# Evolution of thermal limits and stress response in Aphaenogaster

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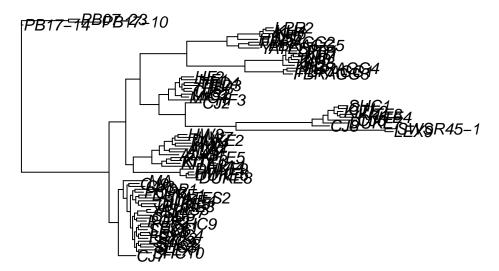
#### Overview

Question: What forces shapes heat tolerance in Aphanogaster?

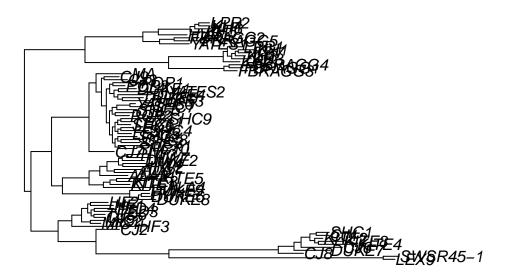
Hypothesis: Common ancestry and/or ecology shape heat tolerance

Methods: Ants were collected in 2014 and acclimated in a common garden experiment

```
dat<-read.csv("2014_sampling_data_sheet.csv")#sampling data
tree<-read.tree("Phylogenetics/20150828_raxml_commongarden_NO_BS.nex")#phylogeny with branch lengths on
#removing a few tips for plotting purposes
tree1<-drop.tip(tree,tip=c("EXIT65","SAL13-14","PMBE","ALA5","LVA2"))
tree2<-root(tree1,node=148,resolve.root=TRUE)
plot(tree2)</pre>
```



```
##kk, I really want a distance matrix for the ingroup, so I need to get rid of pogos
final.tree<-drop.tip(tree,tip=c("EXIT65","SAL13-14","PMBE","ALA5","LVA2","PB17-14","PB17-10","PB07-23")
plot(final.tree)</pre>
```



##kk, do pcoa and add to original dataset
dist.mat<-cophenetic.phylo(final.tree)
knitr::kable(head(dist.mat[1:4,1:6]))</pre>

	LEX9	SWSR45-1	CJ8	DUKE7	CJ6	KITE4
LEX9	0.0000000	0.0199152	0.2593805	0.2786177	0.2875044	0.2975939
SWSR45-1	0.0199152	0.0000000	0.2624274	0.2816646	0.2905513	0.3006407
CJ8	0.2593805	0.2624274	0.0000000	0.0553566	0.0642433	0.0743327
DUKE7	0.2786177	0.2816646	0.0553566	0.0000000	0.0608534	0.0709429

