=1, csh=2, va=c("whole_count", "area"), boot=1000,</pre>
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
raw 2 m$level <- c(2,2)
raw_3_m <- Pst(eye3m, ci=1, csh=3, va=c("whole_count", "area"), boot=1000,
             PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
raw 3 m$level \leftarrow c(3,3)
```

```
raw_4_m <- Pst(eye3m, ci=1, csh=4, va=c("whole_count", "area"), boot=1000,
             PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
raw 4 m$level \leftarrow c(4,4)
raw_values_m <- rbind(raw_0.33_m, raw_0.67_m, raw_1_m,</pre>
                      raw_1.33_m, raw_1.5_m, raw_2_m, raw_3_m, raw_4_m)
#####2) Log-transformed measurements_male####
log 0.33 m <- Pst(eye3m, ci=1, csh=0.33, va=c("whole count log", "area log"),</pre>
boot=1000,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
log 0.33 m$level <- c(0.33,0.33)
log_0.67_m <- Pst(eye3m, ci=1, csh=0.67, va=c("whole_count_log", "area_log"),</pre>
boot=1000,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
log 0.67 m$level <- c(0.67, 0.67)
log_1 m <- Pst(eye3m, ci=1, csh=1, va=c("whole_count_log", "area_log"), boot=</pre>
1000,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
log_1_m$level <- c(1,1)
log 1.33 m <- Pst(eye3m, ci=1, csh=1.33, va=c("whole count log", "area log"),</pre>
boot=1000,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
```

```
log 1.33 m$level <- c(1.33,1.33)
log_1.5_m <- Pst(eye3m, ci=1, csh=1.5, va=c("whole count log", "area log"), b</pre>
oot=1000,
               PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
log_1.5_m$level <- c(1.5,1.5)
log 2 m <- Pst(eye3m, ci=1, csh=2, va=c("whole count log", "area log"), boot=</pre>
1000,
             PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
log 2 m$level <- c(2,2)
log_3_m <- Pst(eye3m, ci=1, csh=3, va=c("whole_count_log", "area_log"), boot=</pre>
1000,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
log_3_m$level <- c(3,3)
log 4 m <- Pst(eye3m, ci=1, csh=4, va=c("whole count log", "area log"), boot=</pre>
1000,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
log 4 m$level <- c(4,4)
log_values_m <- rbind(log_0.33_m, log_0.67_m, log_1_m, log_1.33_m,
                      log_1.5_m, log_2_m, log_3_m, log_4_m)
#####3) allometric-scaled male####
eye3m_al <- (rbind(eye2.1.cp.m2, eye2.1.mp.m2))</pre>
#with allomertic-scaled measurements
all_0.33_m <- Pst(eye3m_al, ci=1, csh=0.33, va=c("whole_count_al", "area_al")
, boot=1000,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
all_0.33_m$level <- c(0.33,0.33)
all_0.67_m <- Pst(eye3m_al, ci=1, csh=0.67, va=c("whole_count_al", "area_al")
, boot=1000,
                Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
all 0.67 m$level <- c(0.67,0.67)
all_1_m <- Pst(eye3m_al, ci=1, csh=1, va=c("whole_count_al", "area_al"), boot
```

```
=1000,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
all_1_m$level <- c(1,1)
all_1.33_m <- Pst(eye3m_al, ci=1, csh=1.33, va=c("whole_count_al", "area_al")
, boot=1000,
                PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
all 1.33 m$level <- c(1.33,1.33)
all 1.5 m <- Pst(eye3m al, ci=1, csh=1.5, va=c("whole count al", "area al"),
boot=1000.
               Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
all_1.5_m$level <- c(1.5,1.5)
all 2 m <- Pst(eye3m al, ci=1, csh=2, va=c("whole count al", "area al"), boot
=1000,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## [1] "Populations sizes are:"
## CP MP
## 15 18
all 2 m$level \leftarrow c(2,2)
all_3_m <- Pst(eye3m_al, ci=1, csh=3, va=c("whole_count_al", "area_al"), boot
=1000,
             Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
all 3 m$level \leftarrow c(3,3)
all 4 m <- Pst(eye3m_al, ci=1, csh=4, va=c("whole_count_al", "area_al"), boot
=1000,
             PW = c("CP", "MP"), pe = 0.95)
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 15 18
all_4_m$level <- c(4,4)
all_values_m <- rbind(all_0.33_m, all_0.67_m, all_1_m,
                      all_1.33_m, all_1.5_m, all_2_m, all_3_m, all_4_m)
####combine pst values into one male data frame with p-values####
pst values m <- rbind(raw values m, log values m, all values m)
#calculate p-values
#use only cydno vs. melp fst values, fst3
#calculate the proportion of the Fst distribution above each Pst value
proportions_m <- lapply(pst_values_m$Pst_Values, function(x) sum(fst3 > x) /
```

```
length(fst3))
# Combine the x values and proportions into a data frame
p.values m <- data.frame(x = pst values m$Pst Values, p value = unlist(propor</pre>
tions m))
pst_values_m <- cbind(pst_values_m, p.values_m)</pre>
#double-check that Pst values are aligned in new dataset
pst_values m$Pst_Values - pst_values m$x #all zeros, everything is correct
0 0 0
## [39] 0 0 0 0 0 0 0 0 0
#export to make a table
write.csv(pst values m,file='~/Documents/LMU/Eye morphology/selection manuscr
ipt/pst table male.csv')
#####1) raw measurements female####
eye3f <- eye2.1[which(eye2.1$sex=="female"),]</pre>
raw_0.33_f <- Pst(eye3f, ci=1, csh=0.33, va=c("whole_count", "area"), boot=10</pre>
00,
                 PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
raw_0.33_f$level <- c(0.33,0.33)
raw_0.67_f <- Pst(eye3f, ci=1, csh=0.67, va=c("whole_count", "area"), boot=10
00,
                  Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
```

```
raw 0.67 f$level <- c(0.67,0.67)
raw_1.0_f <- Pst(eye3f, ci=1, csh=1.0, va=c("whole count", "area"), boot=1000</pre>
                  PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
raw_1.0_f$level <- c(1.0,1.0)
raw_1.33_f <- Pst(eye3f, ci=1, csh=1.33, va=c("whole_count", "area"), boot=10
00,
                 PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
raw 1.33 f$level <- c(1.33,1.33)
raw_1.5_f <- Pst(eye3f, ci=1, csh=1.5, va=c("whole_count", "area"), boot=1000</pre>
               Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
raw_1.5_f$level <- c(1.5,1.5)
raw_2.0_f <- Pst(eye3f, ci=1, csh=2.0, va=c("whole_count", "area"), boot=1000</pre>
               Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
raw 2.0 f$level <- c(2.0,2.0)
raw 3.0 f <- Pst(eye3f, ci=1, csh=3.0, va=c("whole count", "area"), boot=1000
                 Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
raw 3.0 f$level <- c(3.0,3.0)
```

```
raw 4.0 f <- Pst(eye3f, ci=1, csh=4.0, va=c("whole count", "area"), boot=1000
                 Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
raw 4.0 f$level <- c(4.0,4.0)
raw_values_f <- rbind(raw_0.33_f, raw_0.67_f, raw_1.0_f, raw_1.33_f, raw_1.5_
f,
                      raw 2.0 f, raw 3.0 f, raw 4.0 f)
#####2) Log-transformed measurements female####
log 0.33 f <- Pst(eye3f, ci=1, csh=0.33, va=c("whole count log", "area log"),</pre>
boot=1000,
                  Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
log 0.33 f$level <- c(0.33,0.33)
log_0.67_f <- Pst(eye3f, ci=1, csh=0.67, va=c("whole_count_log", "area_log"),</pre>
boot=1000,
                  PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
log 0.67 f$level <- c(0.67,0.67)
log_1.0_f <- Pst(eye3f, ci=1, csh=1.0, va=c("whole count log", "area_log"), b</pre>
oot=1000,
                  PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
log_1.0_f$level <- c(1.0,1.0)
log_1.33_f <- Pst(eye3f, ci=1, csh=1.33, va=c("whole_count_log", "area log"),</pre>
boot=1000.
