

Evolution of Stress response in Aphaenogaster

Andrew Nguyen

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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.

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!
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
```

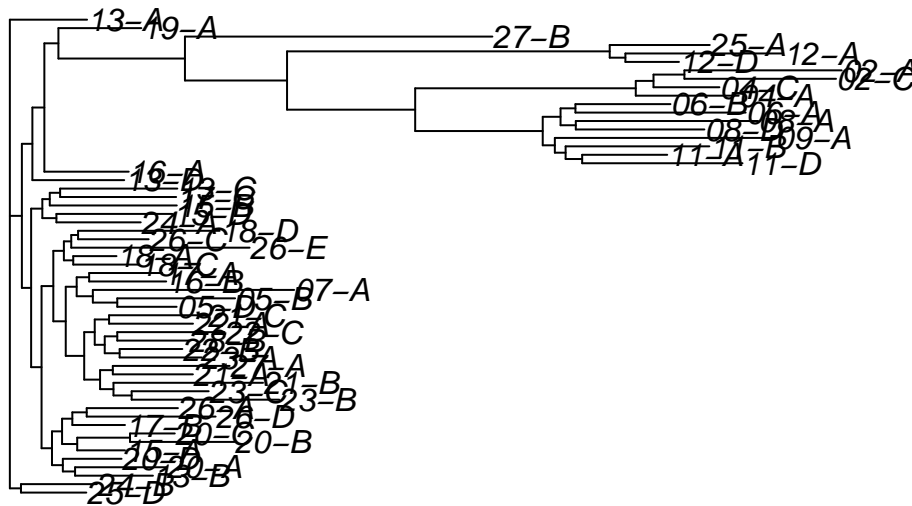
```
summary(as.factor(as.character(merg$Rearing_Temp)))#the number of colonies reared at 20 and 26
```

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

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

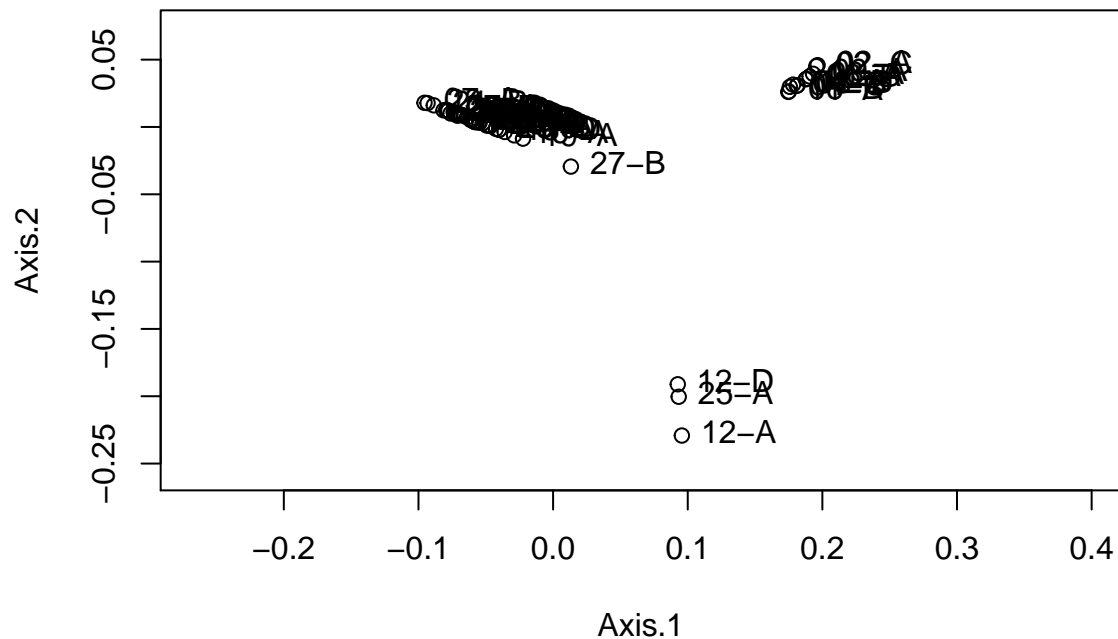
	ID	Colony	Species	Species_2	Site	Site_2	Tree_site	Colony.ID	Rearing_Temp	initial_w
18	ApGXL-11-C	11-C	fulva	fulva	WP	WP	WP3	WP3	26	
34	ApGXL-18-A	18-A	fulva	fulva	Notch	NO	NO1	Notch1	20	
36	ApGXL-18-D	18-D	fulva	fulva	Notch	NO	NO3	Notch2	26	

```
#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)
```



```
write.tree(jsg.tree,"20161012_Hsp_modulation_phylogeny.newick")
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
```

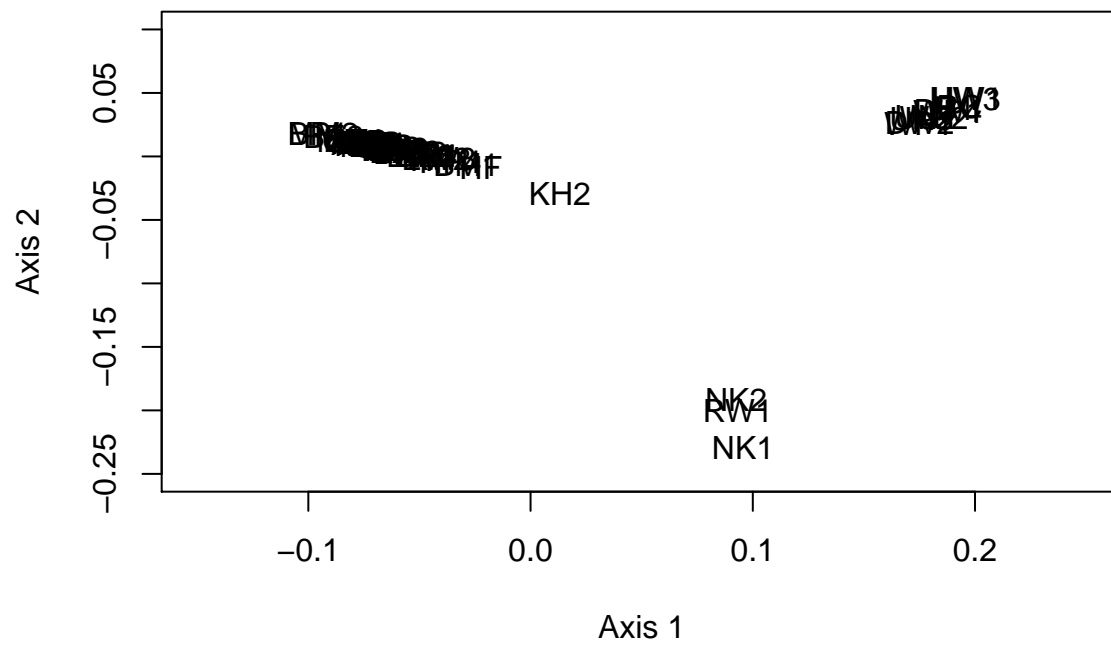
```
## 33 24
```

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

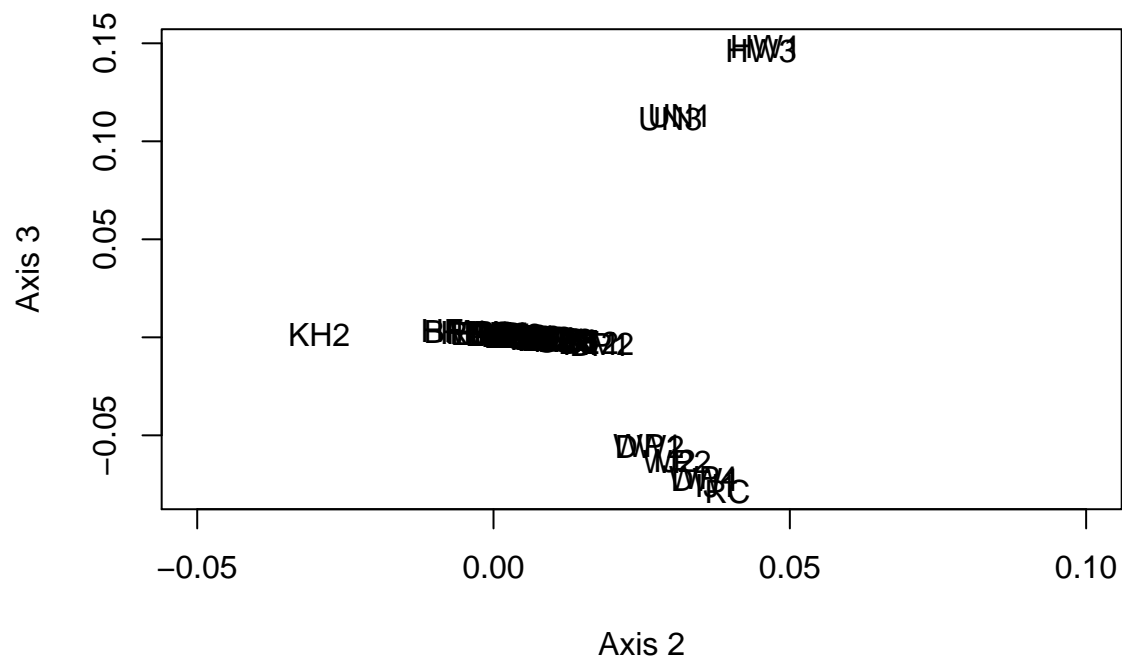
```
ddply(merg,.(Site),summarize,count=length(Colony))#counts of colonies at each site
```

```
##      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)
```

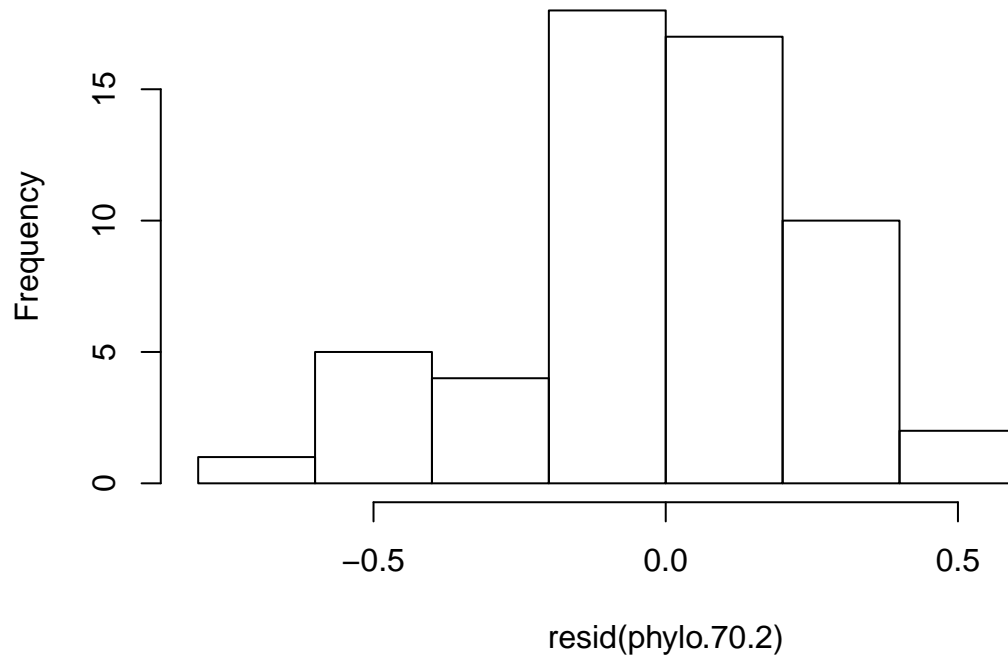


checking axes

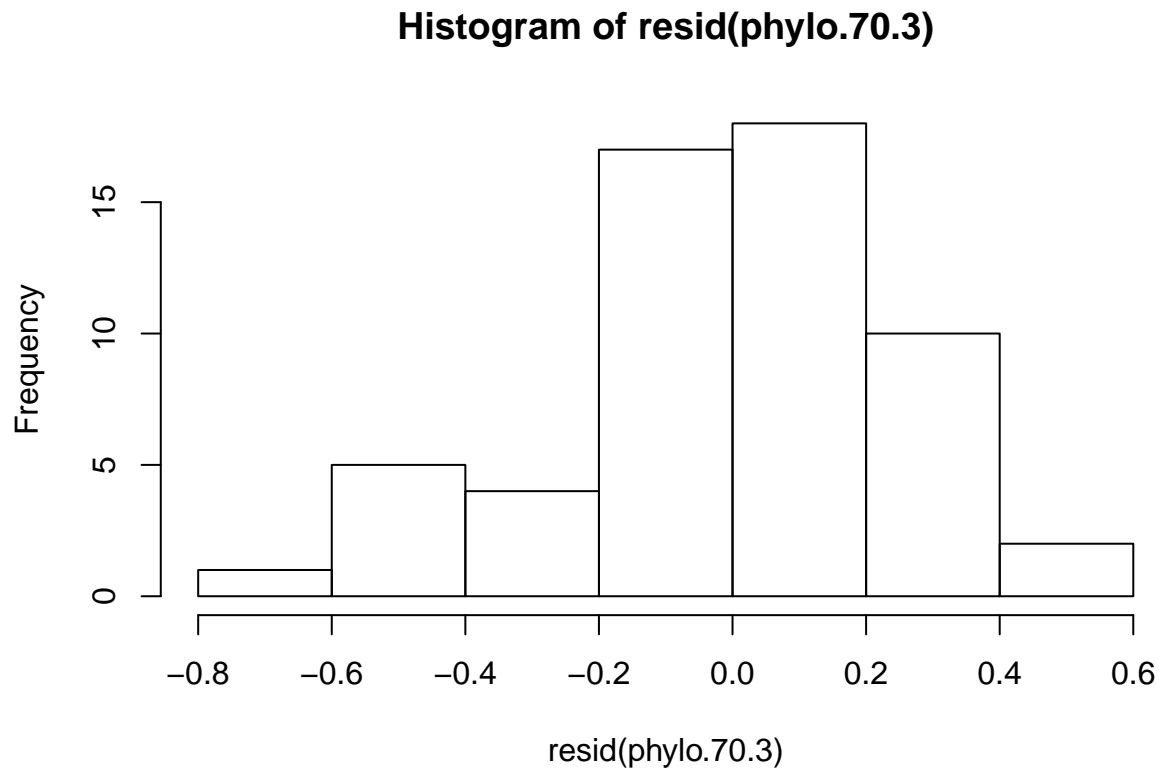
```
#phylo.70.1<-lm(log10(merg$FC_70)~merg$Axis.1)
#hist(resid(phylo.70.1),xlim=c(-.8,.8))
```

