

Evolution of Stress response in Aphaenogaster

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How does the stress response evolve in organisms(ants) that experience varying thermal environments?

Hypothesis: Ants elicit the stress response that match their local thermal environments, indicative of local adaptation.

Prediction: There should be clinal variation in the stress response across *A. picea/rudis*.

Methods: We collected along a N-S transect from Florida to Maine and measured their stress response under a common garden experiment. In total, we sampled 27 unique sites and collected 74 colonies. To determine whether we measured the stress response quantifying the basal and induction gene expression patterns of three heat shock proteins (hsc70, hsp83, hsp40) for each colony. The degree of induction was measured as the relative fold increase of mildly heat treated (37C) compared to control treated (25C) ants.

In total, there were 64 colonies, 37 of which reared at 20C and 27 reared at 26C.

20150824 update: Including phylogenetic relationships into regressions

20150825 meeting: use forward selection with AIC instead of model averaging; Bible (primer of ecological statistics) pg 346

20150826 update: identified error (wrong CT value) for hsp70 basal xp in sample 5-B.

Data parsing

```
dat<-read.csv("../Data/20150810_anbe_curated_dat_v3.csv",skip=6) #xp data
dat2<-read.csv("../Data/2015_JSG_phytotron_bioclim.csv") # full data set with climate factors
#changing factors into characters for Colony so I can merge them!
dat$Colony<-as.factor(as.character(dat$Colony))
dat2$Colony<-as.factor(as.character(dat2$Colony))

#merging dat and dat2 so I can link up xp data with climate factors!
merg<-inner_join(dat2,dat,by="Colony")
```

```
## Warning in inner_join_impl(x, y, by$x, by$y, suffix$x, suffix$y): joining
## factors with different levels, coercing to character vector
```

```
#summary of data
dim(merg) # dimensions of dataset
```

```
## [1] 64 43
```

```
summary(merg$Species_2)# number of species
```

```
## fulva picea rudis
##      3    45    16
```

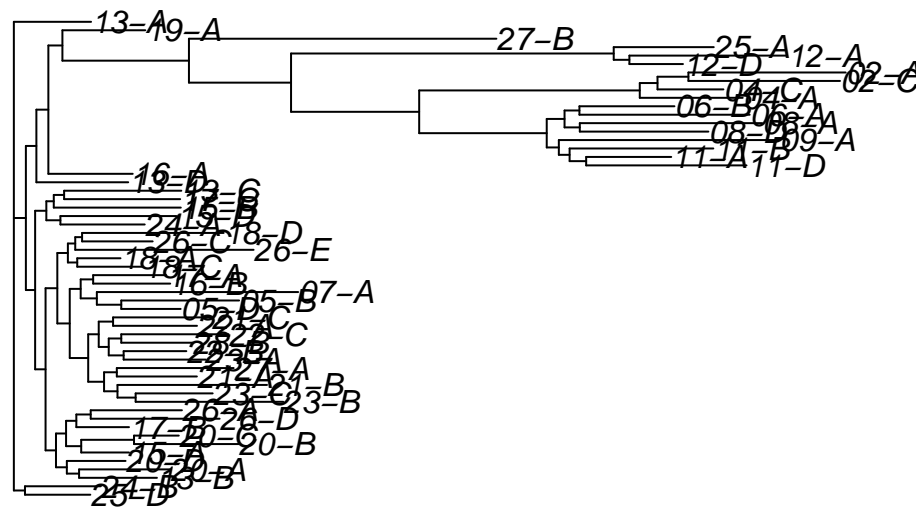
```
summary(as.factor(as.character(merg$Rearing_Temp)))#the number of colonies reared at 20 and 26
```

```
## 20 26
## 37 27
```

```
knitr::kable(subset(merg,merg$Species_2=="fulva")[,1:14] )# just seeing what fulva looks like
```

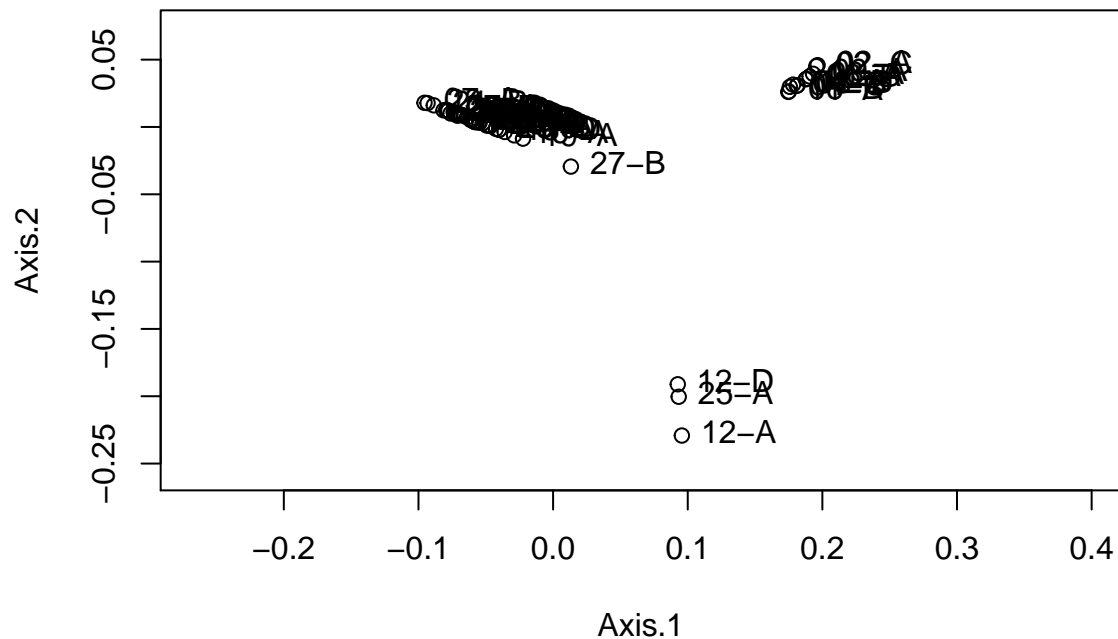
	ID	Colony	Species	Species_2	Site	Site_2	Tree_site	Colony.ID	Rearing_Temp	initial_w
18	ApGXL-11-C	11-C	fulva	fulva	WP	WP	WP3	WP3	26	
34	ApGXL-18-A	18-A	fulva	fulva	Notch	NO	NO1	Notch1	20	
36	ApGXL-18-D	18-D	fulva	fulva	Notch	NO	NO3	Notch2	26	

```
#reading in phylogenetic tree
#jsg.tree<-read.tree("../Phylogenetics/20150824_JSG_phytotron_tree_BL_only_v2")
jsg.tree<-read.tree("../Data/Phylogenetics/20150824_JSG_phytotron_tree_BL_only_v2")
#"28-A"
jsg.tree<-drop.tip(jsg.tree,tip=c("28-A","11-C","25-C","07-B","10-F"))
plot(jsg.tree)
```



```
#newblife<-read.tree("Phylogenetics/20150824_JSG_phytotron_tree_BL_only_v3_20150219_renamed_pub_fig_noB")
#newblife2<-drop.tip(newblife,tip=c("BR1"))
#plot(newblife2)
#write.tree(newblife2,"20150824_JSG_phytotron_tree_BL_only_v3_20150219_renamed_pub_fig_noBR1_v3")
#length(newblife2$tip.label);length(newblife2$tip.label)
pcoa.jsg<-pcoa(cophenetic.phylo(jsg.tree))
biplot(pcoa.jsg) #viewing separation
```

PCoA ordination



```
#setting as dataframe
n.pcoa.jsg<-as.data.frame(pcoa.jsg$vectors[,1:4])
n.pcoa.jsg$Colony<-jsg.tree$tip.label
dim(n.pcoa.jsg)
```

```
## [1] 57 5
```

```
##making full dataset
merg<-inner_join(merg,n.pcoa.jsg,by="Colony")
names(merg)
```

```
## [1] "ID" "Colony" "Species"
## [4] "Species_2" "Site" "Site_2"
## [7] "Tree_site" "Colony.ID" "Rearing_Temp"
## [10] "initial_workers" "initial_mass" "Avg_WORK_wet_weight"
## [13] "lat" "lon" "Elevation"
## [16] "lon.2" "lon.3" "bio1"
## [19] "bio2" "bio3" "bio4"
## [22] "bio5" "bio6" "bio7"
## [25] "bio8" "bio9" "bio10"
## [28] "bio11" "bio12" "bio13"
## [31] "bio14" "bio15" "bio16"
## [34] "bio17" "bio18" "bio19"
## [37] "qpcr_block" "FC_83" "FC_70"
## [40] "FC_40" "B_83" "B_70"
## [43] "B_40" "Axis.1" "Axis.2"
## [46] "Axis.3" "Axis.4"
```

```
names(merg)[44:46]<-c("Axis.1","Axis.2","Axis.3")
dim(merg)# nice!!
```

```
## [1] 57 47
```

```
summary(as.factor(as.character(merg$Rearing_Temp)))
```

```
## 20 26
```

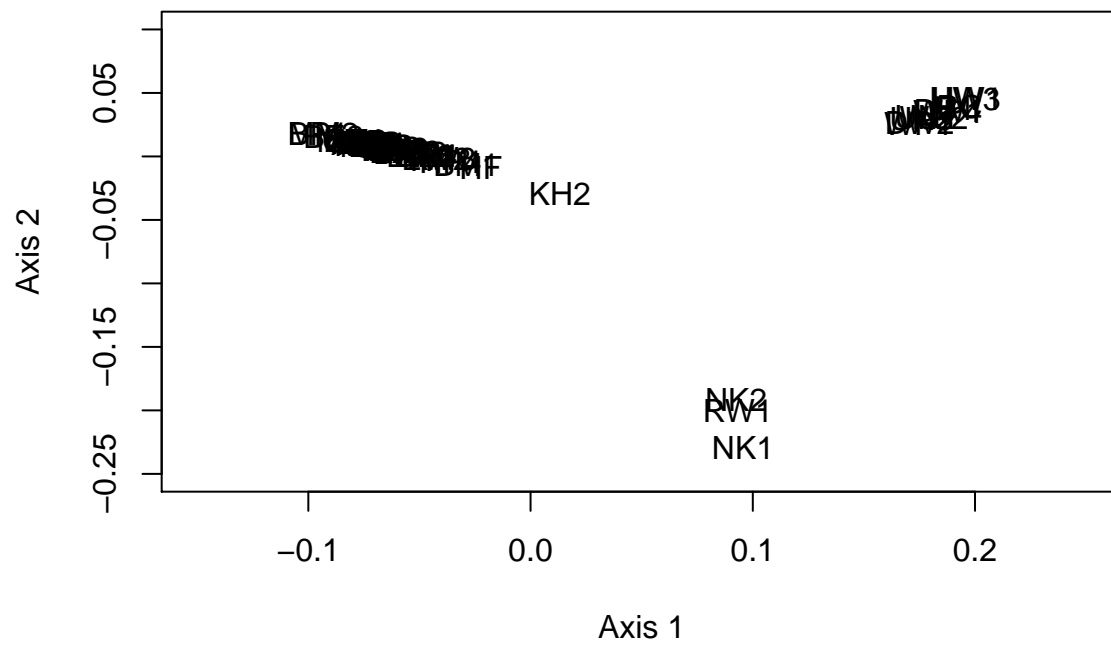
```
## 33 24
```

```
ful<-subset(merg,merg$Species_2=="fulva")
```

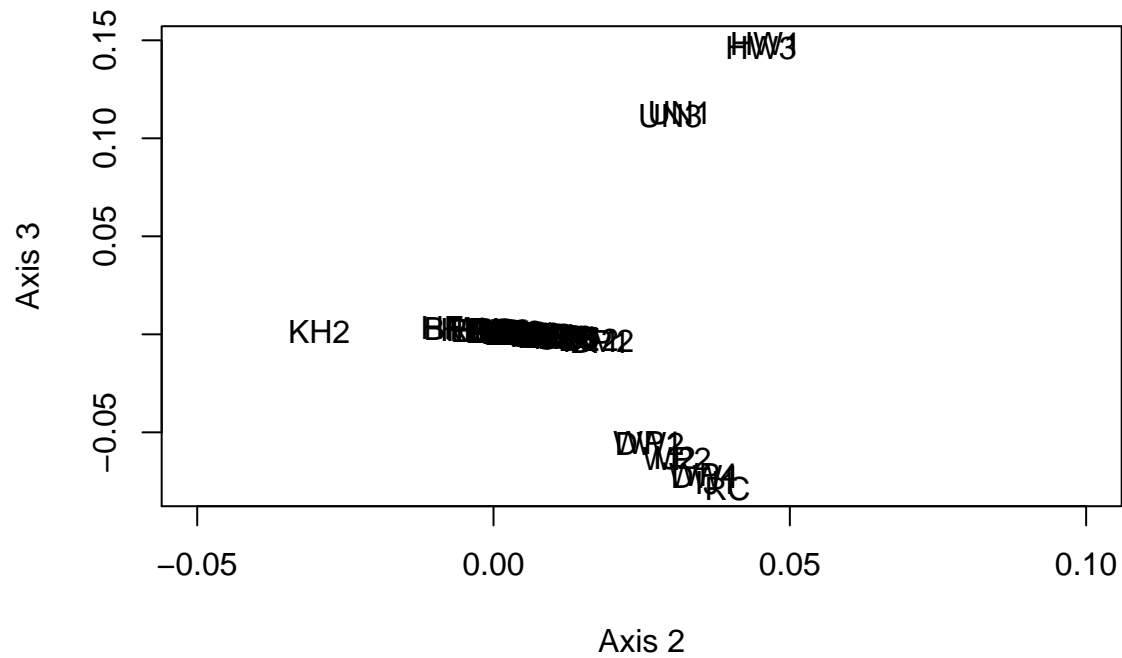
```
ddply(merg,.(Site),summarize,count=length(Colony))#counts of colonies at each site
```

```
##      Site count
## 1     APB      4
## 2     Bard      3
## 3     Bear      3
## 4     Brad      1
## 5     BRM      2
## 6     BRP      1
## 7     DSF      3
## 8      DW      2
## 9      EW      1
## 10     EWO      1
## 11  GSMNP      2
## 12      HF      1
## 13     HSP      4
## 14      HW      2
## 15  Ijams      1
## 16  IJams      1
## 17     KBH      2
## 18      MB      4
## 19      MM      3
## 20    NOCK      2
## 21  Notch      3
## 22      RC      1
## 23      RW      2
## 24     SEB      3
## 25     UNF      2
## 26      WP      3
```

```
plot(merg$Axis.1,merg$Axis.2,xlim=c(-.15,.25),ylim=c(-.25,.1),type="n",xlab="Axis 1",ylab="Axis 2")
text(merg$Axis.1,merg$Axis.2,labels=merg$Tree_site)
```



```
plot(merg$Axis.2,merg$Axis.3,type="n",xlab="Axis 2",ylab="Axis 3",xlim=c(-.05,.1))
text(merg$Axis.2,merg$Axis.3,labels=merg$Tree_site)
```

