Will climate change cause temperature stress?

Curtis A. Provencher

2016-March-29

Contents

Rationale

Anthropogenic warming is likely to drive shifts in phenology, distribution, and performance of species in Eastern deciduous forests. Predicting these ecological cascades will depend on understanding how a primary seed disperser, the keystone ant genus Aphaenogaster, responds to warming. Temperatures surpassing a species' lethal thermal limit will clearly be detrimental, but unfavorably high temperatures may impose stress substantially before that limit is reached; characterizing such sublethal responses will be vital for predicting Aphaenogaster's future performance. Here we test for a physiological stress response in Aphaenogaster workers from a northern and southern deciduous forest under simulated climate warming.

Experimental protocol

loading libraries

```
library(plyr)
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
##
## The following objects are masked from 'package:plyr':
##
##     arrange, count, desc, failwith, id, mutate, rename, summarise,
##     summarize
##
## The following objects are masked from 'package:stats':
##
##     filter, lag
##
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
library(ggplot2)
library(tidyr)
library(MASS)
```

```
##
## Attaching package: 'MASS'
##
## The following object is masked from 'package:dplyr':
##
##     select
```

Loading in the data and accompanying metadata

```
# Date initiated- 4/11/2016
# Date Modified- 4/11/2016
#Affiliations- University of Vermont, University of North Carolina, Harvard Forest, Duke Forest, Univers
#Name and contact info: Curtis Provencher, cprovenc@uvm.edu, cprovenc3@gmail.com. Andrew Nguyen, adnguy
#Study name: The effects of experimental warming on forest ants
#Financial support: National Science Foundation, Division of Environmental Biology (1136644)
#Methods of data collection: Experimental warming chamebrs set up at a northern (Harvard Forest, MA) an
#Experimental units for each variable: Collection Date(YearMonthDay), Site (HF- Harvard Forest, DF- Duk
#Data layout and structure:
warm<-read.csv("../Data//20160411_FinalExperimentalWarmingDataset.csv",skip=10)
```

Quality control of expression values

```
##############################################################################
#Quality control
##############################################################################
#ranges of gene expression
apply(warm[,14:19],2,range,na.rm=TRUE)
```

```
##      CT_18s  CT_40  CT_70  CT_83 CT_actin CT_gapdh
## [1,]  4.972 24.636 19.571 20.215   20.531   20.111
## [2,] 29.813 37.441 32.777 38.100   34.249   34.721
```

```
#filter out very lowly expressed genes
#warm.hsp70<-subset(warm,warm$CT_70<34);dim(warm.hsp70)
warm.long<-gather(warm,Genes,GXP,CT_18s:CT_83) ### converting to long format
qc.samples<-subset(warm.long,warm.long$GXP>34) ###identifying the ones that have too low expression
n.exclude<-qc.samples$n
dim(warm[-n.exclude,]) #ecluding values that are too low in expression
```

```
## [1] 236  24
```

```
warm<-warm[-n.exclude,]
##############################################################################
##############################################################################
```

Visualizing the properties of the dataset

```
str(warm) # visualizing the properties of the dataset
```

```
## 'data.frame':    236 obs. of  24 variables:
##  $ n                      : int  1 3 4 5 6 7 8 9 11 12 ...
##  $ Collection.Date        : int  20130702 20130702 20130702 20130702 20130702 20130702 20130702 201
##  $ Site                   : Factor w/ 2 levels "DF","HF": 1 1 1 1 1 1 1 1 1 1 ...
##  $ Vial.Name              : Factor w/ 170 levels "","DF 1.1","DF 1.2",..: 54 52 79 80 78 83 82 84 8
##  $ Cham                   : int  1 1 2 2 2 3 3 3 4 4 ...
##  $ Sample                 : int  1 3 1 2 3 1 2 3 2 3 ...
```

```
##  $ Window                    : Factor w/ 6 levels "","A","B","C",..: 4 3 3 4 2 3 2 4 5 2 ...
##  $ BaitTemp1                 : num  24.2 25.2 23 23.8 22.4 23 23 22.8 24 23.8 ...
##  $ BaitTemp2                 : num  24.2 25.2 23.2 23.8 23.6 23 22.8 22.8 24.2 23.8 ...
##  $ BaitTemp3                 : num  24.4 25.2 23.2 23.6 23.6 23.6 23 22.8 23.8 24.2 ...
##  $ BaitTemp4                 : num  24.4 25 23.4 23.6 23.6 23.2 23.8 22.4 23.8 23.8 ...
##  $ RNA.conc.                 : Factor w/ 148 levels "","<2","10","10.40",..: 124 15 86 145 130 21 128
##  $ Isolation.Date            : int  20150811 20150731 20150814 20150813 20150813 20150730 20150814 201
##  $ CT_18s                    : num  NA 18.5 13.1 NA NA ...
##  $ CT_40                     : num  28.5 32.1 32.1 NA 31.6 ...
##  $ CT_70                     : num  25.8 26.1 28.4 NA 26.9 ...
##  $ CT_83                     : num  27.4 31.3 31.1 NA 32.7 ...
##  $ CT_actin                  : num  24.4 27.8 33.6 32.7 29.3 ...
##  $ CT_gapdh                  : num  23.5 28.8 30.8 NA 29.2 ...
##  $ RIN_Value                 : num  2.1 3.7 2.8 2.4 7 2.6 2.1 2.4 2.9 1 ...
##  $ CDNA                      : num  2.5 2.5 2.5 2.5 2.5 2.5 2.5 2.5 2.5 2.5 ...
##  $ dilution.factor           : Factor w/ 13 levels "#DIV/0!","10",..: 2 2 2 2 2 2 2 2 2 2 ...
##  $ vol.cDNA.for.dilution     : Factor w/ 13 levels "#DIV/0!","10.86956522",..: 4 4 4 4 4 4 4 4 4 4 ..
##  $ vol.of.water.for.dilution: Factor w/ 13 levels "#DIV/0!","37.5",..: 13 13 13 13 13 13 13 13 13 13
```

```
#calculating # of samples per site per chamber
knitr::kable(ddply(warm,.(Site,as.factor(Cham)),summarize,num=length(n)))
```

Site	as.factor(Cham)	num
DF	1	9
DF	2	12
DF	3	12
DF	4	11
DF	5	11
DF	6	12
DF	7	7
DF	8	9
DF	9	11
DF	10	9
DF	11	12
DF	12	9
DF	13	11
DF	14	9
DF	15	8
HF	1	7
HF	2	8
HF	3	7
HF	4	5
HF	5	8
HF	6	8
HF	7	5
HF	8	7
HF	9	7
HF	10	7
HF	11	7
HF	12	8

```
#number of samples per site!
knitr::kable(ddply(warm,.(Site),summarize,num=length(n)))
```

Site	num
DF	152
HF	84

```
###caulcating the bait temperatures!
warm$baittemp.ave<-apply(warm[,8:11],1,mean,na.rm=TRUE)
#knitr::kable(ddply(warm,.(Site),summarize,range(na.exclude(baittemp.ave))))
range(subset(warm,warm$Site=="DF")$baittemp.ave) # range of temperatures for duke forest
```

```
## [1] 22.30667 32.90000
```

```
range(subset(warm,warm$Site=="HF")$baittemp.ave) # range of temperatures for Harvard forest
```

```
## [1] 23.60 32.95
```

```
dim(warm) # looking at the dimensions...rows,columns
```

```
## [1] 236  25
```

```
# number of samples for hsp70
length(na.exclude(warm$CT_70))
```

```
## [1] 145
```

```
# number of samples for hsp83
length(na.exclude(warm$CT_83))
```

```
## [1] 143
```

```
# number of samples for hsp40
length(na.exclude(warm$CT_40))
```

```
## [1] 139
```