```
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))
```



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), phylo axes
```

```
#with Tmax
```

```
apply(merge[,38:43],2,function(x){summary(stepAIC(lm(log10(x)~merge$bio5+merge$Rearing_Temp+merge$Axis.1+me
```

```
## Start:  AIC=-135.83
## log10(x) ~ merge$bio5 + merge$Rearing_Temp + merge$Axis.1 + merge$Axis.2 +
##      merge$Axis.3
##
##              Df Sum of Sq  RSS   AIC
## - merge$Axis.3    1   0.00006 4.2616 -137.82
## - merge$Axis.2    1   0.00563 4.2671 -137.75
## - merge$Axis.1    1   0.03032 4.2918 -137.42
## - merge$bio5      1   0.05267 4.3142 -137.12
## <none>                        4.2615 -135.83
## - merge$Rearing_Temp 1   0.32622 4.5877 -133.62
##
## Step:  AIC=-137.82
## log10(x) ~ merge$bio5 + merge$Rearing_Temp + merge$Axis.1 + merge$Axis.2
##
```

```

##          Df Sum of Sq    RSS    AIC
## - merg$Axis.2      1  0.00557 4.2671 -139.75
## - merg$Axis.1      1  0.03288 4.2944 -139.39
## - merg$bio5        1  0.05995 4.3215 -139.03
## <none>                4.2616 -137.82
## - merg$Rearing_Temp 1  0.32790 4.5895 -135.60
##
## Step:  AIC=-139.75
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1
##
##          Df Sum of Sq    RSS    AIC
## - merg$Axis.1      1  0.02927 4.2964 -141.36
## - merg$bio5        1  0.05486 4.3220 -141.02
## <none>                4.2671 -139.75
## - merg$Rearing_Temp 1  0.35722 4.6243 -137.17
##
## Step:  AIC=-141.36
## log10(x) ~ merg$bio5 + merg$Rearing_Temp
##
##          Df Sum of Sq    RSS    AIC
## - merg$bio5        1  0.02771 4.3241 -142.99
## <none>                4.2964 -141.36
## - merg$Rearing_Temp 1  0.33717 4.6336 -139.05
##
## Step:  AIC=-142.99
## log10(x) ~ merg$Rearing_Temp
##
##          Df Sum of Sq    RSS    AIC
## <none>                4.3241 -142.99
## - merg$Rearing_Temp 1  0.3481 4.6722 -140.58
## Start:  AIC=-147.19
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##      merg$Axis.3
##
##          Df Sum of Sq    RSS    AIC
## - merg$Axis.1      1  0.009107 3.5000 -149.05
## - merg$Axis.3      1  0.009894 3.5008 -149.03
## - merg$bio5        1  0.016701 3.5076 -148.92
## - merg$Axis.2      1  0.046939 3.5379 -148.43
## <none>                3.4909 -147.19
## - merg$Rearing_Temp 1  0.215627 3.7065 -145.78
##
## Step:  AIC=-149.05
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.2 + merg$Axis.3
##
##          Df Sum of Sq    RSS    AIC
## - merg$Axis.3      1  0.005260 3.5053 -150.96
## - merg$bio5        1  0.008554 3.5086 -150.91
## - merg$Axis.2      1  0.057491 3.5575 -150.12
## <none>                3.5000 -149.05
## - merg$Rearing_Temp 1  0.210727 3.7107 -147.71
##
## Step:  AIC=-150.96
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.2

```



```

##
##           Df Sum of Sq   RSS   AIC
## - merg$bio5      1  0.006235 3.5115 -152.86
## - merg$Axis.2     1  0.059127 3.5644 -152.01
## <none>                3.5053 -150.96
## - merg$Rearing_Temp 1  0.218048 3.7233 -149.52
##
## Step: AIC=-152.86
## log10(x) ~ merg$Rearing_Temp + merg$Axis.2
##
##           Df Sum of Sq   RSS   AIC
## - merg$Axis.2     1  0.065809 3.5773 -153.80
## <none>                3.5115 -152.86
## - merg$Rearing_Temp 1  0.225290 3.7368 -151.31
##
## Step: AIC=-153.8
## log10(x) ~ merg$Rearing_Temp
##
##           Df Sum of Sq   RSS   AIC
## <none>                3.5773 -153.8
## - merg$Rearing_Temp 1  0.18654 3.7639 -152.9
## Start: AIC=-122.77
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##      merg$Axis.3
##
##           Df Sum of Sq   RSS   AIC
## - merg$Axis.3     1  0.01759 5.0640 -124.58
## - merg$Axis.1     1  0.03695 5.0833 -124.37
## - merg$bio5       1  0.09873 5.1451 -123.69
## - merg$Axis.2     1  0.14349 5.1899 -123.20
## <none>                5.0464 -122.77
## - merg$Rearing_Temp 1  0.61137 5.6577 -118.37
##
## Step: AIC=-124.58
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2
##
##           Df Sum of Sq   RSS   AIC
## - merg$Axis.1     1  0.06171 5.1257 -125.90
## - merg$Axis.2     1  0.13474 5.1987 -125.11
## - merg$bio5       1  0.15531 5.2193 -124.89
## <none>                5.0640 -124.58
## - merg$Rearing_Temp 1  0.62522 5.6892 -120.06
##
## Step: AIC=-125.9
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.2
##
##           Df Sum of Sq   RSS   AIC
## - merg$bio5       1  0.11746 5.2431 -126.63
## - merg$Axis.2     1  0.17282 5.2985 -126.04
## <none>                5.1257 -125.90
## - merg$Rearing_Temp 1  0.66713 5.7928 -121.05
##
## Step: AIC=-126.63
## log10(x) ~ merg$Rearing_Temp + merg$Axis.2

```

```

##
##           Df Sum of Sq    RSS    AIC
## <none>                5.2431 -126.63
## - merg$Axis.2         1    0.21853 5.4617 -126.35
## - merg$Rearing_Temp   1    0.63456 5.8777 -122.23
## Start:  AIC=-85.77
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##      merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## - merg$Axis.2         1    0.09471 10.350 -87.247
## - merg$bio5           1    0.14357 10.399 -86.979
## - merg$Axis.3         1    0.17560 10.431 -86.803
## - merg$Rearing_Temp   1    0.34221 10.597 -85.900
## <none>                10.255 -85.771
## - merg$Axis.1         1    0.51791 10.773 -84.963
##
## Step:  AIC=-87.25
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## - merg$bio5           1    0.09885 10.449 -88.705
## - merg$Axis.3         1    0.20541 10.555 -88.127
## - merg$Rearing_Temp   1    0.28656 10.636 -87.690
## <none>                10.350 -87.247
## - merg$Axis.1         1    0.45249 10.802 -86.808
##
## Step:  AIC=-88.71
## log10(x) ~ merg$Rearing_Temp + merg$Axis.1 + merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## - merg$Rearing_Temp   1    0.30750 10.756 -89.052
## <none>                10.449 -88.705
## - merg$Axis.3         1    0.37408 10.823 -88.700
## - merg$Axis.1         1    0.60533 11.054 -87.495
##
## Step:  AIC=-89.05
## log10(x) ~ merg$Axis.1 + merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## <none>                10.756 -89.052
## - merg$Axis.3         1    0.42229 11.178 -88.857
## - merg$Axis.1         1    0.71553 11.472 -87.381
## Start:  AIC=-122.03
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##      merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## - merg$bio5           1    0.0001 5.4282 -124.032
## - merg$Axis.2         1    0.0329 5.4610 -123.689
## - merg$Axis.1         1    0.0409 5.4690 -123.605
## - merg$Axis.3         1    0.0666 5.4947 -123.338
## <none>                5.4281 -122.033
## - merg$Rearing_Temp   1    4.5125 9.9406 -89.546

```

```

##
## Step: AIC=-124.03
## log10(x) ~ merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 + merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## - merg$Axis.2      1    0.0357  5.4639 -125.659
## - merg$Axis.3      1    0.0798  5.5080 -125.200
## - merg$Axis.1      1    0.1695  5.5977 -124.279
## <none>                        5.4282 -124.032
## - merg$Rearing_Temp  1    4.5125  9.9407  -91.545
##
## Step: AIC=-125.66
## log10(x) ~ merg$Rearing_Temp + merg$Axis.1 + merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## - merg$Axis.3      1    0.0784  5.5423 -126.847
## - merg$Axis.1      1    0.1733  5.6372 -125.879
## <none>                        5.4639 -125.659
## - merg$Rearing_Temp  1    4.5377 10.0016  -93.197
##
## Step: AIC=-126.85
## log10(x) ~ merg$Rearing_Temp + merg$Axis.1
##
##           Df Sum of Sq    RSS    AIC
## - merg$Axis.1      1    0.1750  5.7173 -127.075
## <none>                        5.5423 -126.847
## - merg$Rearing_Temp  1    4.4787 10.0209  -95.087
##
## Step: AIC=-127.07
## log10(x) ~ merg$Rearing_Temp
##
##           Df Sum of Sq    RSS    AIC
## <none>                        5.7173 -127.075
## - merg$Rearing_Temp  1    4.7398 10.4571  -94.659
## Start: AIC=-78.04
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##           merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## - merg$bio5      1    0.1636 11.382 -79.225
## <none>                        11.219 -78.036
## - merg$Axis.1      1    0.4666 11.685 -77.754
## - merg$Rearing_Temp  1    0.6847 11.903 -76.718
## - merg$Axis.2      1    0.9679 12.186 -75.402
## - merg$Axis.3      1    3.9432 15.162 -63.168
##
## Step: AIC=-79.23
## log10(x) ~ merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 + merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## <none>                        11.382 -79.225
## - merg$Axis.1      1    0.4311 11.813 -79.144
## - merg$Rearing_Temp  1    0.6969 12.079 -77.897
## - merg$Axis.2      1    0.8346 12.217 -77.263

```

```

## - merg$Axis.3          1      3.9224 15.305 -64.643

## $FC_83
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.61666 -0.14861 -0.03988  0.14529  0.74191
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.36231    0.28484   1.272   0.2087
## merg$Rearing_Temp 0.02638    0.01254   2.104   0.0399 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2804 on 55 degrees of freedom
## Multiple R-squared:  0.07451,    Adjusted R-squared:  0.05768
## F-statistic: 4.428 on 1 and 55 DF,  p-value: 0.03995
##
##
## $FC_70
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.6417 -0.1415  0.0238  0.1711  0.3910
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    2.06670    0.25908   7.977 9.51e-11 ***
## merg$Rearing_Temp -0.01931    0.01140  -1.694   0.096 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.255 on 55 degrees of freedom
## Multiple R-squared:  0.04956,    Adjusted R-squared:  0.03228
## F-statistic: 2.868 on 1 and 55 DF,  p-value: 0.09601
##
##
## $FC_40
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp + merg$Axis.2)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.80392 -0.10073  0.07339  0.22020  0.55569
##
## Coefficients:

```

```

##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)      0.09071    0.32917   0.276   0.7839
## merg$Rearing_Temp 0.03680    0.01453   2.533   0.0143 *
## merg$Axis.2       1.24166    0.83541   1.486   0.1431
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3145 on 53 degrees of freedom
## (1 observation deleted due to missingness)
## Multiple R-squared:  0.166, Adjusted R-squared:  0.1345
## F-statistic: 5.275 on 2 and 53 DF,  p-value: 0.008145
##
##
## $B_83
##
## Call:
## lm(formula = log10(x) ~ merg$Axis.1 + merg$Axis.3)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.89374 -0.32249  0.03374  0.32440  0.77433
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.01073    0.05911   0.182   0.8566
## merg$Axis.1 -1.09010    0.57516  -1.895   0.0634 .
## merg$Axis.3  2.00468    1.37680   1.456   0.1512
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4463 on 54 degrees of freedom
## Multiple R-squared:  0.09566, Adjusted R-squared:  0.06217
## F-statistic: 2.856 on 2 and 54 DF,  p-value: 0.06621
##
##
## $B_70
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.7507 -0.1789 -0.0132  0.2067  0.7046
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  -2.24217    0.32753  -6.846 6.75e-09 ***
## merg$Rearing_Temp 0.09734    0.01442   6.753 9.59e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3224 on 55 degrees of freedom
## Multiple R-squared:  0.4533, Adjusted R-squared:  0.4433
## F-statistic: 45.6 on 1 and 55 DF,  p-value: 9.589e-09