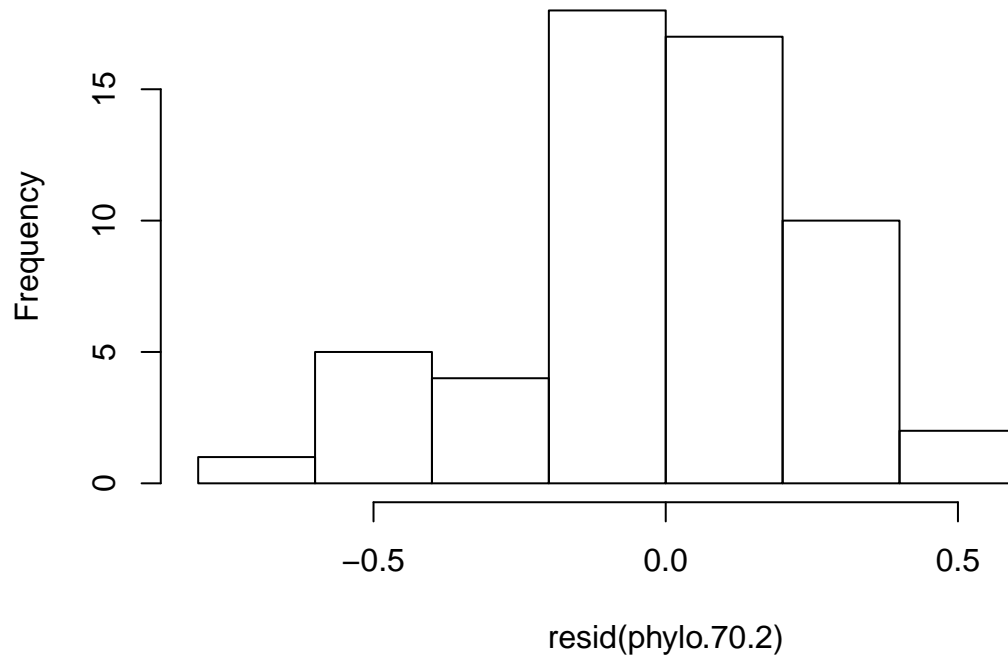


checking axes

```
#phylo.70.1<-lm(log10(merg$FC_70)~merg$Axis.1)
#hist(resid(phylo.70.1),xlim=c(-.8,.8))
```

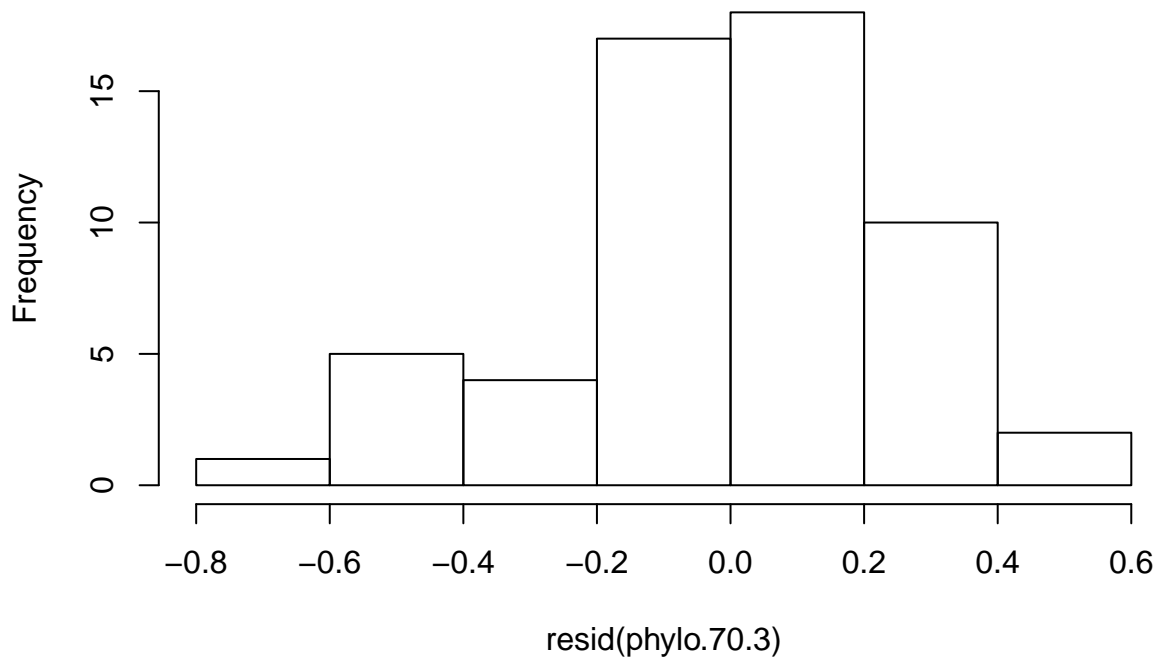
```
phylo.70.2<-lm(log10(merg$FC_70)~merg$Axis.1+merg$Axis.2)
hist(resid(phylo.70.2),xlim=c(-.8,.8))
```

Histogram of resid(phylo.70.2)



```
phylo.70.3<-lm(log10(merg$FC_70)~merg$Axis.1+merg$Axis.2+merg$Axis.3)
hist(resid(phylo.70.3))
```

Histogram of resid(phylo.70.3)



Stats: Using forward selection

response: basal or induction gene xp-log10 transformed to meet the assumptions of normality predictors:
rearing temp, Tmax(bio5)

```
#applying a regression model across columns
#response: basal or induction gene xp
#predictors: rearing temp, bio1(MAT)
mergy<-merg[-57,]

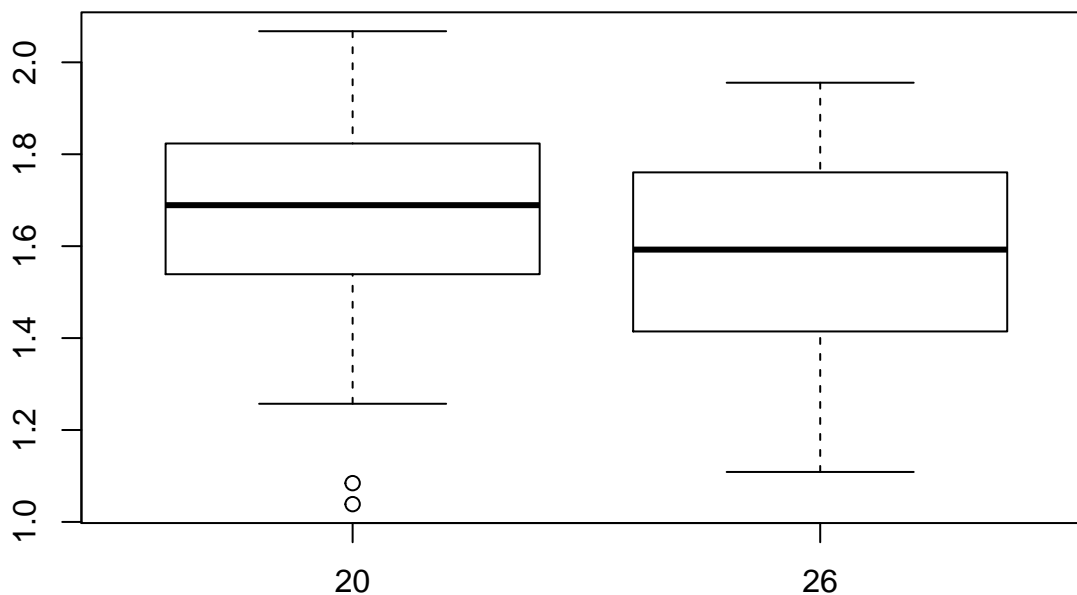
##induction
#hsp70 induction
full.70<-lm(log10(merg$FC_70)~merg$bio5+merg$Rearing_Temp+merg$Axis.1+merg$Axis.2+merg$Axis.3)
summary(stepAIC(full.70,direction="forward"))

## Start:  AIC=-147.19
## log10(merg$FC_70) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 +
##      merg$Axis.2 + merg$Axis.3

##
## Call:
## lm(formula = log10(merg$FC_70) ~ merg$bio5 + merg$Rearing_Temp +
##      merg$Axis.1 + merg$Axis.2 + merg$Axis.3)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.71120 -0.15142  0.02786  0.15764  0.38495
```

```
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    1.664572   0.946685   1.758  0.0847 .
## merg$bio5      0.001626   0.003291   0.494  0.6235
## merg$Rearing_Temp -0.021385  0.012049  -1.775  0.0819 .
## merg$Axis.1    -0.243005  0.666227  -0.365  0.7168
## merg$Axis.2     0.592864  0.715932   0.828  0.4115
## merg$Axis.3    -0.332454  0.874442  -0.380  0.7054
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2616 on 51 degrees of freedom
## Multiple R-squared:  0.07252,    Adjusted R-squared:  -0.01841
## F-statistic: 0.7975 on 5 and 51 DF,  p-value: 0.5566
```

```
boxplot(log10(FC_70)~Rearing_Temp,data=merg)
```



```
#hsp83 induction
full.83<-lm(log10(merg$FC_83)~merg$bio5+merg$Rearing_Temp+merg$Axis.1+merg$Axis.2+merg$Axis.3)
summary(stepAIC(full.83,direction="forward"))
```

```
## Start:  AIC=-135.83
## log10(merg$FC_83) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 +
##      merg$Axis.2 + merg$Axis.3

##
## Call:
## lm(formula = log10(merg$FC_83) ~ merg$bio5 + merg$Rearing_Temp +
##      merg$Axis.1 + merg$Axis.2 + merg$Axis.3)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
```

