

# Sperm size figures

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Wednesday, January 14, 2015

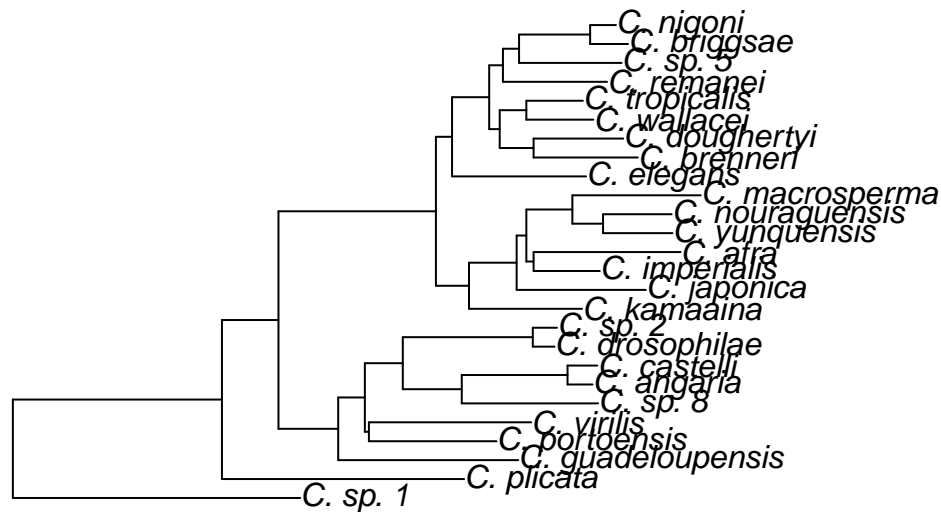
## Sperm size figures

This is the output of an R markdown script - a fancy way of embedding r in a page so we can rerun/tune it as needed and then have the commands

I'm embedding, but hiding a bunch of stuff for readability - the actual code is in the .Rmd file.

```
## Warning: package 'survival' was built under R version 3.1.3
```

Here's the tree from Kiontke et al 2011, figure 3 initial values I recieved had angaria and sp12 flipped - I've corrected it here Here's the tree:



Now read in the compiled data - we have oocytesize, spermsize, primaryspermatocytesize, and oocytearea. The species names in the files are inconsistent - they are fixed now. For now, the oocyte area is using the area formulation as made by the initial sperm size formula. All response variables are logged unless otherwise noted.

I have filtered out some of the data: Any oocytesize that is not stage -1, and any primaryspermatocyte data that is from hermaphrodites rather than males.

We now have clean data, clustered into species, strains or individuals. Variables ending in 1 are in species, 2 are strains and 3 are individuals. The non-clustered data is as is.

## **PIC plots - mean vs CV**

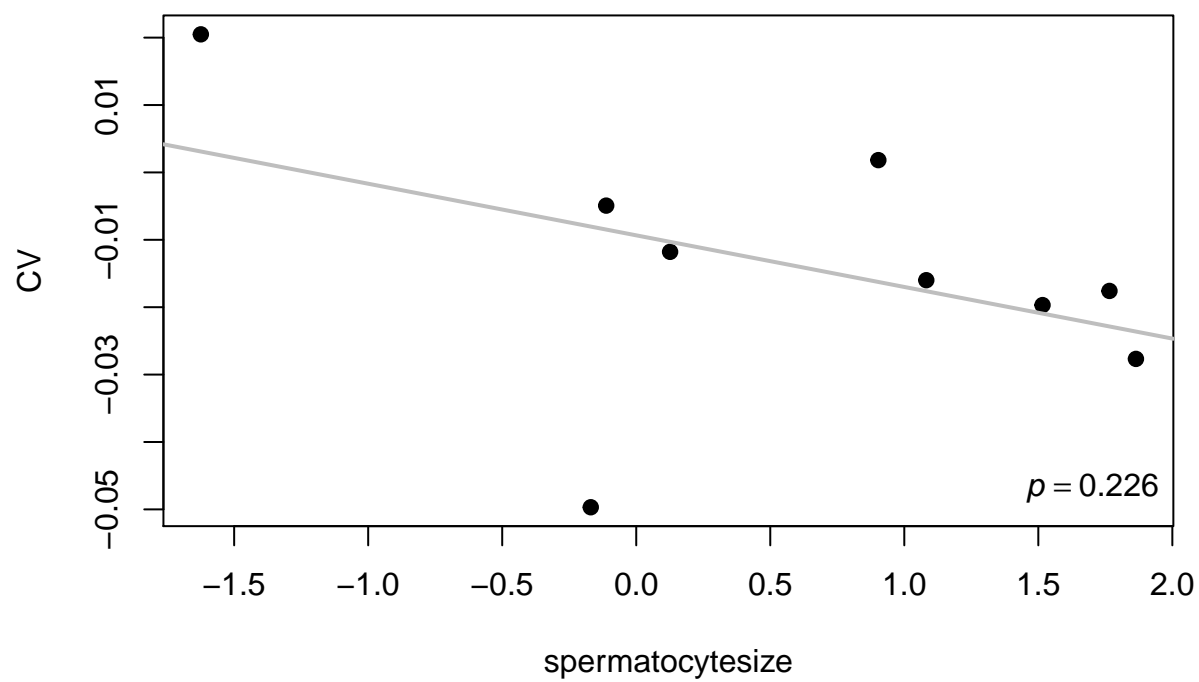
Plotting the phylogenetic independent contrasts (PIC) of means of traits vs CV of traits. All means and CVs are grouped at strain level.

A scatter plot showing the relationship between spermsize (x-axis) and CV (y-axis). The x-axis ranges from approximately -3.5 to 3.0, and the y-axis ranges from -0.10 to 0.25. A grey regression line shows a negative correlation. The p-value is indicated as  $p = 0.0599$ .