aph.pcoa<-pcoa(dist.mat)</pre>

knitr::kable(aph.pcoa\$values)#eigenvalues

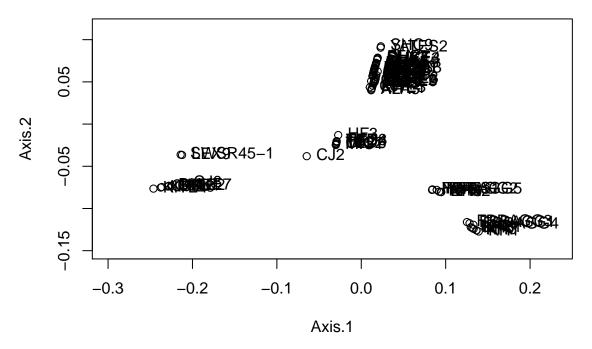
Eigenvalues	Relative_eig	Rel_corr_eig	Broken_stick	Cum_corr_eig	Cumul_br_stick
0.7616536	0.4413092	0.2808578	0.0633374	0.2808578	0.0633374
0.4044550	0.2343450	0.1513638	0.0505169	0.4322216	0.1138544
0.1398667	0.0810400	0.0554436	0.0441067	0.4876651	0.1579611
0.1295289	0.0750503	0.0516959	0.0398332	0.5393610	0.1977942
0.1202895	0.0696969	0.0483463	0.0366280	0.5877073	0.2344223
0.0398956	0.0231159	0.0192014	0.0340639	0.6069087	0.2684862
0.0321383	0.0186212	0.0163892	0.0319272	0.6232979	0.3004134
0.0234180	0.0135686	0.0132278	0.0300957	0.6365257	0.3305091
0.0119497	0.0069237	0.0090703	0.0284931	0.6455960	0.3590022
0.0100711	0.0058353	0.0083892	0.0270686	0.6539852	0.3860709
0.0062277	0.0036084	0.0069959	0.0257866	0.6609811	0.4118574
0.0047220	0.0027360	0.0064500	0.0246211	0.6674311	0.4364785
0.0037188	0.0021547	0.0060864	0.0235527	0.6735175	0.4600312
0.0031812	0.0018432	0.0058915	0.0225665	0.6794089	0.4825977
0.0030826	0.0017861	0.0058557	0.0216508	0.6852647	0.5042485
0.0025997	0.0015063	0.0056807	0.0207961	0.6909453	0.5250445
0.0024562	0.0014232	0.0056286	0.0199948	0.6965740	0.5450393
0.0024353	0.0014110	0.0056210	0.0192406	0.7021950	0.5642799
0.0023255	0.0013474	0.0055813	0.0185284	0.7077763	0.5828083
0.0018983	0.0010999	0.0054264	0.0178536	0.7132026	0.6006619

Eigenvalues	Relative_eig	Rel_corr_eig	Broken_stick	Cum_corr_eig	Cumul_br_stick
0.0016281	0.0009433	0.0053284	0.0172126	0.7185311	0.6178745
0.0015990	0.0009265	0.0053179	0.0166021	0.7238489	0.6344766
0.0013902	0.0008055	0.0052422	0.0160193	0.7290911	0.6504959
0.0012848	0.00074444	0.0052039	0.0154619	0.7342951	0.6659578
0.0012734	0.0007378	0.0051998	0.0149277	0.7394949	0.6808855
0.0012713	0.0007366	0.0051991	0.0144149	0.7446940	0.6953004
0.0012477	0.0007229	0.0051905	0.0139218	0.7498845	0.7092223
0.0012281	0.0007116	0.0051834	0.0134470	0.7550679	0.7226692
0.0011592	0.0006717	0.0051584	0.0129891	0.7602263	0.7356583
0.0011544	0.0006689	0.0051567	0.0125470	0.7653830	0.7482054
0.0010835	0.0006278	0.0051310	0.0121197	0.7705140	0.7603250
0.0009761	0.0005655	0.0050920	0.0117061	0.7756061	0.7720311
0.0009658	0.0005596	0.0050883	0.0113055	0.7806944	0.7833366
0.0008808	0.0005104	0.0050575	0.0109170	0.7857519	0.7942535
0.0008382	0.0004857	0.0050421	0.0105399	0.7907940	0.8047934
0.0008061	0.0004670	0.0050304	0.0101736	0.7958244	0.8149670
0.0007261	0.0004207	0.0050014	0.0098175	0.8008258	0.8247845
0.0006734	0.0003902	0.0049823	0.0094710	0.8058081	0.8342554
0.0006350	0.0003679	0.0049684	0.0091336	0.8107765	0.8433890
0.0006028	0.0003493	0.0049567	0.0088048	0.8157332	0.8521939
0.0005911	0.0003425	0.0049525	0.0084843	0.8206857	0.8606782
0.0005901	0.0003419	0.0049521	0.0081716	0.8256378	0.8688498
0.0005722	0.0003315	0.0049456	0.0078664	0.8305834	0.8767162
0.0005530	0.0003204	0.0049387	0.0075682	0.8355221	0.8842844
0.0005327	0.0003087	0.0049313	0.0072769	0.8404534	0.8915613
0.0005111	0.0002961	0.0049235	0.0069920	0.8453769	0.8985533
0.0004862	0.0002817	0.0049144	0.0067133	0.8502913	0.9052665
0.0004687	0.0002715	0.0049081	0.0064405	0.8551994	0.9117070
0.0004628	0.0002681	0.0049060	0.0061734	0.8601054	0.9178804
0.0004305	0.0002494	0.0048943	0.0059117	0.8649996	0.9237921
0.0004107	0.0002380	0.0048871	0.0056553	0.8698867	0.9294475
0.0003987	0.0002310	0.0048827	0.0054039	0.8747695	0.9348514
0.0003847	0.0002229	0.0048776	0.0051574	0.8796471	0.9400088
0.0003712	0.0002150	0.0048727	0.0049155	0.8845199	0.9449243
0.0003616	0.0002095	0.0048693	0.0046781	0.8893891	0.9496024
0.0003558	0.0002062	0.0048672	0.0044450	0.8942563	0.9540474
0.0003381	0.0001959	0.0048608	0.0042160	0.8991171	0.9582634
0.0003200	0.0001854	0.0048542	0.0039911	0.9039713	0.9622545
0.0003184	0.0001845	0.0048536	0.0037701	0.9088249	0.9660246
0.0002904	0.0001683	0.0048435	0.0035528	0.9136684	0.9695774
0.0002834	0.0001642	0.0048409	0.0033391	0.9185093	0.9729165
0.0002619	0.0001518	0.0048332	0.0031289	0.9233425	0.9760455
0.0002572	0.0001490	0.0048314	0.0029222	0.9281739	0.9789676
0.0002533	0.0001468	0.0048300	0.0027187	0.9330039	0.9816863
0.0002474	0.0001433	0.0048279	0.0025183	0.9378318	0.9842046
0.0002429	0.0001407	0.0048262	0.0023211	0.9426580	0.9865257
0.0002173	0.0001259	0.0048170	0.0021268	0.9474750	0.9886526
0.0002041	0.0001183	0.0048122	0.0019355	0.9522872	0.9905880
0.0001935	0.0001121	0.0048084	0.0017470	0.9570955	0.9923350
0.0001748	0.0001013	0.0048016	0.0015612	0.9618971	0.9938962
0.0001697	0.0000983	0.0047997	0.0013780	0.9666968	0.9952742
0.0001382	0.0000801	0.0047883	0.0011974	0.9714851	0.9964716