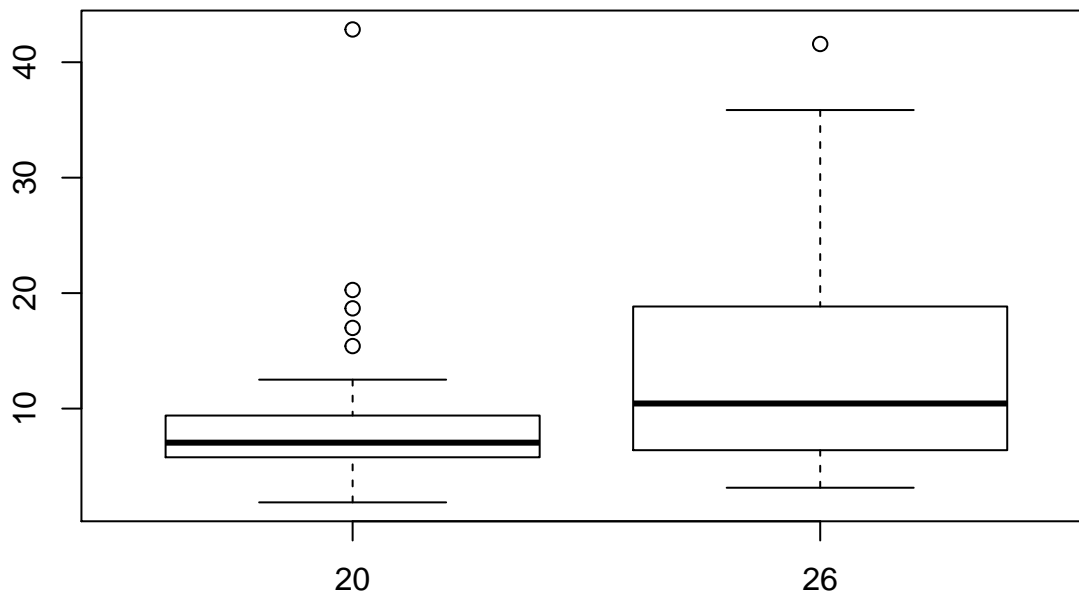


```
## -0.65752 -0.15558 -0.04206 0.14338 0.74010
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    1.161088    1.045965    1.110  0.2722
## merg$bio5      -0.002887    0.003636   -0.794  0.4309
## merg$Rearing_Temp 0.026304    0.013313    1.976  0.0536 .
## merg$Axis.1     0.443423    0.736094    0.602  0.5496
## merg$Axis.2     0.205355    0.791012    0.260  0.7962
## merg$Axis.3     0.026257    0.966145    0.027  0.9784
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2891 on 51 degrees of freedom
## Multiple R-squared:  0.08791,    Adjusted R-squared:  -0.001516
## F-statistic: 0.9831 on 5 and 51 DF,  p-value: 0.4372
```

```
gg<-dredge(full.83)
```

```
## Fixed term is "(Intercept)"
```

```
boxplot(FC_83~Rearing_Temp,data=merg)
```



```
#write.csv(subset(merg,merg$FC_83>10),"test.csv")
#hsp40 induction
full.40<-lm(log10(merg$FC_40)~merg$bio5+merg$Rearing_Temp+merg$Axis.1+merg$Axis.2+merg$Axis.3)
summary(stepAIC(full.40,direction="forward"))
```

```
## Start:  AIC=-122.77
## log10(merg$FC_40) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 +
##      merg$Axis.2 + merg$Axis.3
##
```

```
## Call:
## lm(formula = log10(mergy$FC_40) ~ mergy$bio5 + mergy$Rearing_Temp +
##      mergy$Axis.1 + mergy$Axis.2 + mergy$Axis.3)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.76955 -0.12686  0.05241  0.19614  0.56117
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.994391    1.149811  -0.865   0.3913
## mergy$bio5      0.003960    0.004004   0.989   0.3274
## mergy$Rearing_Temp 0.036410    0.014794   2.461   0.0173 *
## mergy$Axis.1    -0.489558    0.809098  -0.605   0.5479
## mergy$Axis.2     1.036601    0.869371   1.192   0.2387
## mergy$Axis.3     0.443265    1.061906   0.417   0.6782
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3177 on 50 degrees of freedom
## Multiple R-squared:  0.1973, Adjusted R-squared:  0.117
## F-statistic: 2.458 on 5 and 50 DF,  p-value: 0.0456
```

```
#aov_40_induction<-aov(log10(mergy$FC_40)~mergy$Rearing_Temp)
#summary(aov_40_induction)
#plot(aov_40_induction)

#looks good

#removing influential sample # 48
#out_48_for_hsp40<-mergy[-48,]
#sub_40<-lm(log10(out_48_for_hsp40$FC_40)~out_48_for_hsp40$bio5+out_48_for_hsp40$Rearing_Temp+out_48_for_hsp40$Axis.1+out_48_for_hsp40$Axis.2+out_48_for_hsp40$Axis.3)
#summary(stepAIC(sub_40,direction="forward"))

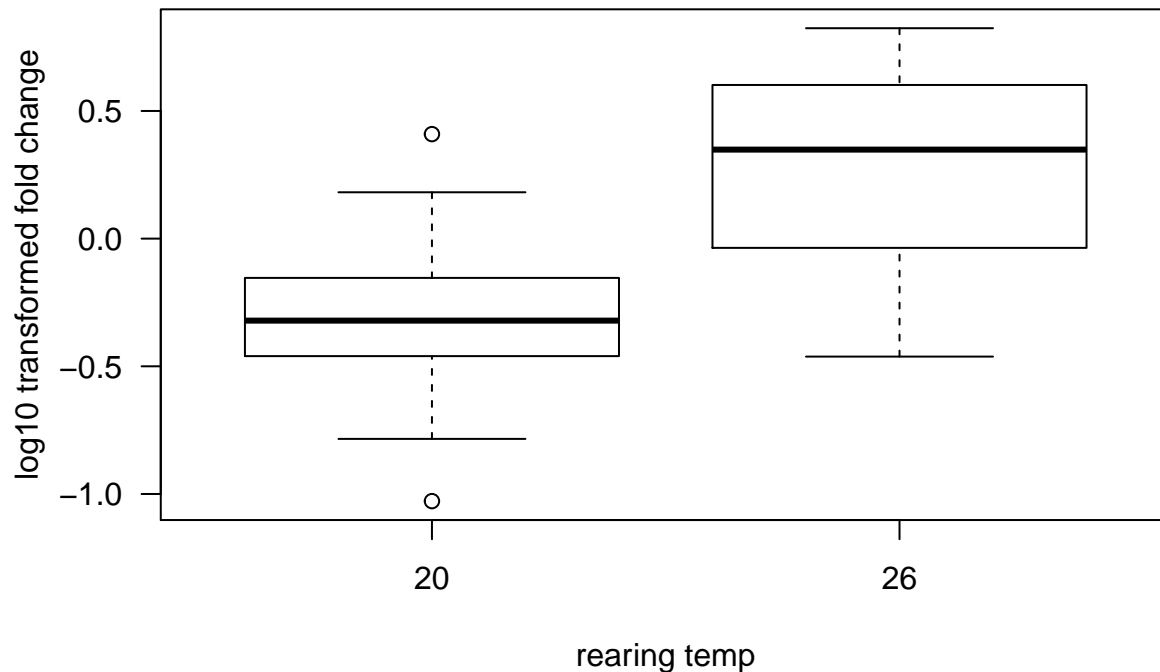
#####

#basal
#hsp70
B.70<-lm(log10(merg$B_70)~merg$bio5+merg$Rearing_Temp+merg$Axis.1+merg$Axis.2+merg$Axis.3)
summary(stepAIC(B.70,direction="forward"))
```

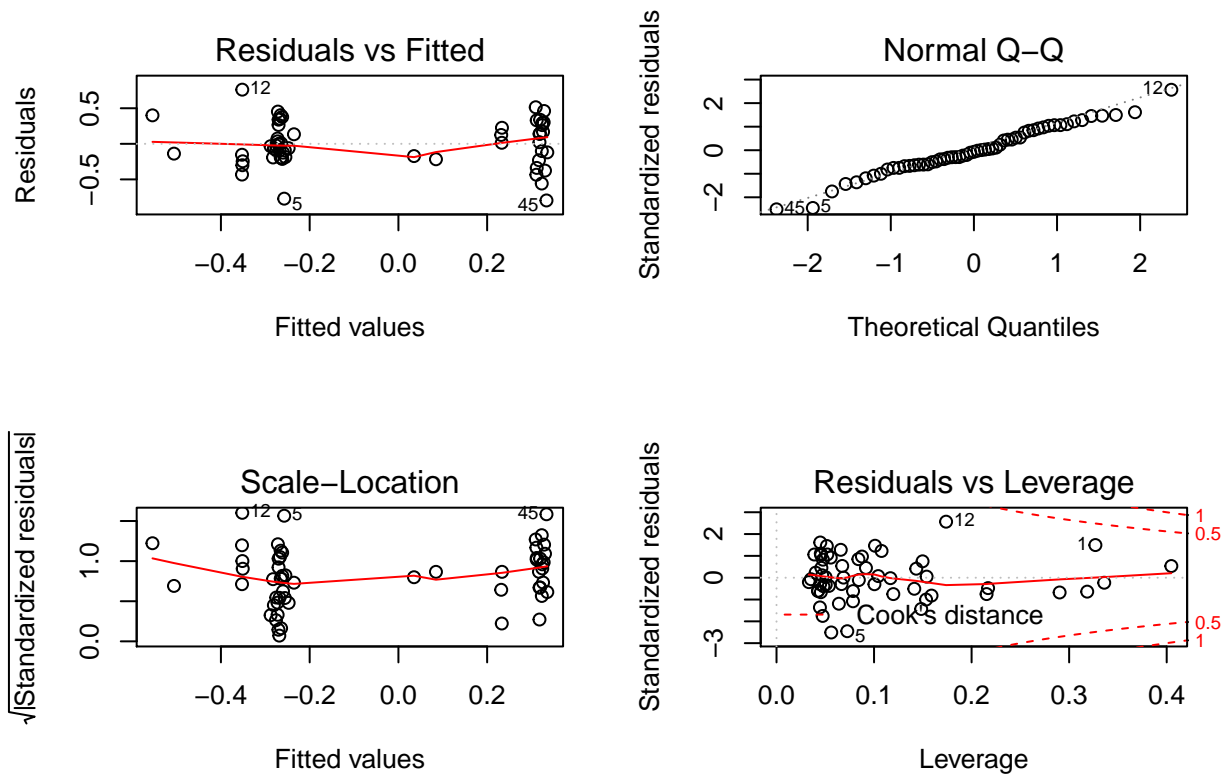
```
## Start:  AIC=-122.03
## log10(merg$B_70) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 +
##      merg$Axis.2 + merg$Axis.3
##
## Call:
## lm(formula = log10(merg$B_70) ~ merg$bio5 + merg$Rearing_Temp +
##      merg$Axis.1 + merg$Axis.2 + merg$Axis.3)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.79568 -0.19170 -0.02223  0.26280  0.76122
```

```
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -2.2229893   1.1804868  -1.883   0.0654 .
## merg$bio5     -0.0001093   0.0041035  -0.027   0.9789
## merg$Rearing_Temp 0.0978311  0.0150248   6.511 3.22e-08 ***
## merg$Axis.1    -0.5150972   0.8307639  -0.620   0.5380
## merg$Axis.2    -0.4962524   0.8927448  -0.556   0.5807
## merg$Axis.3    -0.8625652   1.0904020  -0.791   0.4326
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3262 on 51 degrees of freedom
## Multiple R-squared:  0.4809, Adjusted R-squared:  0.43
## F-statistic: 9.45 on 5 and 51 DF, p-value: 2.019e-06
```

```
boxplot(log10(merg$B_70)~merg$Rearing_Temp,ylab="log10 transformed fold change",las=1,xlab="rearing temp")
```



```
#####
par(mfrow=c(2,2))
plot(B.70)
```



```
par(mfrow=c(1,1))
#hsp83
B.83<-lm(log10(merg$B_83)~merg$bio5+merg$Rearing_Temp+merg$Axis.1+merg$Axis.2+merg$Axis.3)
summary(stepAIC(B.83,direction="forward"))
```

```
## Start: AIC=-85.77
## log10(merg$B_83) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 +
##      merg$Axis.2 + merg$Axis.3
```

```
##
## Call:
## lm(formula = log10(merg$B_83) ~ merg$bio5 + merg$Rearing_Temp +
##      merg$Axis.1 + merg$Axis.2 + merg$Axis.3)
##
```

```
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.94081 -0.28386  0.00283  0.29660  0.80903
##
```

```
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -1.912138   1.622574  -1.178   0.244
## merg$bio5       0.004766   0.005640   0.845   0.402
## merg$Rearing_Temp 0.026941   0.020651   1.305   0.198
## merg$Axis.1    -1.832587   1.141881  -1.605   0.115
## merg$Axis.2    -0.842144   1.227074  -0.686   0.496
## merg$Axis.3     1.400593   1.498753   0.935   0.354
##
```

```
## Residual standard error: 0.4484 on 51 degrees of freedom
```

```
## Multiple R-squared:  0.1378, Adjusted R-squared:  0.05326
## F-statistic:  1.63 on 5 and 51 DF,  p-value: 0.1689
```

```
#boxplot(log10(merg$B_83)~merg$Rearing_Temp) #doesn't look real
#hsp40
```

```
B.40<-lm(log10(B_40)~bio5+Rearing_Temp+Axis.1+Axis.2+Axis.3,data=mergy)
summary(stepAIC(B.40,direction="forward"))
```

```
## Start:  AIC=-78.04
## log10(B_40) ~ bio5 + Rearing_Temp + Axis.1 + Axis.2 + Axis.3

##
## Call:
## lm(formula = log10(B_40) ~ bio5 + Rearing_Temp + Axis.1 + Axis.2 +
##     Axis.3, data = mergy)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.31203 -0.24716  0.02877  0.28990  0.90675
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  -2.247233    1.714370  -1.311 0.195907
## bio5           0.005097    0.005969   0.854 0.397236
## Rearing_Temp  0.038533    0.022057   1.747 0.086787 .
## Axis.1       -1.739635    1.206367  -1.442 0.155525
## Axis.2       -2.692199    1.296234  -2.077 0.042965 *
## Axis.3       -6.637553    1.583304  -4.192 0.000112 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4737 on 50 degrees of freedom
## Multiple R-squared:  0.3375, Adjusted R-squared:  0.2712
## F-statistic: 5.094 on 5 and 50 DF,  p-value: 0.0007505
```

```
#####two panel figurese for hsp70 basal and hsp40 induction!
#side by side plot
#grid.arrange(basal_70,induc_hsp40,ncol=2)
#stacked plot
#new_hsp70<-basal_70+xlabs("")
#grid.arrange(new_hsp70,induc_hsp40,nrow=2)
```