Checking internal control

```
#standard deviation in CT value for each gene
apply(warm[,14:19],2,sd,na.rm=TRUE)
```

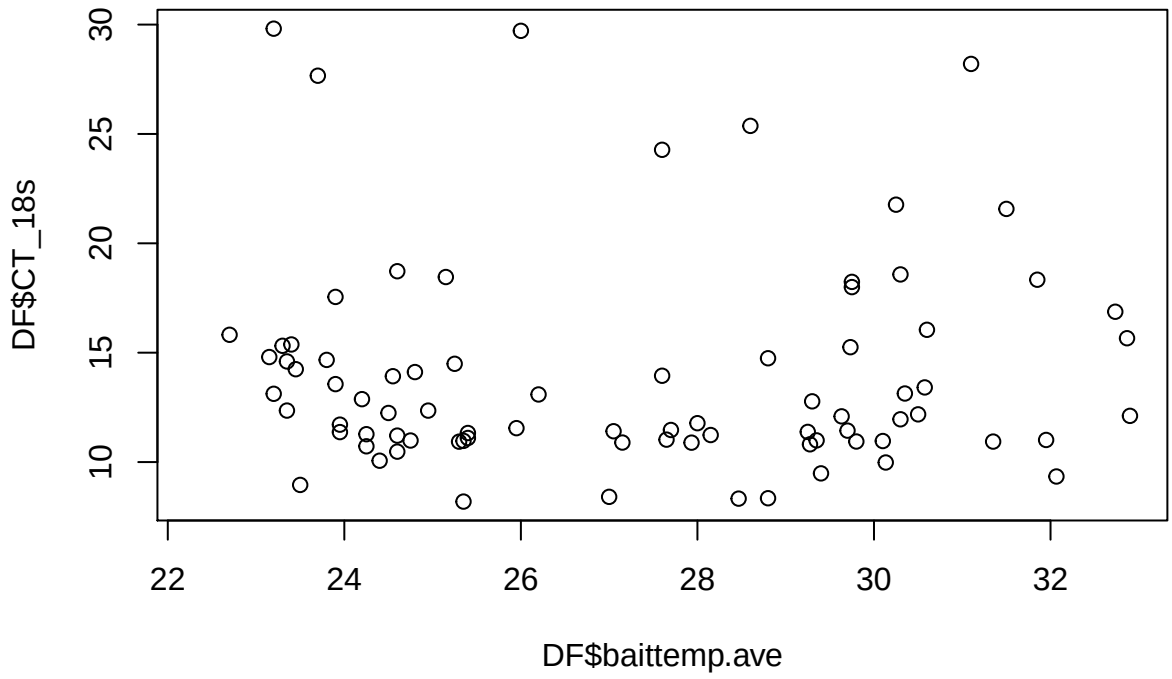
```
##   CT_18s    CT_40    CT_70    CT_83 CT_actin CT_gapdh
## 4.954508 2.438098 2.894001 4.192936 3.138800 3.281183
```

```r
##################################################
##Evaluating 18s rRNA
##################################################
hkg.mod1<-lm(warm$CT_18s~baittemp.ave*Site, data=warm)
summary(hkg.mod1)
```

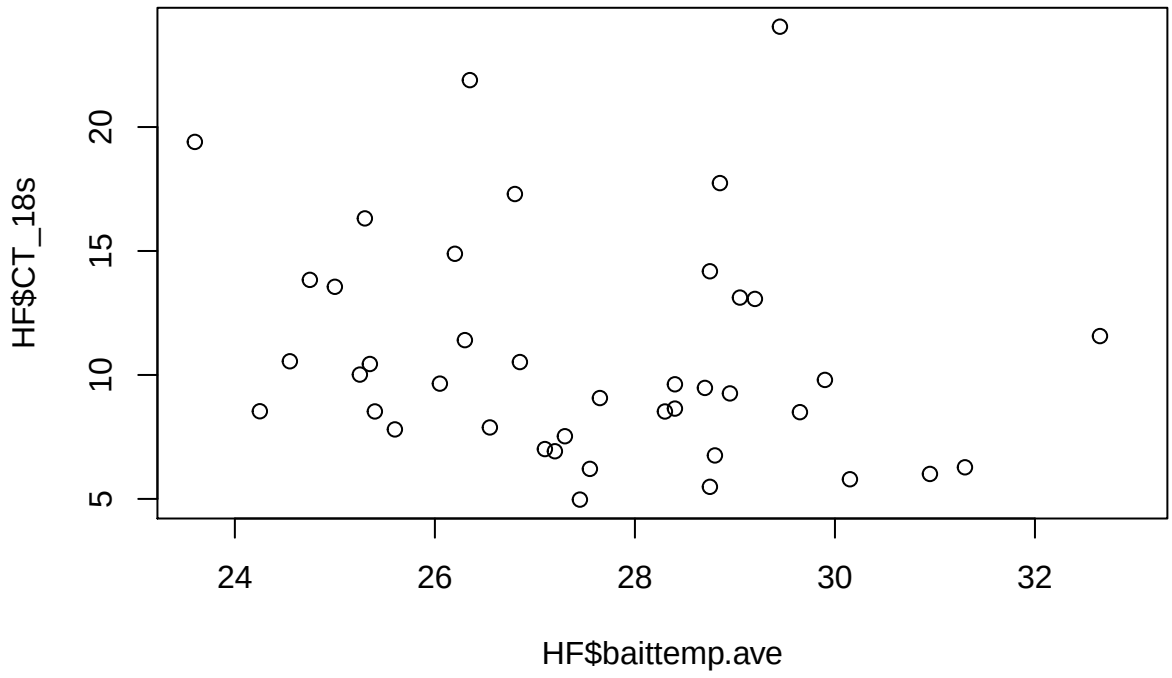
```
##
## Call:
## lm(formula = warm$CT_18s ~ baittemp.ave * Site, data = warm)
##
## Residuals:
##    Min     1Q Median     3Q    Max
## -5.846 -3.062 -1.633  1.599 15.836
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)         13.753124   4.854329   2.833  0.00542 **
## baittemp.ave         0.009657   0.177475   0.054  0.95670
## SiteHF               9.798028  11.108810   0.882  0.37957
## baittemp.ave:SiteHF -0.473518   0.403161  -1.175  0.24255
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.738 on 118 degrees of freedom
##   (114 observations deleted due to missingness)
## Multiple R-squared:  0.1082, Adjusted R-squared:  0.08556
## F-statistic: 4.774 on 3 and 118 DF,  p-value: 0.003547
```

```r
DF<-subset(warm,warm$Site=="DF")
HF<-subset(warm,warm$Site=="HF")

plot(DF$baittemp.ave,DF$CT_18s)
```




```
plot(HF$baittemp.ave,HF$CT_18s)
```