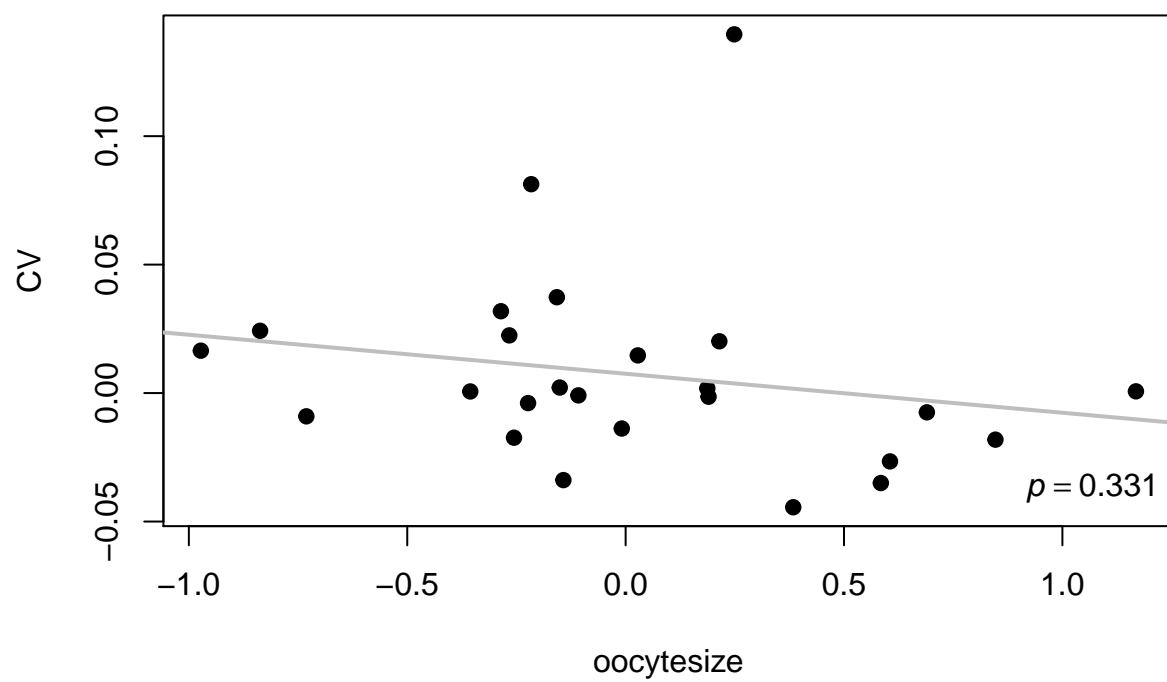
spermsize	CV
-3.2	-0.05
-2.5	0.23
-1.9	0.04
-1.5	0.03
-1.2	0.01
-1.1	0.00
-1.0	-0.05
-0.9	0.02
-0.8	0.03
-0.6	0.01
-0.4	0.04
-0.3	-0.11
0.4	-0.01
0.4	0.00
0.5	-0.05
0.5	0.01
0.7	0.05
1.0	0.04
1.0	0.03
1.2	0.02
1.2	-0.05
1.2	-0.10
2.3	-0.03
2.6	-0.05
2.6	-0.01

A scatter plot showing the relationship between 'oocytesize' (x-axis) and 'CV' (y-axis). The x-axis ranges from -1.5 to 1.5, and the y-axis ranges from -0.02 to 0.08. There are 25 data points represented by black dots. A solid gray regression line shows a negative correlation. The p-value for the regression is indicated as  $p = 0.0997$  in the bottom right corner.

