Supplementary text: Materials and Methods - Figures expression profiles (A case of insect female neoteny: E93 expression and links to the juvenile hormone in mealybugs)

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# Supplementary information 2.3. RNA extraction and quantitative RT-PCR

This document provides the R command lines used to produce the figures for E93 study in the Japanese mealybug. (REF)

# Needed packages

* ggplot2
* plyr
* tidyr

# Files

* sample2profile-Krh1E93.csv: qPCR data for rpL32, Kr-h1 and E93 isoforms for male and female expression profiles
* E93-Pyr-male.csv: qPCR data of JHM treatment on males
* E93-Pyr-N3D0.csv: qPCR data on JHM treatment on females

#if packages not installed  
library(ggplot2)  
library(plyr)  
library(tidyr)

# Expression profile of E93 during the post-oviposition development of male and female mealybugs

We first examined the expression profiles of PkKr-h1 and three identified isoforms of PkE93.

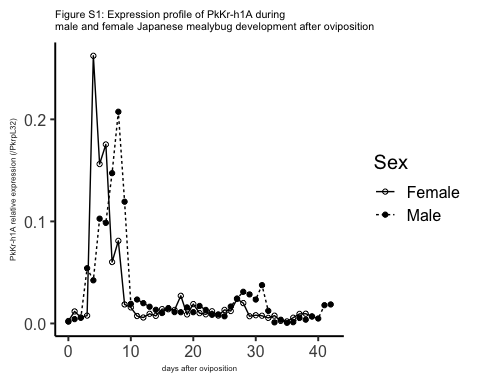
#loading file expression profile  
de<-read.csv(file="sample2profile-Krh1E93.csv",header = TRUE)  
names(de)

## [1] "cDNA.ID" "previous.cDNA.."   
## [3] "Sex" "Day.after.oviposition"  
## [5] "Stage" "rpL32"   
## [7] "Kr.h1" "E93.1"   
## [9] "E93.2" "E93.3"

This file includes the the second derivative maximum (SDM) values for PkKr-h1A, PkE93A, PkE93B, PkE93C and PkrpL32 used for expression profile analysis of males and females. The data was collected from RNA extraction performed on a new independent sampling from a previous study on juvenile hormone variations in the Japanese mealybug (Vea et al., 2016 PLOS ONE):

## PkKr-h1A expression between males and females (Figure S1)

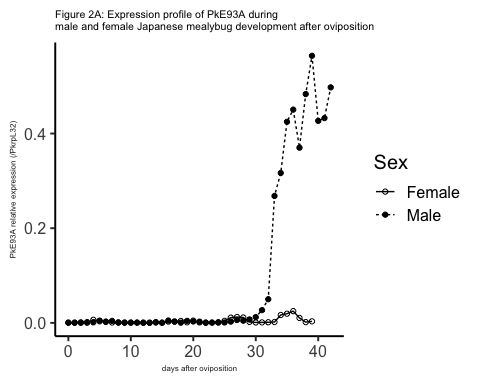
##PkKr-h1A expression profile (Figure S1)  
FigS1<-ggplot(de,aes(x=Day.after.oviposition, y=de$Kr.h1/de$rpL32, fill=Sex)) +   
 geom\_point(aes(shape=Sex)) +   
 geom\_line(aes(linetype=Sex))+  
 scale\_shape\_manual(values=c(1, 19))+ #shape of dots  
 theme(panel.grid.major = element\_blank(), panel.grid.minor = element\_blank(), panel.background = element\_blank(), axis.line = element\_line(colour = "black"))+  
 ylab("PkKr-h1A relative expression (/PkrpL32)") +xlab("days after oviposition") +  
 theme\_classic(15) +   
 ggtitle("Figure S1: Expression profile of PkKr-h1A during\nmale and female Japanese mealybug development after oviposition")+  
 theme(axis.title.x = element\_text(colour = "#242424",size=6), plot.title = element\_text(size=8),axis.title.y = element\_text(colour = "#242424",size=6))  
FigS1



Our independent sampling showed a consistent expression pattern with the sampling carried out for Vea et al., 2016. PkKr-h1A expression shows a diverging expression when N2 start to differente between male and females. Male expression continues and drops later after the prepupal molt, while female N2 have their PkKr-h1 expression progressively decreasing, to remain unusually low until the adult molt.