```

```
##
##
## $B_40
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##     merg$Axis.3)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.38234 -0.22276 -0.00071  0.25240  0.84201
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.84687     0.49824  -1.700  0.09527 .
## merg$Rearing_Temp  0.03887     0.02200   1.767  0.08319 .
## merg$Axis.1     -0.85399     0.61446  -1.390  0.17062
## merg$Axis.2     -2.42734     1.25523  -1.934  0.05870 .
## merg$Axis.3     -6.12416     1.46081  -4.192  0.00011 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4724 on 51 degrees of freedom
## (1 observation deleted due to missingness)
## Multiple R-squared:  0.3278, Adjusted R-squared:  0.2751
## F-statistic: 6.218 on 4 and 51 DF,  p-value: 0.0003732
```

#with MAT

```
apply(merg[,38:43],2,function(x){summary(stepAIC(lm(log10(x)~merg$bio1+merg$Rearing_Temp+merg$Axis.1+me
```

```
## Start:  AIC=-135.49
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##     merg$Axis.3
##
##              Df Sum of Sq    RSS    AIC
## - merg$Axis.3    1  0.000233 4.2866 -137.49
## - merg$Axis.2    1  0.007477 4.2938 -137.40
## - merg$Axis.1    1  0.016119 4.3025 -137.28
## - merg$bio1      1  0.027813 4.3142 -137.12
## <none>                        4.2864 -135.49
## - merg$Rearing_Temp 1  0.309988 4.5963 -133.51
##
## Step:  AIC=-137.49
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2
##
##              Df Sum of Sq    RSS    AIC
## - merg$Axis.2    1  0.008150 4.2947 -139.38
## - merg$Axis.1    1  0.019684 4.3063 -139.23
## - merg$bio1      1  0.034925 4.3215 -139.03
## <none>                        4.2866 -137.49
## - merg$Rearing_Temp 1  0.310900 4.5975 -135.50
##
## Step:  AIC=-139.38
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.1
```

```

##
##           Df Sum of Sq   RSS   AIC
## - merg$Axis.1      1    0.01388 4.3086 -141.20
## - merg$bio1        1    0.02726 4.3220 -141.02
## <none>                4.2947 -139.38
## - merg$Rearing_Temp 1    0.34538 4.6401 -136.97
##
## Step: AIC=-141.2
## log10(x) ~ merg$bio1 + merg$Rearing_Temp
##
##           Df Sum of Sq   RSS   AIC
## - merg$bio1        1    0.01549 4.3241 -142.99
## <none>                4.3086 -141.20
## - merg$Rearing_Temp 1    0.33538 4.6440 -138.93
##
## Step: AIC=-142.99
## log10(x) ~ merg$Rearing_Temp
##
##           Df Sum of Sq   RSS   AIC
## <none>                4.3241 -142.99
## - merg$Rearing_Temp 1    0.3481 4.6722 -140.58
## Start: AIC=-147.22
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##      merg$Axis.3
##
##           Df Sum of Sq   RSS   AIC
## - merg$Axis.3      1    0.010355 3.4999 -149.05
## - merg$Axis.1      1    0.011014 3.5006 -149.04
## - merg$bio1        1    0.018029 3.5076 -148.92
## - merg$Axis.2      1    0.031972 3.5216 -148.70
## <none>                3.4896 -147.22
## - merg$Rearing_Temp 1    0.205434 3.6950 -145.96
##
## Step: AIC=-149.05
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2
##
##           Df Sum of Sq   RSS   AIC
## - merg$Axis.1      1    0.005634 3.5056 -150.96
## - merg$bio1        1    0.010642 3.5106 -150.87
## - merg$Axis.2      1    0.039420 3.5394 -150.41
## <none>                3.4999 -149.05
## - merg$Rearing_Temp 1    0.215299 3.7152 -147.65
##
## Step: AIC=-150.96
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.2
##
##           Df Sum of Sq   RSS   AIC
## - merg$bio1        1    0.005941 3.5115 -152.86
## - merg$Axis.2      1    0.054883 3.5605 -152.07
## <none>                3.5056 -150.96
## - merg$Rearing_Temp 1    0.213708 3.7193 -149.58
##
## Step: AIC=-152.86
## log10(x) ~ merg$Rearing_Temp + merg$Axis.2

```

```

##
##           Df Sum of Sq   RSS   AIC
## - merg$Axis.2      1  0.065809 3.5773 -153.80
## <none>                        3.5115 -152.86
## - merg$Rearing_Temp 1  0.225290 3.7368 -151.31
##
## Step: AIC=-153.8
## log10(x) ~ merg$Rearing_Temp
##
##           Df Sum of Sq   RSS   AIC
## <none>                        3.5773 -153.8
## - merg$Rearing_Temp 1  0.18654 3.7639 -152.9
## Start: AIC=-123.24
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##           merg$Axis.3
##
##           Df Sum of Sq   RSS   AIC
## - merg$bio1      1  0.14064 5.1451 -123.69
## - merg$Axis.3      1  0.15637 5.1608 -123.52
## - merg$Axis.1      1  0.16252 5.1670 -123.45
## <none>                        5.0045 -123.24
## - merg$Axis.2      1  0.33591 5.3404 -121.60
## - merg$Rearing_Temp 1  0.58916 5.5936 -119.01
##
## Step: AIC=-123.69
## log10(x) ~ merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 + merg$Axis.3
##
##           Df Sum of Sq   RSS   AIC
## - merg$Axis.1      1  0.02328 5.1684 -125.44
## - merg$Axis.3      1  0.07416 5.2193 -124.89
## <none>                        5.1451 -123.69
## - merg$Axis.2      1  0.21863 5.3637 -123.36
## - merg$Rearing_Temp 1  0.62034 5.7654 -119.31
##
## Step: AIC=-125.44
## log10(x) ~ merg$Rearing_Temp + merg$Axis.2 + merg$Axis.3
##
##           Df Sum of Sq   RSS   AIC
## - merg$Axis.3      1  0.07474 5.2431 -126.63
## <none>                        5.1684 -125.44
## - merg$Axis.2      1  0.22200 5.3904 -125.08
## - merg$Rearing_Temp 1  0.60223 5.7706 -121.26
##
## Step: AIC=-126.63
## log10(x) ~ merg$Rearing_Temp + merg$Axis.2
##
##           Df Sum of Sq   RSS   AIC
## <none>                        5.2431 -126.63
## - merg$Axis.2      1  0.21853 5.4617 -126.35
## - merg$Rearing_Temp 1  0.63456 5.8777 -122.23
## Start: AIC=-86.08
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##           merg$Axis.3
##

```



```

##              Df Sum of Sq    RSS    AIC
## - merg$Axis.2      1   0.14854 10.347 -87.261
## - merg$Axis.3      1   0.15273 10.351 -87.238
## - merg$bio1        1   0.19986 10.399 -86.979
## <none>                                10.199 -86.085
## - merg$Rearing_Temp 1   0.38051 10.579 -85.997
## - merg$Axis.1      1   0.56628 10.765 -85.005
##
## Step: AIC=-87.26
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.3
##
##              Df Sum of Sq    RSS    AIC
## - merg$bio1        1   0.10131 10.449 -88.705
## - merg$Axis.3      1   0.21298 10.560 -88.099
## - merg$Rearing_Temp 1   0.29988 10.647 -87.632
## <none>                                10.347 -87.261
## - merg$Axis.1      1   0.43480 10.782 -86.914
##
## Step: AIC=-88.71
## log10(x) ~ merg$Rearing_Temp + merg$Axis.1 + merg$Axis.3
##
##              Df Sum of Sq    RSS    AIC
## - merg$Rearing_Temp 1   0.30750 10.756 -89.052
## <none>                                10.449 -88.705
## - merg$Axis.3      1   0.37408 10.823 -88.700
## - merg$Axis.1      1   0.60533 11.054 -87.495
##
## Step: AIC=-89.05
## log10(x) ~ merg$Axis.1 + merg$Axis.3
##
##              Df Sum of Sq    RSS    AIC
## <none>                                10.756 -89.052
## - merg$Axis.3      1   0.42229 11.178 -88.857
## - merg$Axis.1      1   0.71553 11.472 -87.381
## Start: AIC=-122.46
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##      merg$Axis.3
##
##              Df Sum of Sq    RSS    AIC
## - merg$Axis.1      1   0.0001 5.3876 -124.460
## - merg$Axis.2      1   0.0083 5.3958 -124.374
## - merg$Axis.3      1   0.0337 5.4212 -124.105
## - merg$bio1        1   0.0407 5.4282 -124.032
## <none>                                5.3875 -122.461
## - merg$Rearing_Temp 1   4.4356 9.8231 -90.224
##
## Step: AIC=-124.46
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.2 + merg$Axis.3
##
##              Df Sum of Sq    RSS    AIC
## - merg$Axis.2      1   0.0087 5.3963 -126.368
## - merg$Axis.3      1   0.0366 5.4242 -126.074
## <none>                                5.3876 -124.460
## - merg$bio1        1   0.2101 5.5977 -124.279

```

```

## - merg$Rearing_Temp 1 4.4355 9.8231 -92.224
##
## Step: AIC=-126.37
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.3
##
##           Df Sum of Sq  RSS      AIC
## - merg$Axis.3      1    0.0348 5.4311 -128.002
## <none>                        5.3963 -126.368
## - merg$bio1        1    0.2408 5.6372 -125.879
## - merg$Rearing_Temp 1    4.5772 9.9735 -93.358
##
## Step: AIC=-128
## log10(x) ~ merg$bio1 + merg$Rearing_Temp
##
##           Df Sum of Sq  RSS      AIC
## <none>                        5.4311 -128.002
## - merg$bio1        1    0.2862 5.7173 -127.075
## - merg$Rearing_Temp 1    4.5426 9.9737 -95.357
## Start: AIC=-82.24
## log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.1 + merg$Axis.2 +
##      merg$Axis.3
##
##           Df Sum of Sq  RSS      AIC
## <none>                        10.408 -82.236
## - merg$Rearing_Temp 1    0.7812 11.189 -80.183
## - merg$bio1        1    0.9743 11.382 -79.225
## - merg$Axis.1      1    1.3890 11.797 -77.221
## - merg$Axis.2      1    1.5269 11.935 -76.570
## - merg$Axis.3      1    4.8739 15.282 -62.727

## $FC_83
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.61666 -0.14861 -0.03988  0.14529  0.74191
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.36231    0.28484   1.272   0.2087
## merg$Rearing_Temp 0.02638    0.01254   2.104   0.0399 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2804 on 55 degrees of freedom
## Multiple R-squared:  0.07451, Adjusted R-squared:  0.05768
## F-statistic: 4.428 on 1 and 55 DF, p-value: 0.03995
##
##
## $FC_70
##
## Call:

```

```

## lm(formula = log10(x) ~ merg$Rearing_Temp)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.6417 -0.1415  0.0238  0.1711  0.3910
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    2.06670    0.25908   7.977 9.51e-11 ***
## merg$Rearing_Temp -0.01931    0.01140  -1.694   0.096 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.255 on 55 degrees of freedom
## Multiple R-squared:  0.04956,    Adjusted R-squared:  0.03228
## F-statistic: 2.868 on 1 and 55 DF,  p-value: 0.09601
##
##
## $FC_40
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp + merg$Axis.2)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.80392 -0.10073  0.07339  0.22020  0.55569
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.09071    0.32917   0.276   0.7839
## merg$Rearing_Temp 0.03680    0.01453   2.533   0.0143 *
## merg$Axis.2      1.24166    0.83541   1.486   0.1431
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3145 on 53 degrees of freedom
## (1 observation deleted due to missingness)
## Multiple R-squared:  0.166,    Adjusted R-squared:  0.1345
## F-statistic: 5.275 on 2 and 53 DF,  p-value: 0.008145
##
##
## $B_83
##
## Call:
## lm(formula = log10(x) ~ merg$Axis.1 + merg$Axis.3)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.89374 -0.32249  0.03374  0.32440  0.77433
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.01073    0.05911   0.182   0.8566
## merg$Axis.1 -1.09010    0.57516  -1.895   0.0634 .