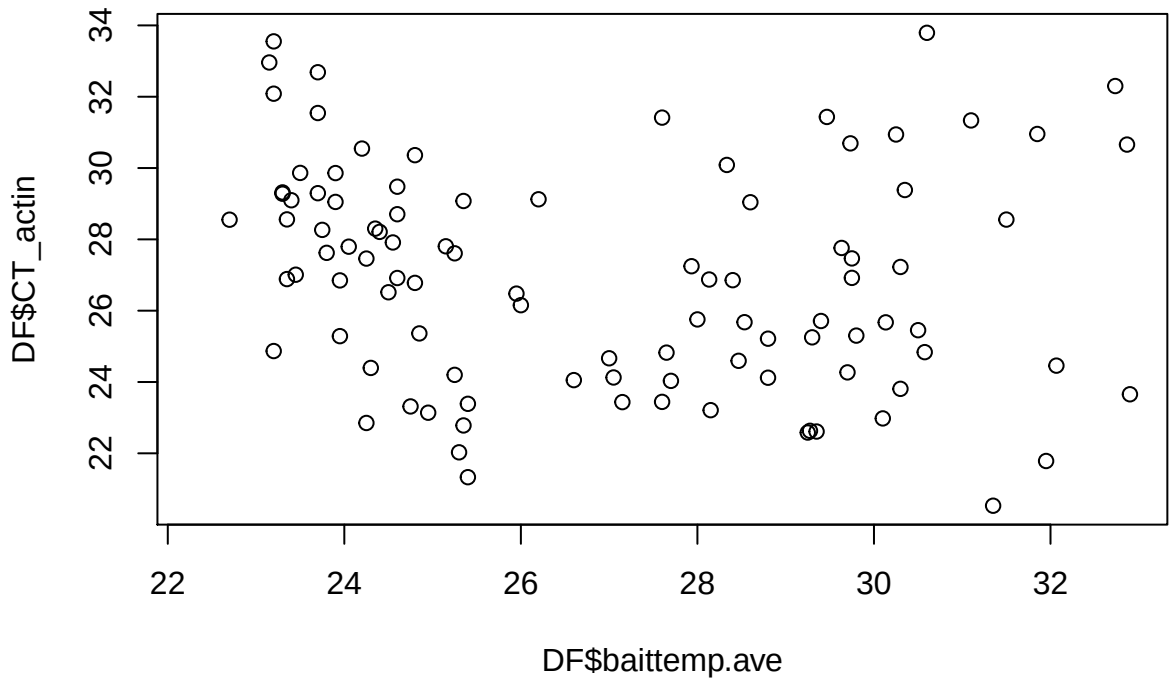

```
#####################################################

#####################################################
##Evaluating actin
#####################################################
hkg.mod2<-lm(warm$CT_actin~baittemp.ave*Site, data=warm)
summary(hkg.mod2)
```

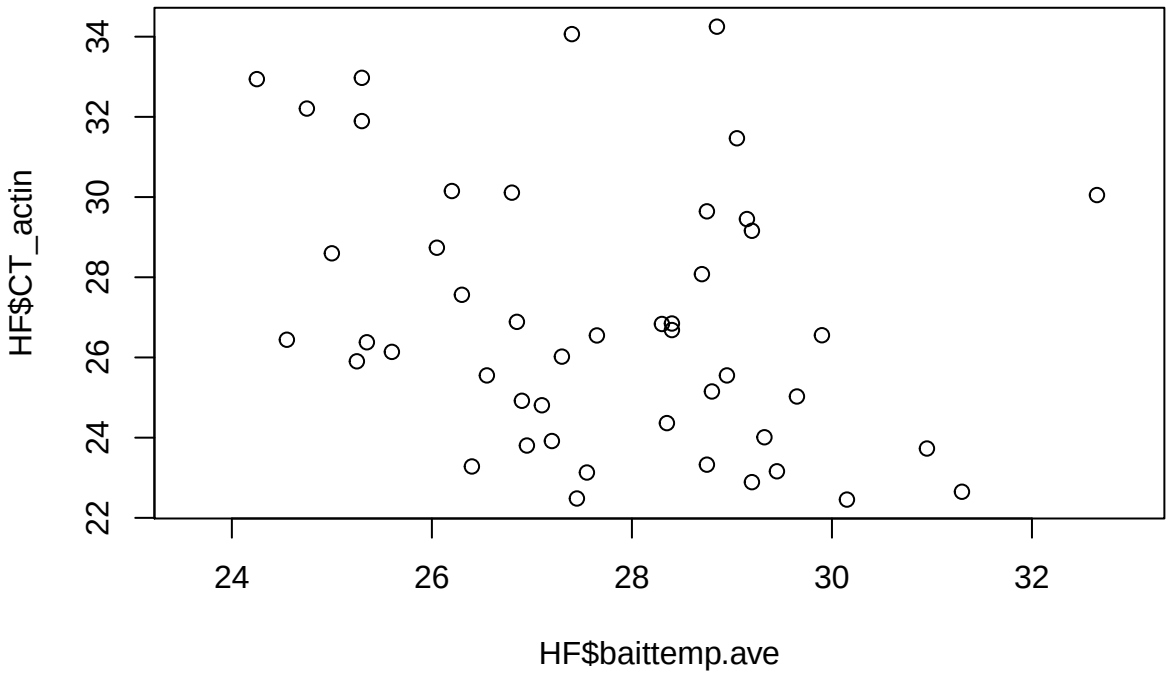
```
##
## Call:
## lm(formula = warm$CT_actin ~ baittemp.ave * Site, data = warm)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -5.9006 -2.3302 -0.5119  2.0468  7.8920
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)          32.1352     2.9093  11.046   <2e-16 ***
## baittemp.ave         -0.1931     0.1075  -1.796   0.0746 .
## SiteHF                9.2652     7.2837   1.272   0.2055
## baittemp.ave:SiteHF  -0.3284     0.2634  -1.247   0.2146
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.086 on 141 degrees of freedom
##   (91 observations deleted due to missingness)
## Multiple R-squared:  0.05324,    Adjusted R-squared:  0.03309
## F-statistic: 2.643 on 3 and 141 DF,  p-value: 0.05168
```

```
DF<-subset(warm,warm$Site=="DF")
HF<-subset(warm,warm$Site=="HF")

plot(DF$baittemp.ave,DF$CT_actin)
```




```
plot(HF$baittemp.ave,HF$CT_actin)
```



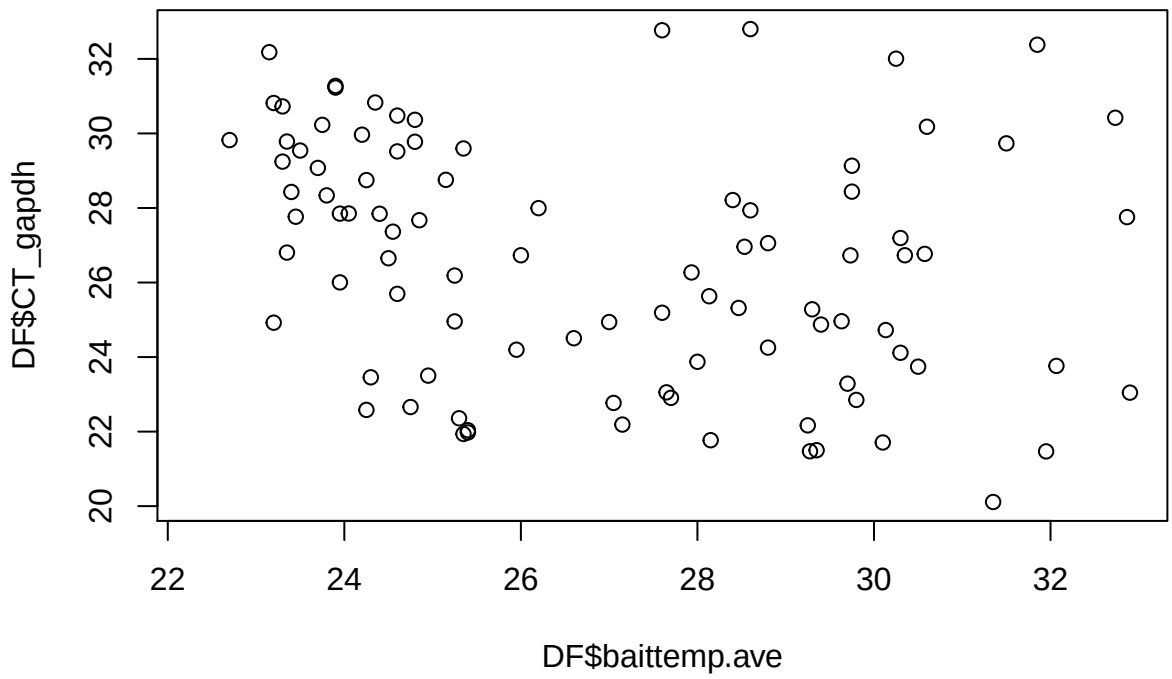

```
#####################################################

#####################################################
##Evaluating gapdh
#####################################################
hkg.mod3<-lm(warm$CT_gapdh~baittemp.ave*Site, data=warm)
summary(hkg.mod3)
```