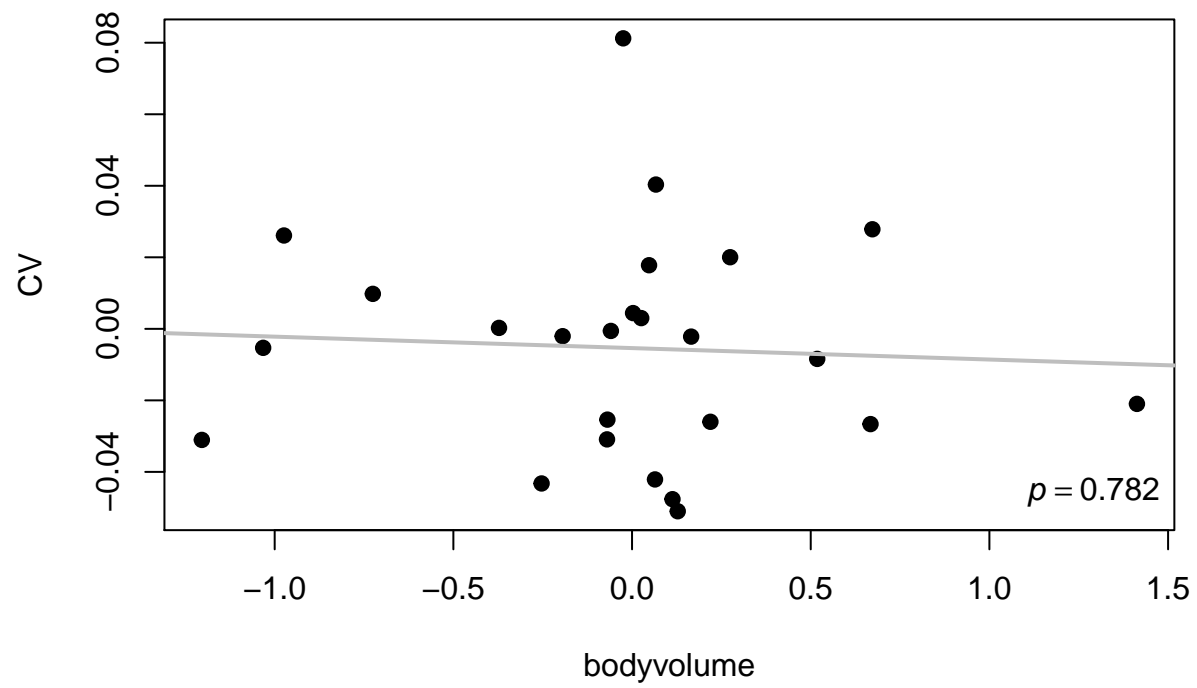
**PIC of spermatocytesize means vs CV**



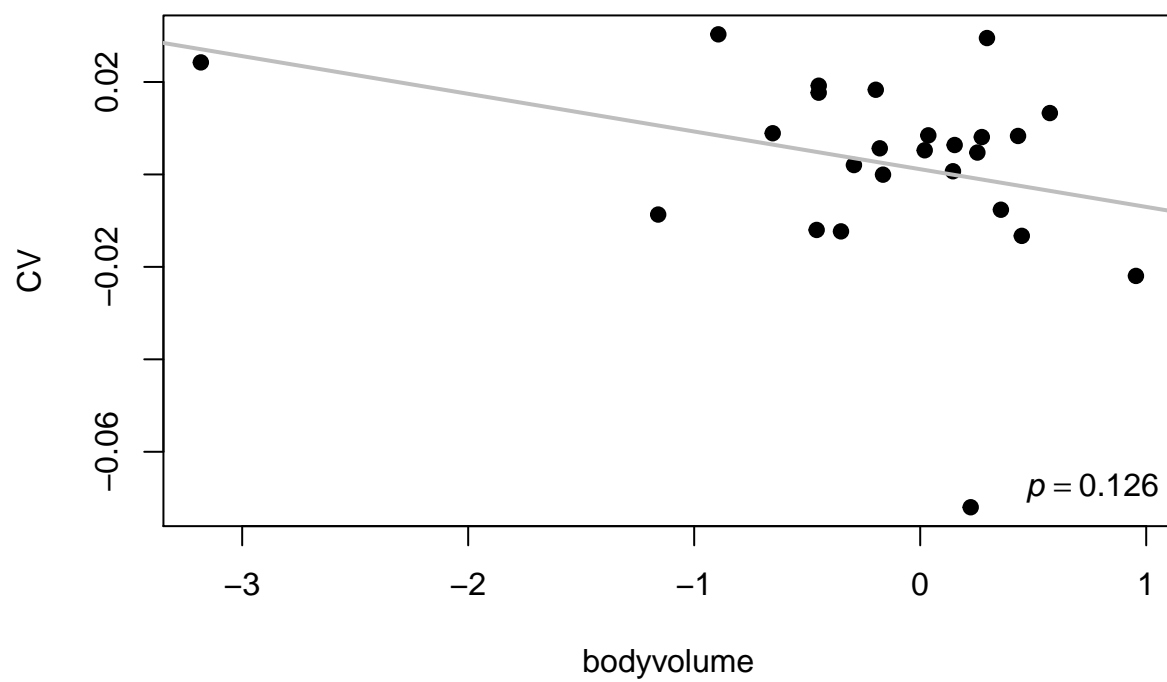
PIC of oocytesizearea means vs CV



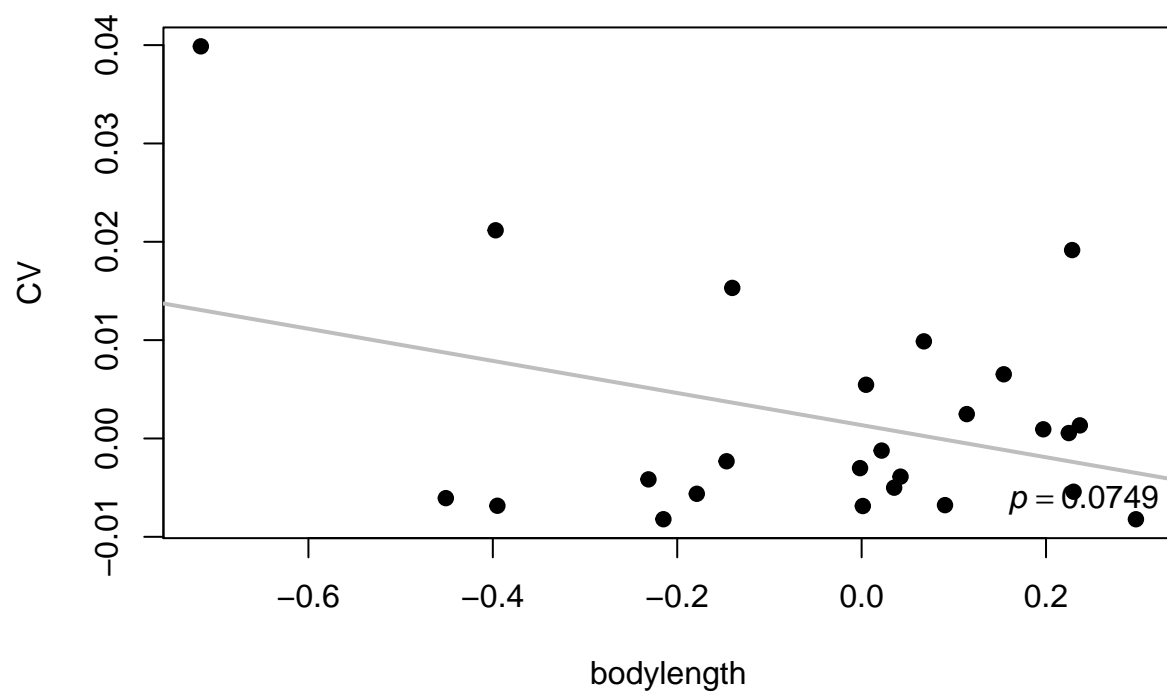
### PIC cv bodyvolume male



PIC cv bodyvolume female