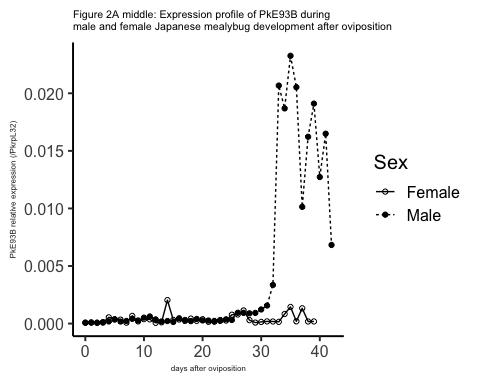
## PkE93 isoform A (Figure 2A-top)

##PkE93A expression profile (Figure 2A)  
Fig2a1<-ggplot(de,aes(x=Day.after.oviposition, y=de$E93.1/de$rpL32, fill=Sex)) +   
 geom\_point(aes(shape=Sex)) +   
 geom\_line(aes(linetype=Sex) )+  
 scale\_shape\_manual(values=c(1, 19))+  
 theme(panel.grid.major = element\_blank(), panel.grid.minor = element\_blank(), panel.background = element\_blank(), axis.line = element\_line(colour = "black"))+  
 ylab("PkE93A relative expression (/PkrpL32)") +xlab("days after oviposition") +  
 theme\_classic(15) +   
 ggtitle("Figure 2A: Expression profile of PkE93A during\nmale and female Japanese mealybug development after oviposition")+  
 theme(axis.title.x = element\_text(colour = "#242424",size=6), plot.title = element\_text(size=8),axis.title.y = element\_text(colour = "#242424",size=6))  
  
Fig2a1

 PkE93A shows sexually dimorphic expression pattern starting from the middle of N2. PkE93A expression rapidly increases and peaks during the pupal stage, while in females, the levels are far lower, but with two small increases at the end of N2 and N3.

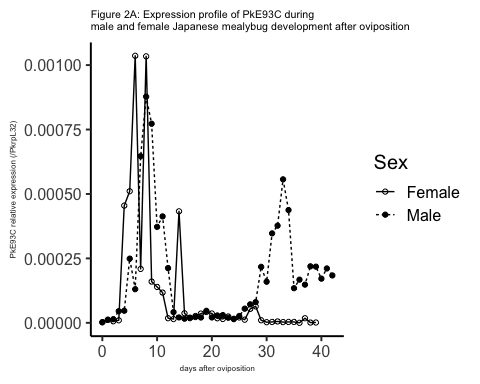
## PkE92 isoform B (Figure 2A-middle)

##PkE93B expression profile (Figure S2)  
Fig2a2<-ggplot(de,aes(x=Day.after.oviposition, y=de$E93.2/de$rpL32, fill=Sex)) +   
 geom\_point(aes(shape=Sex)) +   
 geom\_line(aes(linetype=Sex) )+  
 scale\_shape\_manual(values=c(1, 19))+  
 theme(panel.grid.major = element\_blank(), panel.grid.minor = element\_blank(), panel.background = element\_blank(), axis.line = element\_line(colour = "black"))+  
 ylab("PkE93B relative expression (/PkrpL32)") +xlab("days after oviposition") +  
 theme\_classic(15) +   
 ggtitle("Figure 2A middle: Expression profile of PkE93B during\nmale and female Japanese mealybug development after oviposition")+  
 theme(axis.title.x = element\_text(colour = "#242424",size=6), plot.title = element\_text(size=8),axis.title.y = element\_text(colour = "#242424",size=6))  
  
Fig2a2

 PkE93B shows similar patterns to PkE93A.