Mean expression values

```
## overall mean
knitr::kable(as.data.frame(apply(mergy[,38:43],2,mean)))
```

	apply(mergy[, 38:43], 2, mean)
FC_83	11.218868
FC_70	50.227915
FC_40	10.535062
B_83	1.735492
B_70	1.446917
B_40	1.935067

```
dim(mergy)
```

```
## [1] 56 47
```

```
F<-c(rep("FC_83",56),rep("FC_70",56),rep("FC_40",56))
nn<-data.frame(cbind(F,c(mergy[,38],mergy[,39],mergy[,40])))
nn$V2<-as.numeric(as.character(nn$V2))
summary(aov(V2~F,data=nn))
```

```
##           Df Sum Sq Mean Sq F value Pr(>F)
## F           2  57824   28912   102.3 <2e-16 ***
## Residuals  165  46643     283
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##by rearing temp
c<-ddply(merg,.(Rearing_Temp),summarize, Induction83=mean(FC_83),Basal83=mean(B_83),Induction70=mean(FC_70),Basal70=mean(B_70))

i<-ddply(mergy,.(Rearing_Temp),summarize,Induction40=mean(FC_40),Basal40=mean(B_40))

da<-cbind(c,i[,2:3])

#table of means
knitr::kable(da)
```

Rearing_Temp	Induction83	Basal83	Induction70	Basal70	Induction40	Basal40
20	9.352522	1.262254	55.45230	0.640272	8.059647	1.680941
26	14.320365	2.334319	42.62233	2.565149	14.086744	2.299683

```
#median expression
cc<-ddply(merg,.(Rearing_Temp),summarize, Induction83=median(FC_83),Basal83=median(B_83),Induction70=median(FC_70),Basal70=median(B_70))

ii<-ddply(mergy,.(Rearing_Temp),summarize,Induction40=median(FC_40),Basal40=median(B_40))
#medians
medians<-cbind(cc,ii[,2:3])
```

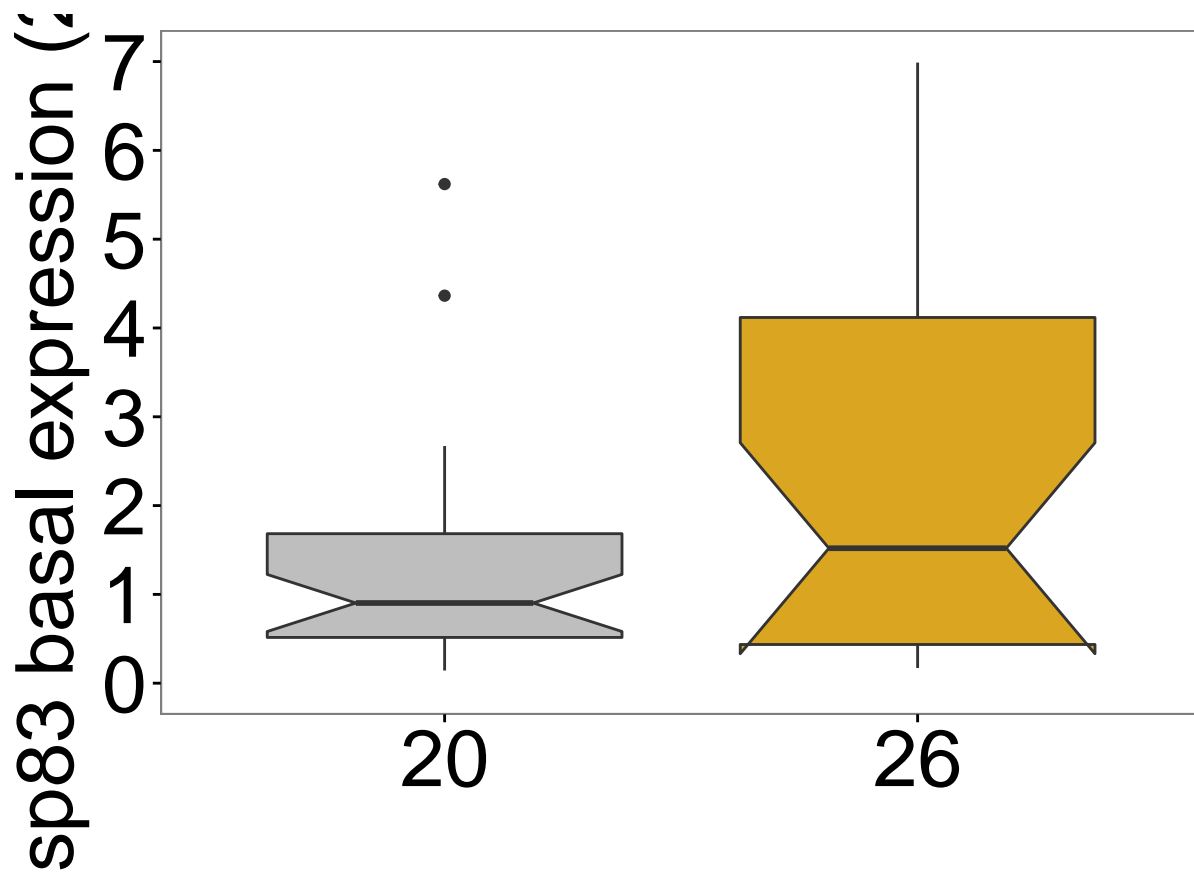
Expression Figures for rearing temp

```
#theme#####
T<-theme_bw()+theme(text=element_text(size=30),axis.text=element_text(size=30),legend.text=element_text(
size=30))

dude<-scale_fill_manual(name = "", values = c("gray", "goldenrod"))
#color
#####
#####ggplot of hsp83 basal
basal_83<-ggplot(data=merg,aes(x=factor(Rearing_Temp),y=B_83,fill=factor(Rearing_Temp)))+geom_boxplot(n
otch=FALSE)

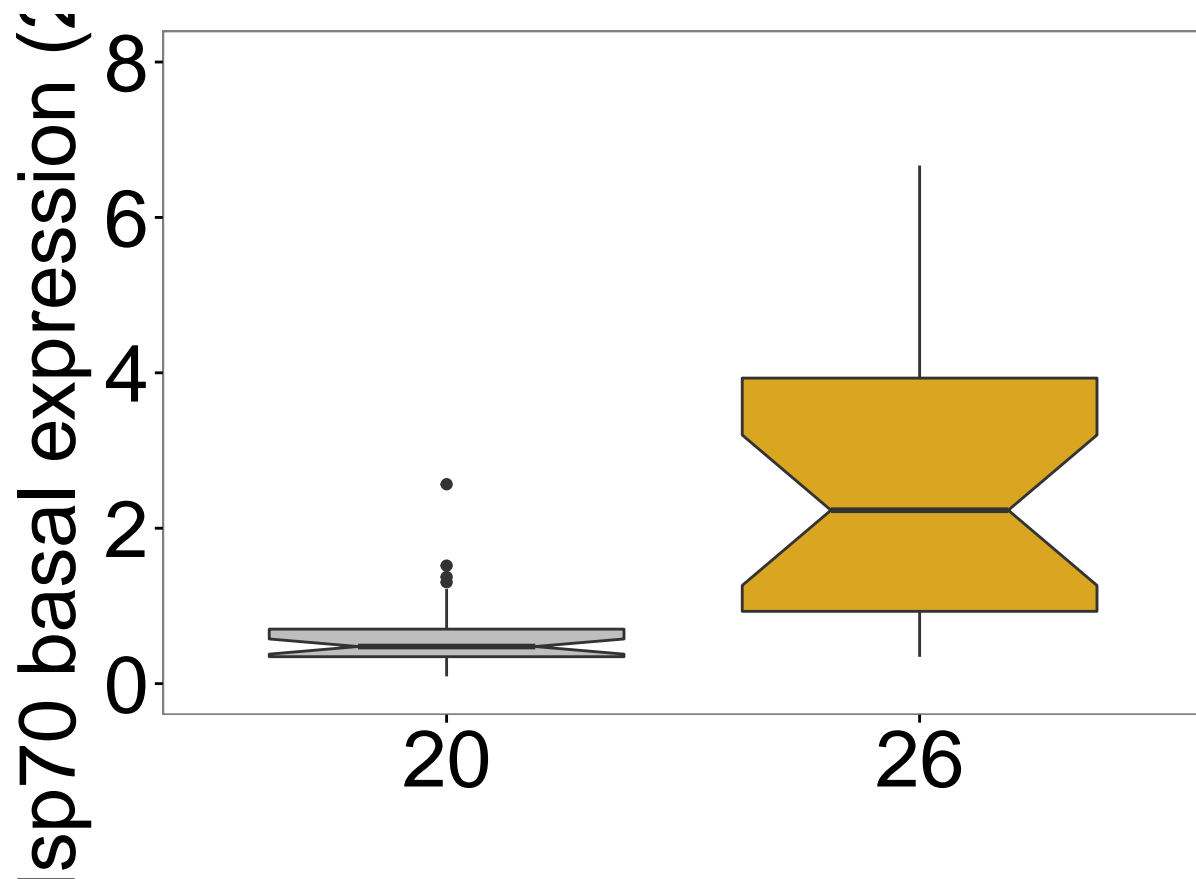
#+xlab("Maintenance Temperature (°C)")
basal_83
```

notch went outside hinges. Try setting notch=FALSE.

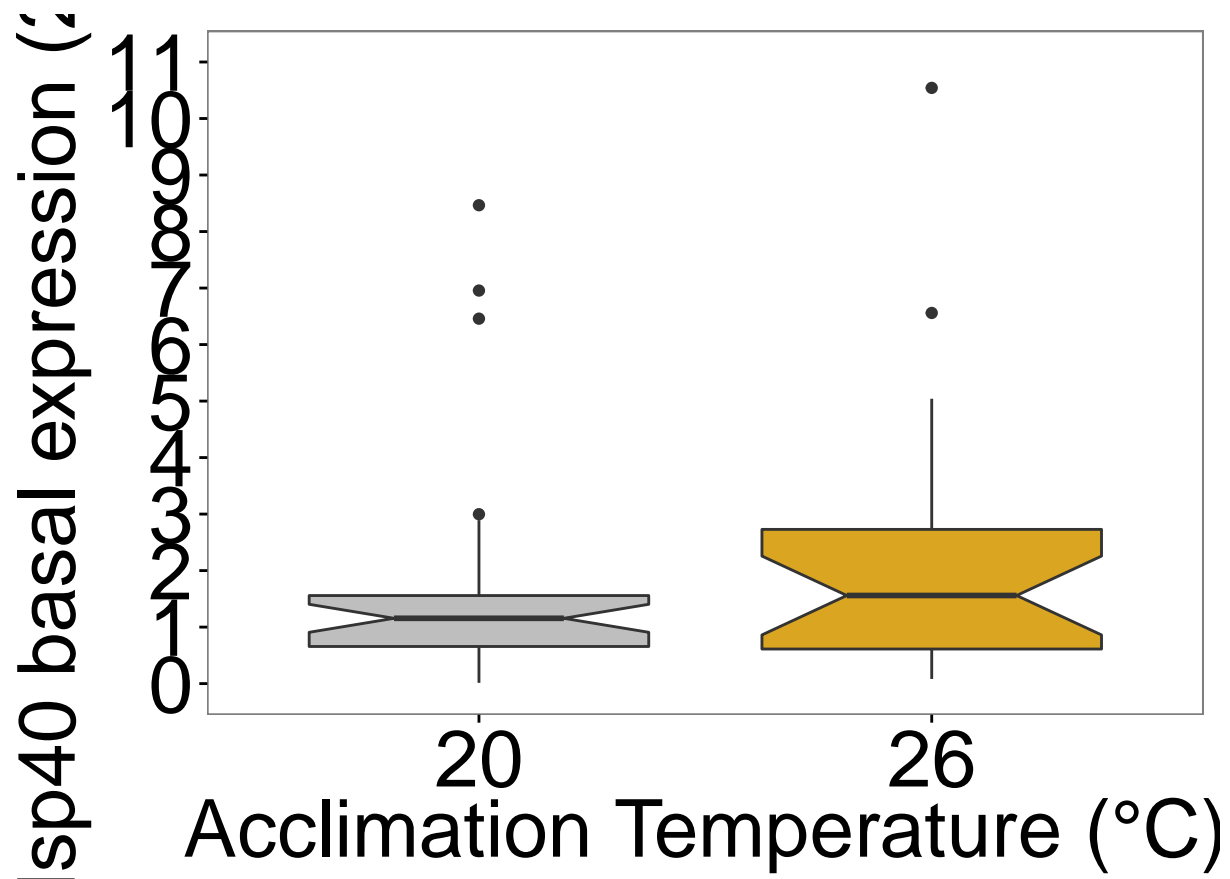


```
#####ggplot of hsp70 basal
basal_70<-ggplot(data=merg,aes(x=factor(Rearing_Temp),y=B_70,fill=factor(Rearing_Temp)))+geom_boxplot(n
otch=FALSE)

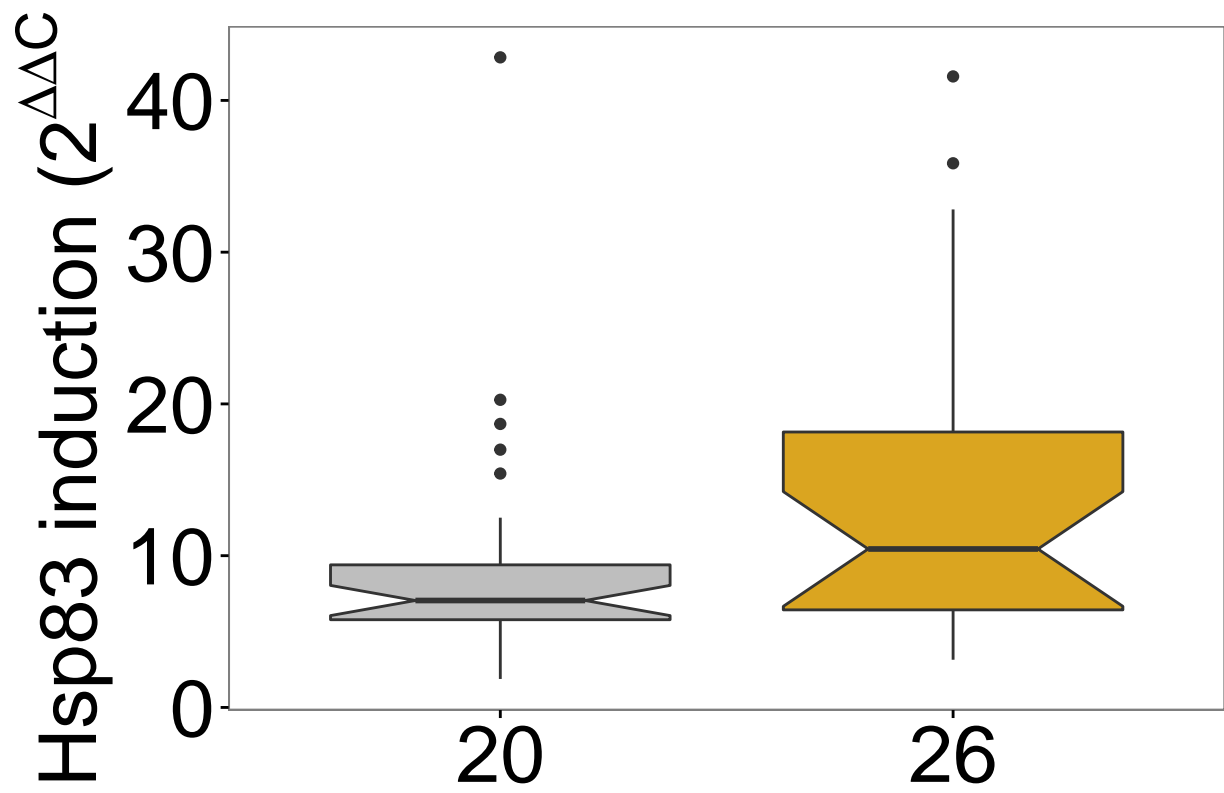
#+xlab("Maintenance Temperature (°C)")
basal_70
```



```
#####ggplot of hsp40 basal
basal_40<-ggplot(data=mergy,aes(x=factor(Rearing_Temp),y=B_40,fill=factor(Rearing_Temp)))+geom_boxplot(
  #+xlab("Maintenance Temperature (°C)")+
  basal_40
```