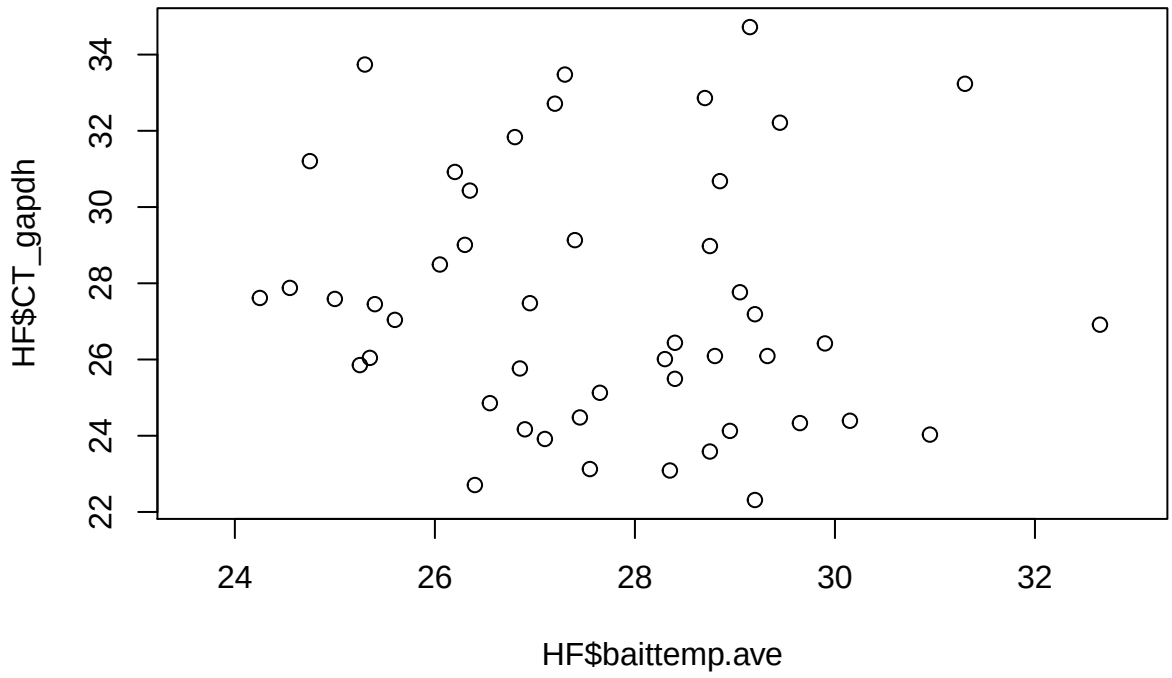
```
##
## Call:
## lm(formula = warm$CT_gapdh ~ baittemp.ave * Site, data = warm)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -5.1416 -2.4008 -0.3861  2.1106  7.5131
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)          35.1825     3.1254  11.257  < 2e-16 ***
## baittemp.ave         -0.3197     0.1153  -2.772  0.00634 **
## SiteHF               -2.5853     7.5508  -0.342  0.73258
## baittemp.ave:SiteHF   0.1348     0.2733   0.493  0.62252
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.193 on 137 degrees of freedom
##   (95 observations deleted due to missingness)
## Multiple R-squared:  0.07328,    Adjusted R-squared:  0.05299
## F-statistic: 3.611 on 3 and 137 DF,  p-value: 0.01501
```

```
DF<-subset(warm,warm$Site=="DF")
HF<-subset(warm,warm$Site=="HF")

plot(DF$baittemp.ave,DF$CT_gapdh)
```




```
plot(HF$baittemp.ave,HF$CT_gapdh)
```



```
#####################################################
```

Statistics

CT values themselves as measures of gene expression. The internal control was 18s rRNA