## PkE92 isoform C (Fig 2A-bottom)

#PkE93C expression profile (Figure 2A)  
Fig2a3<-ggplot(de,aes(x=Day.after.oviposition, y=de$E93.3/de$rpL32, fill=Sex)) +   
 geom\_point(aes(shape=Sex)) +   
 geom\_line(aes(linetype=Sex) )+  
 scale\_shape\_manual(values=c(1, 19))+  
 theme(panel.grid.major = element\_blank(), panel.grid.minor = element\_blank(), panel.background = element\_blank(), axis.line = element\_line(colour = "black"))+  
 ylab("PkE93C relative expression (/PkrpL32)") +xlab("days after oviposition") +  
 theme\_classic(15) +   
 ggtitle("Figure 2A: Expression profile of PkE93C during\nmale and female Japanese mealybug development after oviposition")+  
 theme(axis.title.x = element\_text(colour = "#242424",size=6), plot.title = element\_text(size=8),axis.title.y = element\_text(colour = "#242424",size=6))  
  
Fig2a3



PkE93C has generally very low expression pattern but is expressed during embryogenesis and the differential expression is observed sexually at the end of development too.

# Comparison PkKr-h1A and PkE93A (Figure 2B)

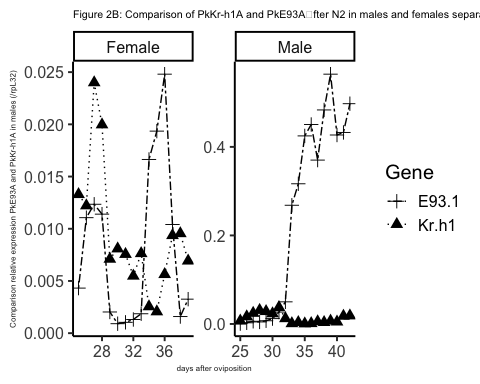
head(de)

## cDNA.ID previous.cDNA.. Sex Day.after.oviposition Stage rpL32 Kr.h1  
## 1 exp1 40 Male 0 ED0 0.0188 3.40e-05  
## 2 exp2 41 Male 1 ED1 0.0111 4.73e-05  
## 3 exp3 42 Male 2 ED2 0.0218 1.22e-04  
## 4 exp4 43 Male 3 ED3 0.0362 1.96e-03  
## 5 exp5 44 Male 4 ED4 0.0279 1.18e-03  
## 6 exp6 45 Male 5 ED5 0.0298 3.06e-03  
## E93.1 E93.2 E93.3  
## 1 6.23e-06 1.28e-06 4.44e-08  
## 2 4.52e-06 8.95e-07 1.26e-07  
## 3 4.64e-06 1.47e-06 3.08e-07  
## 4 5.55e-05 4.80e-06 1.64e-06  
## 5 3.30e-05 5.15e-06 1.31e-06  
## 6 1.42e-04 1.05e-05 7.42e-06

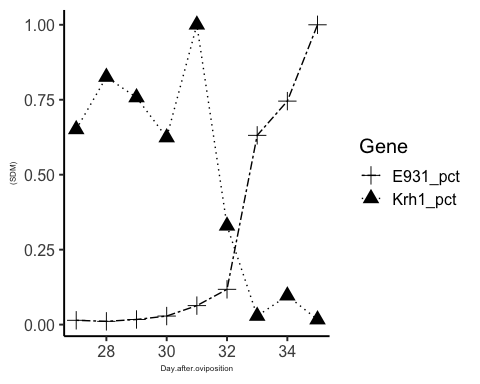
degather<-gather(de,Gene,SDM,7:10)  
head(degather)

## cDNA.ID previous.cDNA.. Sex Day.after.oviposition Stage rpL32 Gene  
## 1 exp1 40 Male 0 ED0 0.0188 Kr.h1  
## 2 exp2 41 Male 1 ED1 0.0111 Kr.h1  
## 3 exp3 42 Male 2 ED2 0.0218 Kr.h1  
## 4 exp4 43 Male 3 ED3 0.0362 Kr.h1  
## 5 exp5 44 Male 4 ED4 0.0279 Kr.h1  
## 6 exp6 45 Male 5 ED5 0.0298 Kr.h1  
## SDM  
## 1 3.40e-05  
## 2 4.73e-05  
## 3 1.22e-04  
## 4 1.96e-03  
## 5 1.18e-03  
## 6 3.06e-03

desub<-subset(degather, Gene=="Kr.h1"|Gene=="E93.1") #subset two genes  
desub<-subset(desub, Day.after.oviposition >= 25) #just after N2  
  