```

```

## merg$Axis.3  2.00468    1.37680    1.456    0.1512
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4463 on 54 degrees of freedom
## Multiple R-squared:  0.09566,    Adjusted R-squared:  0.06217
## F-statistic: 2.856 on 2 and 54 DF,  p-value: 0.06621
##
##
## $B_70
##
## Call:
## lm(formula = log10(x) ~ merg$bio1 + merg$Rearing_Temp)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.81385 -0.20883 -0.03172  0.23678  0.82823
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -2.001005   0.352466  -5.677 5.59e-07 ***
## merg$bio1      -0.002270   0.001346  -1.687  0.0974 .
## merg$Rearing_Temp  0.095560   0.014219   6.721 1.17e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3171 on 54 degrees of freedom
## Multiple R-squared:  0.4806, Adjusted R-squared:  0.4614
## F-statistic: 24.99 on 2 and 54 DF,  p-value: 2.079e-08
##
##
## $B_40
##
## Call:
## lm(formula = log10(x) ~ merg$bio1 + merg$Rearing_Temp + merg$Axis.1 +
##      merg$Axis.2 + merg$Axis.3)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.17493 -0.26532  0.01004  0.24898  1.03464
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -1.746698   0.636020  -2.746  0.00836 **
## merg$bio1      0.009545   0.004412   2.163  0.03531 *
## merg$Rearing_Temp  0.041204   0.021269   1.937  0.05837 .
## merg$Axis.1     -3.327744   1.288247  -2.583  0.01276 *
## merg$Axis.2     -3.592908   1.326576  -2.708  0.00923 **
## merg$Axis.3     -7.393314   1.527899  -4.839 1.29e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4562 on 50 degrees of freedom
## (1 observation deleted due to missingness)

```

```
## Multiple R-squared:  0.3854, Adjusted R-squared:  0.3239
## F-statistic:  6.27 on 5 and 50 DF,  p-value: 0.0001376
```

```
#with no phylo axes 1
```

```
apply(merg[,38:43],2,function(x){summary(stepAIC(lm(log10(x)~merg$bio5+merg$Rearing_Temp+merg$Axis.2+me
```

```
## Start:  AIC=-137.42
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.2 + merg$Axis.3
##
##              Df Sum of Sq    RSS    AIC
## - merg$Axis.2      1  0.001764 4.2936 -139.40
## - merg$Axis.3      1  0.002615 4.2944 -139.39
## - merg$bio5        1  0.024446 4.3163 -139.10
## <none>                4.2918 -137.42
## - merg$Rearing_Temp 1  0.314569 4.6064 -135.39
##
## Step:  AIC=-139.4
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.3
##
##              Df Sum of Sq    RSS    AIC
## - merg$Axis.3      1  0.00281 4.2964 -141.36
## - merg$bio5        1  0.02307 4.3167 -141.09
## <none>                4.2936 -139.40
## - merg$Rearing_Temp 1  0.33992 4.6335 -137.06
##
## Step:  AIC=-141.36
## log10(x) ~ merg$bio5 + merg$Rearing_Temp
##
##              Df Sum of Sq    RSS    AIC
## - merg$bio5        1  0.02771 4.3241 -142.99
## <none>                4.2964 -141.36
## - merg$Rearing_Temp 1  0.33717 4.6336 -139.05
##
## Step:  AIC=-142.99
## log10(x) ~ merg$Rearing_Temp
##
##              Df Sum of Sq    RSS    AIC
## <none>                4.3241 -142.99
## - merg$Rearing_Temp 1  0.3481 4.6722 -140.58
## Start:  AIC=-149.05
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.2 + merg$Axis.3
##
##              Df Sum of Sq    RSS    AIC
## - merg$Axis.3      1  0.005260 3.5053 -150.96
## - merg$bio5        1  0.008554 3.5086 -150.91
## - merg$Axis.2      1  0.057491 3.5575 -150.12
## <none>                3.5000 -149.05
## - merg$Rearing_Temp 1  0.210727 3.7107 -147.71
##
## Step:  AIC=-150.96
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.2
##
##              Df Sum of Sq    RSS    AIC
## - merg$bio5        1  0.006235 3.5115 -152.86
```

```

## - merg$Axis.2      1  0.059127 3.5644 -152.01
## <none>              3.5053 -150.96
## - merg$Rearing_Temp 1  0.218048 3.7233 -149.52
##
## Step: AIC=-152.86
## log10(x) ~ merg$Rearing_Temp + merg$Axis.2
##
##           Df Sum of Sq  RSS    AIC
## - merg$Axis.2      1  0.065809 3.5773 -153.80
## <none>              3.5115 -152.86
## - merg$Rearing_Temp 1  0.225290 3.7368 -151.31
##
## Step: AIC=-153.8
## log10(x) ~ merg$Rearing_Temp
##
##           Df Sum of Sq  RSS    AIC
## <none>              3.5773 -153.8
## - merg$Rearing_Temp 1  0.18654 3.7639 -152.9
## Start: AIC=-124.37
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.2 + merg$Axis.3
##
##           Df Sum of Sq  RSS    AIC
## - merg$Axis.3      1  0.04235 5.1257 -125.90
## - merg$bio5        1  0.08506 5.1684 -125.44
## - merg$Axis.2      1  0.18001 5.2633 -124.42
## <none>              5.0833 -124.37
## - merg$Rearing_Temp 1  0.63486 5.7182 -119.78
##
## Step: AIC=-125.9
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.2
##
##           Df Sum of Sq  RSS    AIC
## - merg$bio5        1  0.11746 5.2431 -126.63
## - merg$Axis.2      1  0.17282 5.2985 -126.04
## <none>              5.1257 -125.90
## - merg$Rearing_Temp 1  0.66713 5.7928 -121.05
##
## Step: AIC=-126.63
## log10(x) ~ merg$Rearing_Temp + merg$Axis.2
##
##           Df Sum of Sq  RSS    AIC
## <none>              5.2431 -126.63
## - merg$Axis.2      1  0.21853 5.4617 -126.35
## - merg$Rearing_Temp 1  0.63456 5.8777 -122.23
## Start: AIC=-84.96
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.2 + merg$Axis.3
##
##           Df Sum of Sq  RSS    AIC
## - merg$Axis.2      1  0.02929 10.802 -86.808
## - merg$bio5        1  0.22258 10.995 -85.797
## <none>              10.773 -84.963
## - merg$Rearing_Temp 1  0.40192 11.175 -84.875
## - merg$Axis.3      1  0.47398 11.247 -84.509
##

```

```

## Step: AIC=-86.81
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## - merg$bio5      1    0.25169 11.054 -87.495
## - merg$Rearing_Temp 1    0.37404 11.176 -86.868
## <none>                10.802 -86.808
## - merg$Axis.3      1    0.48520 11.287 -86.304
##
## Step: AIC=-87.5
## log10(x) ~ merg$Rearing_Temp + merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## - merg$Axis.3      1    0.36702 11.421 -87.633
## <none>                11.054 -87.495
## - merg$Rearing_Temp 1    0.41769 11.472 -87.381
##
## Step: AIC=-87.63
## log10(x) ~ merg$Rearing_Temp
##
##           Df Sum of Sq    RSS    AIC
## <none>                11.421 -87.633
## - merg$Rearing_Temp 1    0.47296 11.894 -87.320
## Start: AIC=-123.6
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.2 + merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## - merg$Axis.2      1    0.0210  5.4900 -125.387
## - merg$Axis.3      1    0.0415  5.5105 -125.174
## - merg$bio5        1    0.1287  5.5977 -124.279
## <none>                5.4690 -123.605
## - merg$Rearing_Temp 1    4.5896 10.0587 -90.873
##
## Step: AIC=-125.39
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.3
##
##           Df Sum of Sq    RSS    AIC
## - merg$Axis.3      1    0.0390  5.5290 -126.983
## - merg$bio5        1    0.1471  5.6372 -125.879
## <none>                5.4900 -125.387
## - merg$Rearing_Temp 1    4.6689 10.1589 -92.308
##
## Step: AIC=-126.98
## log10(x) ~ merg$bio5 + merg$Rearing_Temp
##
##           Df Sum of Sq    RSS    AIC
## - merg$bio5        1    0.1883  5.7173 -127.075
## <none>                5.5290 -126.983
## - merg$Rearing_Temp 1    4.6307 10.1598 -94.303
##
## Step: AIC=-127.07
## log10(x) ~ merg$Rearing_Temp
##
##           Df Sum of Sq    RSS    AIC

```

```

## <none>                                5.7173 -127.075
## - merg$Rearing_Temp  1    4.7398 10.4571  -94.659
## Start:  AIC=-77.75
## log10(x) ~ merg$bio5 + merg$Rearing_Temp + merg$Axis.2 + merg$Axis.3
##
##              Df Sum of Sq    RSS    AIC
## - merg$bio5      1    0.1281 11.813 -79.144
## <none>                                11.685 -77.754
## - merg$Axis.2      1    0.7526 12.438 -76.259
## - merg$Rearing_Temp 1    0.7671 12.452 -76.193
## - merg$Axis.3      1    3.4786 15.164 -65.161
##
## Step:  AIC=-79.14
## log10(x) ~ merg$Rearing_Temp + merg$Axis.2 + merg$Axis.3
##
##              Df Sum of Sq    RSS    AIC
## <none>                                11.813 -79.144
## - merg$Rearing_Temp 1    0.8230 12.636 -77.372
## - merg$Axis.2      1    0.8625 12.676 -77.197
## - merg$Axis.3      1    3.9407 15.754 -65.023

## $FC_83
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.61666 -0.14861 -0.03988  0.14529  0.74191
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.36231    0.28484   1.272   0.2087
## merg$Rearing_Temp 0.02638    0.01254   2.104   0.0399 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2804 on 55 degrees of freedom
## Multiple R-squared:  0.07451,    Adjusted R-squared:  0.05768
## F-statistic: 4.428 on 1 and 55 DF,  p-value: 0.03995
##
##
## $FC_70
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.6417 -0.1415  0.0238  0.1711  0.3910
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    2.06670    0.25908   7.977 9.51e-11 ***

```



```

## merg$Rearing_Temp -0.01931    0.01140  -1.694    0.096 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.255 on 55 degrees of freedom
## Multiple R-squared:  0.04956,    Adjusted R-squared:  0.03228
## F-statistic: 2.868 on 1 and 55 DF,  p-value: 0.09601
##
##
## $FC_40
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp + merg$Axis.2)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.80392 -0.10073  0.07339  0.22020  0.55569
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.09071    0.32917   0.276   0.7839
## merg$Rearing_Temp 0.03680    0.01453   2.533   0.0143 *
## merg$Axis.2      1.24166    0.83541   1.486   0.1431
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3145 on 53 degrees of freedom
## (1 observation deleted due to missingness)
## Multiple R-squared:  0.166,    Adjusted R-squared:  0.1345
## F-statistic: 5.275 on 2 and 53 DF,  p-value: 0.008145
##
##
## $B_83
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.88431 -0.41622  0.04004  0.37877  0.81673
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.68194    0.46292  -1.473   0.146
## merg$Rearing_Temp 0.03075    0.02037   1.509   0.137
##
## Residual standard error: 0.4557 on 55 degrees of freedom
## Multiple R-squared:  0.03977,    Adjusted R-squared:  0.02231
## F-statistic: 2.278 on 1 and 55 DF,  p-value: 0.137
##
##
## $B_70
##
## Call:

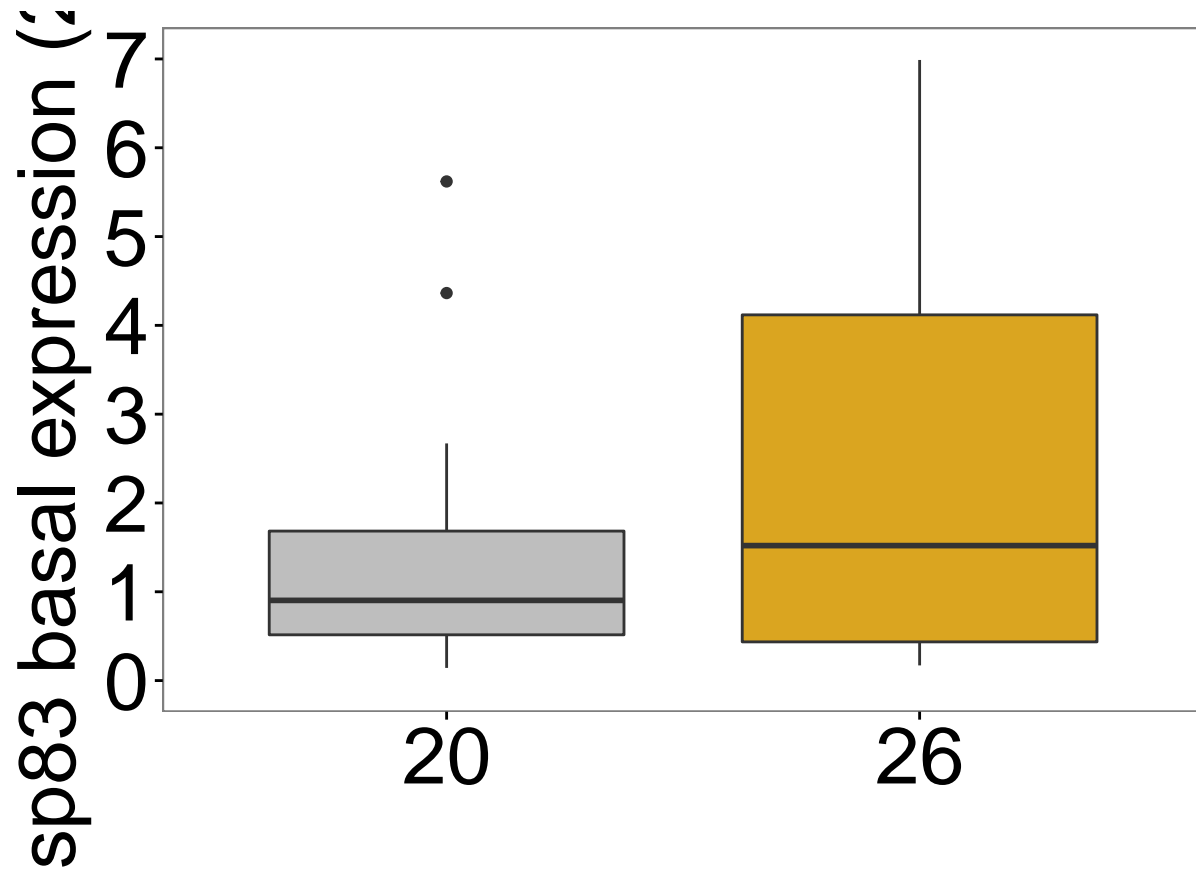
```

```
## lm(formula = log10(x) ~ merg$Rearing_Temp)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.7507 -0.1789 -0.0132  0.2067  0.7046
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -2.24217    0.32753  -6.846 6.75e-09 ***
## merg$Rearing_Temp  0.09734    0.01442   6.753 9.59e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3224 on 55 degrees of freedom
## Multiple R-squared:  0.4533, Adjusted R-squared:  0.4433
## F-statistic: 45.6 on 1 and 55 DF,  p-value: 9.589e-09
##
##
## $B_40
##
## Call:
## lm(formula = log10(x) ~ merg$Rearing_Temp + merg$Axis.2 + merg$Axis.3)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.32651 -0.30517  0.04431  0.29414  0.90335
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.91858    0.49998  -1.837 0.071891 .
## merg$Rearing_Temp  0.04201    0.02207   1.903 0.062535 .
## merg$Axis.2      -2.46702    1.26610  -1.949 0.056758 .
## merg$Axis.3      -6.13824    1.47381  -4.165 0.000118 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4766 on 52 degrees of freedom
## (1 observation deleted due to missingness)
## Multiple R-squared:  0.3024, Adjusted R-squared:  0.2621
## F-statistic: 7.513 on 3 and 52 DF,  p-value: 0.0002872
```

