Will climate change cause temperature stress?

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Rationale

Experimental protocol

Samples

```
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 object is masked from 'package:stats':
##
##     filter
##
## 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
```

Calculating gene expression

GOI= gene of interest
HKG= house keeping gene

$$\frac{2^{(GOI_{mean}-GOI_{sample})}}{2^{(HKG_{mean}-HKG_{sample})}}$$

```r
#warm<-read.csv("2015_warming_prelim.csv") #reading in the data set
warm<-read.csv("20151029_DFHF_gxp_data_v2.csv")
head(warm,4) #big data, so only looking at first 4 lines
```

```
##   n Year Site Vial.Name Cham Sample Window BaitTemp1 BaitTemp2 BaitTemp3
## 1 3 2013   DF    DF A1-A    1      3      B      25.2      25.2      25.2
## 2 4 2013   DF    DF A2-B    2      1      B      23.0      23.2      23.2
## 3 7 2013   DF    DF A3-B    3      1      B      23.0      23.0      23.6
## 4 8 2013   DF    DF A3-A    3      2      A      23.0      22.8      23.0
##   BaitTemp4 RNA.conc. Isolation.Date  CT_18s   CT_40   CT_70   CT_83 CDNA
## 1      25.0      14.8       20150731  18.459  32.099  26.112  31.266  2.5
## 2      23.4      42.1       20150814  13.121  32.058  28.401  31.109  2.5
## 3      23.2      16.5       20150730  29.813      NA  32.777  31.510  2.5
## 4      23.8        65       20150814  14.801  30.751  28.745  31.524  2.5
```

```r
tail(warm,4) # looking at the last 4 lines
```

```
##           n Year Site Vial.Name Cham Sample Window BaitTemp1 BaitTemp2
## 132 226 2013   HF   HF2 10-2   10      2               29.4      29.6
## 133 228 2013   HF   HF2 11-1   11      1               27.8      27.2
## 134 231 2013   HF   HF2 12-1   12      1               29.4      28.4
## 135 233 2013   HF   HF2 12-3   12      3               28.8      28.2
##     BaitTemp3 BaitTemp4 RNA.conc. Isolation.Date    CT_18s    CT_40
## 132      29.8      29.8      44.3       20150827  8.498671 26.30671
## 133      27.6      28.0        55       20150825  9.068080 28.11423
## 134      28.6      28.6        59       20150825  5.487036 26.67928
## 135      28.2      28.4      31.4       20150825  8.642649 27.91521
##        CT_70   CT_83 CDNA
## 132 20.84113 21.060  2.5
## 133 23.25425 24.375  2.5
## 134 21.81287 22.775  2.5
## 135 22.37750 22.076  2.5
```

```r
names(warm) #looking at the names of the headings in data set
```

```
##  [1] "n"              "Year"           "Site"           "Vial.Name"
##  [5] "Cham"           "Sample"         "Window"         "BaitTemp1"
##  [9] "BaitTemp2"      "BaitTemp3"      "BaitTemp4"      "RNA.conc."
## [13] "Isolation.Date" "CT_18s"         "CT_40"          "CT_70"
## [17] "CT_83"          "CDNA"
```

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

```
## [1] 135  18
```

```r
#calculating the mean bait_temp
warm$mean.temps<-rowMeans(warm[,8:11],na.rm=T)
```

```r
gene.means<-apply(na.omit(warm[,14:17]),2,mean) #taking the means of each gene
gene.means
```

```
##    CT_18s    CT_40    CT_70    CT_83
## 12.24230 29.47033 24.78352 27.32496
```

```r
deltaCT<-as.data.frame(t(apply(warm[,14:17],1,function(x){gene.means-x}))) # subtracting the means of e

dd<-as.data.frame(t(apply(deltaCT,1,function(x){2^x})))# raising everything to 2 power
names(dd)<-c('18s',"hsp40","hsp70","hsp83")
#Calculating fold change!
merg<-as.data.frame(cbind(warm,dd[,2:4]/dd[,1]))
```

Stats!!

response:

predictor

```r
#changing collection date into a facto
#merg$Collection.Date<-as.factor(as.character(merg$Collection.Date))

#making plots
merg$color<-ifelse(merg$Site=="DF","red","blue")# making color
merg2<-subset(merg,merg$hsp83<100)
#Doing stats

mod1<-stepAIC(lm(log10(hsp70)~Site*mean.temps,data=merg2,na.rm=T),direction="backward");summary(mod1)
```

```
## Warning in lm.fit(x, y, offset = offset, singular.ok = singular.ok, ...):
## extra argument 'na.rm' is disregarded.
```

```
## Start:  AIC=-86.45
## log10(hsp70) ~ Site * mean.temps
##
##                    Df Sum of Sq    RSS     AIC
## - Site:mean.temps  1   0.42536 59.967 -87.553
## <none>                         59.542 -86.450
```

```
## Warning in lm.fit(x, y, offset = offset, singular.ok = singular.ok, ...):
## extra argument 'na.rm' is disregarded.
```

```
##
## Step:  AIC=-87.55
## log10(hsp70) ~ Site + mean.temps
##
##              Df Sum of Sq    RSS     AIC
## <none>                    59.967 -87.553
## - Site        1    10.658 70.626 -68.940
## - mean.temps  1    12.618 72.586 -65.491
```

```
##
## Call:
## lm(formula = log10(hsp70) ~ Site + mean.temps, data = merg2,
##     na.rm = T)
##
## Residuals:
##      Min      1Q   Median      3Q      Max
## -1.97172 -0.45771 -0.00084  0.38832  1.63251
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -3.0453     0.6407  -4.753 5.49e-06 ***
## SiteHF      -0.6014     0.1286  -4.676 7.58e-06 ***
## mean.temps   0.1185     0.0233   5.087 1.32e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.6982 on 123 degrees of freedom
##   (1 observation deleted due to missingness)
## Multiple R-squared:  0.2776, Adjusted R-squared:  0.2659
## F-statistic: 23.64 on 2 and 123 DF,  p-value: 2.057e-09
```

```r
mod2<-stepAIC(lm(log10(hsp40)~Site*mean.temps,data=merg2),direction="backward");summary(mod2)
```

```
## Start:  AIC=-100.93
## log10(hsp40) ~ Site * mean.temps
##
##                   Df Sum of Sq    RSS     AIC
## - Site:mean.temps  1   0.38054 49.561 -102.00
## <none>                         49.181 -100.94
##
## Step:  AIC=-102
## log10(hsp40) ~ Site + mean.temps
##
##              Df Sum of Sq    RSS     AIC
## <none>                    49.561 -102.002
## - mean.temps  1    3.7383 53.300  -95.203
## - Site        1   10.1617 59.723  -81.435
```

```
##
## Call:
## lm(formula = log10(hsp40) ~ Site + mean.temps, data = merg2)
##
## Residuals:
##      Min      1Q   Median      3Q      Max
## -1.42113 -0.40019 -0.04272  0.34712  2.14357
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -1.64883    0.61577  -2.678  0.00847 **
## SiteHF      -0.59275    0.12051  -4.919 2.85e-06 ***
## mean.temps   0.06719    0.02252   2.983  0.00347 **
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.6481 on 118 degrees of freedom
##    (6 observations deleted due to missingness)
## Multiple R-squared:  0.2138, Adjusted R-squared:  0.2004
## F-statistic: 16.04 on 2 and 118 DF,  p-value: 6.885e-07
```

```r
mod3<-stepAIC(lm(log10(hsp83)~Site*mean.temps,data=merg2),direction="backward");summary(mod3)
```

```
## Start:  AIC=-68.81
## log10(hsp83) ~ Site * mean.temps
##
##                   Df Sum of Sq    RSS     AIC
## - Site:mean.temps  1   0.43955 69.804 -70.010
## <none>                         69.364 -68.812
##
## Step:  AIC=-70.01
## log10(hsp83) ~ Site + mean.temps
##
##              Df Sum of Sq     RSS     AIC
## <none>                    69.804 -70.010
## - Site        1    31.262 101.066 -25.009
## - mean.temps  1    44.474 114.278  -9.405
```

```
##
## Call:
## lm(formula = log10(hsp83) ~ Site + mean.temps, data = merg2)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.92777 -0.40339  0.01246  0.52758  1.62453
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -5.70049    0.68807  -8.285 1.62e-13 ***
## SiteHF      -1.02326    0.13731  -7.452 1.37e-11 ***
## mean.temps   0.22241    0.02502   8.888 6.04e-15 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.7503 on 124 degrees of freedom
## Multiple R-squared:  0.5189, Adjusted R-squared:  0.5111
## F-statistic: 66.86 on 2 and 124 DF,  p-value: < 2.2e-16
```

```r
par(mfrow=c(2,2))
plot(mod3)
```