Hsp70 regression models

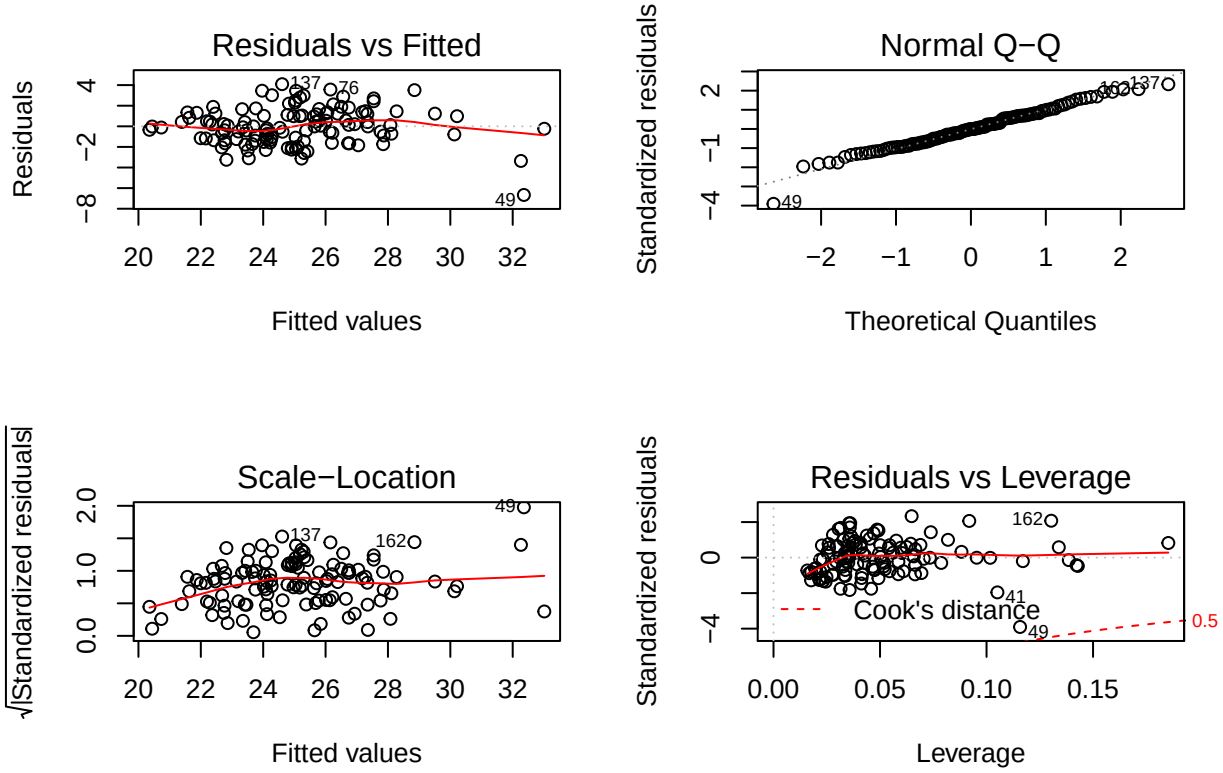
```
#getting rid of 49
#warm<-warm[-49,]
#########################################################
#hsp70 regression model
#########################################################
hsp70.mod<-lm(CT_70~baittemp.ave*Site+RIN_Value+CT_18s,data=warm)
#summary(hsp70.mod)
summary(stepAIC(hsp70.mod,direction="backward"))
```

```
## Start:  AIC=147.56
## CT_70 ~ baittemp.ave * Site + RIN_Value + CT_18s
##
##                      Df Sum of Sq    RSS    AIC
## <none>                              371.78 147.56
## - baittemp.ave:Site  1      7.36 379.14 147.90
## - RIN_Value          1      7.50 379.28 147.94
## - CT_18s             1    357.67 729.45 225.77

##
## Call:
## lm(formula = CT_70 ~ baittemp.ave * Site + RIN_Value + CT_18s,
##     data = warm)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -6.6628 -1.2744 -0.0145  1.2281  4.0905
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)         28.91039    1.94916  14.832  < 2e-16 ***
## baittemp.ave        -0.30529    0.07346  -4.156 6.33e-05 ***
## SiteHF               6.91249    4.38313   1.577    0.118
## RIN_Value           -0.14884    0.09859  -1.510    0.134
## CT_18s               0.38818    0.03723  10.426  < 2e-16 ***
## baittemp.ave:SiteHF -0.23870    0.15961  -1.496    0.138
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.814 on 113 degrees of freedom
##   (117 observations deleted due to missingness)
## Multiple R-squared:  0.6345, Adjusted R-squared:  0.6183
## F-statistic: 39.23 on 5 and 113 DF,  p-value: < 2.2e-16
```

```
#visualizing hsp70 regression model
par(mfrow=c(2,2))
plot(stepAIC(hsp70.mod,direction="backward"))
```

```
## Start:  AIC=147.56
## CT_70 ~ baittemp.ave * Site + RIN_Value + CT_18s
##
##                       Df Sum of Sq    RSS    AIC
## <none>                               371.78 147.56
## - baittemp.ave:Site  1      7.36 379.14 147.90
## - RIN_Value          1      7.50 379.28 147.94
## - CT_18s             1    357.67 729.45 225.77
```




```
par(mfrow=c(1,1))
```

Hsp83 regression models

```
############################################################
#hsp83 regresssion model
############################################################
hsp83.mod<-lm(CT_83~baittemp.ave*Site+RIN_Value+CT_18s,data=warm)
#summary(hsp83.mod)
summary(stepAIC(hsp83.mod,direction="backward"))
```

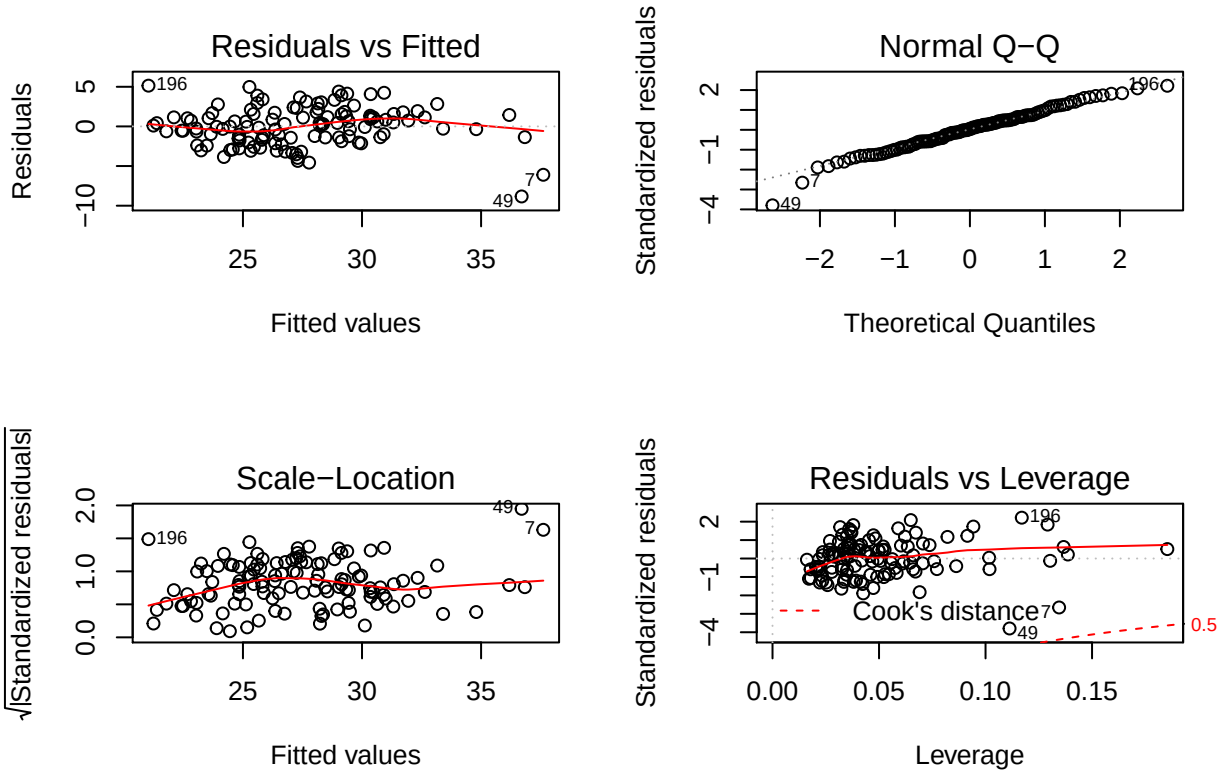
```
## Start:  AIC=219.21
```

```
## CT_83 ~ baittemp.ave * Site + RIN_Value + CT_18s
##
##                     Df Sum of Sq     RSS    AIC
## <none>                             683.14 219.21
## - baittemp.ave:Site  1     29.63  712.77 222.22
## - RIN_Value          1     38.30  721.44 223.65
## - CT_18s             1    600.75 1283.90 291.66
```

```
##
## Call:
## lm(formula = CT_83 ~ baittemp.ave * Site + RIN_Value + CT_18s,
##     data = warm)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -8.8355 -1.4441  0.1035  1.5643  5.1405
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)         35.31227    2.67492  13.201  < 2e-16 ***
## baittemp.ave        -0.49986    0.10039  -4.979 2.34e-06 ***
## SiteHF              15.23281    5.96336   2.554   0.0120 *
## RIN_Value           -0.33674    0.13439  -2.506   0.0137 *
## CT_18s               0.49552    0.04993   9.924  < 2e-16 ***
## baittemp.ave:SiteHF -0.47885    0.21727  -2.204   0.0296 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.47 on 112 degrees of freedom
##   (118 observations deleted due to missingness)
## Multiple R-squared:  0.6653, Adjusted R-squared:  0.6504
## F-statistic: 44.53 on 5 and 112 DF,  p-value: < 2.2e-16
```

```
#visualize hsp83 model
par(mfrow=c(2,2))
plot(stepAIC(hsp83.mod,direction="backward"))
```

```
## Start:  AIC=219.21
## CT_83 ~ baittemp.ave * Site + RIN_Value + CT_18s
##
##                     Df Sum of Sq     RSS    AIC
## <none>                             683.14 219.21
## - baittemp.ave:Site  1     29.63  712.77 222.22
## - RIN_Value          1     38.30  721.44 223.65
## - CT_18s             1    600.75 1283.90 291.66
```




```
par(mfrow=c(1,1))
############################################################
```