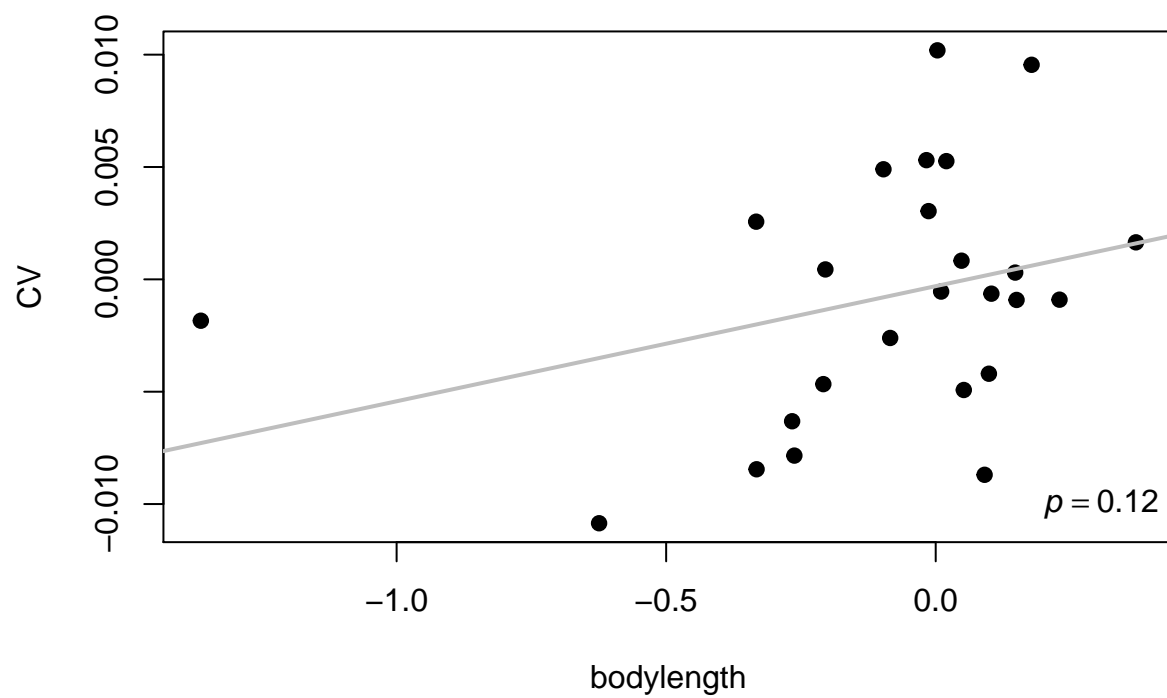


### PIC cv bodylength male



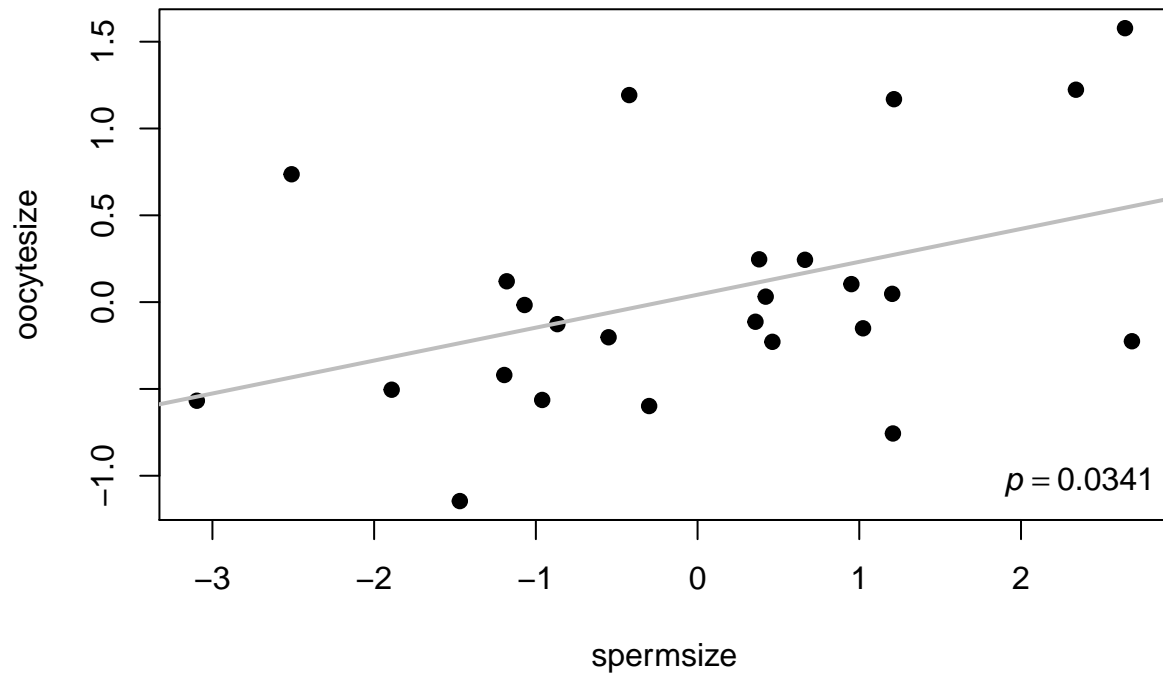


### PIC cv bodylength female

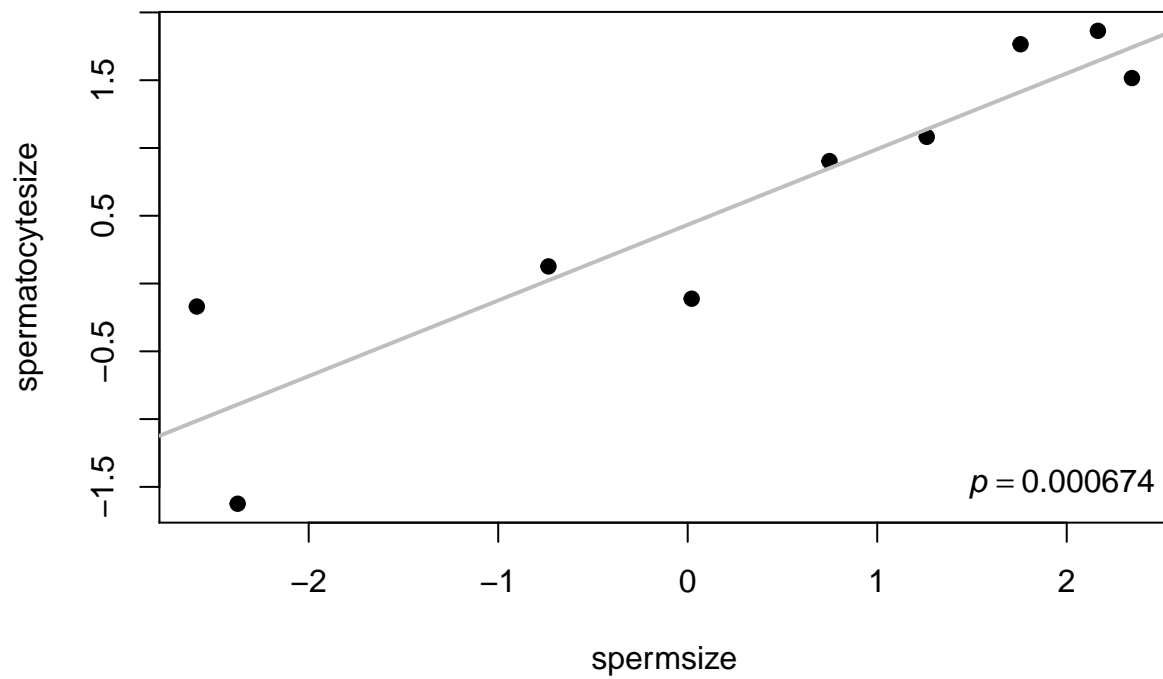


PIC plots - traits vs traits