Residuals vs Fitted

Normal Q–Q

Scale–Location

Residuals vs Leverage
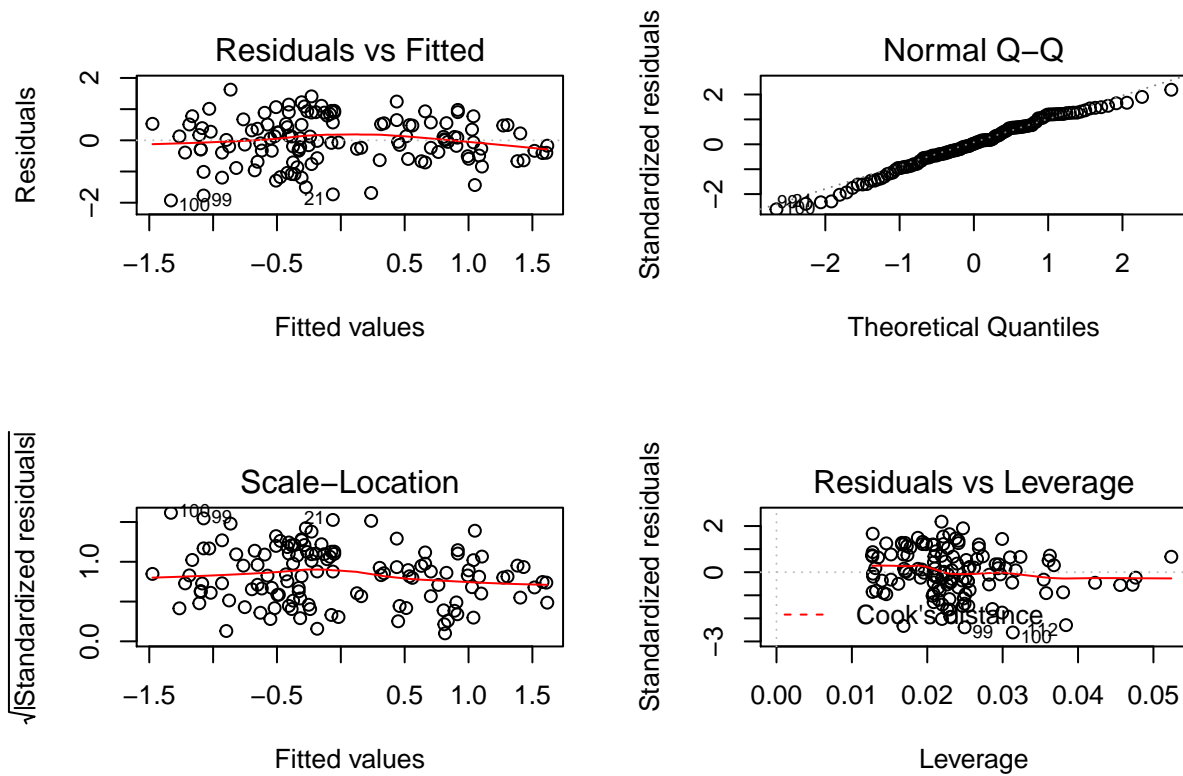
```r
par(mfrow=c(1,1))
#hsp83 plot
plot(merg2$mean.temps,log10(merg2$hsp83),col=merg2$color,pch=16,ylab="Log10 Hsp83 relative expression",)
axis(1,lwd=2);box(lwd=2);axis(2,lwd=2,las=1)
#text(merg2$mean.temps,log10(merg2$hsp83),labels=merg2$Vial.Name)
abline(lm(log10(hsp83)~mean.temps,data=subset(merg2,merg2$Site=="DF")),lty="dotdash",col="red",lwd=3)
abline(lm(log10(hsp83)~mean.temps,data=subset(merg2,merg2$Site=="HF")),lty="dotdash",col="blue",lwd=3)
```
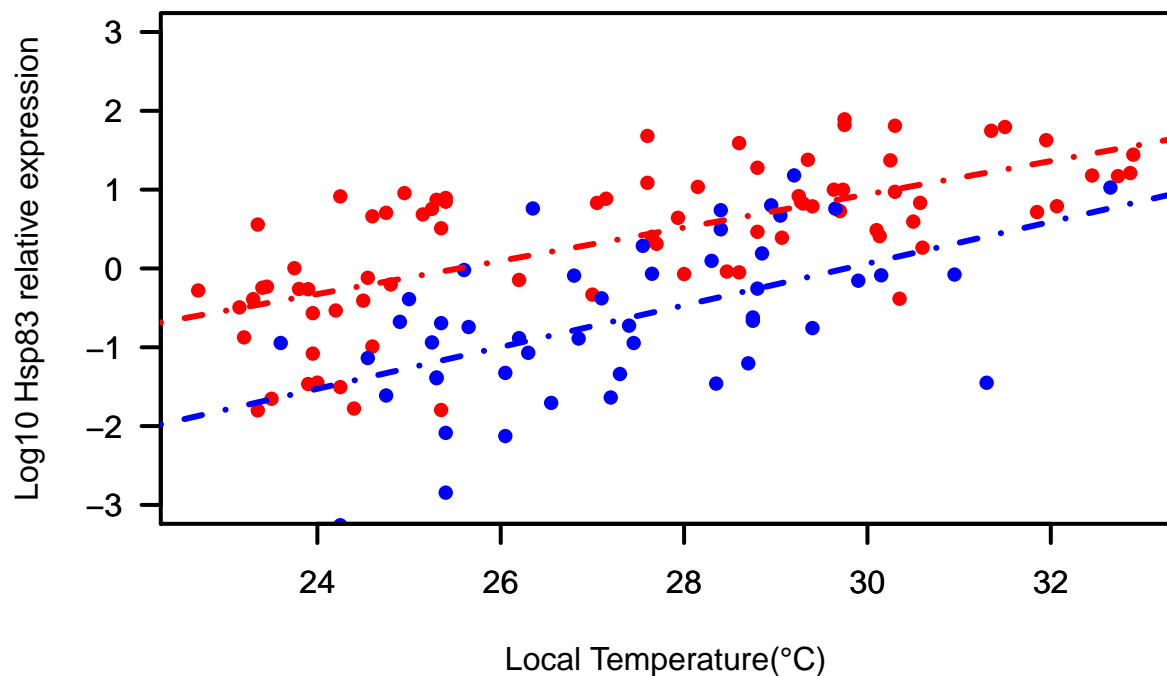
```
#hsp70 plot
plot(merg2$mean.temps,log10(merg2$hsp70),col=merg2$color,pch=16,ylab="Log10 Hsp70 relative expression",
axis(1,lwd=2);box(lwd=2);axis(2,lwd=2,las=1)
#text(merg2$mean.temps,log10(merg2$hsp83),labels=merg2$Vial.Name)
abline(lm(log10(hsp70)~mean.temps,data=subset(merg2,merg2$Site=="DF")),lty="dotdash",col="red",lwd=3)
abline(lm(log10(hsp70)~mean.temps,data=subset(merg2,merg2$Site=="HF")),lty="dotdash",col="blue",lwd=3)
```