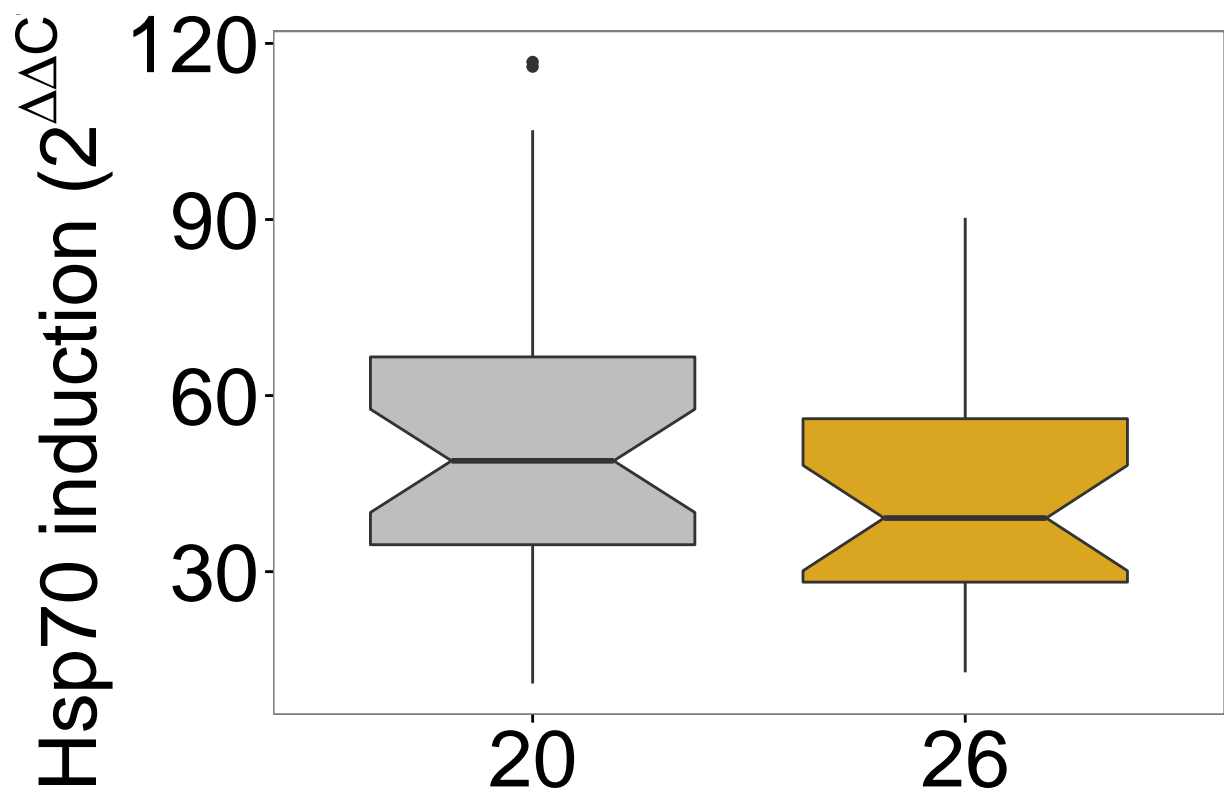



```
#####ggplot of hsp83 induction
induc_hsp83<-ggplot(data=merg,aes(x=factor(Rearing_Temp),y=FC_83,fill=factor(Rearing_Temp)))+geom_boxplot()
induc_hsp83
```



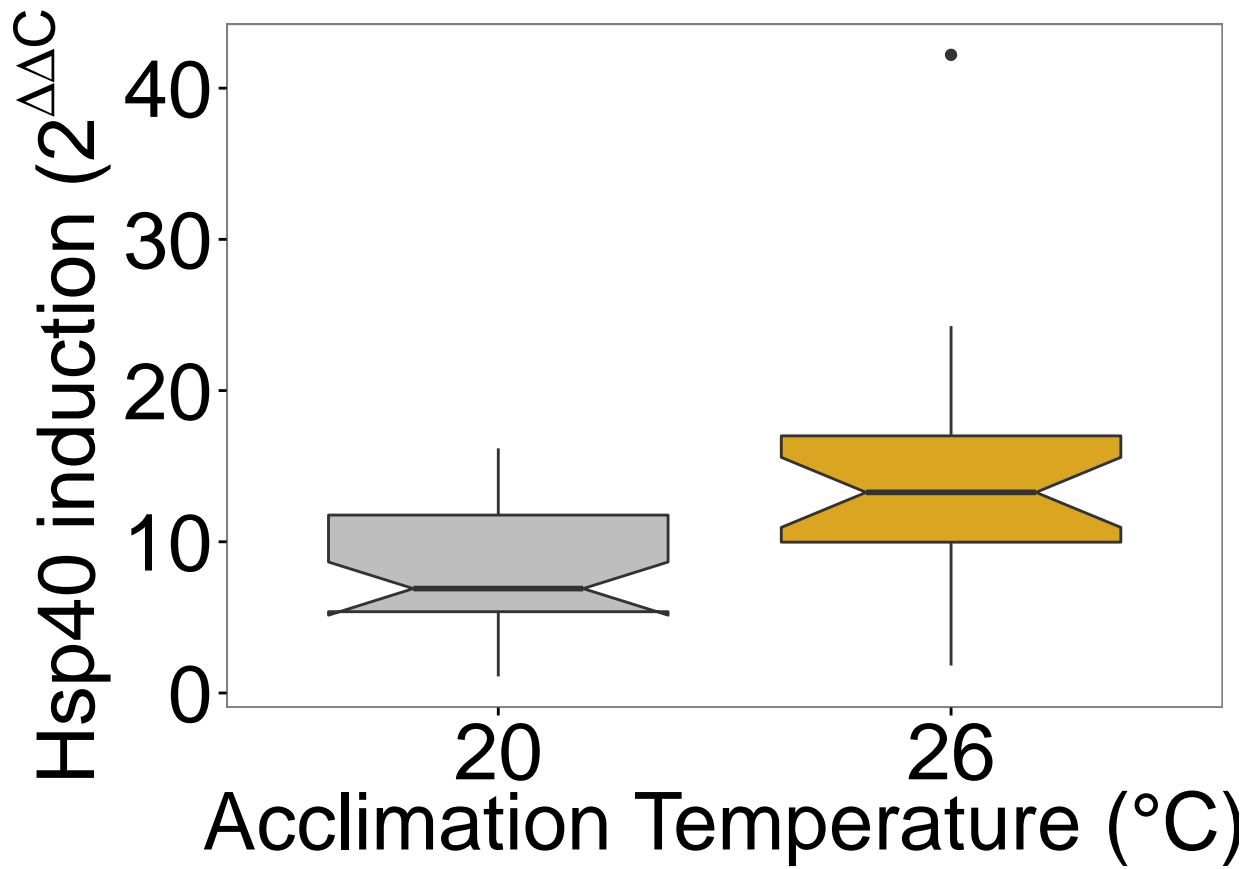
```
#####ggplot of hsp70 induction
```

```
induc_hsp70<-ggplot(data=merg,aes(x=factor(Rearing_Temp),y=FC_70,fill=factor(Rearing_Temp)))+geom_boxplot()
induc_hsp70
```



```
#####ggplot of hsp70 induction
#####ggplot of hsp40 induction
induc_hsp40<-ggplot(data=mergy,aes(x=factor(Rearing_Temp),y=FC_40,fill=factor(Rearing_Temp)))+geom_boxp
induc_hsp40
```

```
## notch went outside hinges. Try setting notch=FALSE.
```



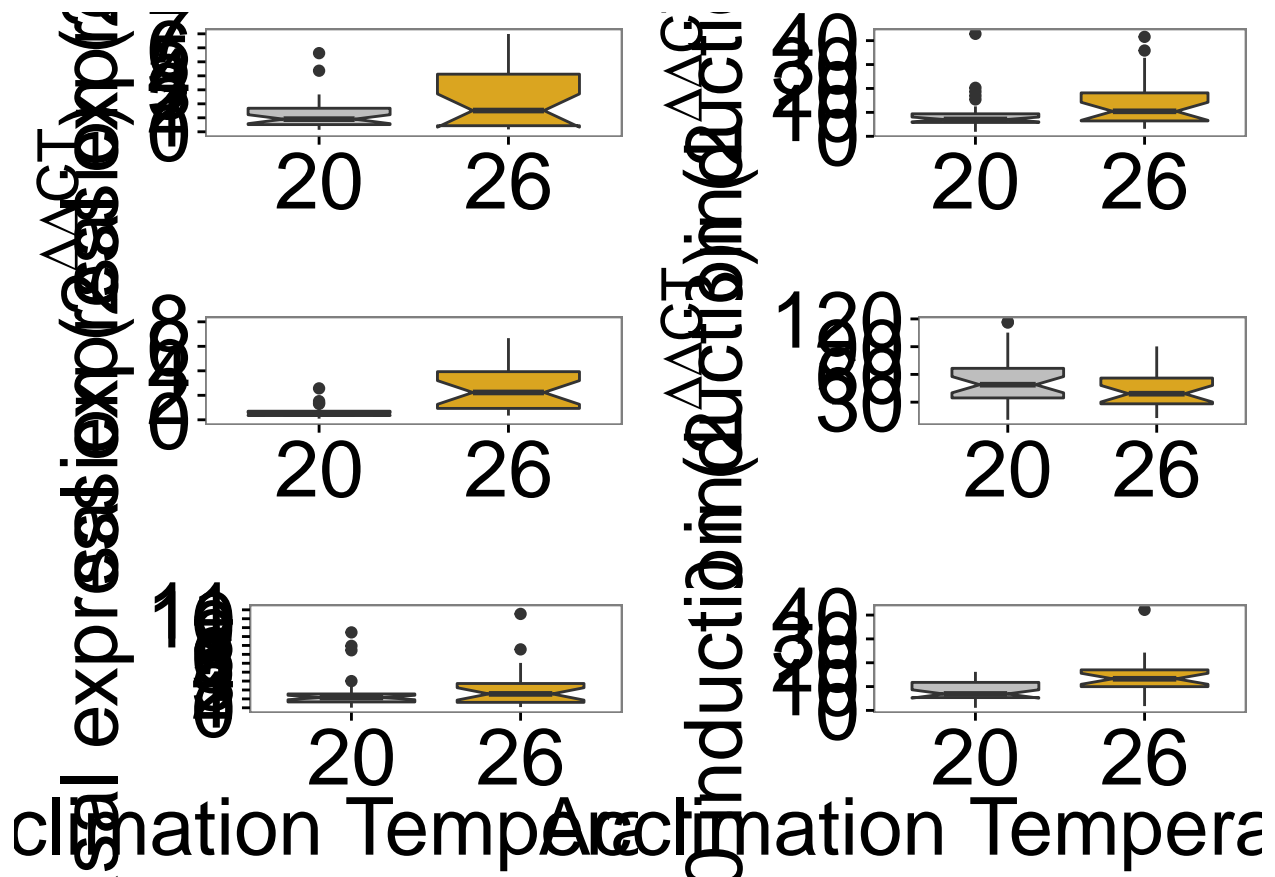
```
#+scale_y_continuous(limits=c(0,8),breaks=seq(0,8,2))
```

```
#grid.arrange(basal_83,basal_70,basal_40,induc_hsp83,induc_hsp70,induc_hsp40,nrow=2,ncol=3)
```

```
grid.arrange(basal_83,induc_hsp83,basal_70,induc_hsp70,basal_40,induc_hsp40,nrow=3,ncol=2)
```

```
## notch went outside hinges. Try setting notch=FALSE.
```