#with both male and female data   
Fig2B<-ggplot(desub,aes(x=Day.after.oviposition, y=(SDM/rpL32), fill=Gene)) +   
 geom\_point(aes(shape=Gene), size=3) +   
 geom\_line(aes(linetype=Gene) )+  
 facet\_wrap(~Sex, scales="free",nrow=1) +  
 scale\_shape\_manual(values=c(3, 17))+  
 scale\_linetype\_manual(values=c("twodash", "dotted"))+  
 theme(panel.grid.major = element\_blank(), panel.grid.minor = element\_blank(), panel.background = element\_blank(), axis.line = element\_line(colour = "black"))+  
ylab("Comparison relative expression PkE93A and PkKr-h1A in males (/rpL32)") +xlab("days after oviposition") +  
 theme\_classic(15) +   
 ggtitle("Figure 2B: Comparison of PkKr-h1A and PkE93A\after N2 in males and females separately")+  
 theme(axis.title.x = element\_text(colour = "#242424",size=6), plot.title = element\_text(size=8),axis.title.y = element\_text(colour = "#242424",size=6))  
  
Fig2B

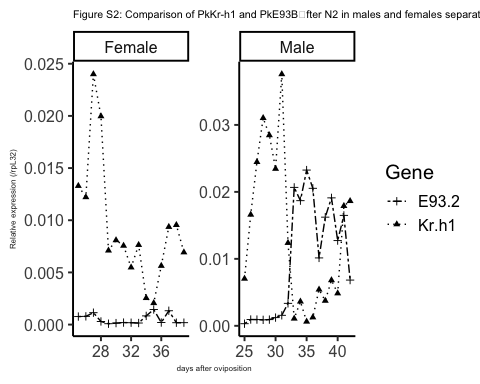


#Fig 2B relative expression of PkE93A and PkKr-h1A in 100%  
maleprepupa<-subset(de,de$Sex=="Male" & de$Day.after.oviposition >=27 &de$Day.after.oviposition <=35)  
  
maleprepupa$E931\_pct<-(maleprepupa$E93.1/maleprepupa$rpL32)/max(maleprepupa$E93.1/maleprepupa$rpL32)  
maleprepupa$Krh1\_pct<-(maleprepupa$Kr.h1/maleprepupa$rpL32)/max(maleprepupa$Kr.h1/maleprepupa$rpL32)  
  
maleprepupagather<-gather(maleprepupa,Gene,SDM,7:12)  
  
maleprepupa\_pct<-subset(maleprepupagather, maleprepupagather$Gene=="E931\_pct" |maleprepupagather$Gene=="Krh1\_pct")  
  
Fig2Bmale<-ggplot(maleprepupa\_pct,aes(x=Day.after.oviposition, y=(SDM), fill=Gene)) +   
 geom\_point(aes(shape=Gene), size=4) +   
 geom\_line(aes(linetype=Gene) )+  
 #facet\_wrap(~Sex, scales="free",nrow=1) +  
 scale\_shape\_manual(values=c(3, 17))+  
 scale\_linetype\_manual(values=c("twodash", "dotted"))+  
 theme(panel.grid.major = element\_blank(), panel.grid.minor = element\_blank(), panel.background = element\_blank(), axis.line = element\_line(colour = "black"))+  
#ylab("Comparison relative expression PkE93A and PkKr-h1A in males (/rpL32)") +xlab("days after oviposition") +  
 theme\_classic(15) +   
 #ggtitle("Figure 2B: Comparison of PkKr-h1A and PkE93A\after N2 in males and females separately")+  
 theme(axis.title.x = element\_text(colour = "#242424",size=6), plot.title = element\_text(size=8),axis.title.y = element\_text(colour = "#242424",size=6))  
  
  
Fig2Bmale



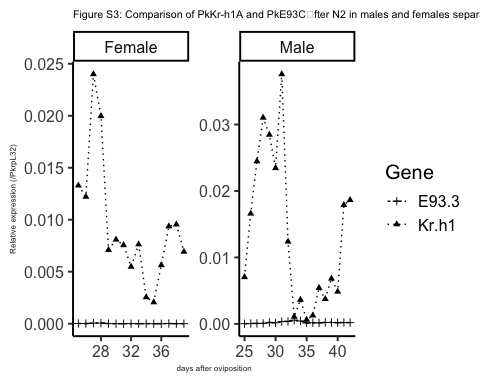
# PkKr-h1A and PkE93B (Figure S2)