Eigenvalues	Relative_eig	Rel_corr_eig	$Broken\_stick$	Cum_corr_eig	Cumul_br_stick
0.0001322	0.0000766	0.0047861	0.0010194	0.9762712	0.9974910
0.0001300	0.0000753	0.0047853	0.0008437	0.9810565	0.9983347
0.0001273	0.0000738	0.0047843	0.0006705	0.9858408	0.9990052
0.0000778	0.0000451	0.0047664	0.0004996	0.9906072	0.9995048
0.0000221	0.0000128	0.0047462	0.0003309	0.9953534	0.9998356
0.0000000	0.0000000	0.0046466	0.0001644	1.0000000	1.0000000
-0.0002527	-0.0001464	0.0000000	0.0000000	1.0000000	1.0000000
-0.0130699	-0.0075728	0.0000000	0.0000000	1.0000000	1.0000000

biplot(aph.pcoa)#biplot of first 2 axes

### **PCoA** ordination



```
#plot(aph.pcoa$vectors[,3],aph.pcoa$vectors[,4])
#write.csv(aph.pcoa$values[,1:2],"aph_pcoa_1_2.csv")
#extracting the new scores/Axis values
#and assigning the right names
sub.pcoa<-as.data.frame(aph.pcoa$vectors);sub.pcoa$Sample<-final.tree$tip.label
##another thing you should try is to
##analyze the raxml ML estimate of pairwise distances

##merging Axes with original datset
#lets check the dimensions of the dataframe
dim(sub.pcoa)</pre>
```