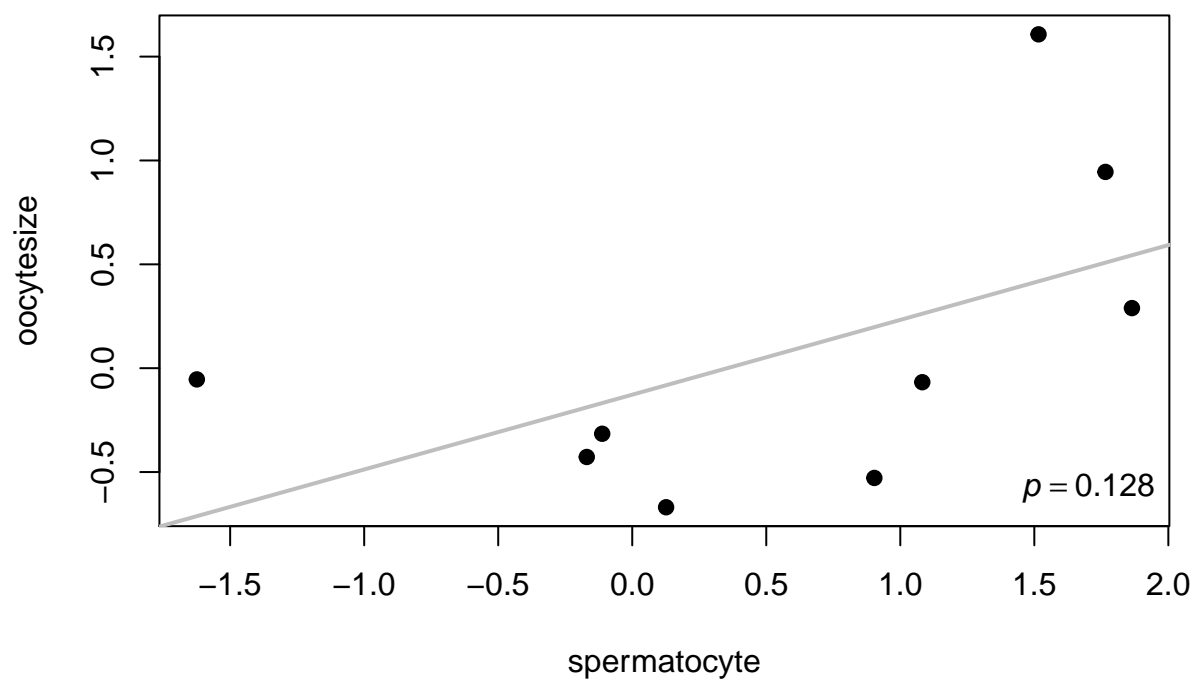
**PIC of spermsize vs oocytesize**



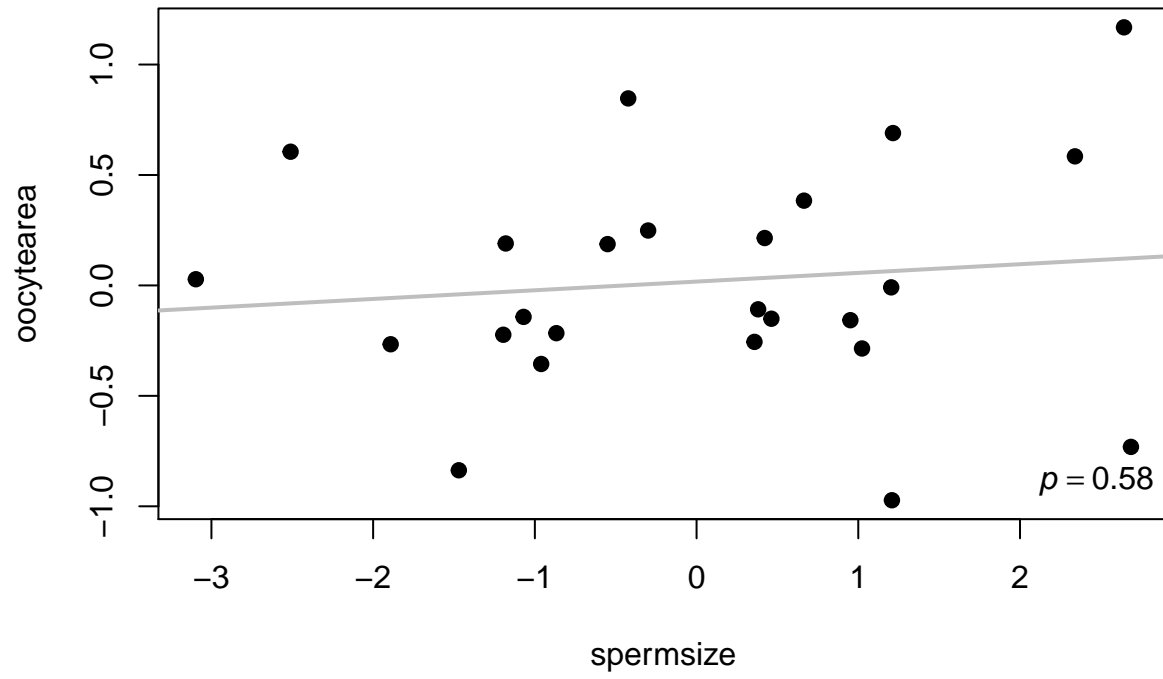
**PIC of spermsize vs spermatocytesize**



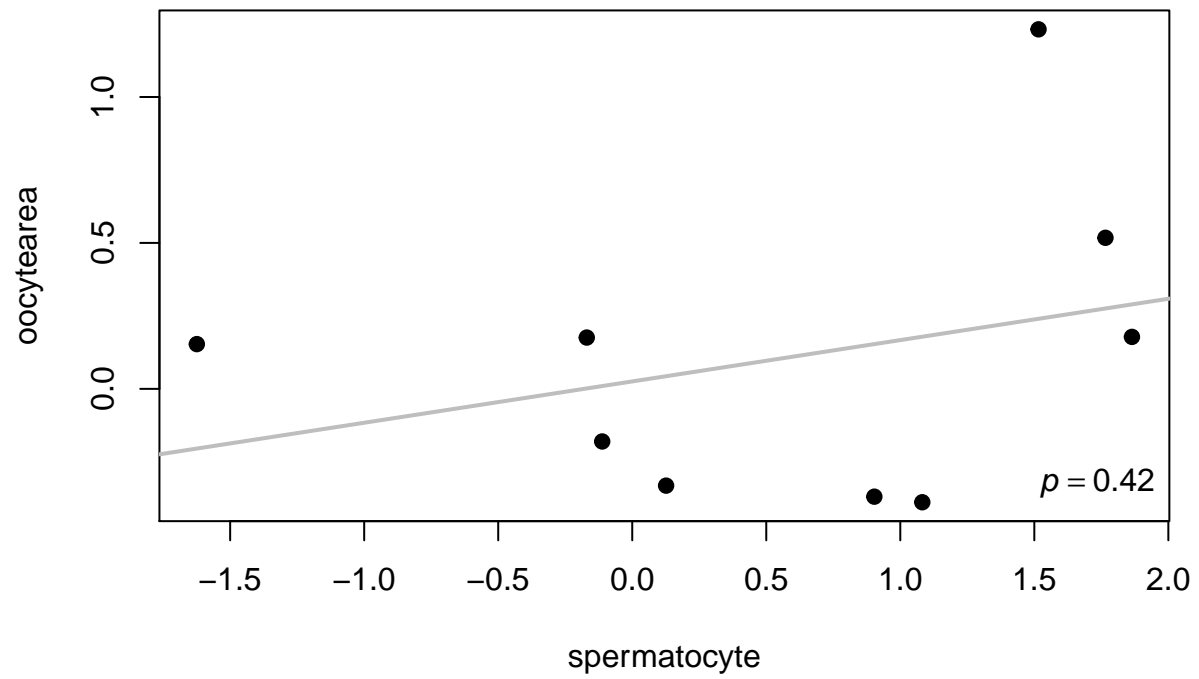
**PIC of spermatocytesize vs oocytesize**