                 Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
```

```
log 1.33 f$level <- c(1.33,1.33)
log_1.5_f <- Pst(eye3f, ci=1, csh=1.5, va=c("whole count log", "area_log"), b</pre>
oot=1000,
               PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
log_1.5_f$level <- c(1.5,1.5)
log 2.0 f <- Pst(eye3f, ci=1, csh=2, va=c("whole count log", "area log"), boo</pre>
t=1000,
               PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
log 2.0 f$level <- c(2.0,2.0)
log_3.0_f <- Pst(eye3f, ci=1, csh=3, va=c("whole_count_log", "area_log"), boo</pre>
t=1000,
                 Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
log_3.0_f$level <- c(3.0,3.0)
log_4.0_f <- Pst(eye3f, ci=1, csh=4, va=c("whole_count_log", "area_log"), boo</pre>
t=1000,
                 PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
log 4.0 f$level <- c(4.0,4.0)
log_values_f <- rbind(log_0.33_f, log_0.67_f, log_1.0_f, log_1.33_f, log_1.5_
f,
                      log_2.0_f, log_3.0_f, log_4.0_f)
#####3) allometric-scaled female####
eye3f_al <- (rbind(eye2.1.cp.f2, eye2.1.mp.f2))
#with allomertic-scaled measurements
all_0.33_f <- Pst(eye3f_al, ci=1, csh=0.33, va=c("whole_count_al", "area_al")
, boot=1000,
                  PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
all 0.33 f$level <- c(0.33,0.33)
all 0.67 f <- Pst(eye3f al, ci=1, csh=0.67, va=c("whole count al", "area al")
, boot=1000,
                  Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
all 0.67 f$level <- c(0.67,0.67)
```

```
all 1.0 f <- Pst(eye3f al, ci=1, csh=1.0, va=c("whole count al", "area al"),
boot=1000,
                  PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
all_1.0_f$level <- c(1.0,1.0)
all_1.33_f <- Pst(eye3f_al, ci=1, csh=1.33, va=c("whole_count_al", "area_al")</pre>
, boot=1000,
               PW = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
all 1.33 f$level <- c(1.33,1.33)
all_1.5_f <- Pst(eye3f_al, ci=1, csh=1.5, va=c("whole count_al", "area_al"),
boot=1000,
                  Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

```
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
all_1.5_f$level <- c(1.5,1.5)
all_2.0_f <- Pst(eye3f_al, ci=1, csh=2, va=c("whole_count_al", "area_al"), bo
ot=1000,
               Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
```

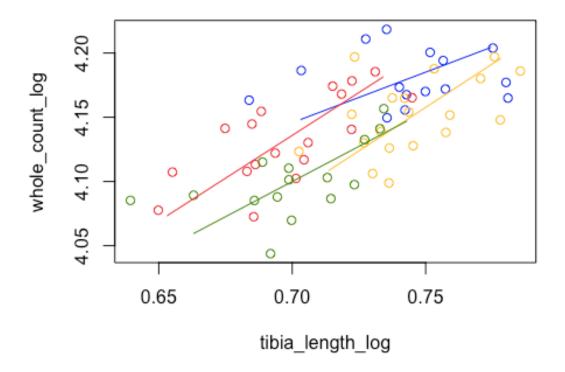
```
## [1] "Populations sizes are:"
## CP MP
## 19 15
all 2.0 f$level <- c(2.0,2.0)
all 3.0 f <- Pst(eye3f_al, ci=1, csh=3, va=c("whole_count_al", "area_al"), bo
ot=1000,
               Pw = c("CP", "MP"), pe = 0.95)
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
all 3.0 f$level <- c(3.0,3.0)
all_4.0_f <- Pst(eye3f_al, ci=1, csh=4, va=c("whole_count_al", "area_al"), bo
ot=1000.
               PW = c("CP", "MP"), pe = 0.95)
```

```
## Warning in dat.fra.prep(data): NAs introduced by coercion
## Warning in sqrt((nna.clm - 1)/nna.clm): NaNs produced
## [1] "Populations sizes are:"
## CP MP
## 19 15
all_4.0_f$level <- c(4.0,4.0)
all_values_f <- rbind(all_0.33_f, all_0.67_f, all_1.0_f, all_1.33_f, all_1.5_
f,
                      all_2.0_f, all_3.0_f, all_4.0_f)
#####combine pst values into one female data frame with p-values####
pst values f <- rbind(raw values f, log values f, all values f)</pre>
#calculate p-values
#use only cydno vs. melp fst values, fst3
#calculate the proportion of the Fst distribution above each Pst value
proportions_f <- lapply(pst_values_f$Pst_Values, function(x) sum(fst3 > x) /
length(fst3))
```

```
# Combine the x_values and proportions into a data frame
p.values f <- data.frame(x = pst values f$Pst Values, p value = unlist(propor
tions f))
pst_values_f <- cbind(pst_values_f, p.values_f)</pre>
#double-check that Pst values are aligned in new dataset
pst_values_f$Pst_Values - pst_values_f$x #all zeros, everything is correct
000
## [39] 0 0 0 0 0 0 0 0 0
#export to make a table
write.csv(pst_values_f,file='~/Documents/LMU/Eye morphology/selection manuscr
ipt/pst table female.csv')
####ALLOMETRY####
library(smatr)
#####1) as an interactive term####
#smatr cannot handle 2 grouping terms, so make an interaction
eye2\$inter <- interaction(eye2\$type, eye2\$sex)
eye2\$inter<- factor(eye2\$inter, c("CP.male", "CP.female",
                              "MP.male", "MP.female"))
#####testing differences in slope (beta)#####
#facet count
a1 <- sma(whole count log~tibia length log*inter, robust=F, data=eye2, multco
mp = T, multcompmethod = "adjust")
multcompmatrix(a1, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
##
             inter 2
             CP.male MP.male MP.female CP.female
## inter 1
   CP.male
##
##
    MP.male
   MP.female
##
##
   CP.female
summary(a1)
```

```
## Call: sma(formula = whole_count_log ~ tibia_length_log * inter, data = eye
2,
      multcomp = T, multcompmethod = "adjust", robust = F)
##
##
## Fit using Standardized Major Axis
## ------
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 2.981 with 3 degrees of freedom
## P-value : 0.39454
## Results of multiple comparisons among groups.
## Test for pair-wise difference in slope :
      inter 1 inter 2
                       Pval
      CP.male CP.female 0.56709 2.28963193
## 1
## 2
      CP.male MP.male 0.52587 2.45762825
      CP.male MP.female 0.93682 0.80736426
## 4 CP.female MP.male 1.00000 0.00278065
## 5 CP.female MP.female 0.98537 0.44349039
## 6
      MP.male MP.female 0.98456 0.45284640
##
## -----
## Coefficients by group in variable "inter"
##
## Group: CP.male
       elevation slope
## estimate
             3.594643 0.7873019
## lower limit 3.243936 0.4465518
## upper limit 3.945351 1.3880679
##
## H0 : variables uncorrelated.
## R-squared : 0.00413478
## P-value : 0.8199
##
## Group: CP.female
            elevation
                        slope
             3.142194 1.3540100
## estimate
## lower limit 2.688987 0.8763565
## upper limit 3.595402 2.0920060
##
## H0 : variables uncorrelated.
## R-squared : 0.2303859
## P-value : 0.037543
##
## Group: MP.male
## elevation slope
```

```
## estimate
                3.201911 1.3342359
## lower limit 2.858373 0.9302675
## upper limit 3.545450 1.9136273
##
## H0 : variables uncorrelated.
## R-squared : 0.5165108
## P-value : 0.00077845
## Group: MP.female
##
               elevation
                             slope
## estimate
                3.333263 1.0952430
## lower limit 2.953909 0.6804684
## upper limit 3.712617 1.7628405
## H0 : variables uncorrelated.
## R-squared : 0.3199202
## P-value : 0.02798
plot(a1)
```



#######**plot facet count major axis regressions**#####
dev.off()

```
## null device
##
             1
par(mgp=c(3.5,0.6,0))
par(mar = c(5, 6, 1.25, 1.25))
par(lwd=1)
plot(a1, col=c("#1F78B4", "#1F78B4", "#FDBF6F", "#FDBF6F"),
     pch=c(17,2,16,1),
     lwd=1,
     cex=0.9
     lty=c(1,2,1,2),
     xlim=c(0.635,0.80), ylim=c(4.03,4.25),
     las=1,cex.axis = 1.3,
     xlab = "",
    ylab = "",
    tck = -0.015
    family = "Times New Roman")
#title(xlab = "log10 (hind tibia length)", line = 2,cex.lab = 1.2,font.lab=2,
family = "Times New Roman")
title(ylab = "log10 (facet count)", line = 3,cex.lab = 1.2,font.lab=2,family
= "Times New Roman")
title("facet count", adj = 0.5, line = 0.6, family = "Times New Roman", cex.m
ain=1.35)
legend("topleft", c("C", "M"),
       col = c("#1F78B4", "#FDBF6F"), pch=c(17,16),
       bty="n")
a2 <- sma(area log~tibia length log*inter, data=eye2, multcomp = T, multcompm
ethod = "adjust")
multcompmatrix(a2, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
##
              inter 2
              CP.male MP.male CP.female MP.female
## inter 1
##
    CP.male
    MP.male
##
     CP.female .