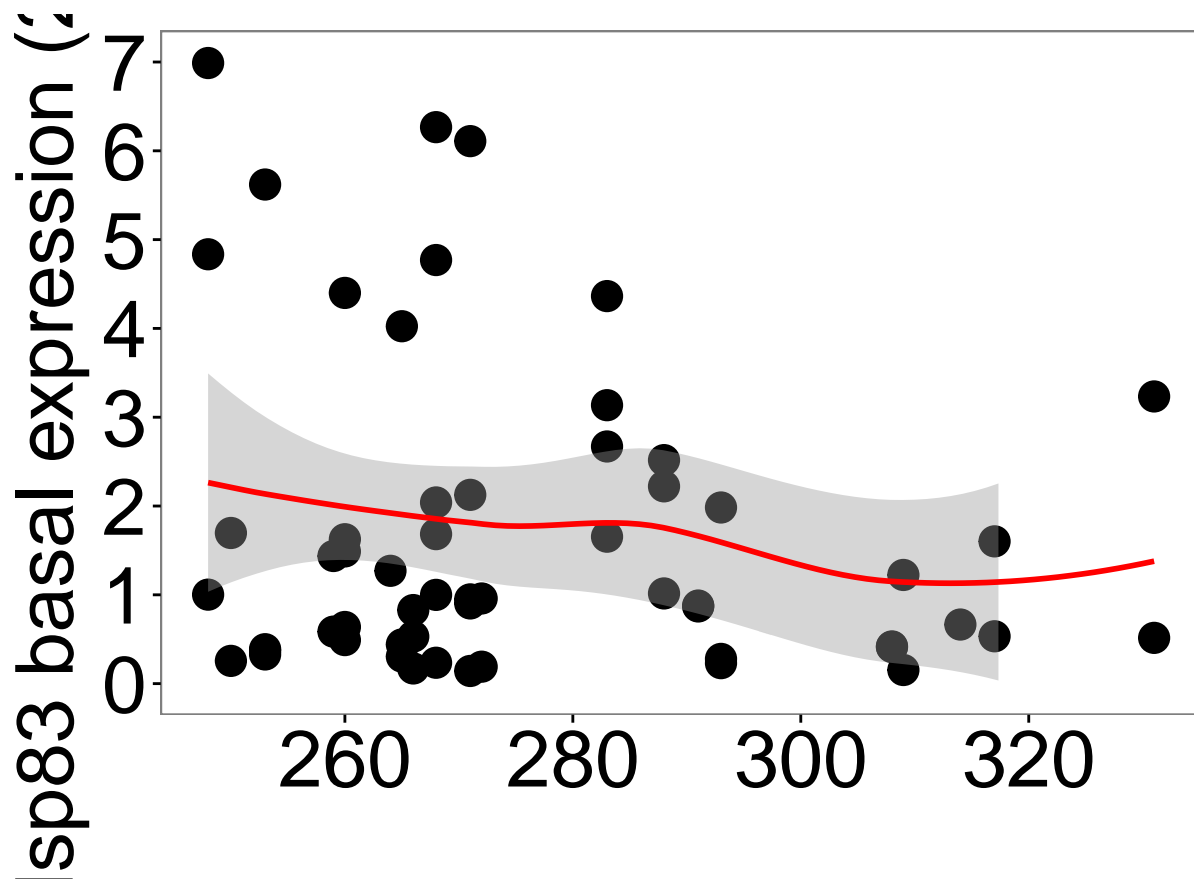
```
## notch went outside hinges. Try setting notch=FALSE.
```



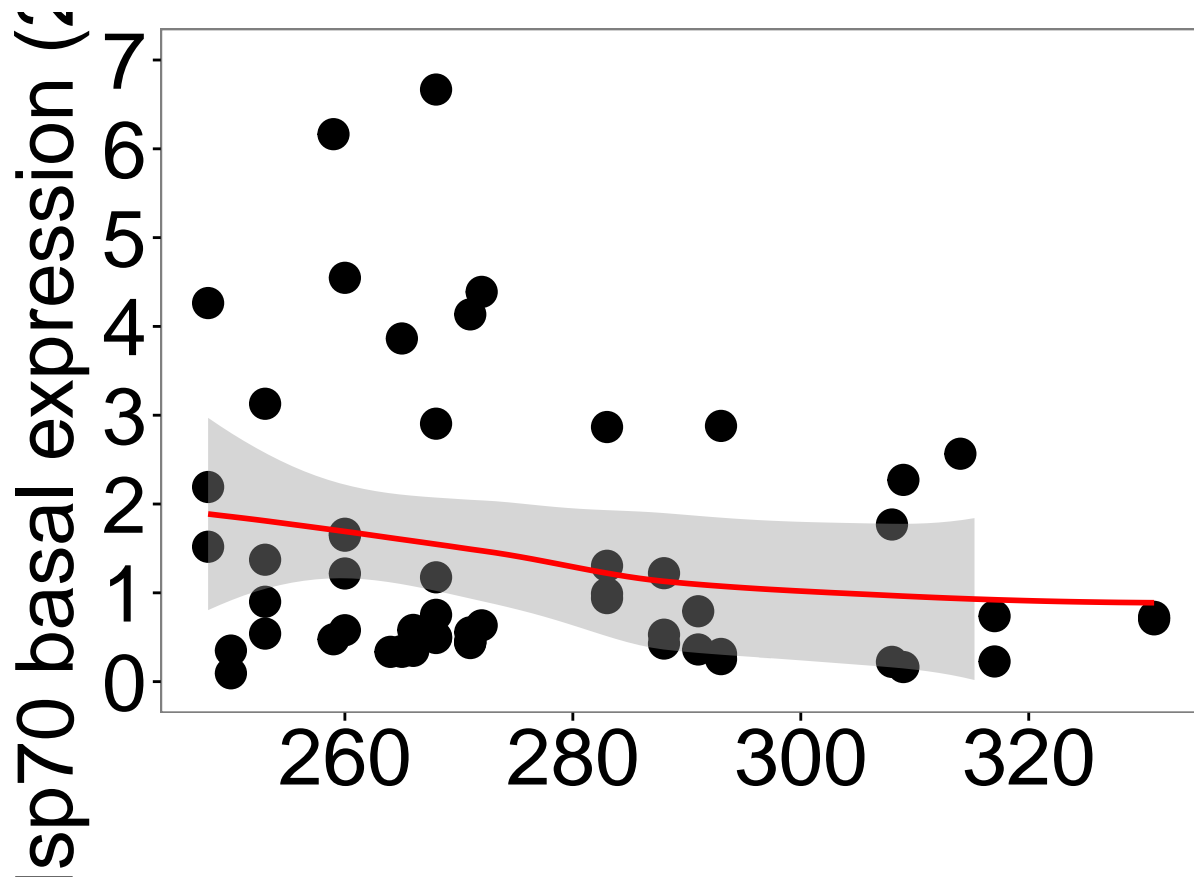
#Expression figures against Tmax

```
T<-theme_bw()+theme(text=element_text(size=30),axis.text=element_text(size=30),legend.text=element_text(size=30))
#loess fit
#loess<-stat_smooth(colour="red",geom="smooth",method="auto",se=FALSE,size=3)
loess<-stat_smooth(colour="red",method="loess",span=1)
#merg$bio5<-merg$bio5/10

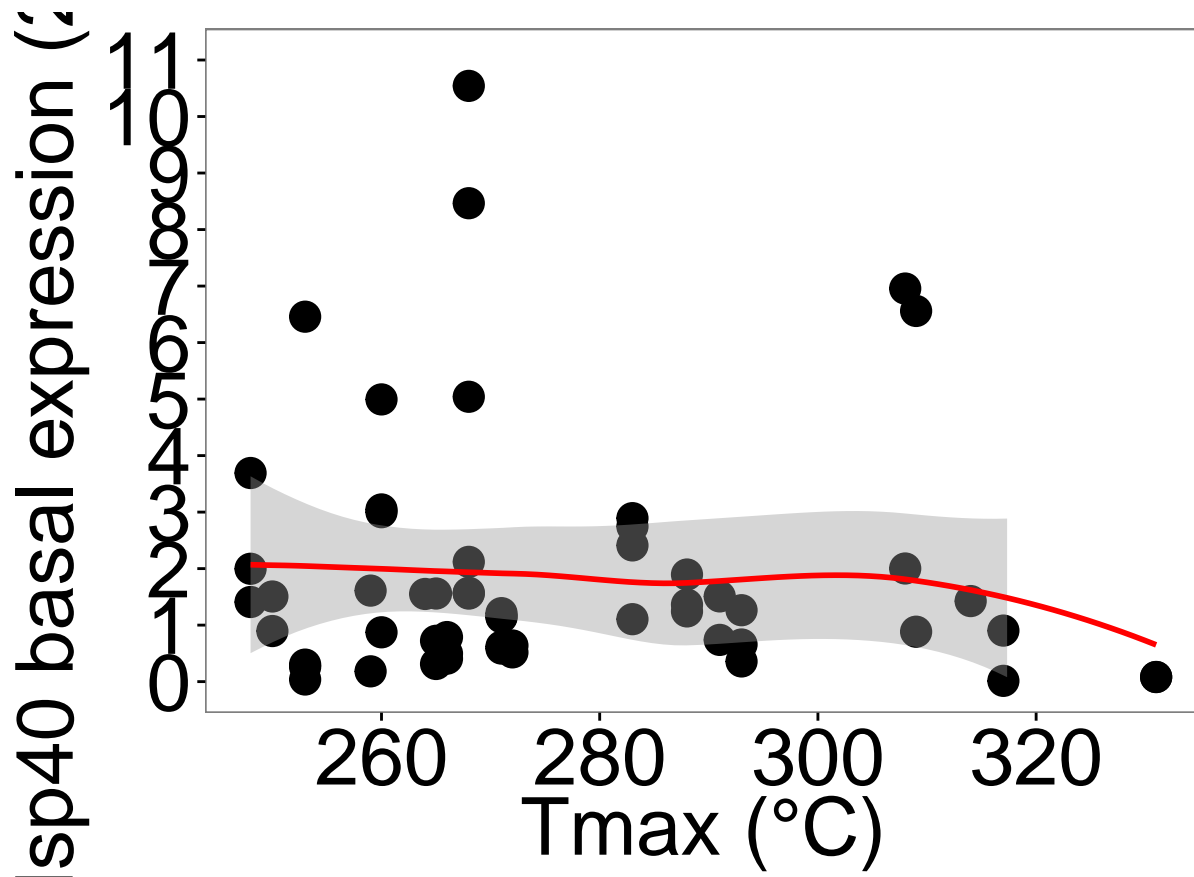
#####hsp83 basal#####
T83<-ggplot(data=merg,aes(x=bio5,y=B_83))+geom_point(size=5)+T+ylab(expression(paste("Hsp83 basal expression",T83)))
```



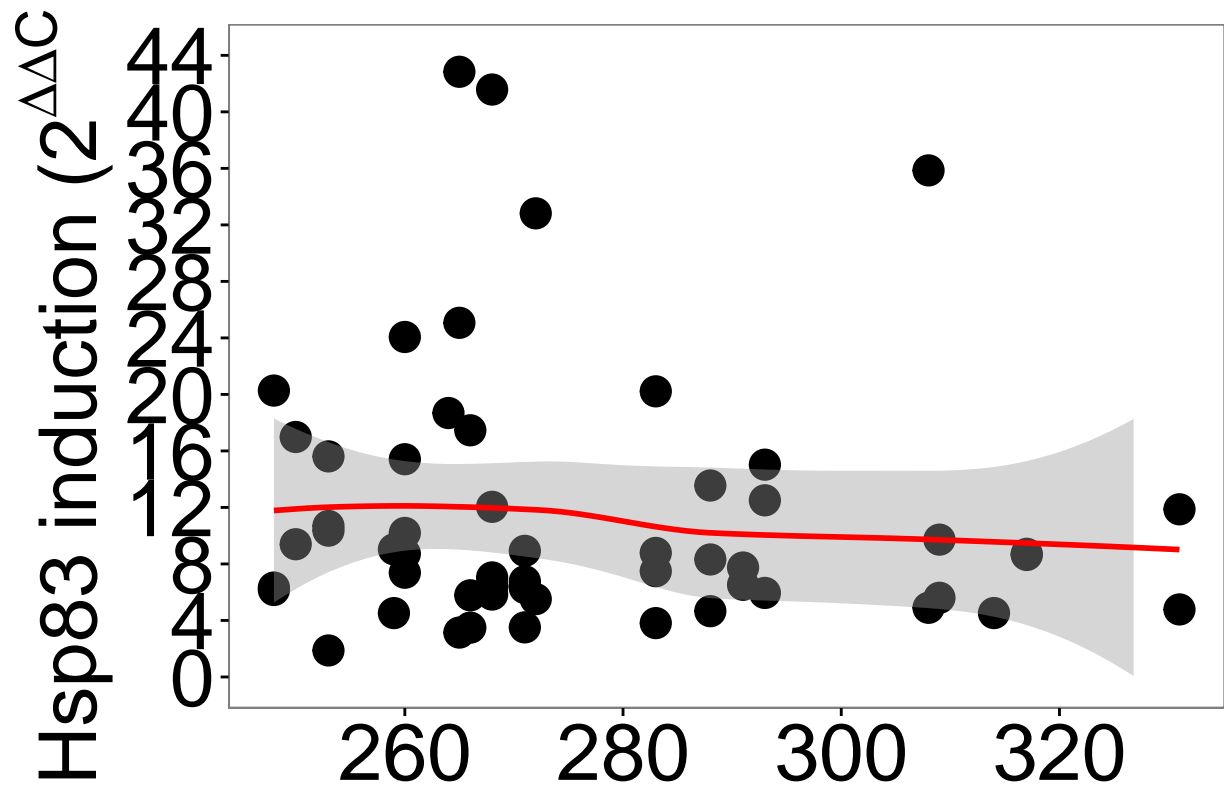
```
#####hsp70 basal#####
T70<-ggplot(data=merg,aes(x=bio5,y=B_70))+geom_point(size=5)+T+ylab(expression(paste("Hsp70 basal expres
T70
```



```
#####hsp40 basal#####
#mergy$bio5<-mergy$bio5/10
T40<-ggplot(data=mergy,aes(x=bio5,y=B_40))+geom_point(size=5)+T+ylab(expression(paste("Hsp40 basal expr
T40
```