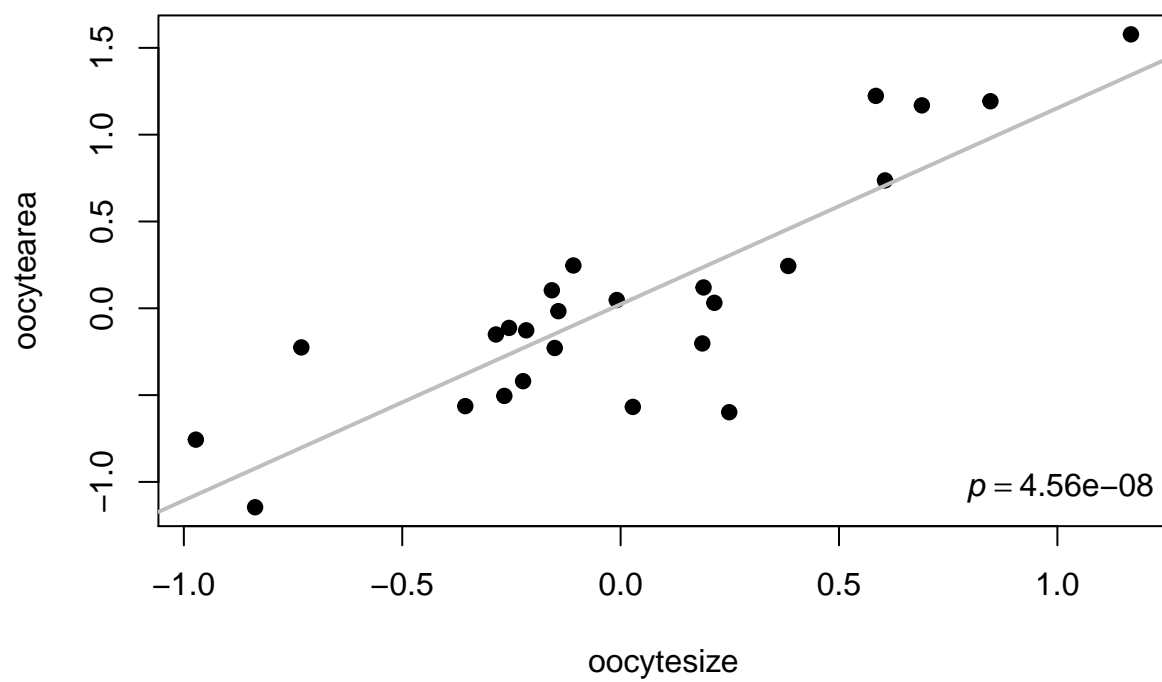
PIC of spermsize vs oocytearea



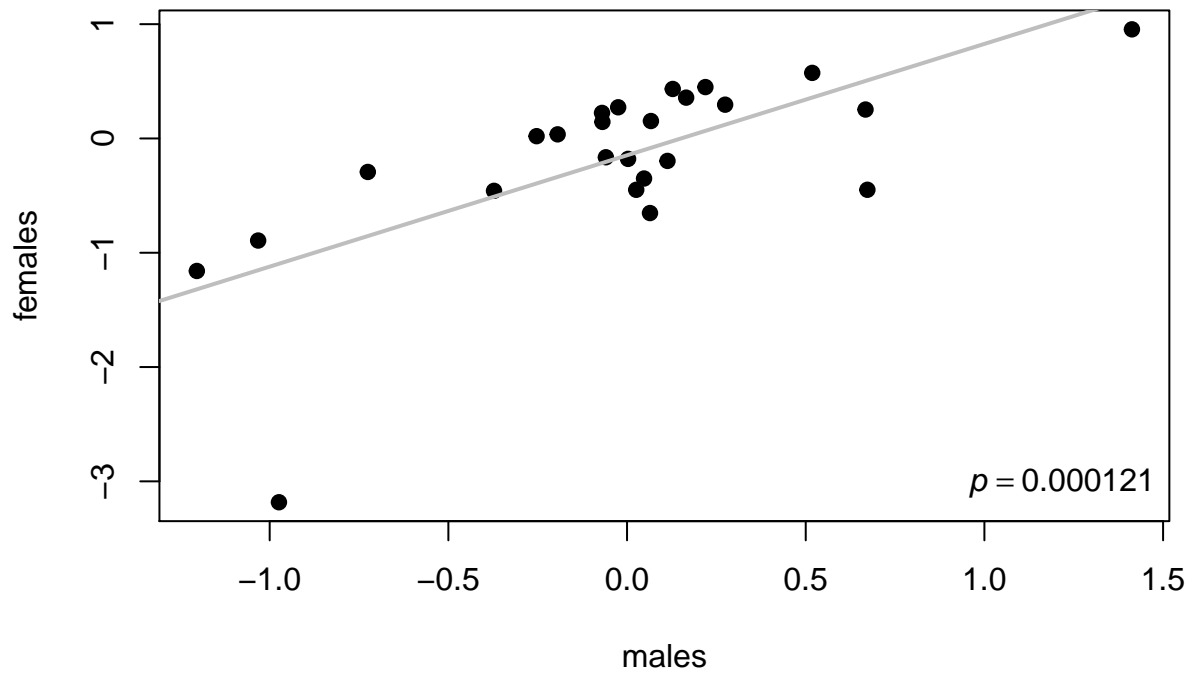
**PIC of spermatocytesize vs oocytearea**



