```
#Below is the code for all statistical procedures used in "Protein stores regulate when reproductive displays begin in the male Caribbean fruit fly"

library("lme4", lib.loc="~/R/win-library/3.5")

library("MASS", lib.loc="C:/Program Files/R/R-3.5.0/library")

library("ggplot2", lib.loc="~/R/win-library/3.5")

library("mosaicData", lib.loc="~/R/win-library/3.5")

library("mosaic", lib.loc="~/R/win-library/3.5")

library(lmerTest)


#set working directory

setwd("Q:/ClancyAShort/Generating-A.suspensa")


#Tests appear in the order that they appear in the manuscript

dataraw1<-read.csv("ComboDat9_9_19.csv")

dataraw<-subset(dataraw1, blockNum!="na", select = Age:std.tub)

dataraw$Age<-as.numeric(dataraw$Age)

feddata<-subset(dataraw, Age>0, select = Age:std.tub)

totprot_lm <- lmer(feddata$tot.prot ~ feddata$Diet+(1|feddata$Cohort))

summary(totprot_lm)#reduced model, feeding verified.

#subset for t tests t

day0<-subset(dataraw, Age==0,select = Age:std.tub)

day1f<-subset(feddata, Age==1,select = Age:std.tub)

day1P<-subset(day1f, Diet=='Prot',select = Age:std.tub)

day1S<-subset(day1f, Diet=='No Prot',select = Age:std.tub)

day2f<-subset(feddata, Age==2,select = Age:std.tub)

day2P<-subset(day2f, Diet=='Prot',select = Age:std.tub)

day2S<-subset(day2f, Diet=='No Prot',select = Age:std.tub)

day3f<-subset(feddata, Age==3,select = Age:std.tub)

day3P<-subset(day3f, Diet=='Prot',select = Age:std.tub)
```

```
day3S<-subset(day3f, Diet=='No Prot',select = Age:std.tub)
day4f<-subset(feddata, Age==4,select = Age:std.tub)
day4P<-subset(day4f, Diet=='Prot',select = Age:std.tub)
day4S<-subset(day4f, Diet=='No Prot',select = Age:std.tub)
day5f<-subset(feddata, Age==5,select = Age:std.tub)
day5P<-subset(day5f, Diet=='Prot',select = Age:std.tub)
day5S<-subset(day5f, Diet=='No Prot',select = Age:std.tub)
day7f<-subset(feddata, Age==7,select = Age:std.tub)
day7P<-subset(day7f, Diet=='Prot',select = Age:std.tub)
day7S<-subset(day7f, Diet=='No Prot',select = Age:std.tub)
day9f<-subset(feddata, Age==9,select = Age:std.tub)
day9P<-subset(day9f, Diet=='Prot',select = Age:std.tub)
day9S<-subset(day9f, Diet=='No Prot',select = Age:std.tub)

#posthoc
t.test(x=day1P$tot.prot,y=day1S$tot.prot,var.equal=F)
t.test(x=day2P$tot.prot,y=day2S$tot.prot,var.equal=F)
t.test(x=day3P$tot.prot,y=day3S$tot.prot,var.equal=F)
t.test(x=day4P$tot.prot,y=day4S$tot.prot,var.equal=F)
t.test(x=day5P$tot.prot,y=day5S$tot.prot,var.equal=F)
t.test(x=day7P$tot.prot,y=day7S$tot.prot,var.equal=F)
t.test(x=day9P$tot.prot,y=day9S$tot.prot,var.equal=F)
prot.ps<-c(0.7804,0.06387,0.3552,0.3688,0.01101,0.8209,0.0897)
p.adjust(prot.ps,method="fdr")

#Methods: Quantification of lsp-2 transcript abundance (examining lsp-2 transcript abundance in head, legs, and abdomen)
data.HLA<-read.csv("HLA data.csv")
HLA_lm <- lm(data.HLA$Transcript ~ data.HLA$Diet*data.HLA$Body)
```

```