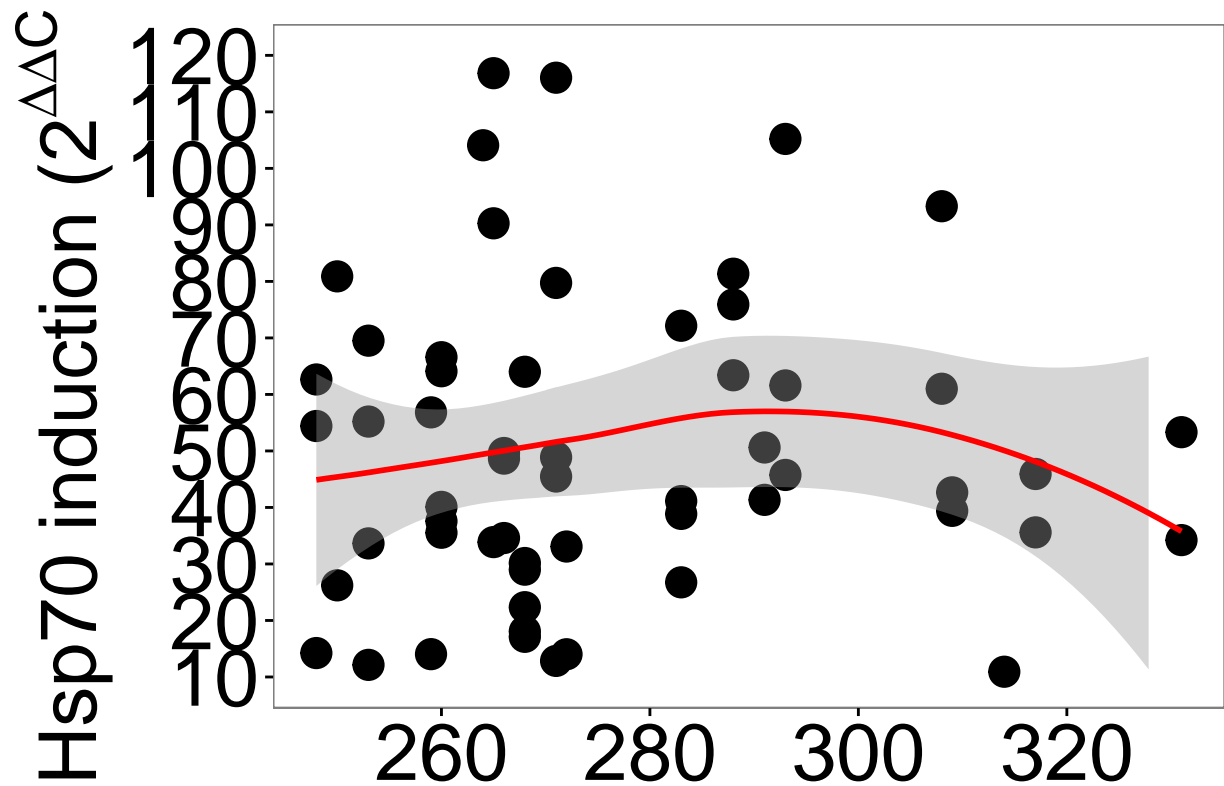


```
#####
#####hsp83 induction#####
T83ind<-ggplot(data=merg,aes(x=bio5,y=FC_83))+geom_point(size=5)+T+ylab(expression(paste("Hsp83 induction")))
T83ind
```

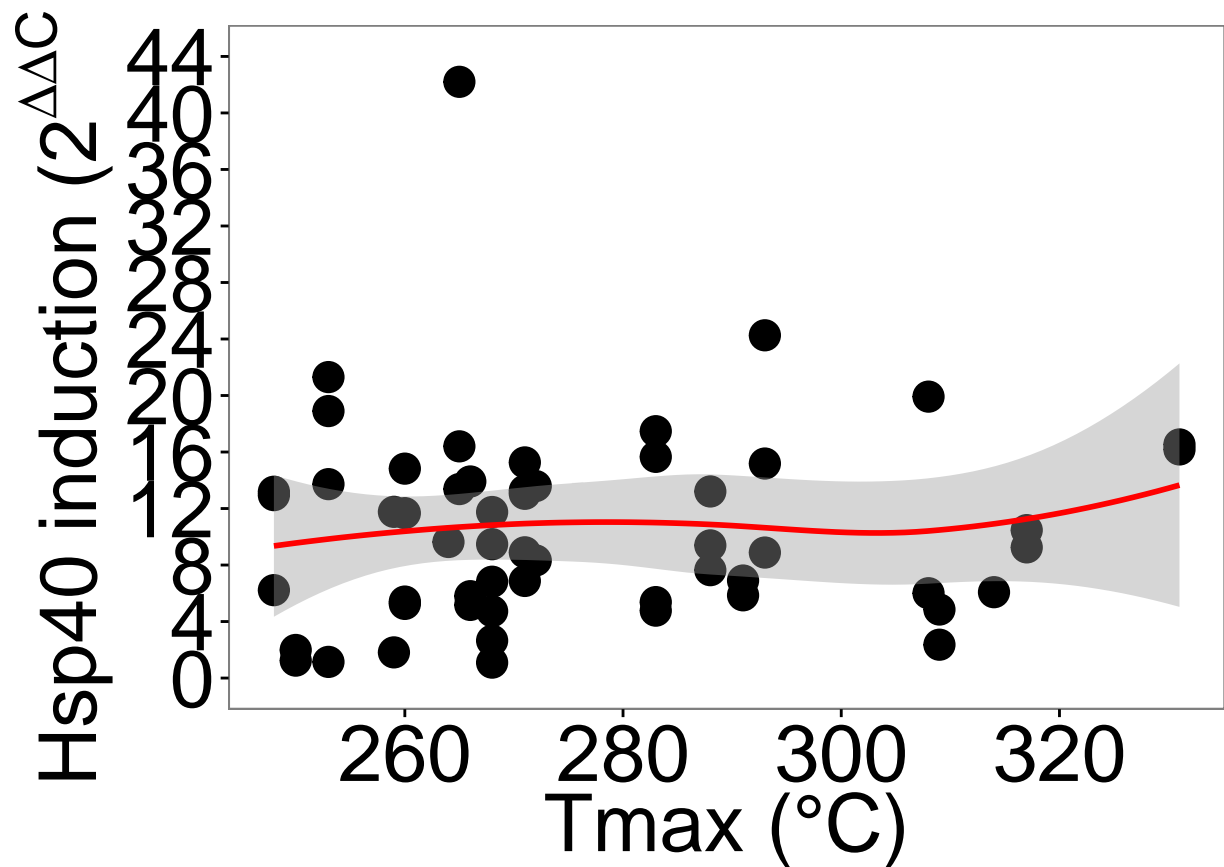


```
#####hsp70 induction#####
```

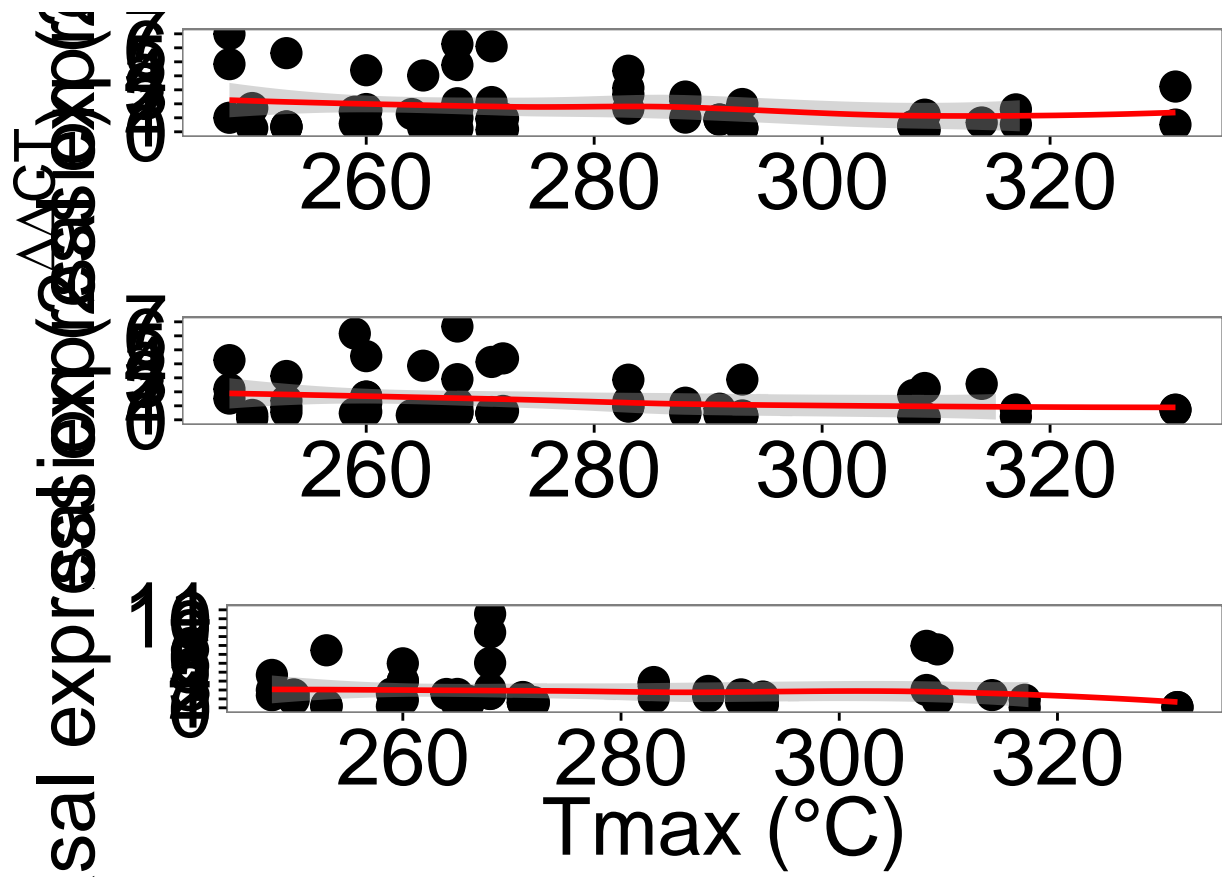
```
T70ind<-ggplot(data=merg,aes(x=bio5,y=FC_70,))+geom_point(size=5)+T+ylab(expression(paste("Hsp70 induction (2^ΔΔC)")))
T70ind
```