#PkKr-h1 and PkE93B (Figure S2)  
desub2<-subset(degather, Gene=="Kr.h1"|Gene=="E93.2") #subset two genes  
desub2<-subset(desub2, Day.after.oviposition >= 25) #just after N2  
#with both male and female data  
FigS2<-ggplot(desub2,aes(x=Day.after.oviposition, y=(SDM/rpL32), fill=Gene)) +   
 geom\_point(aes(shape=Gene)) +   
 geom\_line(aes(linetype=Gene) )+  
 facet\_wrap(~Sex, scales="free",nrow=1) +  
 scale\_shape\_manual(values=c(3, 17))+  
 scale\_linetype\_manual(values=c("twodash", "dotted"))+  
 theme(panel.grid.major = element\_blank(), panel.grid.minor = element\_blank(), panel.background = element\_blank(), axis.line = element\_line(colour = "black"))+  
ylab("Relative expression (/rpL32)") +xlab("days after oviposition") +  
 ggtitle("Figure S2: Comparison of PkKr-h1 and PkE93B\after N2 in males and females separately")+  
 theme\_classic(15) +   
 theme(axis.title.x = element\_text(colour = "#242424",size=6), plot.title = element\_text(size=8),axis.title.y = element\_text(colour = "#242424",size=6))  
FigS2



# PkKr-h1A and PkE93-3 (Figure S3)

#Kr-h1 and E93-3 (Figure S3)  
desub3<-subset(degather, Gene=="Kr.h1"|Gene=="E93.3") #subset two genes  
desub3<-subset(desub3, Day.after.oviposition >= 25) #just after N2  
  
#with both male and female data  
FigS3<-ggplot(desub3,aes(x=Day.after.oviposition, y=(SDM/rpL32), fill=Gene)) +   
 geom\_point(aes(shape=Gene)) +   
 geom\_line(aes(linetype=Gene) )+  
 facet\_wrap(~Sex, scales="free",nrow=1) +  
 scale\_shape\_manual(values=c(3, 17))+  
 scale\_linetype\_manual(values=c("twodash", "dotted"))+  
 theme(panel.grid.major = element\_blank(), panel.grid.minor = element\_blank(), panel.background = element\_blank(), axis.line = element\_line(colour = "black"))+  
ylab("Relative expression (/PkrpL32)") +xlab("days after oviposition") +  
 theme\_classic(15) +  
 ggtitle("Figure S3: Comparison of PkKr-h1A and PkE93C\after N2 in males and females separately")+  
 theme(axis.title.x = element\_text(colour = "#242424",size=6), plot.title = element\_text(size=8),axis.title.y = element\_text(colour = "#242424",size=6))  
FigS3



# JH mimic treatments

JHM treatments were performed on both male and female and we assessed their effect on development and gene expression (see 2.4.JHM assays on male prepupae and female juvenile instars for details on the treatments)

## Effect of pyriproxyfen treatment on gene expression: data

#loading file JHM treatment  
#females  
  
dtf<-read.csv(file="E93-Pyr-N3D0.csv",header = TRUE)  
names(dtf)

## [1] "cDNA." "Compound" "Treatment" "TreatmentStage"  
## [5] "Sex" "DAT" "DayL" "N"   
## [9] "rpL32" "Kr.h1" "E93" "PkE93.1"   
## [13] "PkE93.2" "PkE93.3"

head(dtf)