summary(HLA_lm)
anova(HLA_lm)
TukeyHSD(HLA_lm)
if (require(mosaicData)) {
  model <- lm(data.HLA$Transcript ~ data.HLA$Diet*data.HLA$Body)
  TukeyHSD(model)
}

#Methods: Quantification of lsp-2 transcript abundance (housekeeping gene verification)
dataraw1<-read.csv("ComboDat9_9_19.csv")
dataraw<-subset(dataraw1, blockNum!="na", select = Age:std.tub)
dataraw$Age<-as.numeric(dataraw$Age)
#Need to remove day 0, includes no feeding
feddata<-subset(dataraw, Age>0, select = Age:std.tub)
feddata$Age<-as.numeric(feddata$Age)
RP18_lm1 <- lmer(2^-feddata$RP18 ~ feddata$Age*feddata$Diet+(1|feddata$Cohort))
summary(RP18_lm1)#Full model
RP18_lm2 <- lmer(2^-feddata$RP18 ~ feddata$Age+feddata$Diet+(1|feddata$Cohort))
summary(RP18_lm2)
RP18_lm3 <- lmer(2^-feddata$RP18 ~ feddata$Diet+(1|feddata$Cohort))
summary(RP18_lm3)#housekeeping gene verified

#Methods:Quantification of LSP-2 protein abundance (tubulin verification)
hist(sqrt(feddata$std.tub))
Tub_lm1 <- lmer(sqrt(feddata$std.tub) ~ feddata$Age*feddata$Diet+(1|feddata$Cohort))
summary(Tub_lm1)#Full model
#post hoc
#Tubulin test (Methods)
t.test(x=day1P$std.tub,y=day1S$std.tub,var.equal=F)
```

```
t.test(x=day2P$std.tub,y=day2S$std.tub,var.equal=F)

t.test(x=day3P$std.tub,y=day3S$std.tub,var.equal=F)

t.test(x=day4P$std.tub,y=day4S$std.tub,var.equal=F)

t.test(x=day5P$std.tub,y=day5S$std.tub,var.equal=F)

t.test(x=day7P$std.tub,y=day7S$std.tub,var.equal=F)

t.test(x=day9P$std.tub,y=day9S$std.tub,var.equal=F)

Tub.ps<-c(0.406,0.219,0.111,0.276,0.835,0.0644,0.0460)

p.adjust(Tub.ps,method="fdr")


#Methods:RNAi knockdown of lsp-2(feeding verification)

feed.ver.data<-read.csv("9_16feedtest.csv")

feed.lmer.1<-
lmer(feed.ver.data$TotProt~feed.ver.data$treatment+feed.ver.data$calling+(1|feed.ver.data$cohort))

summary(feed.lmer.1)

extractAIC(feed.lmer.1)

feed.lmer.2<-lmer(feed.ver.data$TotProt~feed.ver.data$treatment+(1|feed.ver.data$cohort))

summary(feed.lmer.2)

extractAIC(feed.lmer.2)#feeding verified


#Results:Age and Diet Explain Male Display Behavior

data.calling<-read.csv("Calling data (3-12-19 cleanup).csv")

data.calling$Age<-as.numeric(data.calling$Age)

call1_glm <- glmer(data.calling$Calling. ~

                  data.calling$Age*data.calling$diet

                 +(1|data.calling$cohort),  family = "binomial")

summary(call1_glm)

extractAIC(call1_glm)

#posthoc analysis

day3<-subset(data.calling, Age==3, select = Age:Calling.)
```

```
summary(day3)

chisq.test(day3$Calling,day3$diet)

day4<-subset(data.calling, Age==4, select = Age:Calling.)

summary(day4)

chisq.test(day4$Calling,day4$diet)

day5<-subset(data.calling, Age==5, select = Age:Calling.)

summary(day5)

chisq.test(day5$Calling,day5$diet)

day7<-subset(data.calling, Age==7, select = Age:Calling.)

summary(day7)

chisq.test(day7$Calling,day7$diet)

day9<-subset(data.calling, Age==9, select = Age:Calling.)