##
    MP.female
##
summary(a2)
## Call: sma(formula = area_log ~ tibia_length_log * inter, data = eye2,
      multcomp = T, multcompmethod = "adjust")
```

```
##
## Fit using Standardized Major Axis
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 8.026 with 3 degrees of freedom
## P-value : 0.045487
##
## Results of multiple comparisons among groups.
## Test for pair-wise difference in slope :
      inter_1 inter_2 Pval TestStat
##
      CP.male CP.female 0.079429 6.07655231
## 2
     CP.male MP.male 0.038657 7.39266161
## 3
      CP.male MP.female 0.416808 2.94858246
              MP.male 0.999985 0.03885275
## 4 CP.female
## 5 CP.female MP.female 0.973795 0.55816819
## 6
      MP.male MP.female 0.910225 0.94558492
##
## ------
## Coefficients by group in variable "inter"
## Group: CP.male
               elevation slope
##
## estimate 0.22086518 0.9387334
## lower limit -0.08562769 0.6134198
## upper limit 0.52735804 1.4365697
##
## H0 : variables uncorrelated.
## R-squared : 0.4645721
## P-value : 0.0051359
##
## Group: CP.female
               elevation slope
##
## estimate -0.44638368 1.811085
## lower limit -0.86747180 1.332310
## upper limit -0.02529556 2.461912
##
## H0 : variables uncorrelated.
## R-squared : 0.6285736
## P-value : 5.157e-05
##
## Group: MP.male
##
               elevation
                         slope
## estimate
             -0.4652718 1.881835
## lower limit -0.8042493 1.458194
## upper limit -0.1262944 2.428555
```

```
##
## H0 : variables uncorrelated.
## R-squared : 0.7633368
## P-value : 2.1756e-06
##
## Group: MP.female
                elevation
                             slope
               -0.2501853 1.518812
## estimate
## lower limit -0.6572024 1.045336
## upper limit 0.1568317 2.206746
## H0 : variables uncorrelated.
## R-squared : 0.5928163
## P-value : 0.00078631
plot(a2)
#######**plot corneal area major axis regressions**######
plot(a2, col=c("#1F78B4", "#1F78B4", "#FDBF6F", "#FDBF6F"),
     pch=c(17,2,16,1),
     lwd=1.
     cex=0.9,
     lty=c(1,2,1,2),
     xlim=c(0.635,0.80), ylim=c(0.73,1.00),
     las=1, cex.axis = 1.3,
     xlab = ""
    ylab = "",
    tck = -0.015,
    family = "Times New Roman")
title(xlab = "log10 (hind tibia length)", line = 2,cex.lab = 1.2,font.lab=2,f
amily = "Times New Roman")
title(ylab = "log10 (corneal area)", line = 3,cex.lab = 1.2,font.lab=2,family
= "Times New Roman")
title("corneal area", adj = 0.5, line = 0.6, family = "Times New Roman", cex.
main=1.35)
legend("topleft", c("C", "M"),
       col = c("#1F78B4", "#FDBF6F"), pch=c(17,16),
       bty="n")
#####testing shift in elevation (grade shift - a)####
#facet count
```

```
a3 <- sma(whole count log~tibia length log+inter, type="elevation",
         data=eye2, multcomp = T, multcompmethod = "adjust")
multcompmatrix(a3, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## inter_1
             CP.male CP.female MP.female MP.male
##
   CP.male
    CP.female *
##
##
    MP.female *
                                        **
                     **
##
   MP.male
summary(a3)
## Call: sma(formula = whole count log ~ tibia length log + inter, data = eye
2,
##
      type = "elevation", multcomp = T, multcompmethod = "adjust")
## Fit using Standardized Major Axis
##
## -----
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 2.981 with 3 degrees of freedom
## P-value : 0.39454
## H0 : no difference in elevation.
## Wald statistic: 23.21 with 3 degrees of freedom
## P-value : 3.6441e-05
## Results of multiple comparisons among groups.
##
## Test for pair-wise difference in elevation :
      inter 1 inter 2
                       Pval
                                 TestStat
      CP.male CP.female 0.0428279 7.2053170
## 1
## 2
      CP.male
              MP.male 0.9988230 0.1757316
      CP.male MP.female 0.0210016 8.5103664
## 3
## 4 CP.female MP.male 0.0019596 12.9098131
## 5 CP.female MP.female 0.9998065 0.0937200
      MP.male MP.female 0.0012326 13.7796832
## -----
## Coefficients by group in variable "inter"
## Group: CP.male
```

```
##
               elevation
## estimate
                3.298465 1.1853030
## lower limit 3.092715 0.9503365
## upper limit 3.504216 1.4802397
##
## H0 : variables uncorrelated.
## R-squared : 0.00413478
## P-value : 0.8199
##
## Group: CP.female
##
               elevation
                             slope
## estimate
                3.267918 1.1853030
## lower limit 3.067266 0.9503365
## upper limit 3.468570 1.4802397
##
## H0 : variables uncorrelated.
## R-squared: 0.2303859
## P-value : 0.037543
##
## Group: MP.male
##
               elevation
                             slope
## estimate
                3.305897 1.1853030
## lower limit 3.117061 0.9503365
## upper limit 3.494733 1.4802397
##
## H0 : variables uncorrelated.
## R-squared : 0.5165108
## P-value : 0.00077845
##
## Group: MP.female
##
               elevation
                             slope
## estimate
                3.270185 1.1853030
## lower limit 3.076896 0.9503365
## upper limit 3.463475 1.4802397
##
## H0 : variables uncorrelated.
## R-squared : 0.3199202
## P-value : 0.02798
plot(a3)
#area
a4 <- sma(area_log~tibia_length_log+inter, type="elevation",
          data=eye2, multcomp = T, multcompmethod = "adjust" )
multcompmatrix(a4, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
              inter 2
```

```
## inter 1 CP.male CP.female MP.female MP.male
##
    CP.male
##
    CP.female
    MP.female ***
                                         ***
##
                               ***
    MP.male
##
summary(a4)
## Call: sma(formula = area_log ~ tibia_length_log + inter, data = eye2,
      type = "elevation", multcomp = T, multcompmethod = "adjust")
##
## Fit using Standardized Major Axis
## ------
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 8.026 with 3 degrees of freedom
## P-value : 0.045487
## H0 : no difference in elevation.
## Wald statistic: 20.12 with 3 degrees of freedom
## P-value : 0.00016011
##
## Results of multiple comparisons among groups.
## Test for pair-wise difference in elevation :
##
      inter 1 inter 2
                             Pval TestStat
      CP.male CP.female 0.31335412 3.51723843
## 1
      CP.male
              MP.male 0.99997969 0.04348447
      CP.male MP.female 8.3887e-05 18.87163877
              MP.male 0.03208414 7.73359385
## 4 CP.female
## 5 CP.female MP.female 0.84596688 1.22774439
      MP.male MP.female 0.00035567 16.12526659
## 6
##
## Coefficients by group in variable "inter"
##
## Group: CP.male
##
                elevation
                           slope
## estimate
              -0.30393283 1.643953
## lower limit -0.51342742 1.383356
## upper limit -0.09443825 1.947097
## H0 : variables uncorrelated.
## R-squared : 0.4645721
## P-value : 0.0051359
##
```

```
## Group: CP.female
##
                elevation
                             slope
               -0.3218331 1.643953
## estimate
## lower limit -0.5262659 1.383356
## upper limit -0.1174003 1.947097
## H0: variables uncorrelated.
## R-squared : 0.6285736
## P-value : 5.157e-05
##
## Group: MP.male
##
                elevation
                             slope
## estimate
               -0.2991812 1.643953
## lower limit -0.4916616 1.383356
## upper limit -0.1067007 1.947097
## H0 : variables uncorrelated.
## R-squared : 0.7633368
## P-value : 2.1756e-06
##
## Group: MP.female
                elevation
                             slope
## estimate
               -0.3378333 1.643953
## lower limit -0.5348932 1.383356
## upper limit -0.1407734 1.947097
##
## H0 : variables uncorrelated.
## R-squared : 0.5928163
## P-value : 0.00078631
plot(a4)
######testing shift along common axis####
#facet count
a5 <- sma(whole count log~tibia length log+inter, type="shift",
          data=eye2, multcomp = T, multcompmethod = "adjust")
multcompmatrix(a5, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
##
              inter 2
               CP.male MP.male MP.female CP.female
## inter_1
                       ***
                               ***
##
    CP.male
                                          ***
               ***
##
    MP.male
##
    MP.female ***
                                          ***
                       ***
                               ***
##
    CP.female
```

```
summary(a5)
## Call: sma(formula = whole count log ~ tibia_length_log + inter, data = eye
2,
      type = "shift", multcomp = T, multcompmethod = "adjust")
##
##
## Fit using Standardized Major Axis
## -----
## Results of comparing lines among groups.
##
## H0 : slopes are equal.
## Likelihood ratio statistic : 2.981 with 3 degrees of freedom
## P-value : 0.39454
## H0 : no shift along common axis.
## Wald statistic: 62.68 with 3 degrees of freedom
## P-value : 1.5743e-13
##
## Results of multiple comparisons among groups.
## Test for pair-wise difference in shift :
      inter 1 inter 2 Pval TestStat
##
      CP.male CP.female 0.28798033 3.680999
## 1
      CP.male MP.male 9.7151e-07 27.441499
## 2
      CP.male MP.female 7.5420e-12 50.395166
## 4 CP.female MP.male 0.00074106 14.737750
## 5 CP.female MP.female 8.8920e-07 27.612733
      MP.male MP.female 0.58922893 2.202177
##
## -----
## Coefficients by group in variable "inter"
##
## Group: CP.male
            elevation
                        slope
## estimate 3.298465 1.1853030
## lower limit 3.243936 0.9503365
## upper limit 3.945351 1.4802397
## H0 : variables uncorrelated.
## R-squared : 0.00413478
## P-value : 0.8199
##
## Group: CP.female
             elevation
           3.267918 1.1853030
## estimate
## lower limit 2.688987 0.9503365
## upper limit 3.595402 1.4802397
```