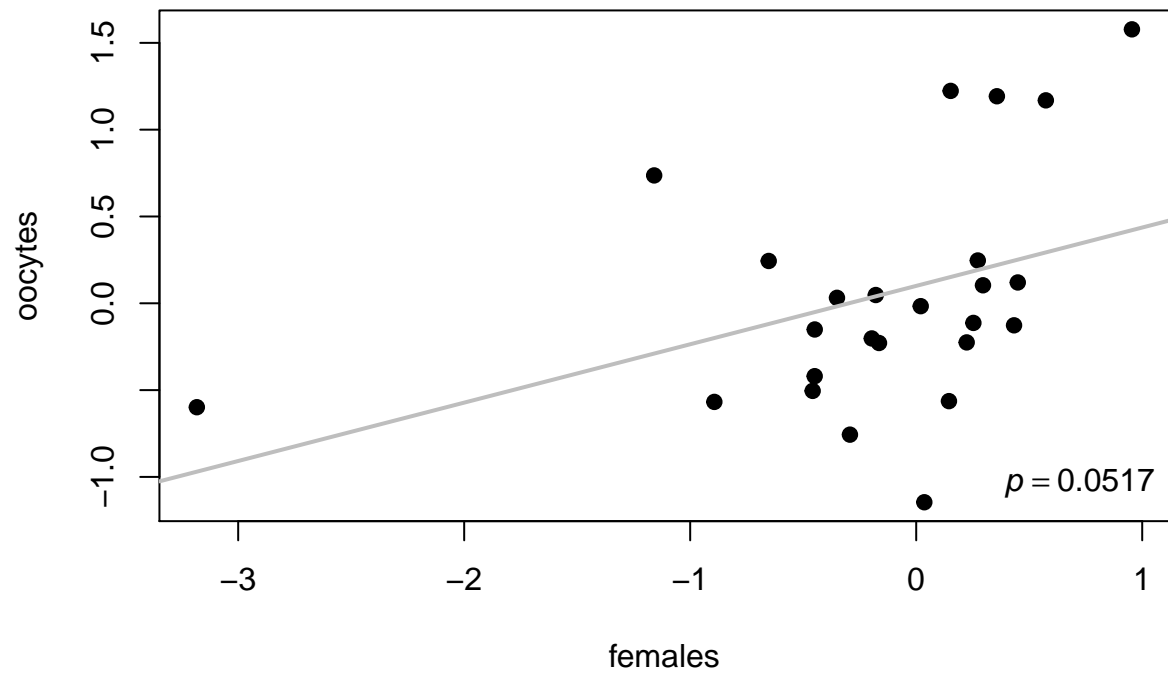
PIC of oocytesize vs oocytearea



Body area male vs female

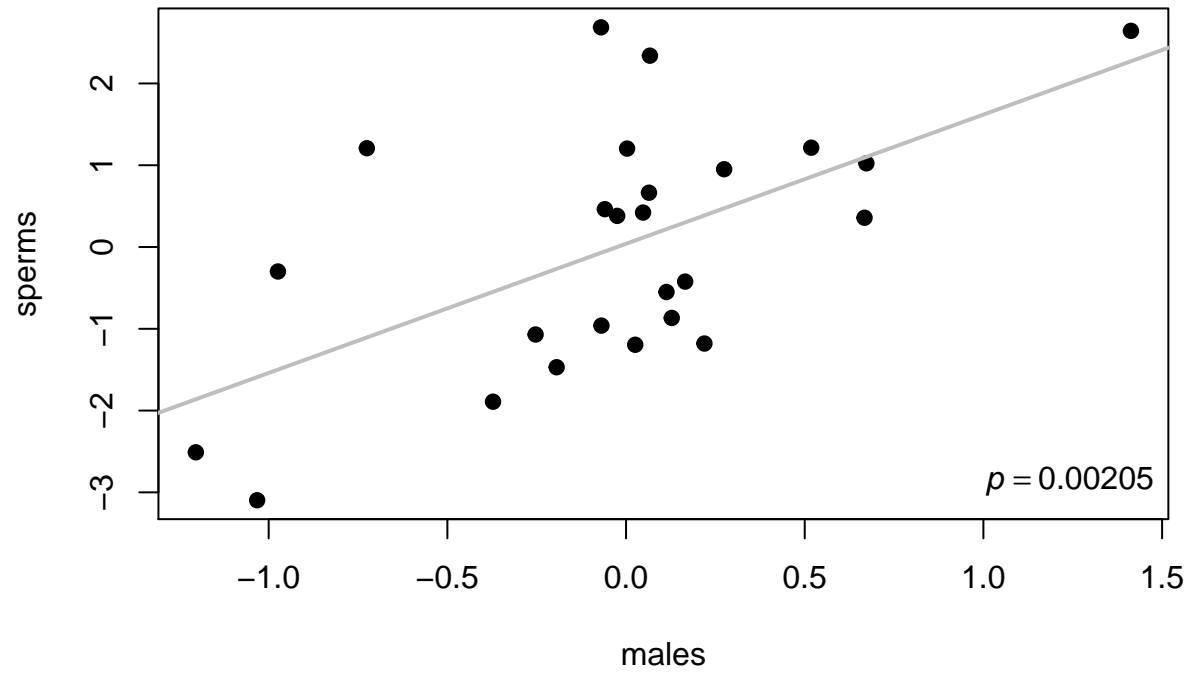


**Body area female vs oocytesize**