Hsp40 regression models

```
############################################################
#hsp40 regression model
############################################################
warm.40<-subset(warm,warm$CT_40!="NA" & RIN_Value !="NA")
hsp40.mod<-lm(CT_40~baittemp.ave*Site+RIN_Value+CT_18s,data=warm.40)
#summary(hsp40.mod)
summary(stepAIC(hsp40.mod,direction="both"))
```

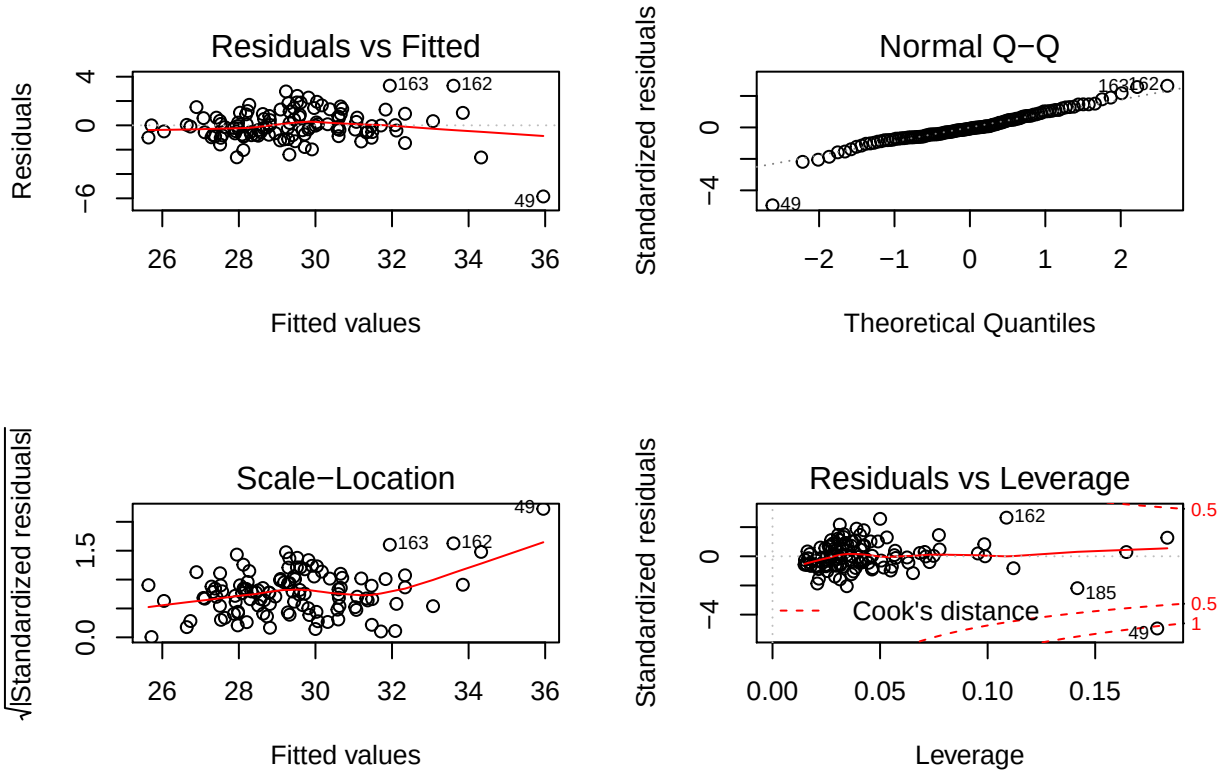
```
## Start:  AIC=65.36
## CT_40 ~ baittemp.ave * Site + RIN_Value + CT_18s
##
##                     Df Sum of Sq    RSS     AIC
## - RIN_Value          1     2.222 183.42  64.738
## <none>                           181.20  65.360
## - baittemp.ave:Site  1     4.439 185.64  66.095
## - CT_18s             1   217.721 398.92 152.536
##
## Step:  AIC=64.74
## CT_40 ~ baittemp.ave + Site + CT_18s + baittemp.ave:Site
##
##                     Df Sum of Sq    RSS     AIC
```

```
## <none>                           183.42  64.738
## + RIN_Value          1     2.222 181.20  65.360
## - baittemp.ave:Site  1     6.019 189.44  66.387
## - CT_18s             1   226.093 409.52 153.497

##
## Call:
## lm(formula = CT_40 ~ baittemp.ave + Site + CT_18s + baittemp.ave:Site,
##     data = warm.40)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -5.8451 -0.7741 -0.1007  0.7691  3.2615
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)         32.66148    1.51298  21.588  < 2e-16 ***
## baittemp.ave        -0.29131    0.05303  -5.493 2.65e-07 ***
## SiteHF               6.49343    3.11716   2.083   0.0396 *
## CT_18s               0.36560    0.03169  11.538  < 2e-16 ***
## baittemp.ave:SiteHF -0.21378    0.11356  -1.883   0.0624 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.303 on 108 degrees of freedom
##   (25 observations deleted due to missingness)
## Multiple R-squared:  0.6659, Adjusted R-squared:  0.6535
## F-statistic: 53.82 on 4 and 108 DF,  p-value: < 2.2e-16
```

```
par(mfrow=c(2,2))
plot(stepAIC(hsp40.mod,direction="backward"))
```

```
## Start:  AIC=65.36
## CT_40 ~ baittemp.ave * Site + RIN_Value + CT_18s
##
##                     Df Sum of Sq    RSS     AIC
## - RIN_Value          1     2.222 183.42  64.738
## <none>                           181.20  65.360
## - baittemp.ave:Site  1     4.439 185.64  66.095
## - CT_18s             1   217.721 398.92 152.536
##
## Step:  AIC=64.74
## CT_40 ~ baittemp.ave + Site + CT_18s + baittemp.ave:Site
##
##                     Df Sum of Sq    RSS     AIC
## <none>                           183.42  64.738
## - baittemp.ave:Site  1     6.019 189.44  66.387
## - CT_18s             1   226.093 409.52 153.497
```




```
par(mfrow=c(1,1))
############################################################
```