summary(day9)

chisq.test(day9$Calling,day9$diet)

call.ps<-c(0.4315,0.003658,0.01459,0.007308,0.2638)

p.adjust(call.ps,method="fdr")


#Results:Age and Diet Explain lsp-2 Transcript and LSP-2 Protein Abundance


#transcript abundance

lsp2_lm.3 <- lmer(feddata$Corrected.biorad.Cq ~
feddata$Age*feddata$Diet*feddata$tot.prot+(1|feddata$Cohort))

summary(lsp2_lm.3)

extractAIC(lsp2_lm.3)#full model

anova(lsp2_lm.3)

lsp2_lm.2 <- lmer(feddata$Corrected.biorad.Cq ~
feddata$Age*feddata$Diet+feddata$tot.prot+feddata$Diet:feddata$tot.prot+(1|feddata$Cohort))

summary(lsp2_lm.2)

extractAIC(lsp2_lm.2)
```

```
anova(lsp2_lm.2)

lsp2_lm.1 <- lmer(feddata$Corrected.biorad.Cq ~
feddata$Age*feddata$Diet+feddata$tot.prot+(1|feddata$Cohort))

summary(lsp2_lm.1)

extractAIC(lsp2_lm.1)

anova(lsp2_lm.1)

lsp2_lm <- lmer(feddata$Corrected.biorad.Cq ~ feddata$Age*feddata$Diet+(1|feddata$Cohort))

summary(lsp2_lm)

extractAIC(lsp2_lm)

anova(lsp2_lm)#reduced model


#transcript post hoc, and stdev

mean(day0$Corrected.biorad.Cq)

sd(day0$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day0$Corrected.biorad.Cq))

t.test(x=day1P$Corrected.biorad.Cq,y=day1S$Corrected.biorad.Cq,var.equal=F)

sd(day1P$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day1P$Corrected.biorad.Cq))

sd(day1S$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day1S$Corrected.biorad.Cq))

t.test(x=day2P$Corrected.biorad.Cq,y=day2S$Corrected.biorad.Cq,var.equal=F)

sd(day2P$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day2P$Corrected.biorad.Cq))

sd(day2S$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day2S$Corrected.biorad.Cq))

t.test(x=day3P$Corrected.biorad.Cq,y=day3S$Corrected.biorad.Cq,var.equal=F)

sd(day3P$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day3P$Corrected.biorad.Cq))

sd(day3S$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day3S$Corrected.biorad.Cq))

t.test(x=day4P$Corrected.biorad.Cq,y=day4S$Corrected.biorad.Cq,var.equal=F)

sd(day4P$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day4P$Corrected.biorad.Cq))

sd(day4S$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day4S$Corrected.biorad.Cq))

t.test(x=day5P$Corrected.biorad.Cq,y=day5S$Corrected.biorad.Cq,var.equal=F)

sd(day5P$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day5P$Corrected.biorad.Cq))

sd(day5S$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day5S$Corrected.biorad.Cq))
```

```
t.test(x=day7P$Corrected.biorad.Cq,y=day7S$Corrected.biorad.Cq,var.equal=F)

sd(day7P$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day7P$Corrected.biorad.Cq))

sd(day7S$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day7S$Corrected.biorad.Cq))

t.test(x=day9P$Corrected.biorad.Cq,y=day9S$Corrected.biorad.Cq,var.equal=F)

sd(day9P$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day9P$Corrected.biorad.Cq))

sd(day9S$Corrected.biorad.Cq, na.rm=TRUE)/sqrt(length(day9S$Corrected.biorad.Cq))

lsp2Abd.ps<-c(0.7494,0.01185,2.427e-05,0.0003482,0.0001186,4.548e-05,0.0002005)

p.adjust(lsp2Abd.ps,method="fdr")

#protein abundance

feddata$Age<-as.numeric(feddata$Age)

LSP2_lm <- lmer(feddata$LSP.2..Norm.. ~
feddata$Age*feddata$Diet*feddata$Corrected.biorad.Cq*feddata$tot.prot+(1|feddata$Cohort))

summary(LSP2_lm)

extractAIC(LSP2_lm)#full model

LSP2_lm2 <- lmer(feddata$LSP.2..Norm.. ~
feddata$Diet+feddata$Age*feddata$Corrected.biorad.Cq*feddata$tot.prot+(1|feddata$Cohort))

summary(LSP2_lm2)

extractAIC(LSP2_lm2)

LSP2_lm3 <- lmer(feddata$LSP.2..Norm.. ~
feddata$Diet+feddata$Age*feddata$Corrected.biorad.Cq+feddata$tot.prot+(1|feddata$Cohort))

summary(LSP2_lm3)

extractAIC(LSP2_lm3)

LSP2_lm4 <- lmer(feddata$LSP.2..Norm.. ~
feddata$Diet+feddata$Age*feddata$Corrected.biorad.Cq+(1|feddata$Cohort))

summary(LSP2_lm4)

extractAIC(LSP2_lm4)

LSP2_lm5 <- lmer(feddata$LSP.2..Norm.. ~
feddata$Diet+feddata$Age+feddata$Corrected.biorad.Cq+(1|feddata$Cohort))

summary(LSP2_lm5)

extractAIC(LSP2_lm5)
```

```
LSP2_lm6 <- lmer(feddata$LSP.2..Norm.. ~
feddata$Age+feddata$Corrected.biorad.Cq+(1|feddata$Cohort))

summary(LSP2_lm6)

extractAIC(LSP2_lm6)#reduced model

#Protein post-hoc and stdev

day0<-subset(dataraw, Age==0,select = Age:LSP.2..Norm..)