Local Temperature (°C)
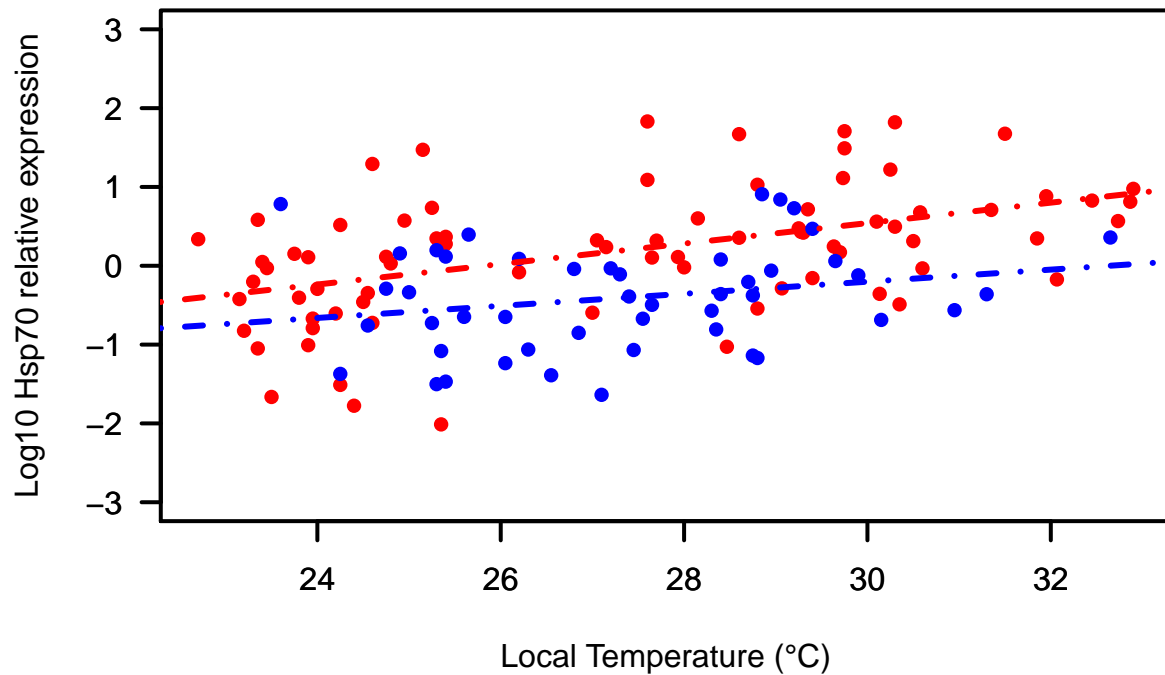
```
#hsp40
plot(merg2$mean.temps,log10(merg2$hsp40),col=merg2$color,pch=16,ylab="Log10 Hsp40 relative expression",
axis(1,lwd=2);box(lwd=2);axis(2,lwd=2,las=1)
#text(merg2$mean.temps,log10(merg2$hsp83),labels=merg2$Vial.Name)
abline(lm(log10(hsp40)~mean.temps,data=subset(merg2,merg2$Site=="DF")),lty="dotdash",col="red",lwd=3)
abline(lm(log10(hsp40)~mean.temps,data=subset(merg2,merg2$Site=="HF")),lty="dotdash",col="blue",lwd=3)
```
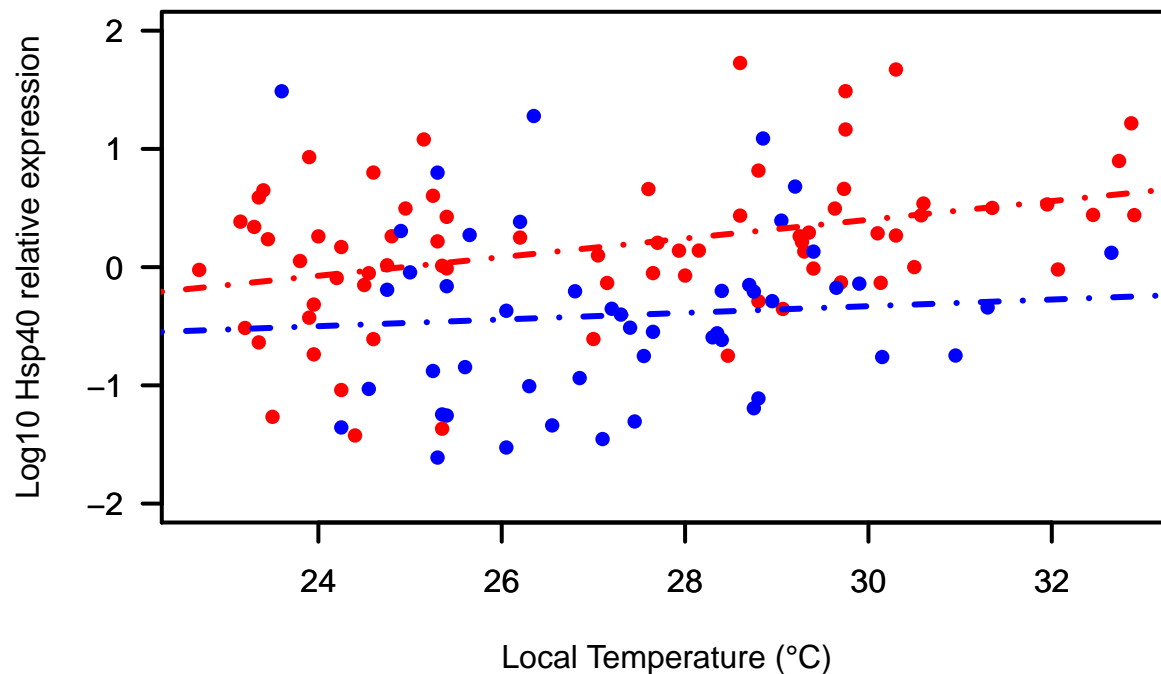
```
#using merg 2, convertin xp to 100/ct
names(merg2)
```

```
##  [1] "n"             "Year"          "Site"          "Vial.Name"
##  [5] "Cham"          "Sample"        "Window"        "BaitTemp1"
##  [9] "BaitTemp2"     "BaitTemp3"     "BaitTemp4"     "RNA.conc."
## [13] "Isolation.Date" "CT_18s"       "CT_40"         "CT_70"
## [17] "CT_83"         "CDNA"          "mean.temps"    "hsp40"
## [21] "hsp70"         "hsp83"         "color"
```

```
diff.way<-as.data.frame(cbind(merg2[,1:7],apply(merg2[,14:17],2,function(x){1000/x}),merg2[,19]))
names(diff.way)[12]<-"mean.temps"
#summary(lm(CT_83~Site*mean.temps+CT_18s+Year,data=diff.way))
#plot(diff.way$mean.temps,diff.way$CT_83)
#summary(lm(CT_70~Site*mean.temps+CT_18s+Year,data=diff.way))
#summary(lm(CT_40~Site*mean.temps+CT_18s+Year,data=diff.way))

#stepwise
library(MASS)
mod11<-stepAIC(lm(CT_83~Site*mean.temps+CT_18s+Year,data=diff.way),direction="backward")
```

```
## Start:  AIC=328.43
## CT_83 ~ Site * mean.temps + CT_18s + Year
##
##                   Df Sum of Sq    RSS    AIC
## - Site:mean.temps  1      9.71 1543.9 327.23
## - Year             1     21.72 1555.9 328.21
## <none>                         1534.2 328.43
## - CT_18s           1   1305.50 2839.7 404.62
##
## Step:  AIC=327.23
```