## [1] 80 78

```
dim(dat)
## [1] 98 21
merg<-inner_join(dat,sub.pcoa,by="Sample")</pre>
## Warning in inner_join_impl(x, y, by$x, by$y): joining factor and character
## vector, coercing into character vector
dim(merg) #79 TAXA
## [1] 80 98
##we actually need climate data from woldclim
w<-getData('worldclim', var='bio', res=2.5)</pre>
dbio1<-extract(w,merg[,c("lon","lat")])</pre>
mergy<-as.data.frame(cbind(merg,dbio1))</pre>
#gonna pca climate data bio1-bio7 (temp related) and bio12-bio15(precip related)
clim<-princomp(scale(cbind(mergy[,99:105],mergy[,110:113])))</pre>
summary(clim)# first 3 pcs epxlain 90% of variation
## Importance of components:
                                        Comp.2
                                                   Comp.3
                                                               Comp.4
                             Comp.1
## Standard deviation
                          2.8351191 1.3732437 0.74429524 0.48698997
## Proportion of Variance 0.7399678 0.1736063 0.05099889 0.02183284
## Cumulative Proportion 0.7399678 0.9135741 0.96457299 0.98640583
##
                               Comp.5
                                           Comp.6
                                                      Comp.7
## Standard deviation
                          0.34151153 0.156179208 0.06556715 0.0383388628
## Proportion of Variance 0.01073695 0.002245519 0.00039577 0.0001353158
## Cumulative Proportion 0.99714278 0.999388302 0.99978407 0.9999193880
##
                                Comp.9
                                             Comp.10 Comp.11
## Standard deviation
                          2.651763e-02 1.313250e-02
## Proportion of Variance 6.473509e-05 1.587686e-05
                                                            0
## Cumulative Proportion 9.999841e-01 1.000000e+00
knitr::kable(clim$loadings[,1:3])
```

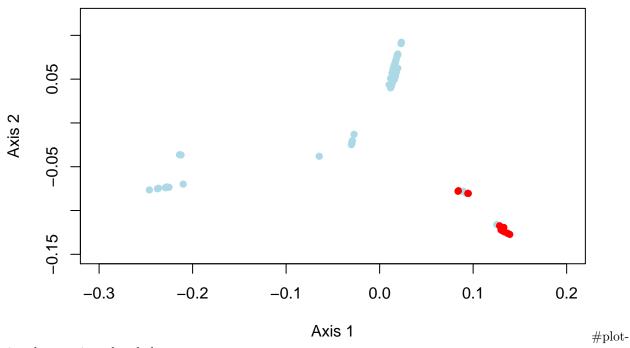
	Comp.1	Comp.2	Comp.3
bio1	0.3449715	-0.0427974	0.1331603
bio2	0.1836921	-0.5291074	0.4283589
bio3	0.3420009	-0.1070520	0.0184678
bio4	-0.3468170	0.0191039	0.0669074
bio5	0.3057476	-0.1648857	0.4178818
bio6	0.3461753	-0.0287588	0.0598595
bio7	-0.3428244	-0.0453872	0.1340204
bio12	0.3133799	-0.1548724	-0.4382364
bio13	0.3153413	0.2386220	-0.3371422
bio14	-0.0828292	-0.6320247	-0.5388643
$\frac{\text{bio}15}{}$	0.2732216	9.4426434	-0.0412163

```
mergy2<-as.data.frame(cbind(mergy,clim$scores[,1:3]))</pre>
Stats:
#evaluating tmax with phylo axes
full<-lm(KO_temp_worker~bio5*Axis.1+bio5*Axis.2+bio5*Axis.3+bio5*Axis.4,data=mergy2)
mod3<-stepAIC(full,direction="forward")</pre>
## Start: AIC=-65.84
## KO_temp_worker ~ bio5 * Axis.1 + bio5 * Axis.2 + bio5 * Axis.3 +
      bio5 * Axis.4
summary(mod3)
##
## Call:
## lm(formula = KO_temp_worker ~ bio5 * Axis.1 + bio5 * Axis.2 +
##
      bio5 * Axis.3 + bio5 * Axis.4, data = mergy2)
##
## Residuals:
##
       Min
                 1Q Median
                                   3Q
                                          Max
## -2.80610 -0.22526 0.08437 0.42006 0.98887
##
## Coefficients:
##
                Estimate Std. Error t value Pr(>|t|)
## (Intercept) 42.162798 1.496260 28.179
                                            <2e-16 ***
## bio5
              -0.002036 0.004770 -0.427
                                              0.671
## Axis.1
             -15.523104 25.506887 -0.609
                                            0.545
## Axis.2
              13.950319 39.503615 0.353
                                              0.725
## Axis.3
             -34.110211 121.704635 -0.280
                                            0.780
## Axis.4
             -50.137455 61.430557 -0.816
                                            0.417
## bio5:Axis.1 0.065741 0.079510 0.827
                                            0.411
## bio5:Axis.2 -0.061812 0.122998 -0.503
                                              0.617
## bio5:Axis.3 0.118997 0.375690 0.317
                                              0.752
## bio5:Axis.4 0.156456 0.191576 0.817
                                              0.417
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 0.6252 on 70 degrees of freedom
## Multiple R-squared: 0.587, Adjusted R-squared: 0.5339
## F-statistic: 11.05 on 9 and 70 DF, p-value: 1.63e-10
#with pca instead of bio5
full.p<-lm(K0_temp_worker~Comp.1*Axis.1+Comp.1*Axis.2+Comp.1*Axis.3+Comp.1*Axis.4,data=mergy2)
mod2<-stepAIC(full.p,direction="forward")</pre>
## Start: AIC=-66.77
## KO_temp_worker ~ Comp.1 * Axis.1 + Comp.1 * Axis.2 + Comp.1 *
      Axis.3 + Comp.1 * Axis.4
```