mean(day0$LSP.2..Norm..)

sd(day0$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day0$LSP.2..Norm..))

t.test(x=day1P$LSP.2..Norm..,y=day1S$LSP.2..Norm..,var.equal=F)

sd(day1P$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day1P$LSP.2..Norm..))

sd(day1S$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day1S$LSP.2..Norm..))

t.test(x=day2P$LSP.2..Norm..,y=day2S$LSP.2..Norm..,var.equal=F)

sd(day2P$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day2P$LSP.2..Norm..))

sd(day2S$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day2S$LSP.2..Norm..))

t.test(x=day3P$LSP.2..Norm..,y=day3S$LSP.2..Norm..,var.equal=F)

sd(day3P$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day3P$LSP.2..Norm..))

sd(day3S$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day3S$LSP.2..Norm..))

t.test(x=day4P$LSP.2..Norm..,y=day4S$LSP.2..Norm..,var.equal=F)

sd(day4P$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day4P$LSP.2..Norm..))

sd(day4S$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day4S$LSP.2..Norm..))

t.test(x=day5P$LSP.2..Norm..,y=day5S$LSP.2..Norm..,var.equal=F)

sd(day5P$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day5P$LSP.2..Norm..))

sd(day5S$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day5S$LSP.2..Norm..))

t.test(x=day7P$LSP.2..Norm..,y=day7S$LSP.2..Norm..,var.equal=F)

sd(day7P$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day7P$LSP.2..Norm..))

sd(day7S$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day7S$LSP.2..Norm..))

t.test(x=day9P$LSP.2..Norm..,y=day9S$LSP.2..Norm..,var.equal=F)

sd(day9P$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day9P$LSP.2..Norm..))

sd(day9S$LSP.2..Norm.., na.rm=TRUE)/sqrt(length(day9S$LSP.2..Norm..))
```

```
LSP2Abd.ps<-c(0.09399,0.1101,0.002347,0.0001841,0.002946,0.01314,0.0009502)

p.adjust(LSP2Abd.ps,method="fdr")


#Results:LSP-2 Abundance Between Dietary Treatments Diverges Before Behavior Diverges

call_glmF <- glmer(feddata$Calling ~
feddata$Age*feddata$Diet*feddata$tot.prot+feddata$Corrected.biorad.Cq+feddata$LSP.2..Norm..

            +(1|feddata$Cohort),  family = "binomial")#full model

summary(call_glmF)

call_glm1 <- glmer(feddata$Calling ~
feddata$Age+feddata$tot.prot+feddata$Diet+feddata$Age:feddata$Diet+feddata$Age:feddata$tot.prot
+

             feddata$Diet:feddata$tot.prot + feddata$Corrected.biorad.Cq+feddata$LSP.2..Norm..