```
## CT_83 ~ Site + mean.temps + CT_18s + Year
##
##               Df Sum of Sq    RSS    AIC
## <none>                     1543.9 327.23
## - Year        1     29.60 1573.5 327.64
## - Site        1    425.40 1969.3 356.14
## - mean.temps  1    796.79 2340.7 378.08
## - CT_18s      1   1504.48 3048.4 411.63
```

```r
summary(mod11)
```

```
##
## Call:
## lm(formula = CT_83 ~ Site + mean.temps + CT_18s + Year, data = diff.way)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -9.5705 -2.1654 -0.1086  2.2795  7.3363
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept) 3255.80850 2127.73130   1.530    0.129
## SiteHF        -4.36140    0.75223  -5.798 5.39e-08 ***
## mean.temps     1.00222    0.12630   7.935 1.15e-12 ***
## CT_18s         0.12906    0.01184  10.904  < 2e-16 ***
## Year          -1.61729    1.05747  -1.529    0.129
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.557 on 122 degrees of freedom
## Multiple R-squared:  0.6322, Adjusted R-squared:  0.6202
## F-statistic: 52.43 on 4 and 122 DF,  p-value: < 2.2e-16
```

```r
#plot(mod11)
```

```r
summary(stepAIC(lm(CT_70~Site*mean.temps+CT_18s+Year,data=diff.way),direction="backward"))
```

```
## Start:  AIC=271.87
## CT_70 ~ Site * mean.temps + CT_18s + Year
##
##                   Df Sum of Sq     RSS    AIC
## - Site:mean.temps  1      5.00  996.01 270.50
## <none>                          991.01 271.87
## - Year             1     53.54 1044.55 276.50
## - CT_18s           1    861.81 1852.82 348.71
##
## Step:  AIC=270.5
## CT_70 ~ Site + mean.temps + CT_18s + Year
##
##               Df Sum of Sq     RSS    AIC
## <none>                      996.01 270.50
## - Year        1     63.48 1059.49 276.29
## - Site        1     85.91 1081.91 278.93
```