```
##
## H0 : variables uncorrelated.
## R-squared: 0.2303859
## P-value : 0.037543
##
## Group: MP.male
              elevation
                             slope
               3.305897 1.1853030
## estimate
## lower limit 2.858373 0.9503365
## upper limit 3.545450 1.4802397
## H0 : variables uncorrelated.
## R-squared : 0.5165108
## P-value : 0.00077845
##
## Group: MP.female
              elevation
                             slope
## estimate
               3.270185 1.1853030
## lower limit 2.953909 0.9503365
## upper limit 3.712617 1.4802397
## H0 : variables uncorrelated.
## R-squared : 0.3199202
## P-value : 0.02798
plot(a5)
#area
a6 <- sma(area log~tibia length log+inter, type="shift",
          data=eye2, multcomp = T, multcompmethod = "adjust")
multcompmatrix(a6, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
##
             inter 2
             CP.male MP.male MP.female CP.female
## inter 1
                       ***
    CP.male
##
               ***
                                         ***
##
    MP.male
                                         ***
   MP.female ***
##
                       ***
                               ***
##
    CP.female
summary(a6)
## Call: sma(formula = area_log ~ tibia_length_log + inter, data = eye2,
      type = "shift", multcomp = T, multcompmethod = "adjust")
##
##
## Fit using Standardized Major Axis
##
```

```
## Results of comparing lines among groups.
##
## H0 : slopes are equal.
## Likelihood ratio statistic : 8.026 with 3 degrees of freedom
## P-value : 0.045487
##
## H0 : no shift along common axis.
## Wald statistic: 62.94 with 3 degrees of freedom
## P-value : 1.3811e-13
##
## Results of multiple comparisons among groups.
## Test for pair-wise difference in shift :
      inter 1 inter 2 Pval
## 1
      CP.male CP.female 0.98634 0.4318941
     CP.male MP.male 1.1129e-06 27.1788341
## 2
## 3 CP.male MP.female 1.7689e-10 44.2110534
## 4 CP.female MP.male 9.4240e-06 23.0594591
## 5 CP.female MP.female 2.9485e-08 34.2231172
      MP.male MP.female 0.88670 1.0548864
##
## Coefficients by group in variable "inter"
## Group: CP.male
##
                elevation
                            slope
## estimate
             -0.30393283 1.643953
## lower limit -0.08562769 1.383356
## upper limit 0.52735804 1.947097
## H0 : variables uncorrelated.
## R-squared : 0.4645721
## P-value : 0.0051359
##
## Group: CP.female
##
                elevation
                             slope
## estimate
             -0.32183311 1.643953
## lower limit -0.86747180 1.383356
## upper limit -0.02529556 1.947097
##
## H0 : variables uncorrelated.
## R-squared : 0.6285736
## P-value : 5.157e-05
##
## Group: MP.male
               elevation slope
## estimate -0.2991812 1.643953
## lower limit -0.8042493 1.383356
```

```
## upper limit -0.1262944 1.947097
##
## H0 : variables uncorrelated.
## R-squared: 0.7633368
## P-value : 2.1756e-06
##
## Group: MP.female
                elevation
                            slope
## estimate -0.3378333 1.643953
## lower limit -0.6572024 1.383356
## upper limit 0.1568317 1.947097
##
## H0 : variables uncorrelated.
## R-squared : 0.5928163
## P-value : 0.00078631
plot(a6)
#####2) as a single term, separated by sex####
#divide datasets by sex
eye2.m <- eye2[which(eye2$sex=="male"),]</pre>
eye2.f <- eye2[which(eye2$sex=="female"),]</pre>
eye.m <- eye[which(eye$sex=="male"),]</pre>
eye.f <- eye[which(eye$sex=="female"),]</pre>
#####testing differences in slope (beta)#####
#facet count - male
b1 <- sma(whole_count_log~tibia_length_log*type, data=eye2.m)
summary(b1)
## Call: sma(formula = whole_count_log ~ tibia_length_log * type, data = eye2
.m)
##
## Fit using Standardized Major Axis
##
## Results of comparing lines among groups.
##
## H0 : slopes are equal.
## Likelihood ratio statistic : 2.458 with 1 degrees of freedom
## P-value : 0.11696
## -----
## Coefficients by group in variable "type"
##
## Group: CP
```

```
##
              elevation slope
## estimate
             3.594643 0.7873019
## lower limit 3.243936 0.4465518
## upper limit 3.945351 1.3880679
##
## H0 : variables uncorrelated.
## R-squared : 0.00413478
## P-value : 0.8199
##
## Group: MP
##
              elevation
                           slope
              3.201911 1.3342359
## estimate
## lower limit 2.858373 0.9302675
## upper limit 3.545450 1.9136273
##
## H0 : variables uncorrelated.
## R-squared : 0.5165108
## P-value : 0.00077845
plot(b1)
#facet count - female
b1.1 <- sma(whole_count_log~tibia_length_log*type, data=eye2.f)
summary(b1.1)
## Call: sma(formula = whole_count_log ~ tibia_length_log * type, data = eye2
.f)
## Fit using Standardized Major Axis
##
## -----
## Results of comparing lines among groups.
##
## H0 : slopes are equal.
## Likelihood ratio statistic : 0.4435 with 1 degrees of freedom
## P-value : 0.50544
## -----
##
## Coefficients by group in variable "type"
##
## Group: CP
              elevation
##
                           slope
## estimate
              3.142194 1.3540100
## lower limit 2.688987 0.8763565
## upper limit 3.595402 2.0920060
##
## H0 : variables uncorrelated.
## R-squared : 0.2303859
## P-value : 0.037543
##
```

```
## Group: MP
##
              elevation
                            slope
              3.333263 1.0952430
## estimate
## lower limit 2.953909 0.6804684
## upper limit 3.712617 1.7628405
##
## H0 : variables uncorrelated.
## R-squared : 0.3199202
## P-value : 0.02798
plot(b1.1)
par(mfrow = c(1, 2))
plot(b1, main = "males")
plot(b1.1,main = "females")
par(mfrow = c(1, 1))
#area - male
b2 <- sma(area_log~tibia_length_log*type, data=eye2.m)
summary(b2)
## Call: sma(formula = area_log ~ tibia_length_log * type, data = eye2.m)
## Fit using Standardized Major Axis
##
## Results of comparing lines among groups.
##
## H0 : slopes are equal.
## Likelihood ratio statistic : 7.393 with 1 degrees of freedom
## P-value : 0.0065491
## -----
## Coefficients by group in variable "type"
##
## Group: CP
                elevation
##
                               slope
## estimate
               0.22086518 0.9387334
## lower limit -0.08562769 0.6134198
## upper limit 0.52735804 1.4365697
##
## H0 : variables uncorrelated.
## R-squared : 0.4645721
## P-value : 0.0051359
##
## Group: MP
##
               elevation
             -0.4652718 1.881835
## estimate
## lower limit -0.8042493 1.458194
## upper limit -0.1262944 2.428555
```

```
##
## H0 : variables uncorrelated.
## R-squared : 0.7633368
## P-value : 2.1756e-06
plot(b2)
#area - female
b2.1 <- sma(area_log~tibia_length_log*type, data=eye2.f)
summary(b2.1)
## Call: sma(formula = area_log ~ tibia_length_log * type, data = eye2.f)
## Fit using Standardized Major Axis
##
## -----
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 0.5582 with 1 degrees of freedom
## P-value : 0.455
## Coefficients by group in variable "type"
## Group: CP
##
               elevation
                            slope
## estimate
            -0.44638368 1.811085
## lower limit -0.86747180 1.332310
## upper limit -0.02529556 2.461912
##
## H0 : variables uncorrelated.
## R-squared: 0.6285736
## P-value : 5.157e-05
##
## Group: MP
##
               elevation slope
             -0.2501853 1.518812
## estimate
## lower limit -0.6572024 1.045336
## upper limit 0.1568317 2.206746
## H0 : variables uncorrelated.
## R-squared : 0.5928163
## P-value : 0.00078631
plot(b2.1)
#####testing shift in elevation (grade shift - a)####
```

```
#facet count - male
b3 <- sma(whole count log~tibia length log+type, type="elevation", data=eye2.