#### summary(mod2)

##

```
## Call:
## lm(formula = KO_temp_worker ~ Comp.1 * Axis.1 + Comp.1 * Axis.2 +
      Comp.1 * Axis.3 + Comp.1 * Axis.4, data = mergy2)
##
##
## Residuals:
      Min
               1Q Median
                              3Q
## -2.7089 -0.2207 0.1097 0.3931 1.0009
## Coefficients:
##
                Estimate Std. Error t value Pr(>|t|)
                41.53081 0.08553 485.586 < 2e-16 ***
## (Intercept)
               -0.04840
## Comp.1
                           0.03218 -1.504
                                             0.1371
                6.12891 0.95460 6.420 1.40e-08 ***
## Axis.1
## Axis.2
                -5.86597 1.39994 -4.190 8.01e-05 ***
## Axis.3
                 2.32195
                           3.07497
                                    0.755
                                            0.4527
## Axis.4
                -2.01123 2.18512 -0.920
                                            0.3605
## Comp.1:Axis.1 0.24294 0.37578 0.646
                                             0.5201
## Comp.1:Axis.2 -0.03910
                         0.50817 -0.077
                                             0.9389
## Comp.1:Axis.3 0.94549
                           0.85296
                                    1.108
                                             0.2714
## Comp.1:Axis.4 1.25035
                           0.71879
                                     1.740
                                             0.0863 .
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 0.6215 on 70 degrees of freedom
## Multiple R-squared: 0.5918, Adjusted R-squared: 0.5393
## F-statistic: 11.27 on 9 and 70 DF, p-value: 1.115e-10
#plotting axis 1 and 2
plot(aph.pcoa$vectors[,1],aph.pcoa$vectors[,2],col=match$color,pch=16,xlim=c(-.3,.2),ylim=c(-.15,.12),x
```



ting data against the phylogeny

plot(plot.tree2)

```
library(phytools)
library(picante)
```

```
## Loading required package: nlme
##
## Attaching package: 'nlme'
##
## The following object is masked from 'package:dplyr':
##
## collapse
##
## the following object is masked from 'package:raster':
##
## getData

trans<-read.tree("Phylogenetics/20150831_transformed_bl_raxml.newick")
plot.tree1<-drop.tip(trans,tip=c("LVA2","PMBE","PB17-14","PB17-10","PB07-23"))
#plot.tree2<-rotate(plot.tree1,node=84)</pre>
```

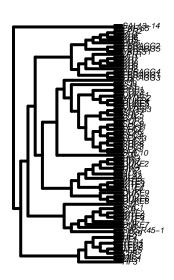
```
#matching species with thermal trait data-pain in the ass
id<-as.data.frame(plot.tree2$tip.label)
names(id)<-"Sample"
match<-inner_join(id,dat,by="Sample")

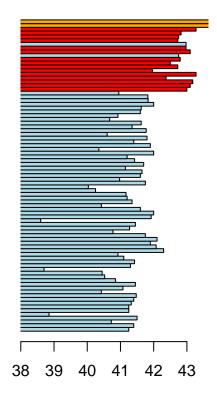
## Warning in inner_join_impl(x, y, by$x, by$y): joining factors with
## different levels, coercing to character vector

match$color<-ifelse(match$habitat=="flat woods","red","lightblue")
match[82:83,]$color<-c("orange","orange") # color for messor
dim(match)</pre>
```