9

```
## - mean.temps   1     433.74 1429.75 314.05
## - CT_18s        1     982.73 1978.74 355.00


##
## Call:
## lm(formula = CT_70 ~ Site + mean.temps + CT_18s + Year, data = diff.way)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -7.5938 -1.6997  0.0345  1.9859  6.0100
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 4781.25519 1716.65320   2.785  0.00621 **
## SiteHF         -1.99364    0.61712  -3.231  0.00159 **
## mean.temps      0.73953    0.10188   7.259  4.1e-11 ***
## CT_18s          0.10632    0.00973  10.926  < 2e-16 ***
## Year           -2.36929    0.85317  -2.777  0.00636 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.869 on 121 degrees of freedom
##   (1 observation deleted due to missingness)
## Multiple R-squared:  0.6192, Adjusted R-squared:  0.6066
## F-statistic: 49.19 on 4 and 121 DF,  p-value: < 2.2e-16
```

```r
summary(stepAIC(lm(CT_40~Site*mean.temps+CT_18s+Year,data=diff.way),direction="backward"))
```

```
## Start:  AIC=92.71
## CT_40 ~ Site * mean.temps + CT_18s + Year
##
##                   Df Sum of Sq    RSS     AIC
## <none>                         235.77  92.713
## - Site:mean.temps  1     4.407 240.17  92.953
## - Year             1     4.969 240.73  93.236
## - CT_18s           1   298.559 534.32 189.711


##
## Call:
## lm(formula = CT_40 ~ Site * mean.temps + CT_18s + Year, data = diff.way)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.2715 -1.0592  0.1469  1.0081  3.0808
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)       1425.35888  902.51666   1.579   0.1170
## SiteHF              -6.46000    3.38607  -1.908   0.0589 .
## mean.temps           0.36114    0.06265   5.764 7.01e-08 ***
## CT_18s               0.06178    0.00512  12.068  < 2e-16 ***
## Year                -0.69847    0.44867  -1.557   0.1223
## SiteHF:mean.temps    0.18419    0.12563   1.466   0.1454
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.432 on 115 degrees of freedom
##   (6 observations deleted due to missingness)
## Multiple R-squared:  0.7016, Adjusted R-squared:  0.6886
## F-statistic: 54.07 on 5 and 115 DF,  p-value: < 2.2e-16
```

```r
#only analyzing 2013
diff.way2<-subset(diff.way,diff.way$Year=="2013")

#summary(lm(CT_83~Site*mean.temps+CT_18s,data=diff.way2))
#summary(lm(CT_70~Site*mean.temps+CT_18s,data=diff.way2))
#summary(lm(CT_40~Site*mean.temps+CT_18s,data=diff.way2))

#stepwise
mod22<-stepAIC(lm(CT_83~Site*mean.temps+CT_18s,data=diff.way2),direction="backward");summary(mod22)
```

```