m)
summary(b3)
## Call: sma(formula = whole_count_log ~ tibia_length_log + type, data = eye2
.m,
      type = "elevation")
##
##
## Fit using Standardized Major Axis
## -----
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 2.458 with 1 degrees of freedom
## P-value : 0.11696
## -----
##
## H0 : no difference in elevation.
## Wald statistic: 0.1757 with 1 degrees of freedom
## P-value : 0.67507
## Coefficients by group in variable "type"
##
## Group: CP
##
            elevation slope
## estimate 3.326171 1.1480723
## lower limit 3.051381 0.8316018
## upper limit 3.600961 1.5644850
## H0 : variables uncorrelated.
## R-squared : 0.00413478
## P-value : 0.8199
##
## Group: MP
##
            elevation slope
## estimate 3.331892 1.1480723
## lower limit 3.079316 0.8316018
## upper limit 3.584467 1.5644850
## H0 : variables uncorrelated.
## R-squared : 0.5165108
## P-value : 0.00077845
plot(b3)
#facet count - female
b3.1 <- sma(whole count log~tibia length log+type, type="elevation", data=eye
```

```
2.f)
summary(b3.1)
## Call: sma(formula = whole_count_log ~ tibia_length_log + type, data = eye2
.f,
      type = "elevation")
##
##
## Fit using Standardized Major Axis
##
## Results of comparing lines among groups.
##
## H0 : slopes are equal.
## Likelihood ratio statistic : 0.4435 with 1 degrees of freedom
## P-value : 0.50544
## -----
##
## H0 : no difference in elevation.
## Wald statistic: 0.09372 with 1 degrees of freedom
## P-value : 0.7595
## Coefficients by group in variable "type"
## Group: CP
##
              elevation
                            slope
## estimate
              3.238105 1.2253080
## lower limit 2.936997 0.8928909
## upper limit 3.539213 1.6915754
##
## H0 : variables uncorrelated.
## R-squared: 0.2303859
## P-value : 0.037543
##
## Group: MP
##
              elevation
                            slope
## estimate
              3.242166 1.2253080
## lower limit 2.952253 0.8928909
## upper limit 3.532079 1.6915754
##
## H0 : variables uncorrelated.
## R-squared : 0.3199202
## P-value : 0.02798
plot(b3.1)
plot(eye2.f$whole_count_log~eye2.f$tibia_length_log,
    col = eye2.f$type,
    pch = 16)
```

```
#area - male
b4 <- sma(area log~tibia length log+type, type="elevation", data=eye2.m)
summary(b4)
## Call: sma(formula = area_log ~ tibia_length_log + type, data = eye2.m,
##
      type = "elevation")
##
## Fit using Standardized Major Axis
##
## -----
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 7.393 with 1 degrees of freedom
## P-value : 0.0065491
##
## H0 : no difference in elevation.
## Wald statistic: 0.04348 with 1 degrees of freedom
## P-value : 0.83482
## -----
##
## Coefficients by group in variable "type"
##
## Group: CP
                           slope
##
                elevation
## estimate -0.27058881 1.599145
## lower limit -0.55421065 1.209623
## upper limit 0.01303303 2.041624
## H0 : variables uncorrelated.
## R-squared : 0.4645721
## P-value : 0.0051359
##
## Group: MP
##
                elevation
## estimate -0.26789644 1.599145
## lower limit -0.52876075 1.209623
## upper limit -0.00703214 2.041624
##
## H0 : variables uncorrelated.
## R-squared : 0.7633368
## P-value : 2.1756e-06
plot(b4)
#area - female
b4.1 <- sma(area log~tibia length log+type, type="elevation", data=eye2.f)
summary(b4.1)
```

```
## Call: sma(formula = area_log ~ tibia_length_log + type, data = eye2.f,
      type = "elevation")
##
##
## Fit using Standardized Major Axis
##
## ------
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 0.5582 with 1 degrees of freedom
## P-value : 0.455
##
## H0 : no difference in elevation.
## Wald statistic: 1.228 with 1 degrees of freedom
## P-value : 0.26785
## Coefficients by group in variable "type"
##
## Group: CP
##
               elevation
                           slope
## estimate -0.3509322 1.683000
## lower limit -0.6531155 1.330451
## upper limit -0.0487490 2.134163
## H0 : variables uncorrelated.
## R-squared: 0.6285736
## P-value : 5.157e-05
##
## Group: MP
              elevation slope
## estimate -0.3651822 1.683000
## lower limit -0.6562231 1.330451
## upper limit -0.0741414 2.134163
##
## H0 : variables uncorrelated.
## R-squared : 0.5928163
## P-value : 0.00078631
plot(b4.1)
#####testing shift along common axis####
#facet count - male
b5 <- sma(whole_count_log~tibia_length_log+type, type="shift", data=eye2.m)
summary(b5)
```

```
## Call: sma(formula = whole count log ~ tibia length log + type, data = eye2
.m,
      type = "shift")
##
##
## Fit using Standardized Major Axis
## -----
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 2.458 with 1 degrees of freedom
## P-value : 0.11696
## H0 : no shift along common axis.
## Wald statistic: 27.44 with 1 degrees of freedom
## P-value : 1.6192e-07
##
## Coefficients by group in variable "type"
##
## Group: CP
              elevation
##
                            slope
            3.326171 1.1480723
## estimate
## lower limit 3.243936 0.8316018
## upper limit 3.945351 1.5644850
##
## H0 : variables uncorrelated.
## R-squared : 0.00413478
## P-value : 0.8199
##
## Group: MP
## elevation slope
## estimate 3.331892 1.1480723
## lower limit 2.858373 0.8316018
## upper limit 3.545450 1.5644850
##
## H0 : variables uncorrelated.
## R-squared : 0.5165108
## P-value : 0.00077845
plot(b5)
#facet count - female
b5.1 <- sma(whole_count_log~tibia_length_log+type, type="shift", data=eye2.f)
summary(b5.1)
## Call: sma(formula = whole_count_log ~ tibia_length_log + type, data = eye2
.f,
## type = "shift")
```

```
##
## Fit using Standardized Major Axis
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 0.4435 with 1 degrees of freedom
## P-value : 0.50544
## H0 : no shift along common axis.
## Wald statistic: 27.61 with 1 degrees of freedom
## P-value : 1.482e-07
## Coefficients by group in variable "type"
##
## Group: CP
             elevation slope
##
## estimate 3.238105 1.2253080
## lower limit 2.688987 0.8928909
## upper limit 3.595402 1.6915754
## H0 : variables uncorrelated.
## R-squared : 0.2303859
## P-value : 0.037543
##
## Group: MP
             elevation slope
## estimate 3.242166 1.2253080
## lower limit 2.953909 0.8928909
## upper limit 3.712617 1.6915754
## H0 : variables uncorrelated.
## R-squared : 0.3199202
## P-value : 0.02798
plot(b5.1)
#area - male
b6 <- sma(area_log~tibia_length_log+type, type="shift", data=eye2.m)
summary(b6)
## Call: sma(formula = area_log ~ tibia_length_log + type, data = eye2.m,
      type = "shift")
##
## Fit using Standardized Major Axis
##
```

```
## ------
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 7.393 with 1 degrees of freedom
## P-value : 0.0065491
## H0 : no shift along common axis.
## Wald statistic: 27.18 with 1 degrees of freedom
## P-value : 1.8548e-07
##
## Coefficients by group in variable "type"
##
## Group: CP
##
                elevation
                            slope
## estimate
             -0.27058881 1.599145
## lower limit -0.08562769 1.209623
## upper limit 0.52735804 2.041624
##
## H0 : variables uncorrelated.
## R-squared : 0.4645721
## P-value : 0.0051359
##
## Group: MP
               elevation
##
                           slope
            -0.2678964 1.599145
## estimate
## lower limit -0.8042493 1.209623
## upper limit -0.1262944 2.041624
## H0 : variables uncorrelated.
## R-squared: 0.7633368
## P-value : 2.1756e-06
plot(b6)
#area - female
b6.1 <- sma(area_log~tibia_length_log+type, type="shift", data=eye2.f)
summary(b6.1)
## Call: sma(formula = area_log ~ tibia_length_log + type, data = eye2.f,
##
      type = "shift")
## Fit using Standardized Major Axis
##
## Results of comparing lines among groups.
##
## H0 : slopes are equal.
```

```
## Likelihood ratio statistic : 0.5582 with 1 degrees of freedom
## P-value : 0.455
##
## H0 : no shift along common axis.
## Wald statistic: 34.22 with 1 degrees of freedom
## P-value : 4.9142e-09
##
## Coefficients by group in variable "type"
##
## Group: CP
##
                 elevation
                              slope
## estimate -0.35093225 1.683000
## lower limit -0.86747180 1.330451
## upper limit -0.02529556 2.134163
##
## H0 : variables uncorrelated.
## R-squared : 0.6285736
## P-value : 5.157e-05
##
## Group: MP
##
              elevation slope
## estimate -0.3651822 1.683000
## lower limit -0.6572024 1.330451
## upper limit 0.1568317 2.134163
## H0 : variables uncorrelated.
## R-squared : 0.5928163
## P-value : 0.00078631
plot(b6.1)
#####FDR P-VALUE CORRECTION #####
pvalues2 <- c(0.11696,
              0.0065491,
              0.50544,
              0.455,
              0.67507,
              0.7595,
              0.26785,
              1.6192e-07,
              1.482e-07,
              4.9142e-09)
adjusted.p2 <- p.adjust(pvalues2, method = "fdr", n = length(pvalues2))</pre>
adjusted.p2
```

```
## [1] 2.339200e-01 1.637275e-02 6.318000e-01 6.318000e-01 7.500778e-01
## [6] 7.595000e-01 4.464167e-01 5.397333e-07 5.397333e-07 4.914200e-08
#####3) now including F1 hybrids####
eye$inter <- interaction(eye$type, eye$sex)</pre>
#facet count
c1 <- sma(whole_count_log~tibia_length_log*inter, data=eye, multcomp = T, mul</pre>
tcompmethod = "adjust")
multcompmatrix(c1, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
                 inter_2
##
                  CP.female MPxCP.male MP.female CPxMP.male MP.male CP.male
## inter 1
    CP.female
##
    MPxCP.male
##
##
    MP.female
##
    CPxMP.male
##
    MP.male
##
    CP.male
   CPxMP.female
##
    MPxCP.female
##
##
                 inter_2
                 CPxMP.female MPxCP.female
## inter 1
    CP.female
##
    MPxCP.male
##
    MP.female
##
    CPxMP.male
##
##
    MP.male
## CP.male
##
    CPxMP.female -
   MPxCP.female
summary(c1)
## Call: sma(formula = whole_count_log ~ tibia_length_log * inter, data = eye
       multcomp = T, multcompmethod = "adjust")
##
## Fit using Standardized Major Axis
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 7.464 with 7 degrees of freedom
## P-value : 0.3822
##
```