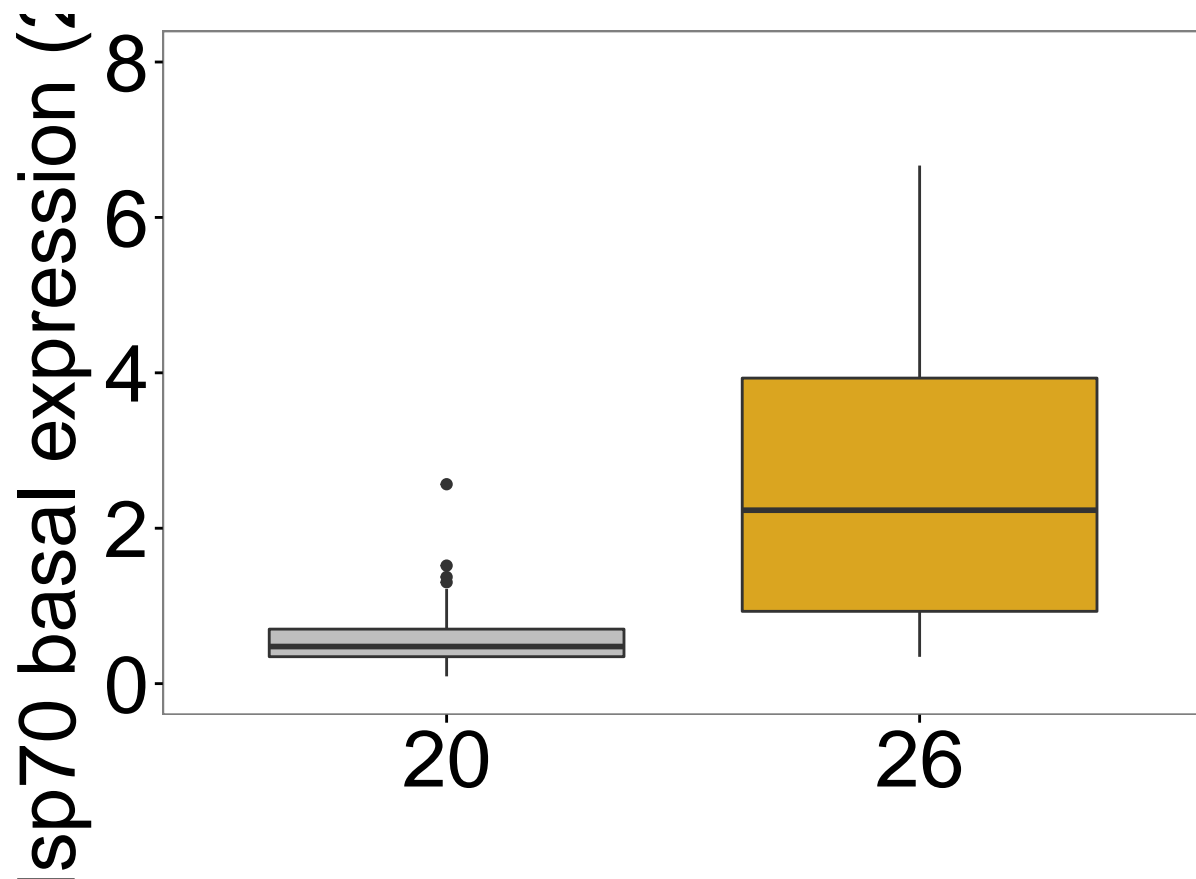
Expression Figures for rearing temp

```
#theme#####
T<-theme_bw()+theme(text=element_text(size=30),axis.text=element_text(size=30),legend.text=element_text
dude<-scale_fill_manual(name = "", values = c("gray", "goldenrod"))
#color
#####
#####ggplot of hsp83 basal
#add notches: geom_boxplot(notch=TRUE)
```

```
basal_83<-ggplot(data=merg,aes(x=factor(Rearing_Temp),y=B_83,fill=factor(Rearing_Temp)))+geom_boxplot()
# + xlab("Maintenance Temperature (°C)")
basal_83
```

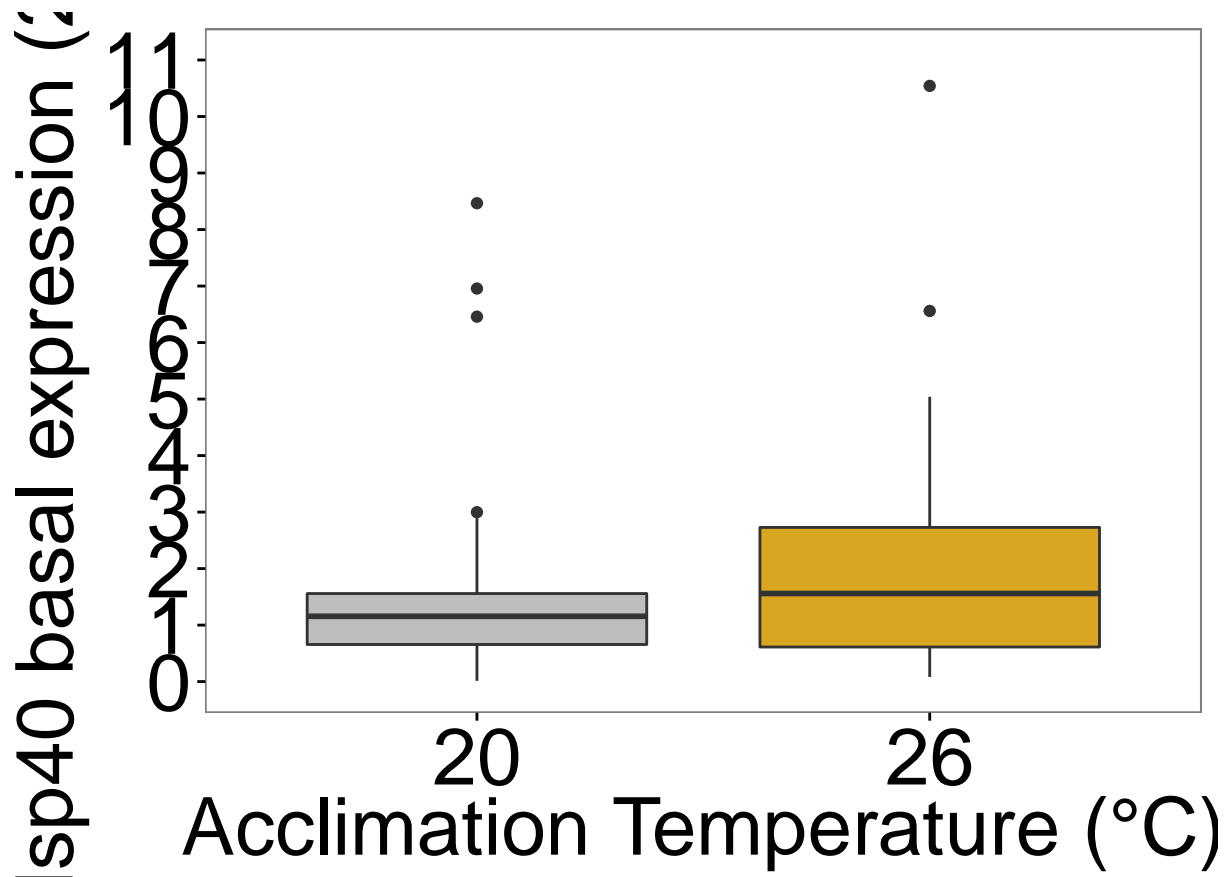


```
#####ggplot of hsp70 basal
basal_70<-ggplot(data=merg,aes(x=factor(Rearing_Temp),y=B_70,fill=factor(Rearing_Temp)))+geom_boxplot()
# + xlab("Maintenance Temperature (°C)")
basal_70
```



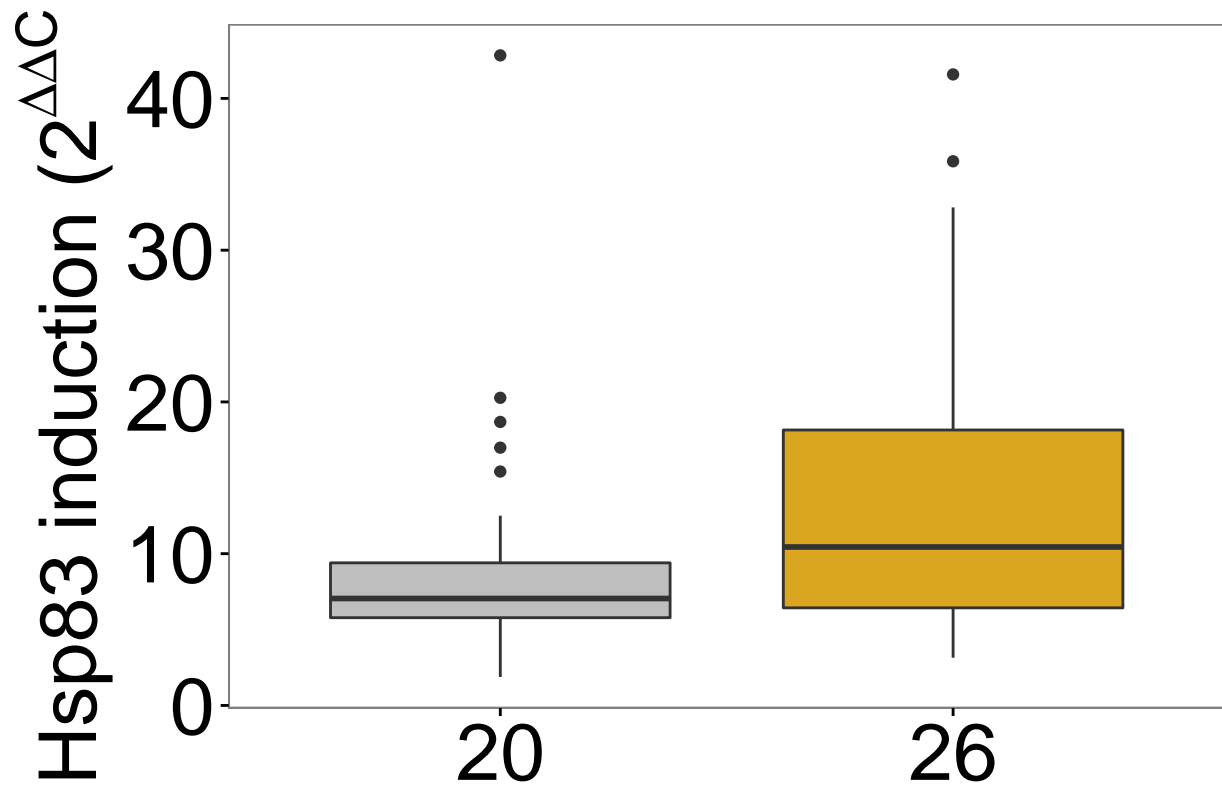
```
#####ggplot of hsp40 basal
basal_40<-ggplot(data=merg,aes(x=factor(Rearing_Temp),y=B_40,fill=factor(Rearing_Temp)))+geom_boxplot()+
  #+xlab("Maintenance Temperature (°C)")+
  basal_40
```

```
## Warning: Removed 1 rows containing non-finite values (stat_boxplot).
```