            +(1|feddata$Cohort),  family = "binomial")

summary(call_glm1)

call_glm2 <- glmer(feddata$Calling ~
feddata$Age+feddata$tot.prot+feddata$Diet+feddata$Age:feddata$Diet+feddata$Age:feddata$tot.prot
+

             feddata$Diet:feddata$tot.prot + feddata$Corrected.biorad.Cq+(1|feddata$Cohort),  family =
"binomial")

summary(call_glm2)

call_glm3 <- glmer(feddata$Calling ~
feddata$Age+feddata$tot.prot+feddata$Diet+feddata$Age:feddata$Diet+

             feddata$Diet:feddata$tot.prot + feddata$Corrected.biorad.Cq+(1|feddata$Cohort),  family =
"binomial")

summary(call_glm3)

call_glm4 <- glmer(feddata$Calling ~
feddata$Age+feddata$tot.prot+feddata$Diet+feddata$Age:feddata$Diet+

             feddata$Diet:feddata$tot.prot + (1|feddata$Cohort),  family = "binomial")

summary(call_glm4)

call_glm5 <- glmer(feddata$Calling ~
feddata$Age+feddata$tot.prot+feddata$Diet+feddata$Age:feddata$Diet+

             (1|feddata$Cohort),  family = "binomial")
```

```
summary(call_glm5)

call_glm <- glmer(feddata$Calling ~ feddata$Age*feddata$Diet

            +(1|feddata$Cohort),  family = "binomial")#Reduced model

summary(call_glm)


#correlation

feddata$Prob_calling <-predict(call_glm,type="response",interval="confidence", level=.95)

cor.test(feddata$LSP.2..Norm.., feddata$Prob_calling)


#Results: lsp-2 Knockdown Mimics the Protein-Deprived Mating Phenotype

Raw.dsrna<-read.csv("RNAiCalling1_8_2018.csv")

Raw.dsrna$Age<-as.factor(Raw.dsrna$Age)

Day4i<-subset(Raw.dsrna, Age!="7",select = Tag:secRand)

Pday4i<-subset(Day4i, Diet=='Prot',select = Tag:secRand)

Sday4i<-subset(Day4i, Diet=='No Prot',select = Tag:secRand)

Day7i<-subset(Raw.dsrna, Age!="4",select = Tag:secRand)

Pday7i<-subset(Day7i, Diet=='Prot',select = Tag:secRand)

Sday7i<-subset(Day7i, Diet=='No Prot',select = Tag:secRand)

#test whether dsRNA injection reduces lsp-2 transcript abundance

transcripta <- lmer(Raw.dsrna$X2.negDeltDeltCt ~ Raw.dsrna$Age * Raw.dsrna$Diet *
Raw.dsrna$Treatment+ (1|Raw.dsrna$Cohort))

summary(transcripta)

extractAIC(transcripta)#full model

transcriptb<-
lmer(Raw.dsrna$X2.negDeltDeltCt~Raw.dsrna$Age*Raw.dsrna$Diet+Raw.dsrna$Diet:Raw.dsrna$Treat
ment+Raw.dsrna$Treatment+(1|Raw.dsrna$Cohort))

summary(transcriptb)

extractAIC(transcriptb)
```

```
transcriptc<-
lmer(Raw.dsrna$X2.negDeltDeltCt~Raw.dsrna$Age*Raw.dsrna$Diet+Raw.dsrna$Treatment+(1|Raw.dsr
na$Cohort))

summary(transcriptc)

extractAIC(transcriptc)

transcriptd<-
lmer(Raw.dsrna$X2.negDeltDeltCt~Raw.dsrna$Age+Raw.dsrna$Diet+Raw.dsrna$Treatment+(1|Raw.dsr
na$Cohort))

summary(transcriptd)

extractAIC(transcriptd)#reduced model

#post-hoc

t.test(Pday4i$negDeltCq~Pday4i$Treatment,var.equal=F)

t.test(Sday4i$negDeltCq~Sday4i$Treatment,var.equal=F)

t.test(Pday7i$negDeltCq~Pday7i$Treatment,var.equal=F)

t.test(Sday7i$negDeltCq~Sday7i$Treatment,var.equal=F)

#test whether calling behavior is effected by dsRNA treatment

#because day 4 had no calling in the protein deprived group, day 4 and 7 need to be analyzed in
seperate analyses

chisq.test(Pday4i$Calling,Pday4i$Treatment)

D7glm<-glmer(Day7i$Calling~Day7i$Diet*Day7i$Treatment+(1|Day7i$Cohort),family="binomial")

summary(D7glm)

D7glm<-glmer(Day7i$Calling~Day7i$Diet+Day7i$Treatment+(1|Day7i$Cohort),family="binomial")

summary(D7glm)

D7glm<-glmer(Day7i$Calling~Day7i$Diet+(1|Day7i$Cohort),family="binomial")

summary(D7glm) # Reduced
```