## Start:  AIC=292.35
## CT_83 ~ Site * mean.temps + CT_18s
##
##                   Df Sum of Sq    RSS    AIC
## - Site:mean.temps  1     12.61 1425.2 291.33
## <none>                         1412.6 292.35
## - CT_18s           1    954.83 2367.4 347.66
##
## Step:  AIC=291.33
## CT_83 ~ Site + mean.temps + CT_18s
##
##              Df Sum of Sq    RSS    AIC
## <none>                    1425.2 291.33
## - Site        1    371.55 1796.8 315.05
## - mean.temps  1    802.57 2227.8 338.91
## - CT_18s      1   1122.32 2547.5 353.80


##
## Call:
## lm(formula = CT_83 ~ Site + mean.temps + CT_18s, data = diff.way2)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -9.1938 -2.1649 -0.2561  2.2322  7.3032
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -0.19050    3.62803  -0.053    0.958
## SiteHF      -4.11972    0.78002  -5.282 6.79e-07 ***
## mean.temps   1.04565    0.13471   7.762 5.28e-12 ***
## CT_18s       0.11909    0.01297   9.179 3.67e-15 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.65 on 107 degrees of freedom
```

```
## Multiple R-squared:  0.6113, Adjusted R-squared:  0.6004
## F-statistic:  56.1 on 3 and 107 DF,  p-value: < 2.2e-16
```

```r
mod23<-stepAIC(lm(CT_70~Site*mean.temps+CT_18s,data=diff.way2),direction="backward");summary(mod23)
```

```
## Start:  AIC=241.03
## CT_70 ~ Site * mean.temps + CT_18s
##
##                    Df Sum of Sq     RSS    AIC
## - Site:mean.temps  1      4.56  903.10 239.59
## <none>                          898.54 241.03
## - CT_18s           1    657.71 1556.25 299.45
##
## Step:  AIC=239.59
## CT_70 ~ Site + mean.temps + CT_18s
##
##              Df Sum of Sq     RSS    AIC
## <none>                     903.10 239.59
## - Site        1     71.55  974.65 245.98
## - mean.temps  1    456.80 1359.90 282.62
## - CT_18s      1    755.42 1658.52 304.45


##
## Call:
## lm(formula = CT_70 ~ Site + mean.temps + CT_18s, data = diff.way2)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -7.9951 -1.6203 -0.0211  2.0202  6.2053
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 11.06602    2.90769   3.806 0.000237 ***
## SiteHF      -1.84195    0.63560  -2.898 0.004565 **
## mean.temps   0.78889    0.10774   7.322 4.96e-11 ***
## CT_18s       0.09983    0.01060   9.416 1.16e-15 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.919 on 106 degrees of freedom
##   (1 observation deleted due to missingness)
## Multiple R-squared:  0.6172, Adjusted R-squared:  0.6064
## F-statistic: 56.98 on 3 and 106 DF,  p-value: < 2.2e-16
```

```r
mod24<-stepAIC(lm(CT_40~Site*mean.temps+CT_18s,data=diff.way2),direction="backward");summary(mod24)
```

```
## Start:  AIC=73.76
## CT_40 ~ Site * mean.temps + CT_18s
##
##                    Df Sum of Sq     RSS     AIC
## <none>                          192.72  73.764
## - Site:mean.temps  1     5.347 198.07  74.637
## - CT_18s           1   206.876 399.59 148.332
```

```
## 
## Call:
## lm(formula = CT_40 ~ Site * mean.temps + CT_18s, data = diff.way2)
## 
## Residuals:
##     Min      1Q  Median      3Q     Max
## -2.9316 -1.1450  0.0463  0.9872  3.1693
## 
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)       19.276165   1.744573  11.049  < 2e-16 ***