## cDNA. Compound Treatment TreatmentStage Sex DAT DayL N rpL32  
## 1 203 Methanol Control N3D0 female 1 A 1 0.00281  
## 2 204 Methanol Control N3D0 female 1 A 1 0.00405  
## 3 205 Methanol Control N3D0 female 1 A 1 0.01160  
## 4 206 Methanol Control N3D0 female 1 A 1 0.00519  
## 5 281 Methanol Control N3D0 female 2 B 1 0.01247  
## 6 282 Methanol Control N3D0 female 2 B 1 0.00844  
## Kr.h1 E93 PkE93.1 PkE93.2 PkE93.3  
## 1 4.220e-05 9.750e-06 1.765e-06 3.333e-07 6.944e-08  
## 2 6.700e-05 1.810e-05 1.847e-06 3.363e-07 9.378e-08  
## 3 9.580e-05 3.810e-05 5.162e-06 1.081e-06 1.435e-07  
## 4 7.690e-05 2.780e-05 3.619e-06 5.923e-07 1.668e-07  
## 5 4.730e-05 2.261e-05 1.528e-06 4.653e-07 1.791e-07  
## 6 3.376e-05 1.156e-05 2.448e-07 1.614e-07 1.413e-07

dtf<-gather(dtf,Gene,SDM,10:14)  
head(dtf)

## cDNA. Compound Treatment TreatmentStage Sex DAT DayL N rpL32 Gene  
## 1 203 Methanol Control N3D0 female 1 A 1 0.00281 Kr.h1  
## 2 204 Methanol Control N3D0 female 1 A 1 0.00405 Kr.h1  
## 3 205 Methanol Control N3D0 female 1 A 1 0.01160 Kr.h1  
## 4 206 Methanol Control N3D0 female 1 A 1 0.00519 Kr.h1  
## 5 281 Methanol Control N3D0 female 2 B 1 0.01247 Kr.h1  
## 6 282 Methanol Control N3D0 female 2 B 1 0.00844 Kr.h1  
## SDM  
## 1 4.220e-05  
## 2 6.700e-05  
## 3 9.580e-05  
## 4 7.690e-05  
## 5 4.730e-05  
## 6 3.376e-05

dtf<-subset(dtf, Gene=="Kr.h1" | Gene=="PkE93.1"| Gene=="PkE93.2"| Gene=="PkE93.3")  
dtf$Gene

## [1] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [8] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [15] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [22] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [29] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [36] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [43] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [50] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [57] "Kr.h1" "Kr.h1" "Kr.h1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [64] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [71] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [78] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [85] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [92] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [99] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [106] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [113] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.2"  
## [120] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [127] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [134] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [141] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [148] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [155] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [162] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [169] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [176] "PkE93.2" "PkE93.2" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [183] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [190] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [197] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [204] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [211] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [218] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [225] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [232] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"

dtf$DAT

## [1] 1 1 1 1 2 2 2 3 3 3 4 4 4 5 5 5 6 6 6 7 7 7 8 8 8 8 8 8 8 1 1 1 1 2 2  
## [36] 2 3 3 3 4 4 4 5 5 5 6 6 6 7 7 7 8 8 8 8 8 8 8 8 1 1 1 1 2 2 2 3 3 3 4  
## [71] 4 4 5 5 5 6 6 6 7 7 7 8 8 8 8 8 8 8 1 1 1 1 2 2 2 3 3 3 4 4 4 5 5 5 6  
## [106] 6 6 7 7 7 8 8 8 8 8 8 8 8 1 1 1 1 2 2 2 3 3 3 4 4 4 5 5 5 6 6 6 7 7 7  
## [141] 8 8 8 8 8 8 8 1 1 1 1 2 2 2 3 3 3 4 4 4 5 5 5 6 6 6 7 7 7 8 8 8 8 8 8  
## [176] 8 8 1 1 1 1 2 2 2 3 3 3 4 4 4 5 5 5 6 6 6 7 7 7 8 8 8 8 8 8 8 1 1 1 1  
## [211] 2 2 2 3 3 3 4 4 4 5 5 5 6 6 6 7 7 7 8 8 8 8 8 8 8 8

#males  
dtm<-read.csv(file="E93-Pyr-male.csv",header = TRUE)  
names(dtm)

## [1] "Sample.ID" "treatment" "Day" "DayL" "Gene" "SDM.Gene"   
## [7] "SDM.rpL32"

head(dtm)