```
## Results of multiple comparisons among groups.
##
## Test for pair-wise difference in slope :
                         inter 2
##
           inter 1
                                    Pval
                                             TestStat
## 1
         CP.female CPxMP.female 1.00000 0.0552550141
## 2
         CP.female
                      MP.female 1.00000 0.4434903863
## 3
         CP.female MPxCP.female 0.94757 2.7064376571
## 4
         CP.female
                         CP.male 0.97990 2.2896319310
## 5
         CP.female
                     CPxMP.male 1.00000 0.0006193157
## 6
         CP.female
                        MP.male 1.00000 0.0027806498
## 7
         CP.female
                     MPxCP.male 0.99960 1.3585339811
      CPxMP.female
                      MP.female 1.00000 0.6865808043
## 8
## 9
      CPxMP.female MPxCP.female 0.91895 2.9509245933
## 10 CPxMP.female
                         CP.male 0.96172 2.5542830939
## 11 CPxMP.female
                     CPxMP.male 1.00000 0.0665304562
## 12 CPxMP.female
                        MP.male 1.00000 0.0893557790
## 13 CPxMP.female
                     MPxCP.male 0.99812 1.6362292468
## 14
         MP.female MPxCP.female 0.99997 1.0257654093
## 15
         MP.female
                         CP.male 1.00000 0.8073642614
## 16
         MP.female
                     CPxMP.male 1.00000 0.4144393854
## 17
         MP.female
                        MP.male 1.00000 0.4528463996
## 18
         MP.female
                     MPxCP.male 1.00000 0.2917443153
## 19 MPxCP.female
                        CP.male 1.00000 0.0072575172
## 20 MPxCP.female
                     CPxMP.male 0.95418 2.6390570492
## 21 MPxCP.female
                        MP.male 0.92155 2.9310307543
## 22 MPxCP.female
                     MPxCP.male 1.00000 0.1987820145
## 23
                     CPxMP.male 0.98295 2.2302342983
           CP.male
## 24
                        MP.male 0.96927 2.4576282480
           CP.male
## 25
           CP.male
                     MPxCP.male 1.00000 0.1232918867
## 26
        CPxMP.male
                        MP.male 1.00000 0.0006597410
        CPxMP.male
                     MPxCP.male 0.99971 1.3122193965
## 27
## 28
           MP.male
                     MPxCP.male 0.99932 1.4444363378
##
## Coefficients by group in variable "inter"
##
## Group: CP.female
##
               elevation
                              slope
## estimate
                3.142194 1.3540100
## lower limit
                2.688987 0.8763565
## upper limit
               3.595402 2.0920060
##
## H0 : variables uncorrelated.
## R-squared: 0.2303859
## P-value : 0.037543
##
## Group: CPxMP.female
               elevation
                              slope
## estimate
                3.042379 1.4666891
## lower limit 2.440755 0.8623369
```

```
## upper limit 3.644004 2.4945900
##
## H0 : variables uncorrelated.
## R-squared: 0.1375647
## P-value : 0.17351
##
## Group: MP.female
##
               elevation
                             slope
## estimate
                3.333263 1.0952430
## lower limit 2.953909 0.6804684
## upper limit 3.712617 1.7628405
##
## H0 : variables uncorrelated.
## R-squared : 0.3199202
## P-value : 0.02798
## Group: MPxCP.female
##
               elevation
                             slope
## estimate
                3.577457 0.7615128
## lower limit 3.263320 0.4413273
## upper limit 3.891595 1.3139949
## H0 : variables uncorrelated.
## R-squared: 0.08552689
## P-value : 0.29017
##
## Group: CP.male
##
               elevation
                             slope
## estimate
                3.594643 0.7873019
## lower limit 3.243936 0.4465518
## upper limit 3.945351 1.3880679
## H0 : variables uncorrelated.
## R-squared : 0.00413478
## P-value : 0.8199
##
## Group: CPxMP.male
##
               elevation
                             slope
                3.160335 1.3437926
## estimate
## lower limit 2.708533 0.8652938
## upper limit 3.612138 2.0868965
##
## H0 : variables uncorrelated.
## R-squared: 0.4245295
## P-value : 0.0084988
##
## Group: MP.male
               elevation
                             slope
## estimate
                3.201911 1.3342359
## lower limit 2.858373 0.9302675
```

```
## upper limit 3.545450 1.9136273
##
## H0 : variables uncorrelated.
## R-squared : 0.5165108
## P-value : 0.00077845
##
## Group: MPxCP.male
               elevation
                             slope
## estimate
               3.481762 0.9030686
## lower limit 3.102523 0.5239273
## upper limit 3.861001 1.5565765
##
## H0 : variables uncorrelated.
## R-squared : 0.08947516
## P-value : 0.27879
plot(c1)
eye.m <- eye[which(eye$sex=="male"),]
c1m <- sma(whole_count_log~tibia_length_log*inter, data=eye.m, multcomp = T,</pre>
multcompmethod = "adjust")
multcompmatrix(c1m, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
               inter 2
## inter 1
                CP.male CPxMP.male MPxCP.male MP.male
##
    CP.male
    CPxMP.male
##
    MPxCP.male
##
    MP.male
##
summary(c1m)
## Call: sma(formula = whole_count_log ~ tibia_length_log * inter, data = eye
.m,
       multcomp = T, multcompmethod = "adjust")
##
## Fit using Standardized Major Axis
##
## Results of comparing lines among groups.
##
## H0 : slopes are equal.
## Likelihood ratio statistic : 3.77 with 3 degrees of freedom
## P-value : 0.28744
##
## Results of multiple comparisons among groups.
```

```
##
## Test for pair-wise difference in slope :
        inter 1
                  inter 2
                             Pval
##
                                     TestStat
## 1
        CP.male CPxMP.male 0.58208 2.230234298
## 2
       CP.male
                  MP.male 0.52587 2.457628248
## 3
        CP.male MPxCP.male 0.99957 0.123291887
## 4 CPxMP.male
                  MP.male 1.00000 0.000659741
## 5 CPxMP.male MPxCP.male 0.82484 1.312219397
       MP.male MPxCP.male 0.79064 1.444436338
##
## ------
## Coefficients by group in variable "inter"
##
## Group: CP.male
##
              elevation
                            slope
## estimate
               3.594643 0.7873019
## lower limit 3.243936 0.4465518
## upper limit 3.945351 1.3880679
##
## H0 : variables uncorrelated.
## R-squared : 0.00413478
## P-value : 0.8199
##
## Group: CPxMP.male
##
              elevation
                            slope
## estimate
               3.160335 1.3437926
## lower limit 2.708533 0.8652938
## upper limit 3.612138 2.0868965
##
## H0 : variables uncorrelated.
## R-squared : 0.4245295
## P-value : 0.0084988
##
## Group: MP.male
##
              elevation
                            slope
## estimate
               3.201911 1.3342359
## lower limit 2.858373 0.9302675
## upper limit 3.545450 1.9136273
## H0 : variables uncorrelated.
## R-squared : 0.5165108
## P-value : 0.00077845
##
## Group: MPxCP.male
              elevation
                            slope
               3.481762 0.9030686
## estimate
## lower limit 3.102523 0.5239273
## upper limit 3.861001 1.5565765
##
## H0 : variables uncorrelated.
```

```
## R-squared : 0.08947516
## P-value : 0.27879
plot(c1m)
eye.f <- eye[which(eye$sex=="female"),]</pre>
c1f <- sma(whole_count_log~tibia_length_log*inter, data=eye.f, multcomp = T,</pre>
multcompmethod = "adjust")
multcompmatrix(c1f, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
                 inter 2
                  CP.female MPxCP.female CPxMP.female MP.female
## inter 1
##
    CP.female
   MPxCP.female
##
##
    CPxMP.female
    MP.female
##
summary(c1f)
## Call: sma(formula = whole_count_log ~ tibia_length_log * inter, data = eye
.f,
       multcomp = T, multcompmethod = "adjust")
##
## Fit using Standardized Major Axis
## Results of comparing lines among groups.
##
## H0 : slopes are equal.
## Likelihood ratio statistic : 3.692 with 3 degrees of freedom
## P-value : 0.29675
##
## Results of multiple comparisons among groups.
## Test for pair-wise difference in slope :
##
          inter 1
                       inter 2
                                  Pval
                                         TestStat
## 1
        CP.female CPxMP.female 0.99996 0.05525501
                    MP.female 0.98537 0.44349039
## 2
        CP.female
        CP.female MPxCP.female 0.46836 2.70643766
## 4 CPxMP.female
                     MP.female 0.95666 0.68658080
## 5 CPxMP.female MPxCP.female 0.41633 2.95092459
        MP.female MPxCP.female 0.89316 1.02576541
##
## Coefficients by group in variable "inter"
##
```