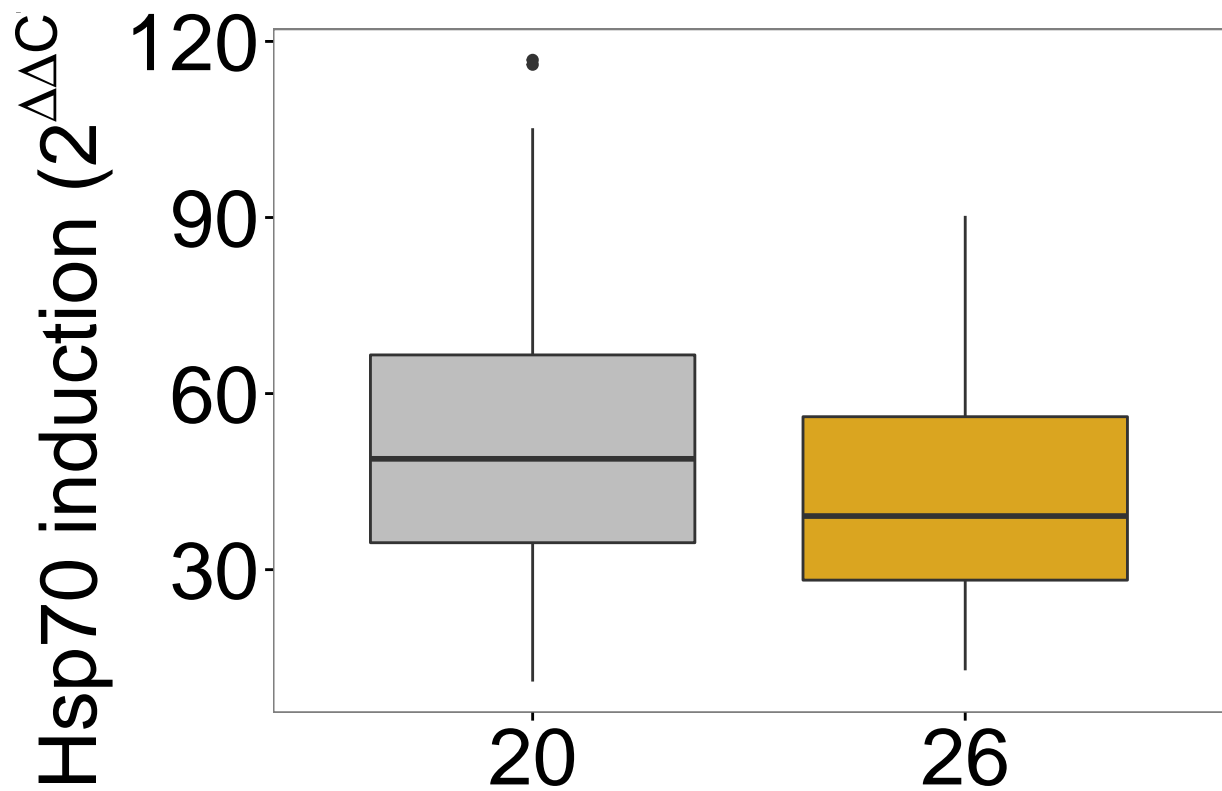
```
#####ggplot of hsp83 induction
```

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



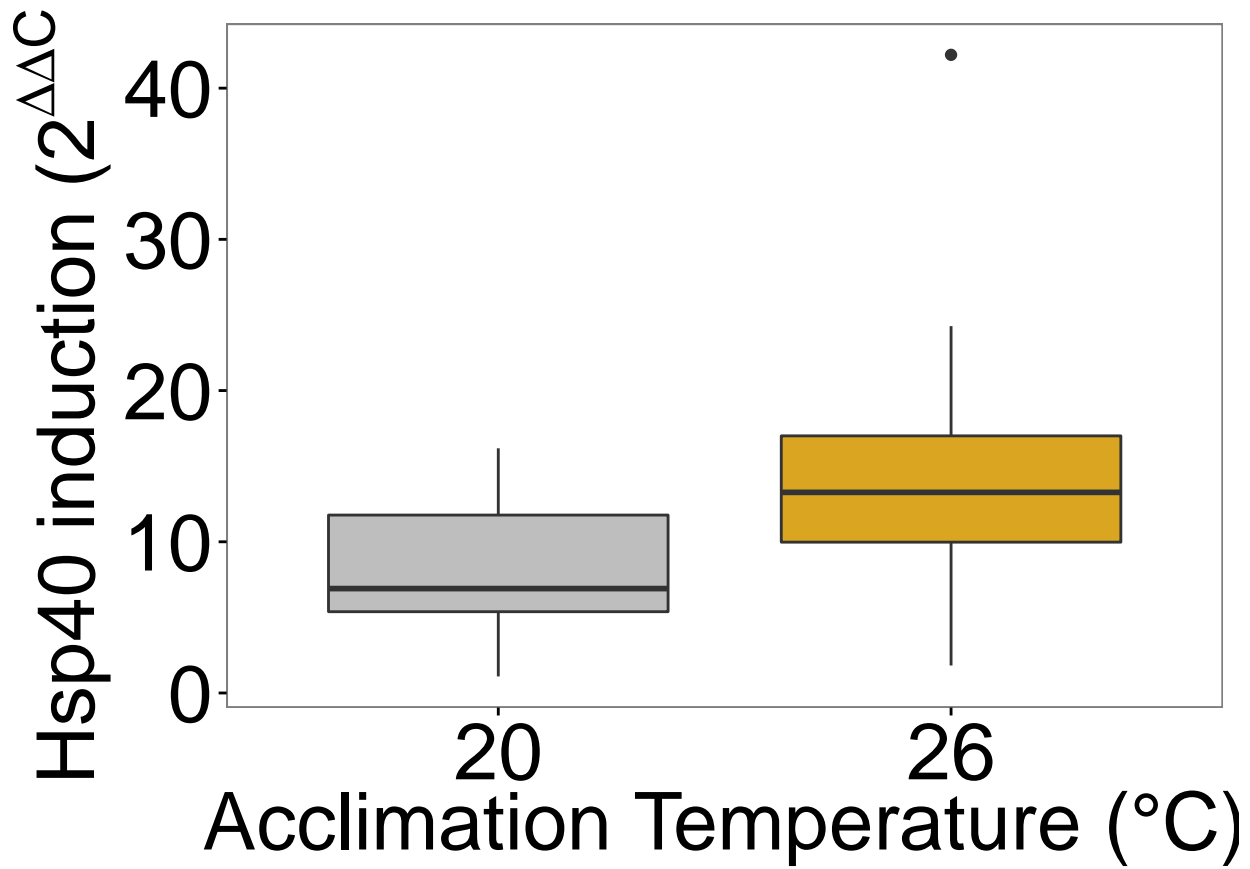
```
#####ggplot of hsp70 induction
```

```
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=merg,aes(x=factor(Rearing_Temp),y=FC_40,fill=factor(Rearing_Temp)))+geom_boxplot()
induc_hsp40
```

```
## Warning: Removed 1 rows containing non-finite values (stat_boxplot).
```



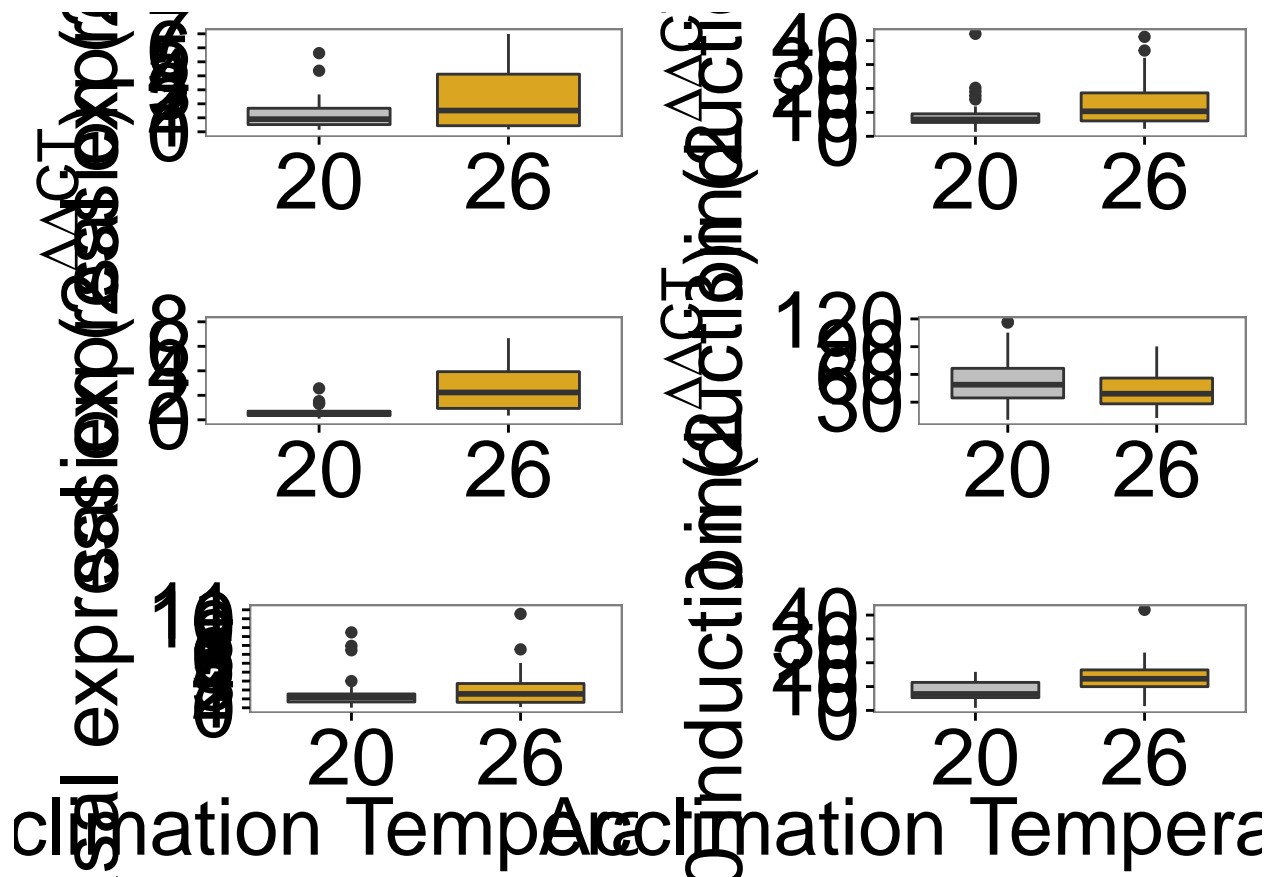
```
#+scale_y_continuous(limits=c(0,8),breaks=seq(0,8,2))
```

```
#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)
```

```
## Warning: Removed 1 rows containing non-finite values (stat_boxplot).
```

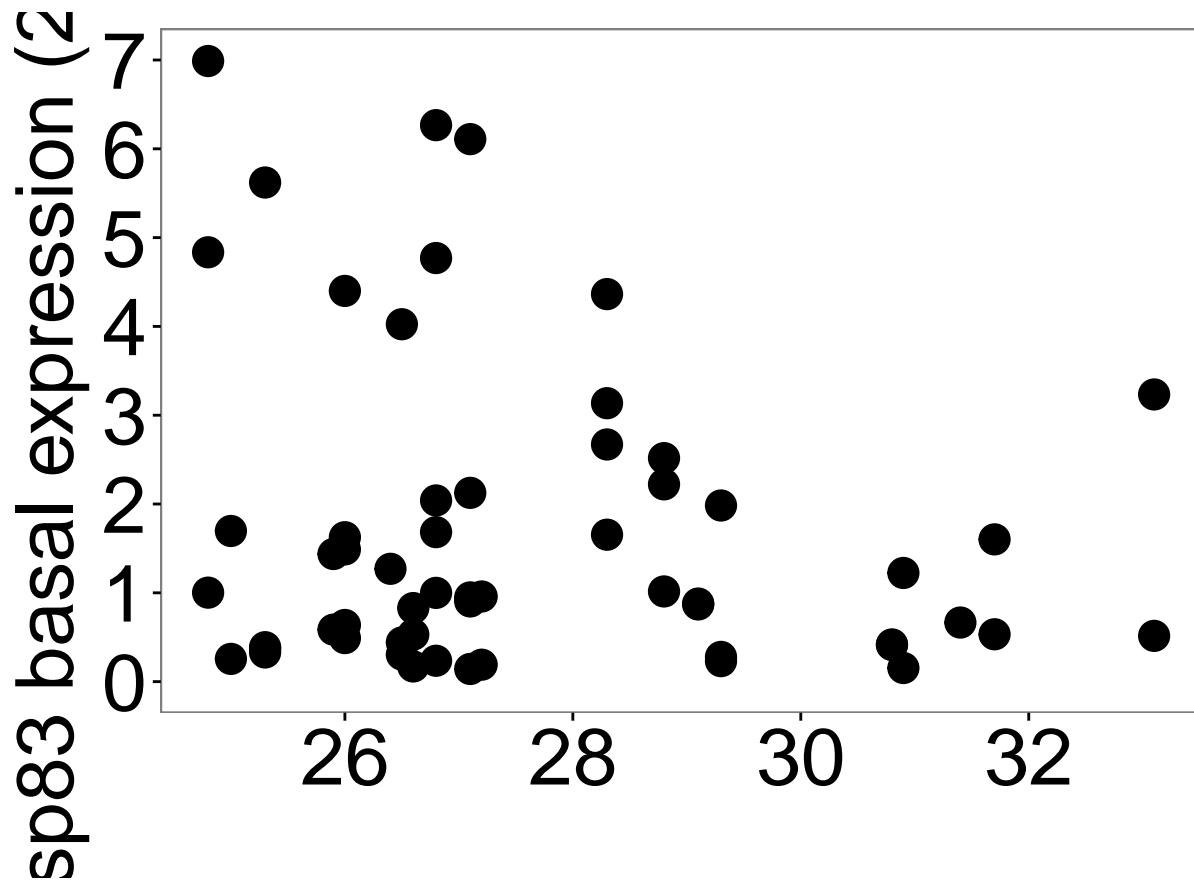
```
## Warning: Removed 1 rows containing non-finite values (stat_boxplot).
```