## Sample.ID treatment Day DayL Gene SDM.Gene SDM.rpL32  
## 1 299 Control 1 A Kr-h1 3.91e-05 0.00116  
## 2 300 Control 2 B Kr-h1 1.05e-05 0.00148  
## 3 301 Control 3 C Kr-h1 2.82e-06 0.00221  
## 4 302 Control 4 D Kr-h1 3.03e-06 0.00206  
## 5 303 Control 4 D Kr-h1 7.78e-06 0.00238  
## 6 304 Mimic 4 D Kr-h1 1.50e-04 0.00197

dtm<-subset(dtm, Gene=="Kr-h1" | Gene=="PkE93-1"| Gene=="PkE93-2"| Gene=="PkE93-3")  
dtm$Gene

## [1] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1   
## [9] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1   
## [17] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1   
## [25] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1   
## [33] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1   
## [41] Kr-h1 Kr-h1 Kr-h1 Kr-h1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [49] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [57] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [65] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [73] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [81] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [89] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [97] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [105] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [113] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [121] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [129] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [137] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [145] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [153] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [161] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [169] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## Levels: Kr-h1 PkE93-1 PkE93-2 PkE93-3

dtm$DayL

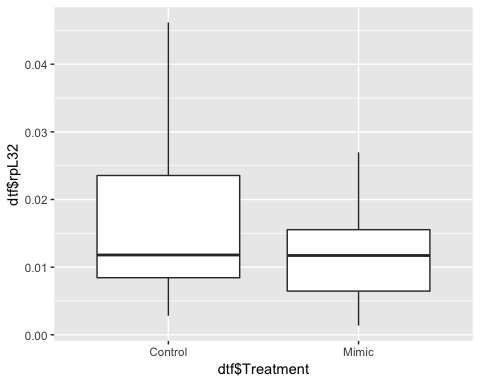
## [1] A B C D D D D F E E E E E E E E E E E E D D F F F D D F F B B B B B C  
## [36] C C C C A A A A A A B C D D D D F E E E E E E E E E E E E D D F F F D  
## [71] D F F B B B B B C C C C C A A A A A A B C D D D D F E E E E E E E E E  
## [106] E E E D D F F F D D F F B B B B B C C C C C A A A A A A B C D D D D F  
## [141] E E E E E E E E E E E E D D F F F D D F F B B B B B C C C C C A A A A  
## [176] A  
## Levels: A B C D E F

Plot rpl32 per treatments

#female data  
head(dtf)

## cDNA. Compound Treatment TreatmentStage Sex DAT DayL N rpL32 Gene  
## 1 203 Methanol Control N3D0 female 1 A 1 0.00281 Kr.h1  
## 2 204 Methanol Control N3D0 female 1 A 1 0.00405 Kr.h1  
## 3 205 Methanol Control N3D0 female 1 A 1 0.01160 Kr.h1  
## 4 206 Methanol Control N3D0 female 1 A 1 0.00519 Kr.h1  
## 5 281 Methanol Control N3D0 female 2 B 1 0.01247 Kr.h1  
## 6 282 Methanol Control N3D0 female 2 B 1 0.00844 Kr.h1  
## SDM  
## 1 4.220e-05  
## 2 6.700e-05  
## 3 9.580e-05  
## 4 7.690e-05  
## 5 4.730e-05  
## 6 3.376e-05

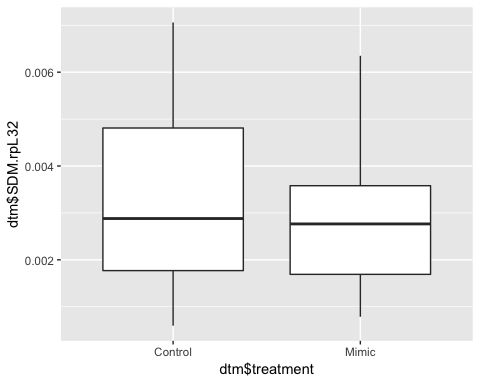
rpl32f<-ggplot(dtf)+  
geom\_boxplot(aes(y=dtf$rpL32, x=dtf$Treatment))  
rpl32f



#male data  
head(dtm)