Plotting gxp values

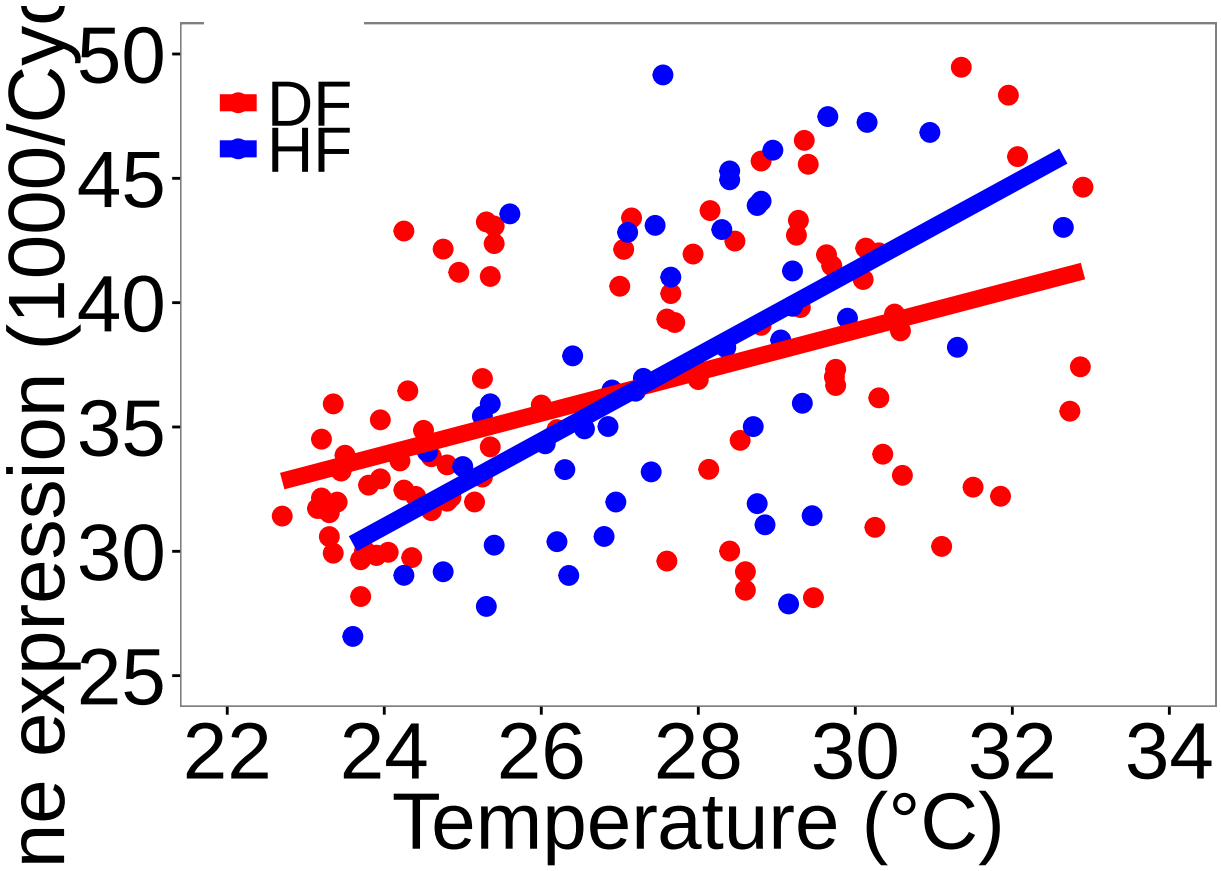
```
#the overall theme for ggplot
T<-theme_bw()+theme(text=element_text(size=30),axis.text=element_text(size=30), panel.grid.major=elemen
```

hsp83 plot

```
ggplot(warm,aes(x=baittemp.ave,y=(1000/CT_83),colour=factor(Site)))+geom_point(size=3)+T+geom_smooth(me
```

```
## Warning: Removed 93 rows containing non-finite values (stat_smooth).
```

```
## Warning: Removed 93 rows containing missing values (geom_point).
```

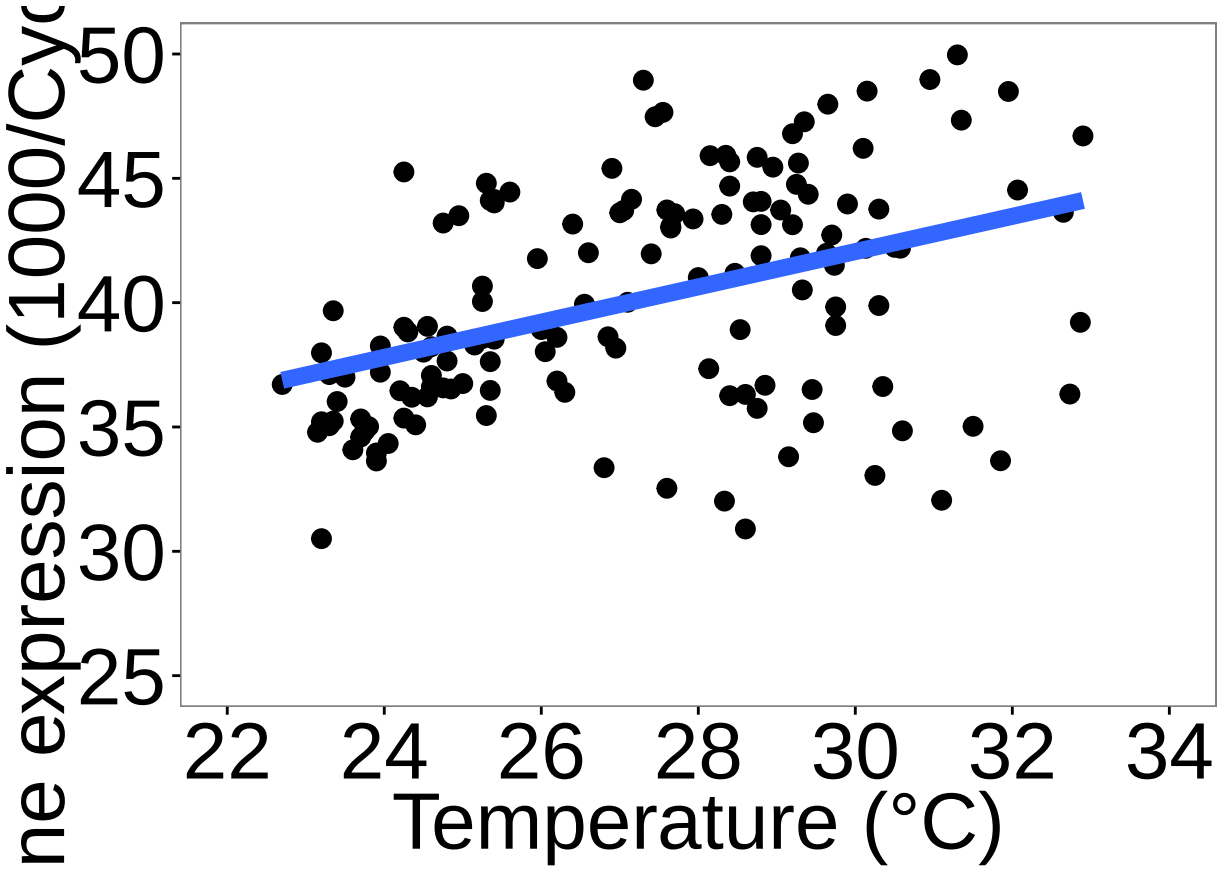


hsp70 plot

```
ggplot(warm,aes(x=baittemp.ave,y=(1000/CT_70)))+geom_point(size=3)+T+geom_smooth(method="lm",se=FALSE,s
```