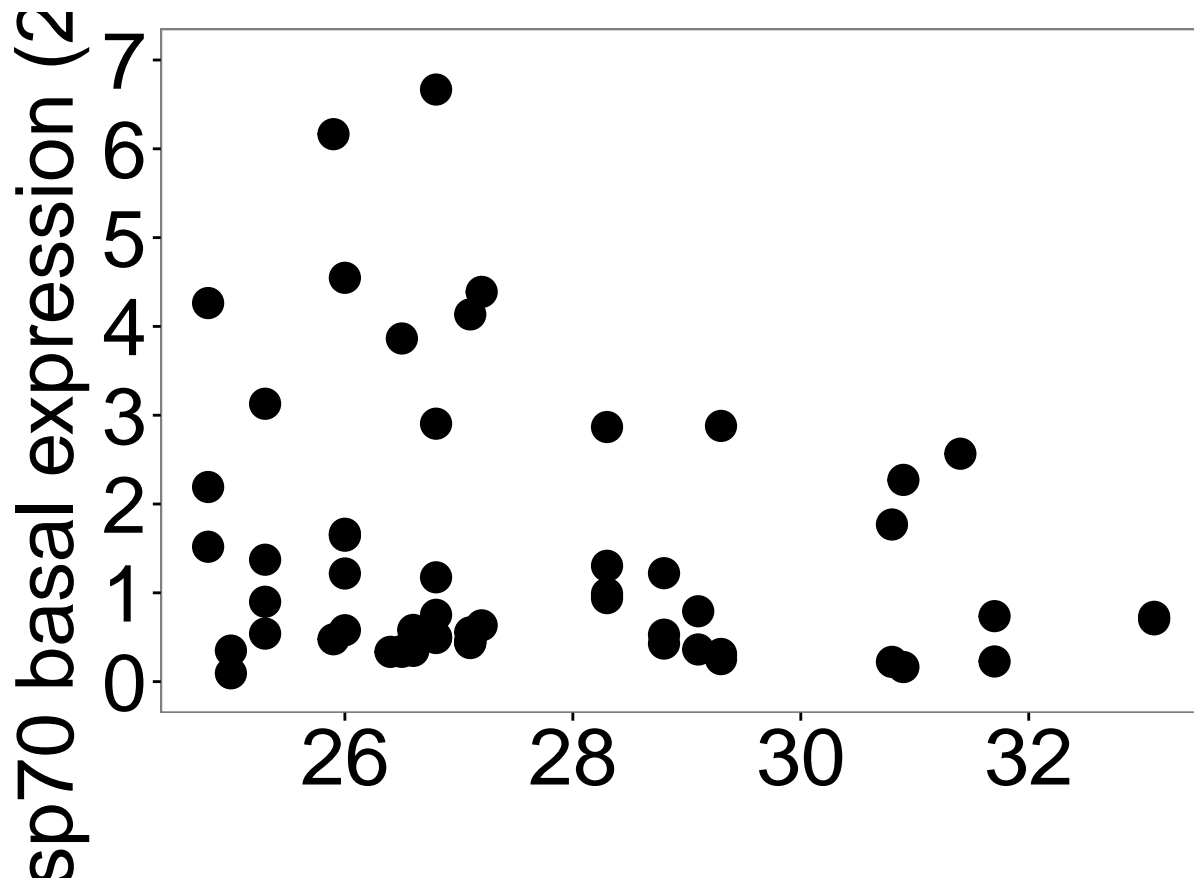
#Expression figures against Tmax

```
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=bio5/10,y=B_83))+geom_point(size=5)+T+ylab(expression(paste("Hsp83 basal expression")))
#loess
T83
```

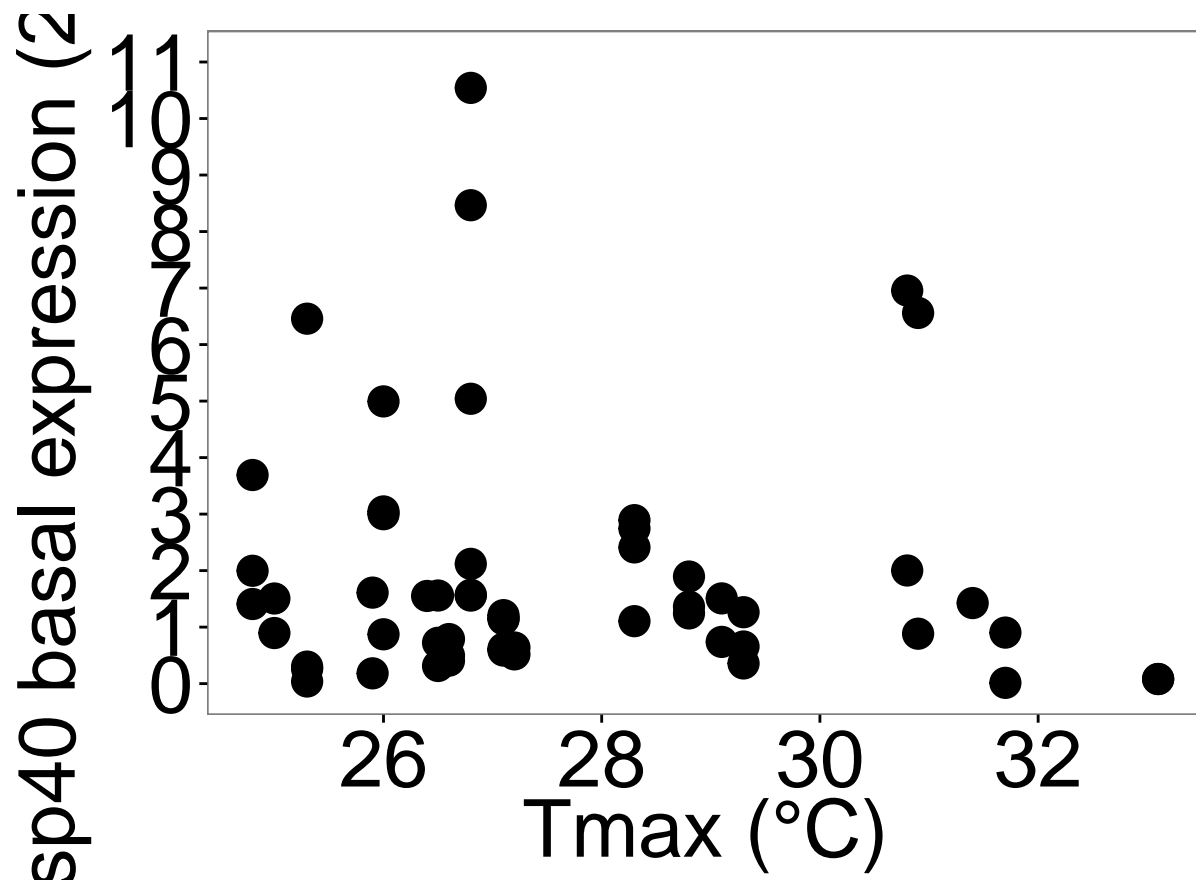



```
#####hsp70 basal#####
T70<-ggplot(data=merg,aes(x=bio5/10,y=B_70))+geom_point(size=5)+T+ylab(expression(paste("Hsp70 basal exp
#+loess
T70
```

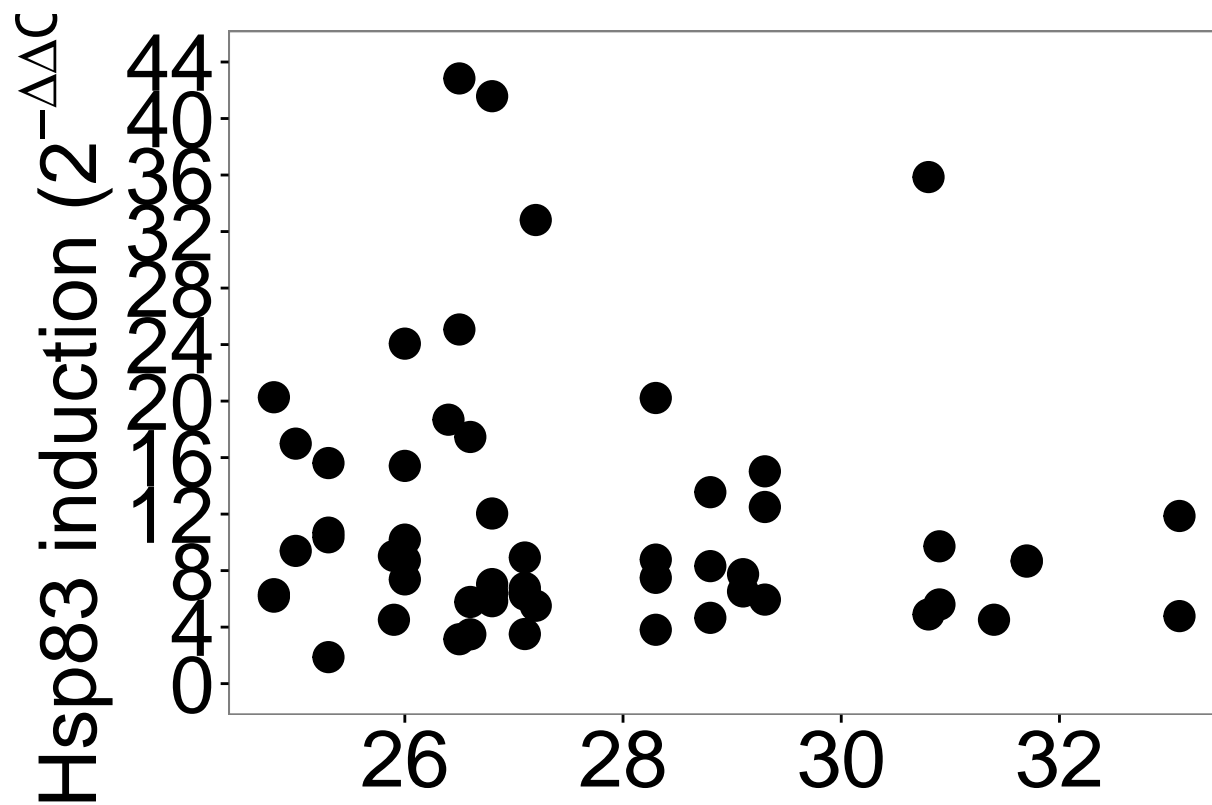


```
#####hsp40 basal#####
#merg$bio5<-merg$bio5/10
T40<-ggplot(data=merg,aes(x=bio5/10,y=B_40))+geom_point(size=5)+T+ylab(expression(paste("Hsp40 basal exp
#+loess
T40
```

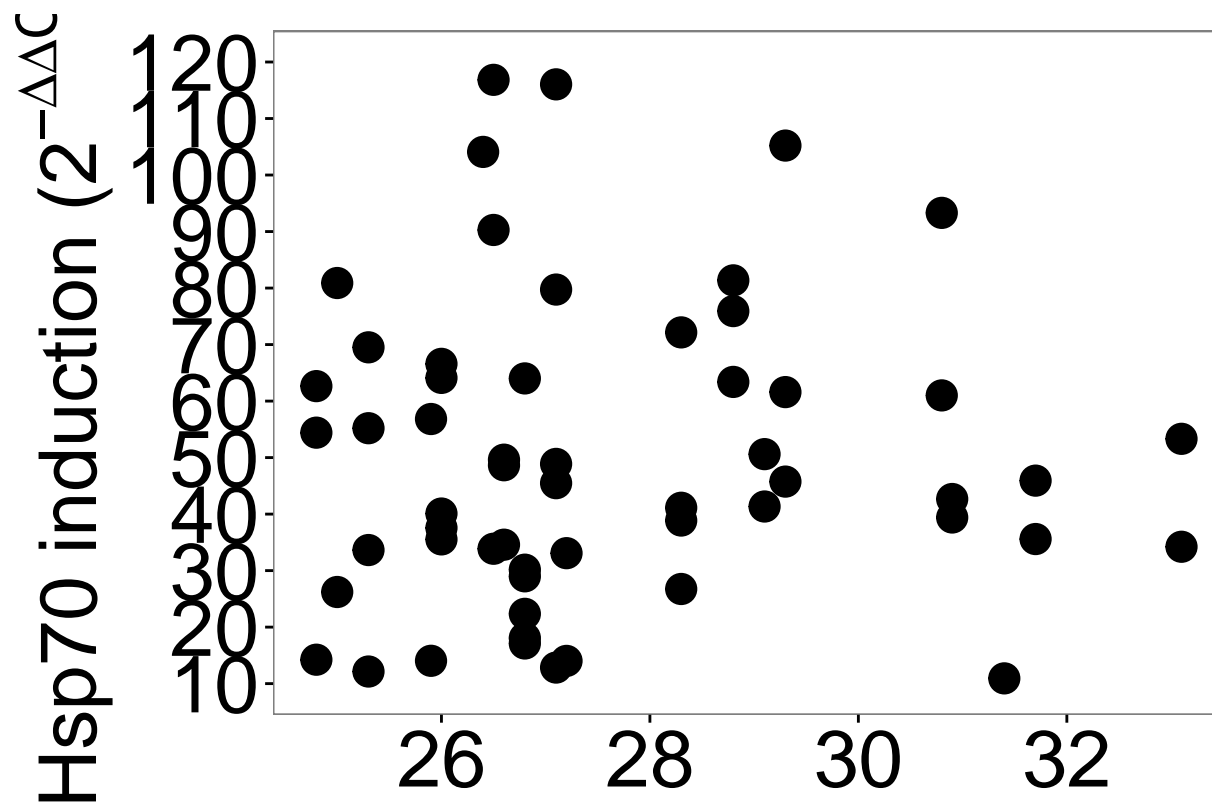
```
## Warning: Removed 1 rows containing missing values (geom_point).
```



```
#####
#####hsp83 induction#####
T83ind<-ggplot(data=merg,aes(x=bio5/10,y=FC_83))+geom_point(size=5)+T+ylab(expression(paste("Hsp83 indu
#+loess
T83ind
```