## SiteHF            -6.912472   3.326799  -2.078   0.0403 *
## mean.temps         0.382791   0.064131   5.969 3.65e-08 ***
## CT_18s             0.055341   0.005341  10.361  < 2e-16 ***
## SiteHF:mean.temps  0.205811   0.123564   1.666   0.0989 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Residual standard error: 1.388 on 100 degrees of freedom
##   (6 observations deleted due to missingness)
## Multiple R-squared:  0.7073, Adjusted R-squared:  0.6956
## F-statistic: 60.42 on 4 and 100 DF,  p-value: < 2.2e-16
```

```r
#adjusting sizing
sizzy<- theme_bw()+
  theme(axis.title.y=element_text(size=rel(2)),axis.title.x=element_text(size=rel(3)))+
  theme(axis.text.y=element_text(size=rel(2)),axis.text.x=element_text(size=rel(2)),legend.position=c(.8
  theme(legend.title=element_blank(),legend.text=element_text(size=28),panel.grid.major=element_blank()

diff.way$Year<-as.factor(as.character(diff.way$Year))

#hsp83 fig
ggplot(diff.way2,aes(x=mean.temps,y=CT_83,colour=Site,shape=Site))+geom_point(size=3)+scale_x_continuou
```
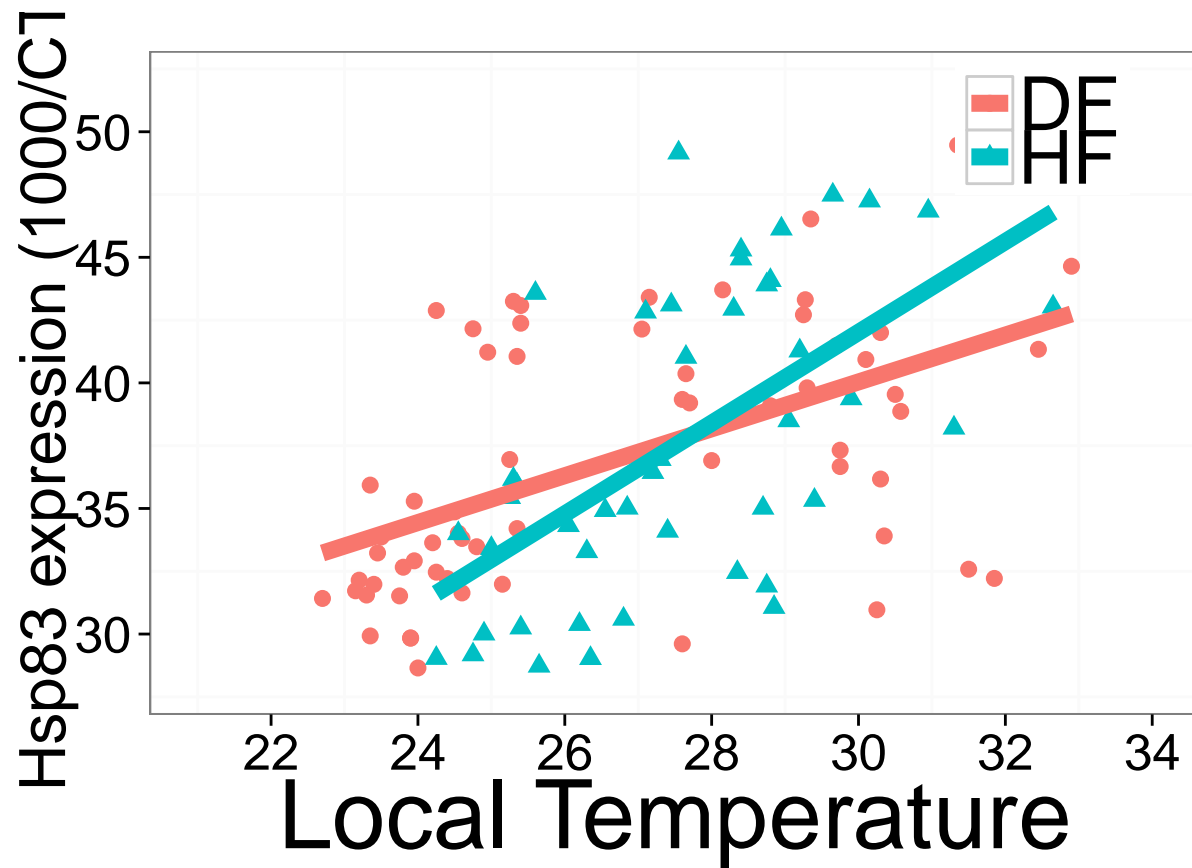
```
## Warning in loop_apply(n, do.ply): Removed 4 rows containing missing values
## (stat_smooth).
```
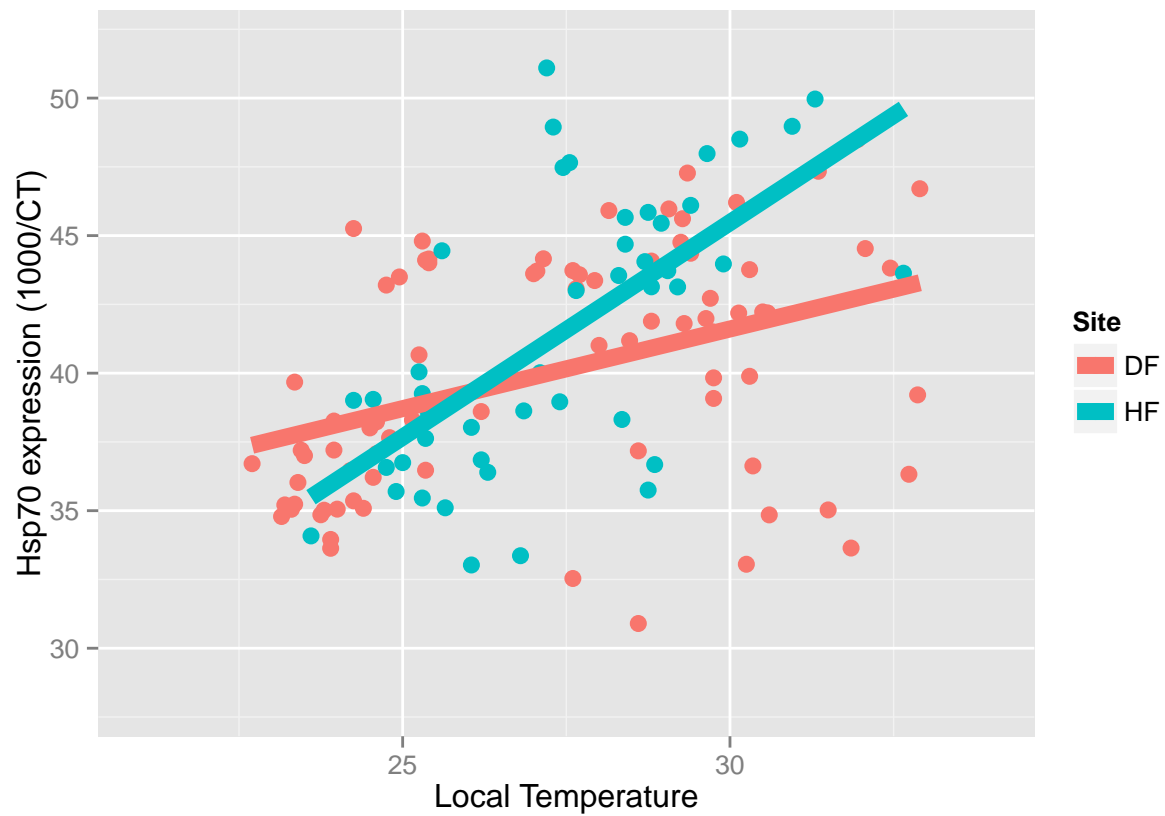
```
## Warning in loop_apply(n, do.ply): Removed 4 rows containing missing values
## (geom_point).
```

```
ggplot(diff.way,aes(x=mean.temps,y=CT_70,colour=Site))+geom_point(size=3)+scale_x_continuous("Local Temp
```

```
## Warning in loop_apply(n, do.ply): Removed 1 rows containing missing values
## (stat_smooth).
```
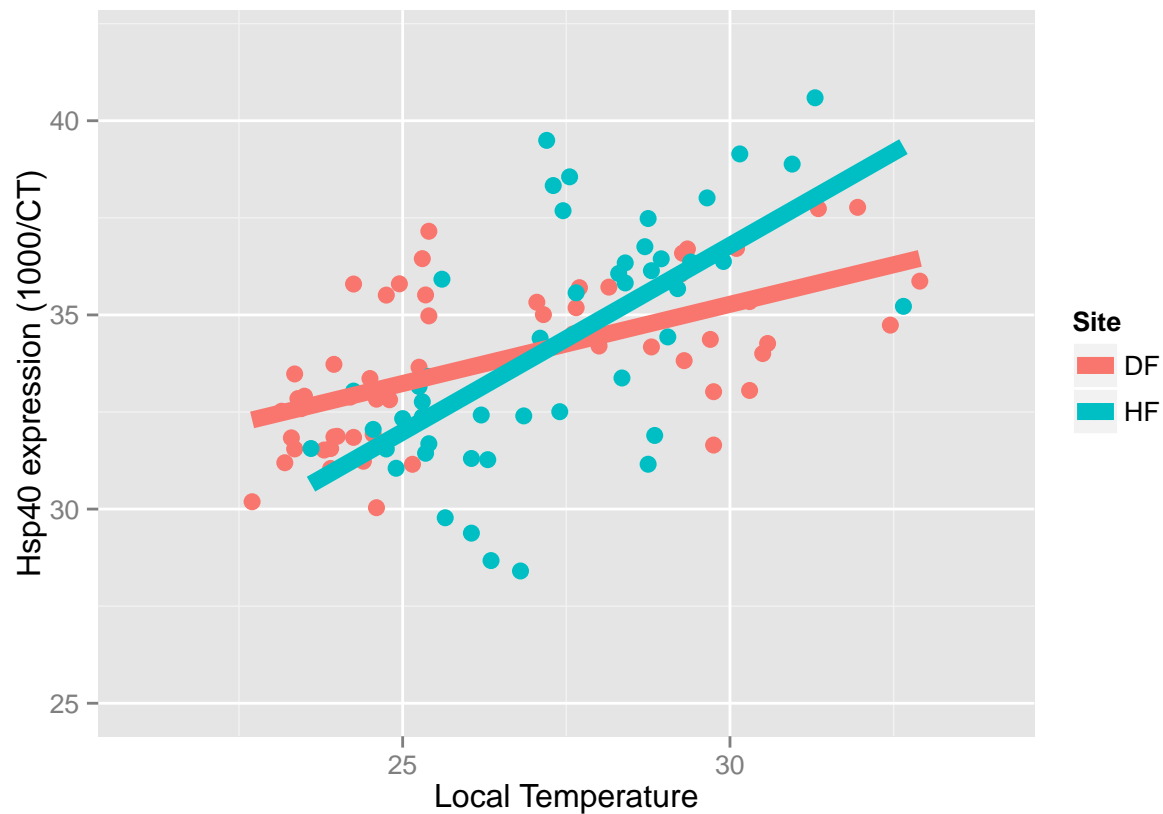
```
## Warning in loop_apply(n, do.ply): Removed 1 rows containing missing values
## (geom_point).
```

```
#hsp40
ggplot(diff.way2,aes(x=mean.temps,y=CT_40,colour=Site))+geom_point(size=3)+scale_x_continuous("Local Tem
```

```
## Warning in loop_apply(n, do.ply): Removed 6 rows containing missing values
## (stat_smooth).
```

```
## Warning in loop_apply(n, do.ply): Removed 6 rows containing missing values
## (geom_point).
```

```
sessionInfo()
```

```
## R version 3.2.2 (2015-08-14)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: OS X 10.10.5 (Yosemite)
##
## 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_7.3-43  tidyr_0.2.0   ggplot2_1.0.1 dplyr_0.4.1   plyr_1.8.2
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.11.6      knitr_1.10.5    magrittr_1.5    munsell_0.4.2
##  [5] colorspace_1.2-6 stringr_1.0.0   tools_3.2.2     parallel_3.2.2
##  [9] grid_3.2.2       gtable_0.1.2    DBI_0.3.1       htmltools_0.2.6
## [13] yaml_2.1.13      assertthat_0.1  digest_0.6.8    reshape2_1.4.1
## [17] formatR_1.2      evaluate_0.7    rmarkdown_0.6.1 labeling_0.3
## [21] stringi_0.4-1    scales_0.2.4    proto_0.3-10
```