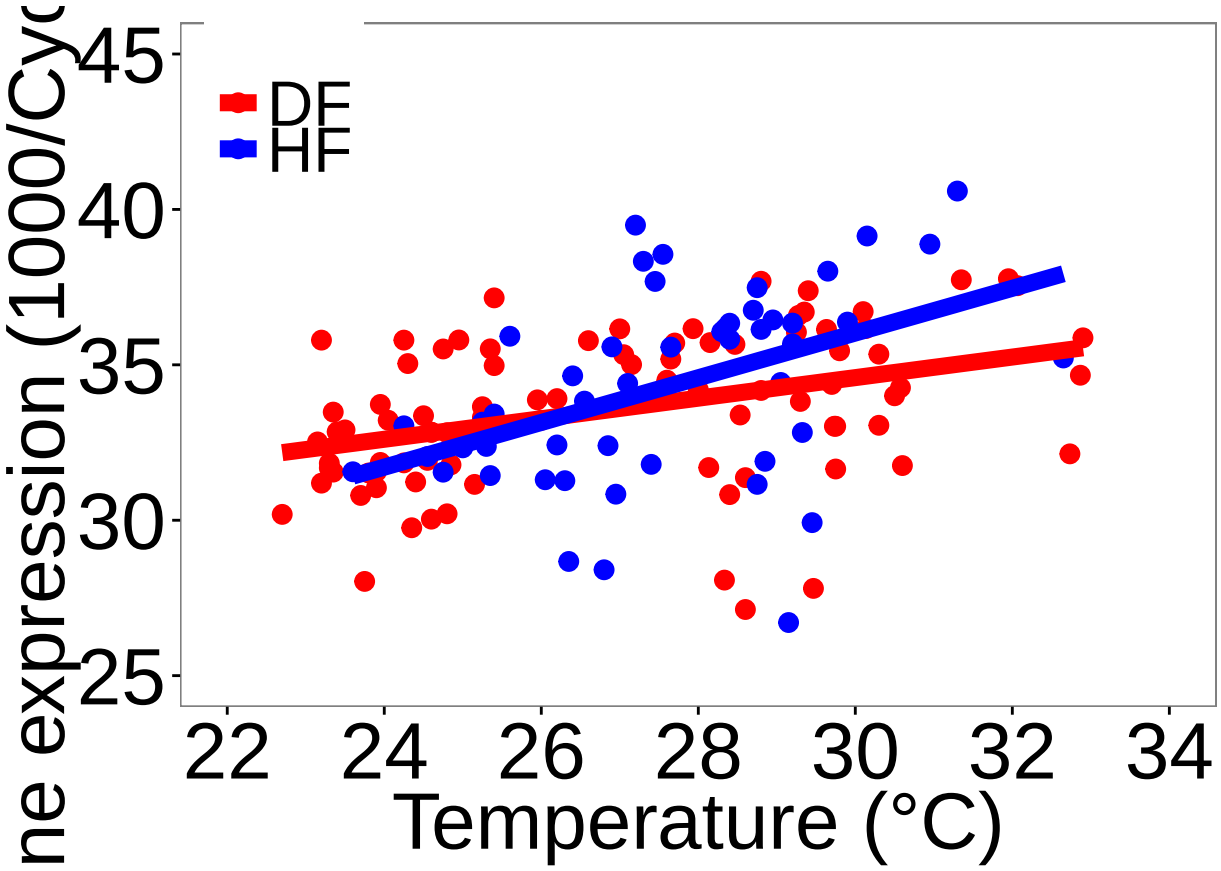
```
## Warning: Removed 92 rows containing non-finite values (stat_smooth).
```

```
## Warning: Removed 92 rows containing missing values (geom_point).
```

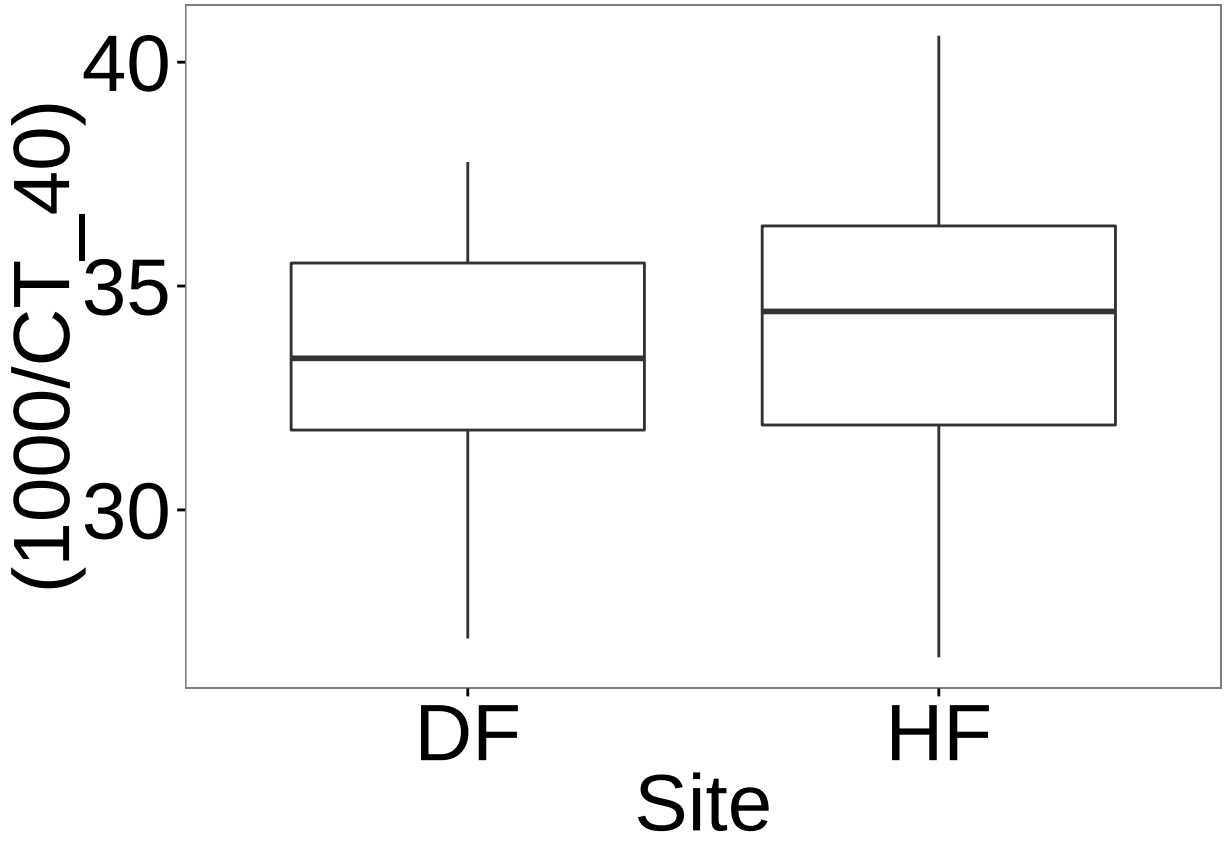


hsp40 plot

```
ggplot(warm.40,aes(x=baittemp.ave,y=(1000/CT_40),colour=factor(Site)))+geom_point(size=3)+T+geom_smooth
```




```
ggplot(warm.40,aes(x=Site,y=(1000/CT_40)))+geom_boxplot()+T # gxp by by site
```




```
sessionInfo()
```

```
## R version 3.2.3 (2015-12-10)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: OS X 10.11.1 (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] MASS_7.3-45   tidyr_0.4.1   ggplot2_2.0.0 dplyr_0.4.3   plyr_1.8.3
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.3      knitr_1.10.5     magrittr_1.5     munsell_0.4.2
##  [5] colorspace_1.2-6 R6_2.1.2         stringr_1.0.0    highr_0.5
##  [9] tools_3.2.3      parallel_3.2.3   grid_3.2.3       gtable_0.1.2
## [13] DBI_0.3.1        htmltools_0.2.6  yaml_2.1.13      lazyeval_0.1.10
## [17] assertthat_0.1   digest_0.6.8     formatR_1.2      evaluate_0.7.2
## [21] rmarkdown_0.7    labeling_0.3     stringi_1.0-1    scales_0.3.0
```