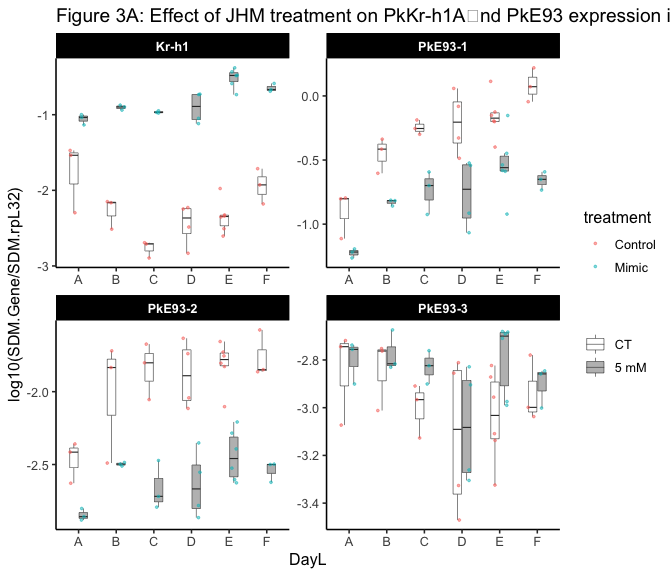
## Sample.ID treatment Day DayL Gene SDM.Gene SDM.rpL32  
## 1 299 Control 1 A Kr-h1 3.91e-05 0.00116  
## 2 300 Control 2 B Kr-h1 1.05e-05 0.00148  
## 3 301 Control 3 C Kr-h1 2.82e-06 0.00221  
## 4 302 Control 4 D Kr-h1 3.03e-06 0.00206  
## 5 303 Control 4 D Kr-h1 7.78e-06 0.00238  
## 6 304 Mimic 4 D Kr-h1 1.50e-04 0.00197

rpl32m<-ggplot(dtm)+  
geom\_boxplot(aes(y=dtm$SDM.rpL32, x=dtm$treatment))  
rpl32m

 The treatment did not affect rpl32 expression

Graphs of the effect of JHM treatments on Kr-h1 and E93 gene expression and statistical tests were obtained using the log10-transformed data (see statistical analysis file for more details [add a link to the file after github upload]).

#Fig 3A: male normalized data by PkrpL32 transformed by log 10  
boxplot3A<-ggplot(dtm,aes(x=DayL,y=log10(SDM.Gene/SDM.rpL32))) +   
 geom\_boxplot(aes(fill=treatment),width=0.5, size=0.2, outlier.shape=NA) +   
 geom\_jitter(aes(color=treatment),position=position\_jitterdodge(dodge.width=0.6),alpha=0.5,size=0.6) +  
 facet\_wrap(~Gene,scales="free",nrow=2) +   
 theme\_classic(base\_size = 12) +  
 ggtitle("Figure 3A: Effect of JHM treatment on PkKr-h1A\and PkE93 expression in males transformed by log 10")+  
 scale\_fill\_manual(name="", # Legend label, use darker colors  
 breaks=c("Control", "Mimic"),  
 labels=c("CT","5 mM"),  
 values=c("white", "grey")) +  
 theme(strip.background=element\_rect(fill="black")) +  
 theme(strip.text=element\_text(color="white",face="bold"))  
   
 boxplot3A



#Fig 3B: females absolute values normalized by rpL32 and transformed by log10  
dtflogbox <- ggplot(dtf,aes(x=DayL,y=log10(SDM/rpL32))) +   
 geom\_boxplot(width=0.5, size=0.2, outlier.size = 0.1, aes(fill=Treatment)) +   
 geom\_jitter(aes(color=Treatment),position=position\_jitterdodge(dodge.width=0.6),alpha=0.5,size=0.6) +  
 facet\_wrap(~Gene, scales="free",nrow=2) +   
 theme\_classic(base\_size = 12) +  
 scale\_fill\_manual(name="", # Legend label, use darker colors  
 breaks=c("Control", "Mimic"),  
 labels=c("CT","20 mM"),  
 values=c("white", "grey")) +  
 ggtitle("Figure 3B: Effect of JHM treatment on PkKr-h1A and\ PkE93 expression in females transformed log10")+  
 theme(strip.background=element\_rect(fill="black"))+   
 theme(strip.text=element\_text(color="white", face="bold"))  
  
dtflogbox