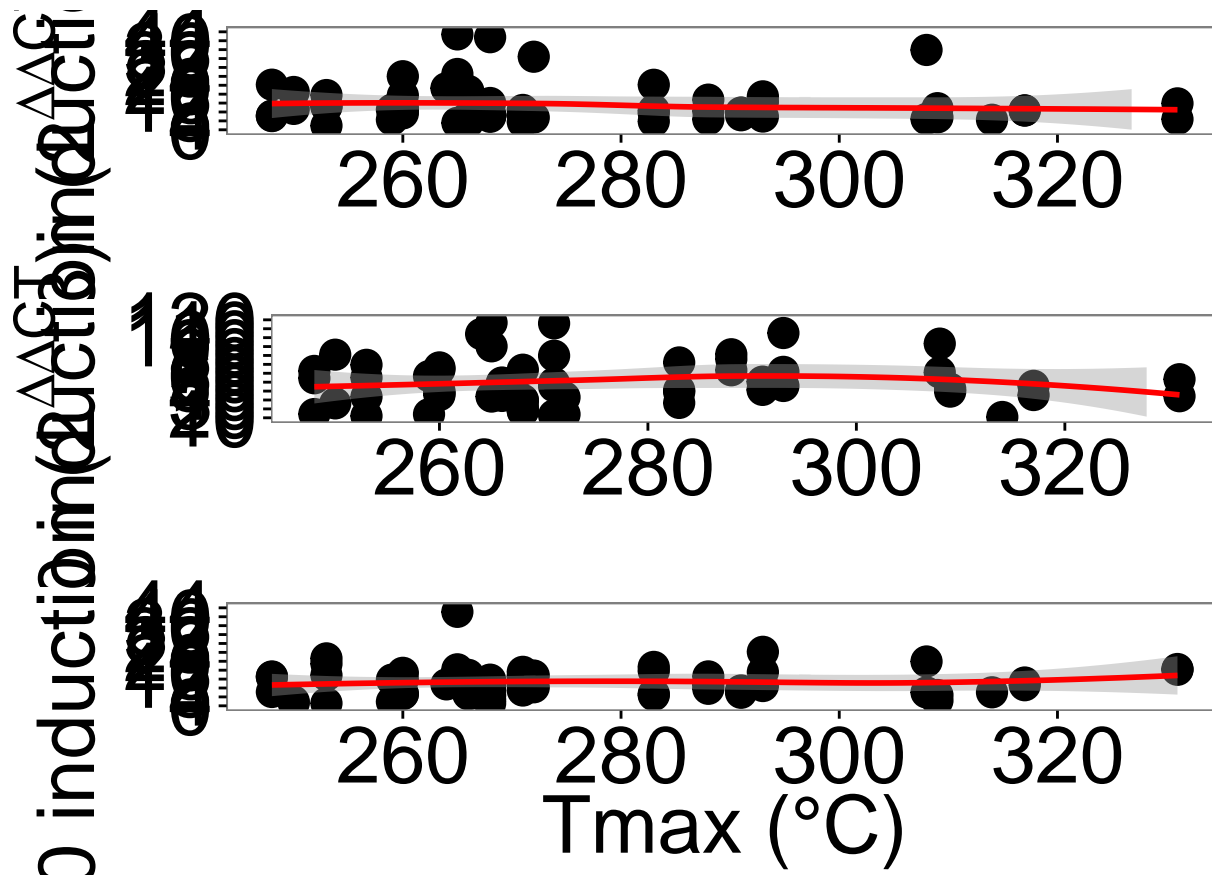

```
#####hsp40 induction#####
T40ind<-ggplot(data=mergy,aes(x=bio5,y=FC_40))+geom_point(size=5)+T+ylab(expression(paste("Hsp40 induc
T40ind
```



```
#grid.arrange(T83,T70,T40,T83ind,T70ind,T40ind,nrow=2,ncol=3)
grid.arrange(T83,T70,T40,nrow=3)
```



```
grid.arrange(T83ind,T70ind,T40ind,nrow=3)
```



#Hsp40 vs Axis 3

```
plot(B_40~Axis.3,data=mergy)
text(mergy$Axis.3,mergy$B_40,labels=mergy$Colony)
#
#hist(mergy$Axis.3)
#regular zoom
plot(mergy$Axis.3,mergy$Axis.2,type="n")
text(mergy$Axis.3,mergy$Axis.2,labels=mergy$Colony)

#zoomed in
plot(mergy$Axis.3,mergy$Axis.2,ylim=c(0.02,0.05))
text(mergy$Axis.3,mergy$Axis.2,labels=mergy$Colony)
#data parsing
mergy<-subset(merg,merg$Axis.2> -0.1)
sub<-subset(merg,merg$Axis.2< -0.1)
sub$axis3_desig<-rep("zAxis 2 A. picea",3)
mergy$axis3_desig<-ifelse(mergy$Axis.3<= -0.044,"North",ifelse(mergy$Axis.3>0.05,"South","A. picea"))
mergy<-rbind(mergy,sub)
mergy<-mergy[-54,]
boxplot(log10(B_40)~axis3_desig,data=mergy)
summary(aov(log10(B_40)~axis3_desig,data=mergy))

newb<-subset(mergy,mergy$axis3_desig=="South")
newb2<-subset(mergy,mergy$axis3_desig=="North")
newb3<-as.data.frame(rbind(newb,newb2))
summary(aov(log10(B_40)~axis3_desig,data=mergy))
```

```

boxplot(B_40~axis3_desig,data=newb3,las=1,xlab="A. rudis clade (Axis 3)",ylab="log10 Hsp40 Basal expres

###ggplot fig
Axis3_b40<-ggplot(data=newb3,aes(x=factor(axis3_desig),y=B_40,fill=factor(axis3_desig)))+geom_boxplot()
#+xlab("A. rudis clade (Axis 3)")
Axis3_b40

###with all 3
Axis3_b40_v2<-ggplot(data=mergy,aes(x=factor(axis3_desig),y=B_40,color=factor(axis3_desig)))+geom_jitter(
  ylab(expression(paste("Hsp40 basal expression (",2~paste(Delta,Delta,"CT"),")")))+
  scale_y_continuous(limits=c(0,7),breaks=seq(0,7,1))+
  scale_colour_manual(name = "", values = c("black","deepskyblue4", "firebrick"))+guides(fill=FALSE)+xlab("A. rudis clade (Axis 3)")
Axis3_b40_v2

###v3

#grab medians
meds <- c(by(mergy$B_40, mergy$axis3_desig, median))
means <- c(by(mergy$B_40, mergy$axis3_desig, mean))

Axis3_b40_v3<-ggplot(data=mergy,aes(x=factor(axis3_desig),y=B_40,fill=factor(axis3_desig)))+geom_boxplot(
  ylab(expression(paste("Hsp40 basal expression (",2~paste(Delta,Delta,"CT"),")")))+scale_x_discrete(exp
  scale_y_continuous(limits=c(-1,11),breaks=seq(0,11,1))+
  scale_fill_manual(name = "", values = c("gray","deepskyblue4", "firebrick","purple"))+guides(fill=FALSE)
Axis3_b40_v3

boxplot(B_40~axis3_desig,data=mergy)

```

hsp40 vs axis2

```

#regular zoom
plot(mergy$Axis.3,mergy$Axis.2,type="n")
text(mergy$Axis.3,mergy$Axis.2,labels=mergy$Colony)

mergy$desig_axis2<-ifelse(mergy$Axis.2<= -.1,"hybrids","picea/rudis")

par(mar=c(5,5,5,5))
boxplot(B_40~desig_axis2,data=mergy,ylab="hsp40 basal expression",xlab="Axis 2 designations")#looking
boxplot(log10(B_40)~desig_axis2,data=mergy,ylab="Log10 hsp40 basal expression",xlab="Axis 2 designation

```

older stats

making maps for a talk

field collections site map

```
w <- getData('worldclim', var='bio', res=2.5)

plot(w, 5, xlim=c(-87,-65), ylim=c(30,48), axes=F, legend=T, col=colorRampPalette(c("skyblue","white")))
#map("world",c("USA","Canada"),add=TRUE)
map("state", c('florida', 'south carolina', 'north carolina', 'georgia', 'virginia', 'west virginia', 'texas'))
rect(-150,25,-55,5,col="white",border="white")
rect(-65,50,-50,25,col="white",border="white")
rect(-87,50,-100,25,col="white",border="white")
bb8<-read.csv("../Data/20160219_site_for_map_excludingsome_v2.csv")
colnm<-ifelse(merg$Axis.1>.17,"#D55E00","#0072B2")
points(merg$lon.2,merg$lat,pch=20,col=colnm,cex=1.75)
#points(bb8$lon.2,bb8$lat,pch=20,cex=1.5)
text(bb8$lon.3,bb8$lat+.4,labels=bb8$Site_2,cex=.75,font=2)
#plotting points with the size related to thermal niche breadth
#add color by species
#http://www.cookbook-r.com/Graphs/Colors_(ggplot2)/#a-colorblind-friendly-palette

#text(merg$lon.2,merg$lat+.5,labels=merg$Site_2,cex=.75)

cpsit<-ddply(merg,.(Site_2),summarize,Longitude=mean(lon.2),Latitude=mean(lat),Tmax=mean(bio5))
head(cpsit)
```

```
##   Site_2 Longitude Latitude Tmax
## 1    AP -73.85635  42.71930  283
## 2    BA -73.91630  42.01740  288
## 3    BE -71.34803  43.09943  271
## 4    BM -74.02140  41.40405  271
## 5    BP -81.95380  35.92640  260
## 6    BR -68.51740  44.98180  260
```

```
dim(cpsit)
```

```
## [1] 24  4
```

```
cpsit
```

```
##   Site_2 Longitude Latitude Tmax
## 1    AP -73.85635  42.71930  283
## 2    BA -73.91630  42.01740  288
## 3    BE -71.34803  43.09943  271
## 4    BM -74.02140  41.40405  271
```

```
## 5      BP -81.95380 35.92640 260
## 6      BR -68.51740 44.98180 260
## 7      DF -75.01010 41.30233 260
## 8      DW -83.94955 35.91995 309
## 9      EW -73.19690 44.43970 272
## 10     GP -83.49340 35.63650 250
## 11     HF -72.18980 42.53130 264
## 12     HP -75.71751 41.02210 268
## 13     HW -81.73115 33.55605 331
## 14     IJ -83.86400 35.95570 308
## 15     KH -69.92110 44.56755 259
## 16     MB -72.64000 44.50000 253
## 17     MM -71.13913 44.11107 265
## 18     NK -75.25890 40.43940 291
## 19     NO -73.01277 42.49833 248
## 20     RC -79.07720 36.03640 314
## 21     RW -73.48550 44.49060 268
## 22     SE -70.58310 43.92370 266
## 23     UN -79.97450 35.36930 317
## 24     WP -76.07883 39.72570 293
```

```
new<-cpsit[order(cpsit$Tmax),]
new
```

```
##      Site_2 Longitude Latitude Tmax
## 19      NO -73.01277 42.49833 248
## 10      GP -83.49340 35.63650 250
## 16      MB -72.64000 44.50000 253
## 15      KH -69.92110 44.56755 259
## 5       BP -81.95380 35.92640 260
## 6       BR -68.51740 44.98180 260
## 7       DF -75.01010 41.30233 260
## 11      HF -72.18980 42.53130 264
## 17      MM -71.13913 44.11107 265
## 22      SE -70.58310 43.92370 266
## 12      HP -75.71751 41.02210 268
## 21      RW -73.48550 44.49060 268
## 3       BE -71.34803 43.09943 271
## 4       BM -74.02140 41.40405 271
## 9       EW -73.19690 44.43970 272
## 1       AP -73.85635 42.71930 283
## 2       BA -73.91630 42.01740 288
## 18      NK -75.25890 40.43940 291
## 24      WP -76.07883 39.72570 293
## 14      IJ -83.86400 35.95570 308
## 8       DW -83.94955 35.91995 309
## 20      RC -79.07720 36.03640 314
## 23      UN -79.97450 35.36930 317
## 13      HW -81.73115 33.55605 331
```

```
#write.csv(new,"Sampling_sites_table.csv")
```

20160825__sampling map

```
fgl<-read.csv("20160825_final_dataset_HSP_modulation.csv")
fgl$tree_color<-as.character(fgl$tree_color)
```

```
plot(w, 5, xlim=c(-87,-65), ylim=c(30,48), axes=FALSE, legend=FALSE, col=colorRampPalette(c("skyblue", "white"))
#map("world", c("USA", "Canada"), add=TRUE)
map("state", c('florida', 'south carolina', 'north carolina', 'georgia', 'virginia', 'west virginia', 'texas'), fill="white",
rect(-150,25,-55,5,col="white",border="white")
rect(-65,50,-50,25,col="white",border="white")
rect(-87,50,-100,25,col="white",border="white")

points(fgl$lon.2,fgl$lat,col=fgl$tree_color,pch=19)
#la<-subset(bb8,bb8$Site=="LA")
points(-79.181,37.4211,pch=19,col="blue")
#text(fgl$lon.3,fgl$lat+.4,labels=fgl$Site_2,cex=.75,font=2)
text(bb8$lon.3,bb8$lat+.4,labels=bb8$Site_2,cex=.75,font=2)
```

```
sessionInfo()
```

```
## R version 3.3.1 (2016-06-21)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: OS X 10.11.6 (El Capitan)
##
## locale:
##  [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
##
## other attached packages:
##  [1] mapdata_2.2-6  maps_3.1.1    maptools_0.8-39  dismo_1.1-1
##  [5] rgdal_1.1-10   raster_2.5-8  sp_1.2-3         gridExtra_2.2.1
##  [9] ggplot2_2.1.0  MASS_7.3-45   ape_3.5          MuMIn_1.15.6
## [13] dplyr_0.5.0    plyr_1.8.4
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.7    formatR_1.4    highr_0.6        tools_3.3.1
##  [5] digest_0.6.10  evaluate_0.9    tibble_1.2        gtable_0.2.0
##  [9] nlme_3.1-128   lattice_0.20-33 Matrix_1.2-6      DBI_0.5-1
## [13] yaml_2.1.13    stringr_1.1.0  knitr_1.14        stats4_3.3.1
## [17] grid_3.3.1     R6_2.1.3       foreign_0.8-66    rmarkdown_1.0
## [21] magrittr_1.5    scales_0.4.0    htmltools_0.3.5   assertthat_0.1
## [25] colorspace_1.2-6 labeling_0.3     stringi_1.1.2     lazyeval_0.2.0
## [29] munsell_0.4.3
```