```
## Group: CP.female
##
               elevation
                             slope
                3.142194 1.3540100
## estimate
## lower limit 2.688987 0.8763565
## upper limit 3.595402 2.0920060
## H0: variables uncorrelated.
## R-squared : 0.2303859
## P-value : 0.037543
##
## Group: CPxMP.female
##
              elevation
                             slope
## estimate
                3.042379 1.4666891
## lower limit 2.440755 0.8623369
## upper limit 3.644004 2.4945900
## H0 : variables uncorrelated.
## R-squared : 0.1375647
## P-value : 0.17351
##
## Group: MP.female
               elevation
                             slope
## estimate
                3.333263 1.0952430
## lower limit 2.953909 0.6804684
## upper limit 3.712617 1.7628405
##
## H0 : variables uncorrelated.
## R-squared : 0.3199202
## P-value : 0.02798
##
## Group: MPxCP.female
               elevation
## estimate
                3.577457 0.7615128
## lower limit 3.263320 0.4413273
## upper limit 3.891595 1.3139949
##
## H0 : variables uncorrelated.
## R-squared: 0.08552689
## P-value : 0.29017
plot(c1f)
#######**plot facet count major axis regressions: C, M, F1**#####
dev.off()
## null device
##
             1
```

```
par(mgp=c(3.5,0.6,0))
par(mar = c(5, 6, 2, 1.25))
par(lwd=1)
eye$inter<- factor(eye$inter, c("CP.male", "CP.female",</pre>
                                 "CPxMP.male", "CPxMP.female",
                                 "MPxCP.male", "MPxCP.female",
                                 "MP.male", "MP.female"))
#males
plot(c1, col=c("#1F78B4","#1F78B4", "#FF7F00", "#FF7F00", "grey40","grey40",
"#FDBF6F", "#FDBF6F"),
     #pch=c(17,2, 15,0, 18,5, 19,1),
     pch="",
     1wd=3,
     cex=0.9,
     lty=c(1,0,1,0,1,0,1,0),
     xlab = "",
    ylab = "log10 (facet count)",
    cex.axis = 1.65,cex.lab = 1.7,font.lab = 2, las=1,
    xlim=c(0.635,0.80), ylim=c(4.03,4.25),
    family = "Times New Roman", tck=-0.015)
legend("topleft", c("C", "CxM", "MxC", "M"),
       col = c("#1F78B4","#FF7F00", "grey40", "#FDBF6F"), lty= 1, lwd=3,
       bty="n")
plot(c1m, col=c("#1F78B4", "#FF7F00", "grey40", "#FDBF6F"),
     #pch=c(17,15,18,19),
     pch=19.
     1wd=3,
     cex=0.25,
     lty=c(1,1,1,1),
    xlim=c(0.635,0.80), ylim=c(4.03,4.25),
     las=1, cex.axis = 1.3,
    xlab = "",
    ylab = "",
    tck = -0.015,
    family = "Times New Roman")
title(xlab = "log10 (hind tibia length)", line = 2,cex.lab = 1.2,font.lab=2,f
amily = "Times New Roman")
title(ylab = "log10 (facet count)", line = 3,cex.lab = 1.2,font.lab=2,family
= "Times New Roman")
title("males", adj = 0.5, line = 0.6, family = "Times New Roman", cex.main=1.
35)
legend("topleft", c("C", "CxM", "MxC", "M"),
       col = c("#1F78B4","#FF7F00", "grey40", "#FDBF6F"), lty= 1, lwd=3,
```

```
bty="n")
#females
plot(c1, col=c("#1F78B4","#1F78B4", "#FF7F00", "#FF7F00", "grey40","grey40",
"#FDBF6F", "#FDBF6F"),
     \#pch=c(17,2, 15,0, 18,5, 19,1),
     pch="",
     1wd=3,
     cex=0.9,
     lty=c(0,1,0,1,0,1,0,1),
     xlab = "",
    ylab = "log10 (facet count)",
     cex.axis = 1.65,cex.lab = 1.7,font.lab = 2, las=1,
     xlim=c(0.635,0.80), ylim=c(4.03,4.25),
    family = "Times New Roman", tck=-0.015)
legend("topleft", c("C", "CxM", "MxC", "M"),
       col = c("#1F78B4","#FF7F00", "grey40", "#FDBF6F"), lty= 1, lwd=3,
       bty="n")
plot(c1f, col=c("#1F78B4", "#FF7F00", "grey40", "#FDBF6F"),
     #pch=c(17,15,18,19),
     pch=19,
     1wd=3,
     cex=0.25,
     lty=c(1,1,1,1),
    xlim=c(0.635,0.80), ylim=c(4.03,4.25),
    las=1,cex.axis = 1.3,
    xlab = "",
    ylab = "",
    tck = -0.015,
    family = "Times New Roman")
title(xlab = "log10 (hind tibia length)", line = 2,cex.lab = 1.2,font.lab=2,f
amily = "Times New Roman")
title(ylab = "log10 (facet count)", line = 3,cex.lab = 1.2,font.lab=2,family
= "Times New Roman")
title("females", adj = 0.5, line = 0.6, family = "Times New Roman", cex.main=
1.35)
legend("topleft", c("C", "CxM", "MxC", "M"),
       col = c("#1F78B4","#FF7F00", "grey40", "#FDBF6F"), lty= 1, lwd=3,
       bty="n")
#area
d1 <- sma(area log~tibia length log*inter, data=eye, multcomp = T, multcompme
```

```
thod = "adjust")
multcompmatrix(d1, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
                 inter 2
                  CP.male CPxMP.male MP.female MP.male CP.female CPxMP.female
## inter 1
     CP.male
##
##
    CPxMP.male
##
    MP.female
##
    MP.male
##
    CP.female
    CPxMP.female
##
    MPxCP.female
##
    MPxCP.male
##
                 inter 2
##
                  MPxCP.female MPxCP.male
## inter 1
##
    CP.male
    CPxMP.male
##
##
    MP.female
##
    MP.male
    CP.female
##
    CPxMP.female
##
    MPxCP.female -
##
##
    MPxCP.male
summary(d1)
## Call: sma(formula = area log ~ tibia length log * inter, data = eye,
       multcomp = T, multcompmethod = "adjust")
##
## Fit using Standardized Major Axis
##
## Results of comparing lines among groups.
##
## H0 : slopes are equal.
## Likelihood ratio statistic : 11.56 with 7 degrees of freedom
## P-value : 0.11589
##
## Results of multiple comparisons among groups.
## Test for pair-wise difference in slope :
##
           inter 1
                        inter 2
                                   Pval
                                            TestStat
## 1
           CP.male
                      CP.female 0.32038 6.076552e+00
## 2
           CP.male
                     CPxMP.male 0.11628 8.108833e+00
           CP.male CPxMP.female 0.56824 4.735240e+00
## 3
## 4
          CP.male MPxCP.male 0.99969 1.319598e+00
```