#### ## [1] 83 22

```
x<-as.vector(match$KO_temp_worker)
names(x)<-match$Sample
x<-x[match(plot.tree2$tip.label,names(x))]
#xx<-match.phylo.data(plot.tree2, x)
## create a split plot
layout(matrix(c(1,2),1,2),c(0.7,0.3))
## plot our tree
plot(plot.tree1,edge.width=3,cex=.5)
## add bar plot
par(mar=c(4.1,0,1.1,1.1))
barplot(x[plot.tree1$tip.label],horiz=TRUE,width=1,space=0,
    ylim=c(1,length(plot.tree1$tip.label))-0.5,names="",xlim=c(38,43),col=match$color)</pre>
```



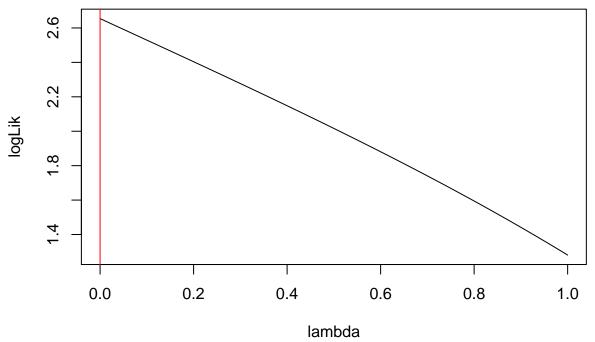


#### **PGLS**

```
library(caper)
new.dat<-inner_join(mergy2,match,by="Sample")</pre>
#traits$KO<-as.numeric(as.character(traits$KO))</pre>
cdobj<-comparative.data(plot.tree2, new.dat,Sample)</pre>
pgls_mod<-pgls(KO_temp_worker.x~ Comp.1+Comp.2+Comp.3, data=cdobj, lambda="ML")
summary(pgls_mod)
##
## Call:
## pgls(formula = KO_temp_worker.x ~ Comp.1 + Comp.2 + Comp.3, data = cdobj,
       lambda = "ML")
##
##
## Residuals:
##
        Min
                       Median
                                              Max
                  1Q
                                     ЗQ
##
   -0.04508 -0.03980 0.00000 0.00000 0.06013
##
## Branch length transformations:
##
## kappa [Fix] : 1.000
## lambda [ ML] : 0.000
##
      lower bound : 0.000, p = 1
      upper bound : 1.000, p = 0.097407
##
```

```
95.0% CI
                 : (NA, NA)
## delta [Fix]
                : 1.000
##
## Coefficients:
##
                Estimate Std. Error t value Pr(>|t|)
## (Intercept) 41.976787
                          0.528833 79.3763 0.00802 **
## Comp.1
                0.089100
                           0.107335 0.8301 0.55893
## Comp.2
                           0.222492 0.4333 0.73969
                0.096408
## Comp.3
               -0.835868
                          0.448776 -1.8626 0.31368
## ---
                  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Signif. codes:
##
## Residual standard error: 0.08504 on 1 degrees of freedom
## Multiple R-squared: 0.9796, Adjusted R-squared: 0.9184
## F-statistic: 16.01 on 3 and 1 DF, p-value: 0.1812
```