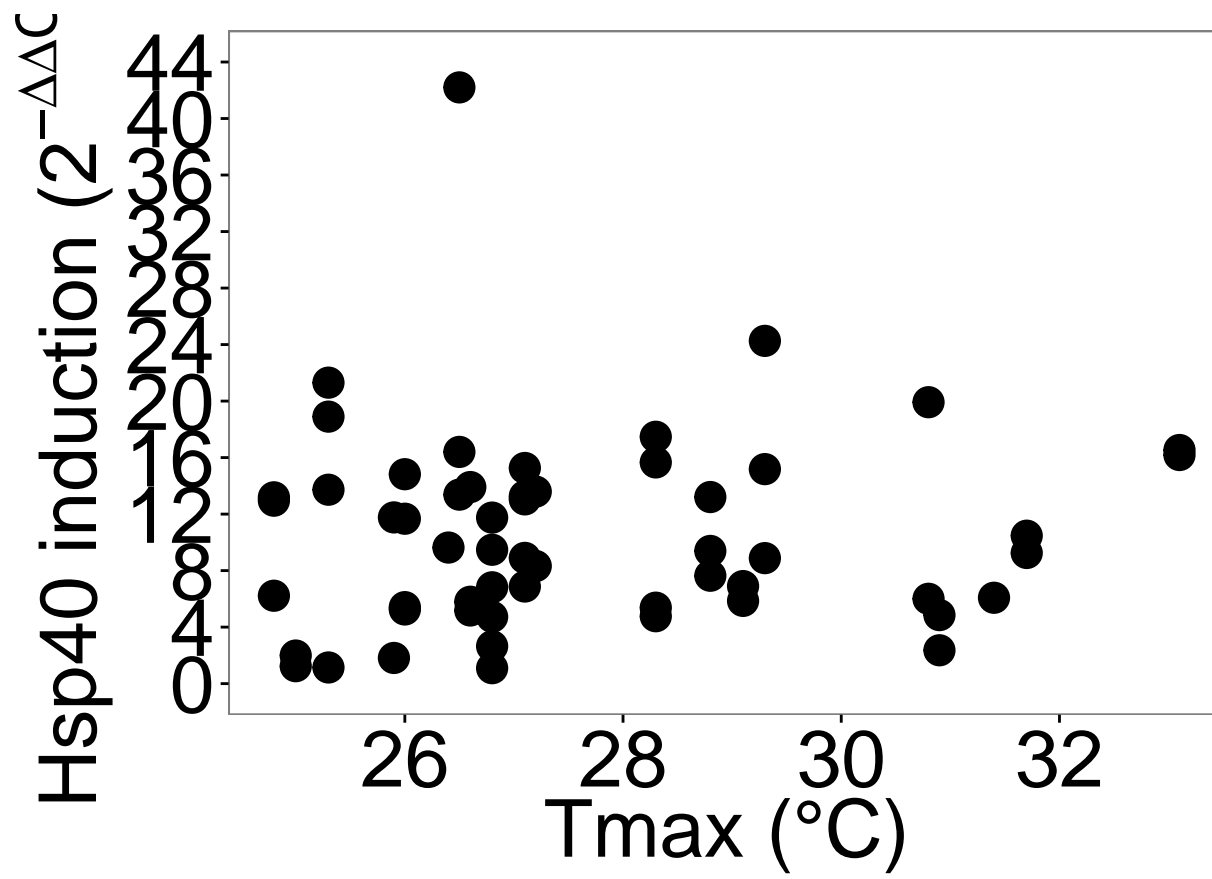


```
#####hsp70 induction#####
T70ind<-ggplot(data=merg,aes(x=bio5/10,y=FC_70,))+geom_point(size=5)+T+ylab(expression(paste("Hsp70 ind
#+loess
T70ind
```



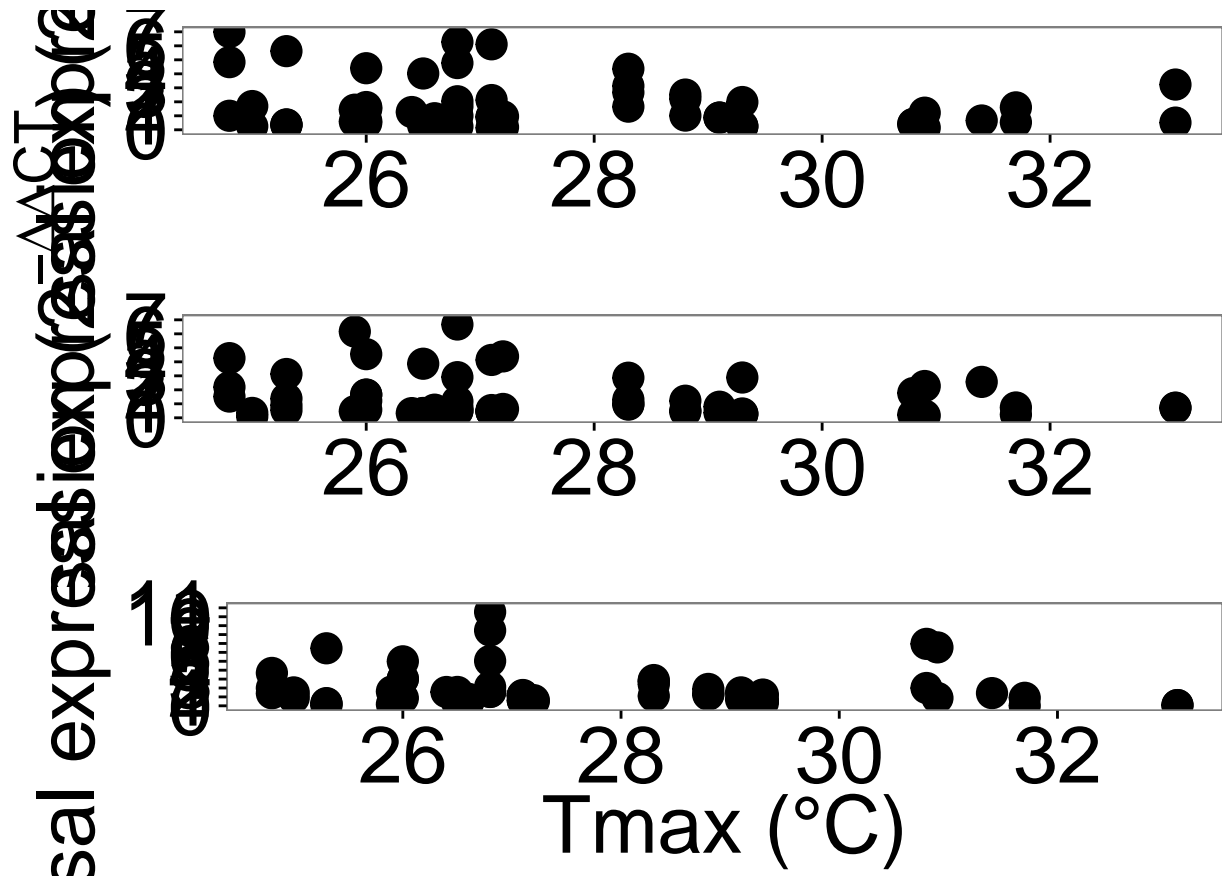
```
#####hsp40 induction#####
T40ind<-ggplot(data=merg,aes(x=bio5/10,y=FC_40))+geom_point(size=5)+T+ylab(expression(paste("Hsp40 indu
#+loess
T40ind
```

```
## Warning: Removed 1 rows containing missing values (geom_point).
```



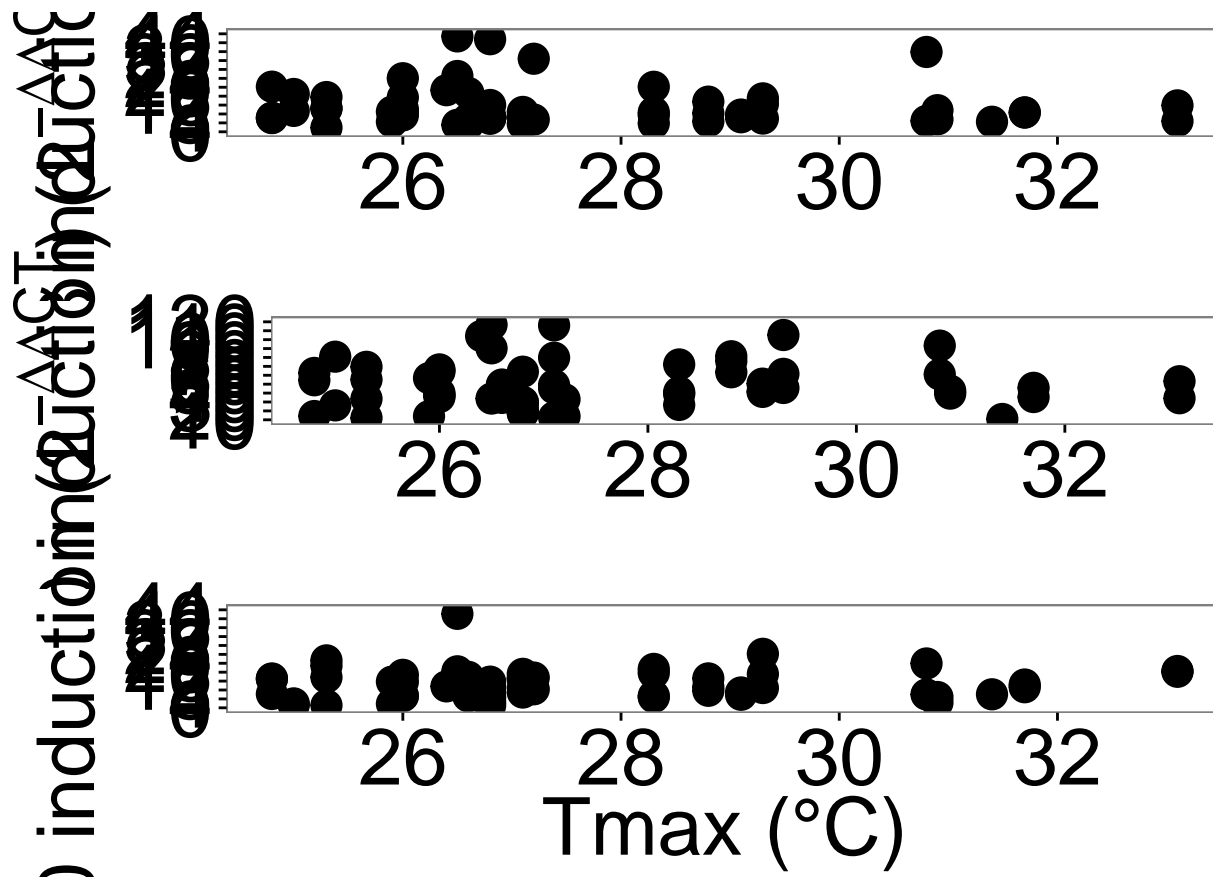
```
#grid.arrange(T83, T70, T40, T83ind, T70ind, T40ind, nrow=2, ncol=3)
grid.arrange(T83, T70, T40, nrow=3)
```

```
## Warning: Removed 1 rows containing missing values (geom_point).
```



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

```
## Warning: Removed 1 rows containing missing values (geom_point).
```



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")
colm<-ifelse(merg$Axis.1>.17,"#D55E00","#0072B2")
points(merg$lon.2,merg$lat,pch=20,col=colm,cex=1.75)
#points(bb8$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
```

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

```

## Running under: OS X 10.12.1 (Sierra)
##
## 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          dplyr_0.5.0
## [13] plyr_1.8.4
##
## loaded via a namespace (and not attached):
## [1] Rcpp_0.12.7      knitr_1.14       magrittr_1.5      munsell_0.4.3
## [5] colorspace_1.2-6 lattice_0.20-33   R6_2.1.3          highr_0.6
## [9] stringr_1.1.0    tools_3.3.1      grid_3.3.1        nlme_3.1-128
## [13] gtable_0.2.0     DBI_0.5-1        htmltools_0.3.5   lazyeval_0.2.0
## [17] yaml_2.1.13      assertthat_0.1    digest_0.6.10     tibble_1.2
## [21] formatR_1.4       evaluate_0.9      rmarkdown_1.0     labeling_0.3
## [25] stringi_1.1.2    scales_0.4.0     foreign_0.8-66

```