```
## 5
          CP.male MPxCP.female 0.94996 2.682725e+00
## 6
          CP.male
                        MP.male 0.16804 7.392662e+00
## 7
          CP.male
                      MP.female 0.91925 2.948582e+00
## 8
         CP.female
                     CPxMP.male 1.00000 5.589360e-01
         CP.female CPxMP.female 1.00000 1.755995e-02
## 9
## 10
         CP.female
                     MPxCP.male 0.99908 1.498170e+00
## 11
        CP.female MPxCP.female 1.00000 3.952514e-01
        CP.female
## 12
                        MP.male 1.00000 3.885275e-02
## 13
        CP.female
                      MP.female 1.00000 5.581682e-01
## 14
        CPxMP.male CPxMP.female 1.00000 2.315879e-01
## 15
        CPxMP.male
                     MPxCP.male 0.90691 3.038767e+00
## 16
       CPxMP.male MPxCP.female 0.99930 1.451255e+00
## 17
       CPxMP.male
                        MP.male 1.00000 3.961990e-01
## 18
       CPxMP.male
                      MP.female 0.99557 1.831142e+00
## 19 CPxMP.female
                     MPxCP.male 0.99977 1.276150e+00
## 20 CPxMP.female MPxCP.female 1.00000 4.061232e-01
## 21 CPxMP.female
                        MP.male 1.00000 5.021207e-05
## 22 CPxMP.female
                      MP.female 1.00000 5.225954e-01
        MPxCP.male MPxCP.female 1.00000 2.662393e-01
## 23
                        MP.male 0.98889 2.088041e+00
## 24
       MPxCP.male
## 25
       MPxCP.male
                     MP.female 1.00000 2.617518e-01
## 26 MPxCP.female
                        MP.male 1.00000 6.708751e-01
## 27 MPxCP.female
                     MP.female 1.00000 1.963364e-03
## 28
          MP.male
                     MP.female 0.99999 9.455849e-01
##
## -----
## Coefficients by group in variable "inter"
##
## Group: CP.male
##
                 elevation
                               slope
## estimate
               0.22086518 0.9387334
## lower limit -0.08562769 0.6134198
## upper limit 0.52735804 1.4365697
##
## H0 : variables uncorrelated.
## R-squared : 0.4645721
## P-value : 0.0051359
##
## Group: CP.female
                 elevation
                              slope
## estimate
               -0.44638368 1.811085
## lower limit -0.86747180 1.332310
## upper limit -0.02529556 2.461912
##
## H0 : variables uncorrelated.
## R-squared : 0.6285736
## P-value : 5.157e-05
##
## Group: CPxMP.male
                elevation
                              slope
```

```
## estimate -0.71608306 2.166657
## lower limit -1.34718526 1.475379
## upper limit -0.08498085 3.181827
##
## H0 : variables uncorrelated.
## R-squared: 0.5680501
## P-value : 0.0011742
## Group: CPxMP.female
##
                elevation
                             slope
## estimate
               -0.5253307 1.878349
## lower limit -1.2020595 1.172645
## upper limit 0.1513980 3.008748
## H0 : variables uncorrelated.
## R-squared : 0.3346212
## P-value : 0.023879
##
## Group: MPxCP.male
##
                 elevation
                              slope
## estimate
               -0.08807595 1.318846
## lower limit -0.51496747 0.859966
## upper limit 0.33881557 2.022585
##
## H0 : variables uncorrelated.
## R-squared : 0.4588542
## P-value : 0.0055297
##
## Group: MPxCP.female
                elevation
                              slope
## estimate
               -0.2677770 1.5376629
## lower limit -0.7647541 0.9951569
## upper limit 0.2292001 2.3759139
##
## H0 : variables uncorrelated.
## R-squared : 0.4385127
## P-value : 0.0071532
##
## Group: MP.male
                elevation
                             slope
## estimate
               -0.4652718 1.881835
## lower limit -0.8042493 1.458194
## upper limit -0.1262944 2.428555
##
## H0 : variables uncorrelated.
## R-squared : 0.7633368
## P-value : 2.1756e-06
##
## Group: MP.female
                elevation
                             slope
```

```
## estimate -0.2501853 1.518812
## lower limit -0.6572024 1.045336
## upper limit 0.1568317 2.206746
##
## H0 : variables uncorrelated.
## R-squared: 0.5928163
## P-value : 0.00078631
plot(d1)
eye.m <- eye[which(eye$sex=="male"),]
d1m <- sma(area_log~tibia_length_log*inter, data=eye.m, multcomp = T, multcom</pre>
pmethod = "adjust")
multcompmatrix(d1m, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
              inter 2
## inter 1
               CP.male CPxMP.male MP.male MPxCP.male
               - *
    CP.male
    CPxMP.male *
##
##
    MP.male
##
    MPxCP.male
summary(d1m)
## Call: sma(formula = area_log ~ tibia_length_log * inter, data = eye.m,
      multcomp = T, multcompmethod = "adjust")
##
##
## Fit using Standardized Major Axis
## -----
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 10.58 with 3 degrees of freedom
## P-value : 0.0142
##
## Results of multiple comparisons among groups.
## Test for pair-wise difference in slope :
##
       inter 1
                  inter 2 Pval TestStat
       CP.male CPxMP.male 0.026141 8.108833
## 1
       CP.male MPxCP.male 0.822965 1.319598
## 2
       CP.male MP.male 0.038657 7.392662
## 4 CPxMP.male MPxCP.male 0.398758 3.038767
## 5 CPxMP.male MP.male 0.989091 0.396199
## 6 MPxCP.male MP.male 0.618722 2.088041
```

```
##
## Coefficients by group in variable "inter"
## Group: CP.male
##
                 elevation
                                slope
## estimate
                0.22086518 0.9387334
## lower limit -0.08562769 0.6134198
## upper limit 0.52735804 1.4365697
##
## H0 : variables uncorrelated.
## R-squared : 0.4645721
## P-value : 0.0051359
##
## Group: CPxMP.male
                 elevation
## estimate
               -0.71608306 2.166657
## lower limit -1.34718526 1.475379
## upper limit -0.08498085 3.181827
##
## H0 : variables uncorrelated.
## R-squared : 0.5680501
## P-value : 0.0011742
##
## Group: MPxCP.male
##
                 elevation
                               slope
## estimate
               -0.08807595 1.318846
## lower limit -0.51496747 0.859966
## upper limit 0.33881557 2.022585
##
## H0 : variables uncorrelated.
## R-squared: 0.4588542
## P-value : 0.0055297
##
## Group: MP.male
##
                elevation
                              slope
## estimate
               -0.4652718 1.881835
## lower limit -0.8042493 1.458194
## upper limit -0.1262944 2.428555
## H0 : variables uncorrelated.
## R-squared: 0.7633368
## P-value : 2.1756e-06
plot(d1m)
eye.f <- eye[which(eye$sex=="female"),]</pre>
d1f <- sma(area_log~tibia_length_log*inter, data=eye.f, multcomp = T, multcom</pre>
```

```
pmethod = "adjust")
multcompmatrix(d1f, sort=T)
## Multiple comparison matrix.
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
                inter 2
## inter 1
                 CP.female MP.female CPxMP.female MPxCP.female
    CP.female
##
##
    MP.female
##
   CPxMP.female
##
    MPxCP.female
summary(d1f)
## Call: sma(formula = area_log ~ tibia_length_log * inter, data = eye.f,
      multcomp = T, multcompmethod = "adjust")
##
##
## Fit using Standardized Major Axis
## Results of comparing lines among groups.
## H0 : slopes are equal.
## Likelihood ratio statistic : 0.9642 with 3 degrees of freedom
## P-value : 0.80991
##
## Results of multiple comparisons among groups.
## Test for pair-wise difference in slope :
##
         inter 1 inter 2
                                Pval
                                        TestStat
       CP.female CPxMP.female 1.00000 0.017559948
## 1
       CP.female MPxCP.female 0.98916 0.395251399
                    MP.female 0.97380 0.558168187
       CP.female
## 4 CPxMP.female MPxCP.female 0.98836 0.406123187
## 5 CPxMP.female MP.female 0.97777 0.522595433
## 6 MPxCP.female MP.female 1.00000 0.001963364
##
## ------
## Coefficients by group in variable "inter"
##
## Group: CP.female
                elevation
                           slope
## estimate -0.44638368 1.811085
## lower limit -0.86747180 1.332310
## upper limit -0.02529556 2.461912
##
## H0 : variables uncorrelated.
## R-squared : 0.6285736
```

```
## P-value : 5.157e-05
##
## Group: CPxMP.female
                elevation
                             slope
               -0.5253307 1.878349
## estimate
## lower limit -1.2020595 1.172645
## upper limit 0.1513980 3.008748
## H0 : variables uncorrelated.
## R-squared : 0.3346212
## P-value : 0.023879
##
## Group: MPxCP.female
                elevation
                              slope
## estimate
               -0.2677770 1.5376629
## lower limit -0.7647541 0.9951569
## upper limit 0.2292001 2.3759139
##
## H0 : variables uncorrelated.
## R-squared : 0.4385127
## P-value : 0.0071532
##
## Group: MP.female
##
                elevation
                             slope
## estimate
               -0.2501853 1.518812
## lower limit -0.6572024 1.045336
## upper limit 0.1568317 2.206746
##
## H0 : variables uncorrelated.
## R-squared: 0.5928163
## P-value : 0.00078631
plot(d1f)
#######**plot corneal area major axis regressions: C, M, F1**#####
dev.off()
## null device
##
             1
par(mgp=c(3.5,0.6,0))
par(mar = c(5, 6, 2, 1.25))
par(lwd=1)
eye$inter<- factor(eye$inter, c("CP.male", "CP.female",</pre>
                                 "CPxMP.male", "CPxMP.female",
                                "MPxCP.male", "MPxCP.female",
                                 "MP.male", "MP.female"))
```

```
#males
plot(d1m, col=c("#1F78B4", "#FF7F00", "grey40", "#FDBF6F"),
     #pch=c(17,15,18,19),
     pch=19,
     1wd=3,
     cex=0.25,
     lty=c(1,1,1,1),
     xlim=c(0.635,0.80), ylim=c(0.73,1.00),
     las=1,cex.axis = 1.3,
     xlab = "",
     ylab = "".
     tck=-0.015,
     family = "Times New Roman")
title(xlab = "log10 (hind tibia length)", line = 2,cex.lab = 1.2,font.lab=2,f
amily = "Times New Roman")
title(ylab = "log10 (corneal area)", line = 3,cex.lab = 1.2,font.lab=2,family
= "Times New Roman")
title("males", adj = 0.5, line = 0.6, family = "Times New Roman", cex.main=1.
35)
legend("topleft", c("C", "CxM", "MxC", "M"),
       col = c("#1F78B4","#FF7F00", "grey40", "#FDBF6F"), lty= 1, lwd=3,
       bty="n")
#females
plot(d1f, col=c("#1F78B4", "#FF7F00", "grey40", "#FDBF6F"),
     #pch=c(17,15,18,19),
     pch=19,
     1wd=3,
     cex=0.25,
     lty=c(1,1,1,1),
     xlim=c(0.635,0.80), ylim=c(0.73,1.00),
     las=1,cex.axis = 1.3,
     xlab = "",
    ylab = "",
     tck = -0.015,
     family = "Times New Roman")
title(xlab = "log10 (hind tibia length)", line = 2,cex.lab = 1.2,font.lab=2,f
amily = "Times New Roman")
title(ylab = "log10 (corneal area)", line = 3,cex.lab = 1.2,font.lab=2,family
= "Times New Roman")
title("females", adj = 0.5, line = 0.6, family = "Times New Roman", cex.main=
1.35)
```