#### plot(pgls.profile(pgls\_mod))



Data: cdobj; Model: KO\_temp\_worker.x ~ Comp.1 + Comp.2 + Comp.3

#### pgls.profile(pgls\_mod)\$ci\$opt

```
## lambda
## 1e-06
```

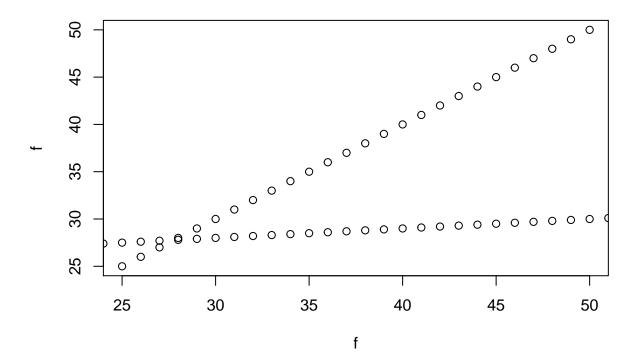
no sig effect

## PHylogenetic anova

```
#phylo anova
#removing outgroup and ma
new.tree<-drop.tip(plot.tree1,tip=c("ALA5","EXIT65","SAL13-14")) #taking out ma and outgroups
new.data2<-new.dat[match(new.tree$tip.label,new.dat$Sample),] #making sure sample order
new.data<-droplevels(new.data2)
h<-phylANOVA(new.tree,new.data$habitat.x,new.data$KO_temp_worker.x,p.adj="hochberg")
## Warning: no labels for x. Assuming order of tree$tip.label.
## Warning: no labels for y. Assuming order of tree$tip.label.
## $F
## [1] 62.51083
## $Pf
## [1] 0.022
##
## $T
##
                   deciduous forest flat woods
                     0.000000 -7.906379
## deciduous forest
## flat woods
                          7.906379 0.000000
##
## $method
## [1] "hochberg"
##
## $Pt
                   deciduous forest flat woods
## deciduous forest 1.000 0.022
## flat woods
                            0.022
                                      1.000
```

## heat shock figure

```
f<-seq(25,50,1)
s<-seq(25,50,.1)
c<-seq(0,250,1)
plot(f,f)
points(c,s)</pre>
```



#### sessionInfo()

```
## R version 3.2.2 (2015-08-14)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: OS X 10.10.4 (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] grid
                 stats
                           graphics grDevices utils
                                                          datasets methods
## [8] base
##
## other attached packages:
   [1] caper_0.5.2
                          picante_1.6-2
                                             nlme_3.1-121
##
   [4] phytools_0.4-56
                          mvSLOUCH_1.1.5
                                             corpcor_1.6.8
                          numDeriv_2014.2-1 ouch_2.9-2
   [7] mvtnorm_1.0-2
## [10] subplex_1.1-6
                          dplyr_0.4.1
                                             plyr_1.8.2
## [13] MuMIn_1.13.4
                          ggplot2_1.0.1
                                             QuantPsyc_1.5
## [16] boot_1.3-17
                                             maps_2.3-9
                          ape_3.2
## [19] foreign_0.8-65
                          maptools_0.8-36
                                             dismo_1.0-12
                          raster_2.3-40
  [22] rgdal_0.9-2
                                             sp_1.1-0
  [25] MASS_7.3-43
                          vegan_2.2-1
                                             permute_0.8-3
##
  [28] lattice_0.20-33
##
## loaded via a namespace (and not attached):
   [1] phangorn_1.99-13
                                reshape2_1.4.1
##
   [3] splines_3.2.2
                                colorspace_1.2-6
##
   [5] expm_0.99-1.1
                                htmltools_0.2.6
##
   [7] yaml_2.1.13
                                mgcv_1.8-7
   [9] survival_2.38-3
                                DBI_0.3.1
                                munsell_0.4.2
## [11] stringr_1.0.0
```

```
## [13] gtable_0.1.2
                                evaluate_0.7
## [15] knitr_1.10.5
                                parallel_3.2.2
## [17] highr_0.5
                                proto_0.3-10
## [19] Rcpp_0.11.6
                                scales_0.2.4
                                plotrix_3.5-12
## [21] formatR_1.2
## [23] clusterGeneration_1.3.4 scatterplot3d_0.3-36
## [25] mnormt_1.5-3
                                digest_0.6.8
## [27] stringi_0.4-1
                                msm_1.5
## [29] animation_2.3
                                tools_3.2.2
## [31] magrittr_1.5
                                cluster_2.0.3
## [33] Matrix_1.2-2
                                nnls_1.4
## [35] assertthat_0.1
                                rmarkdown_0.6.1
## [37] igraph_0.7.1
```