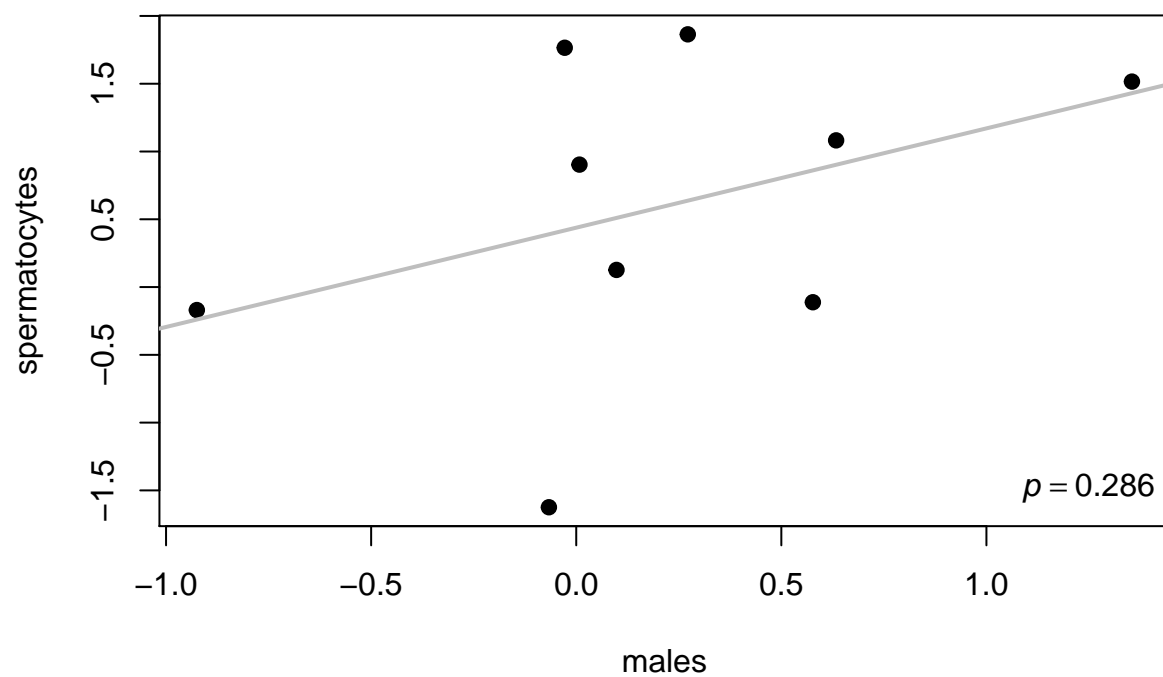




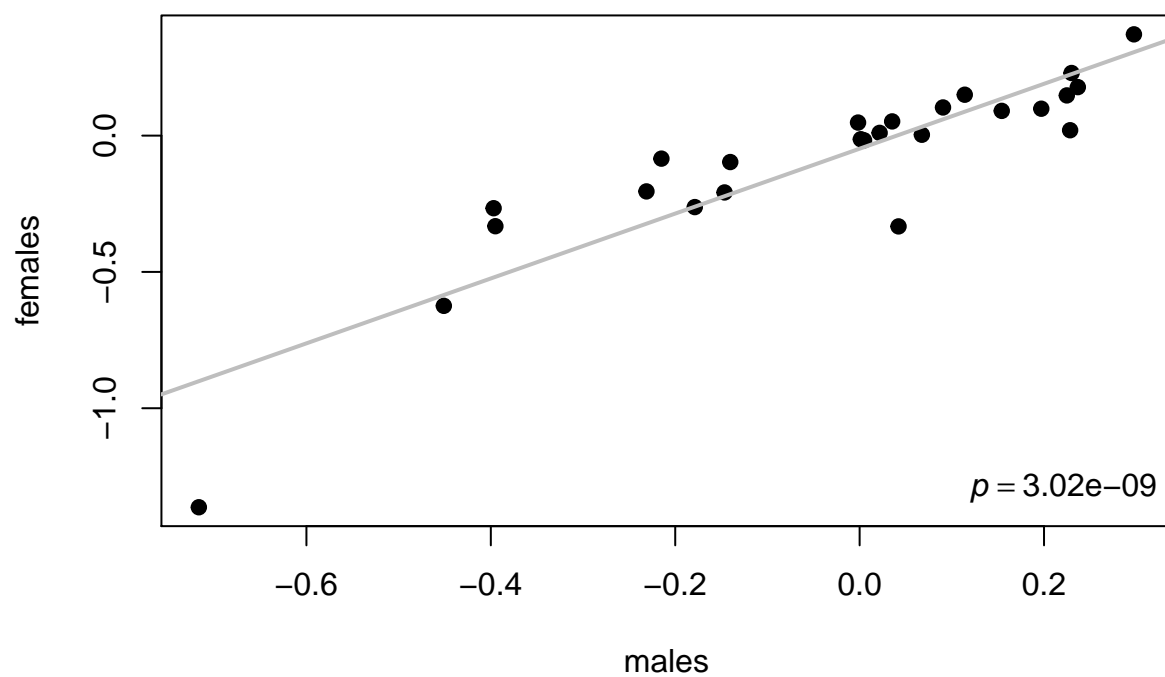
Body area male vs spermsize



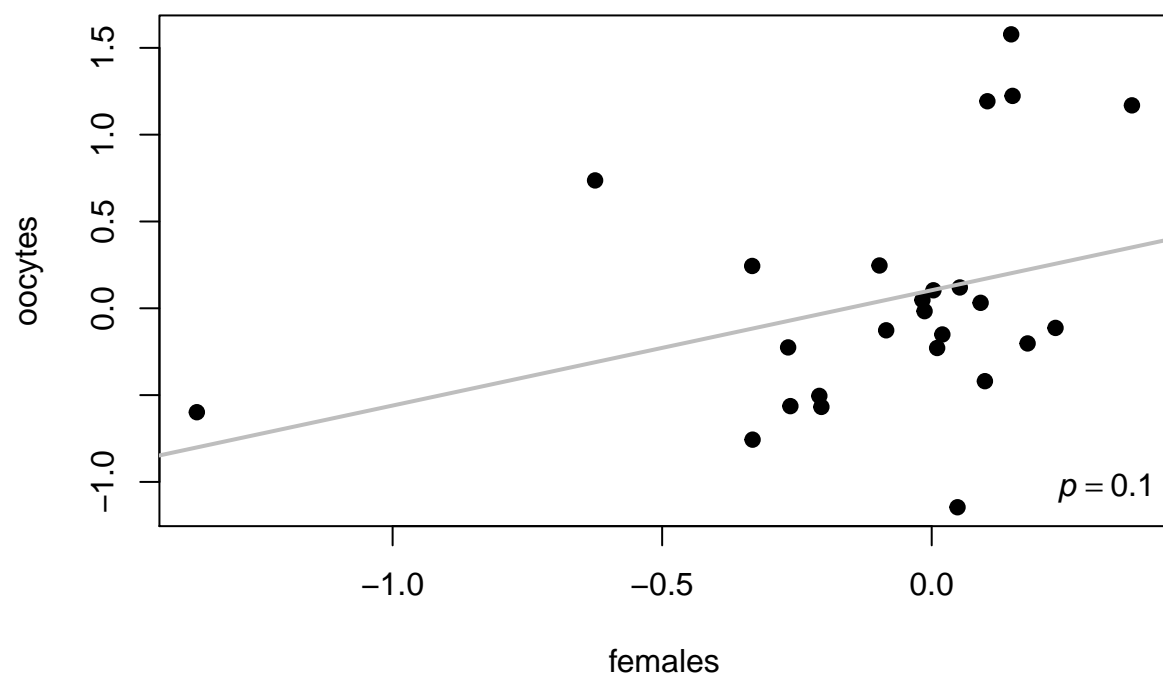
Body area male vs spermatocytesize



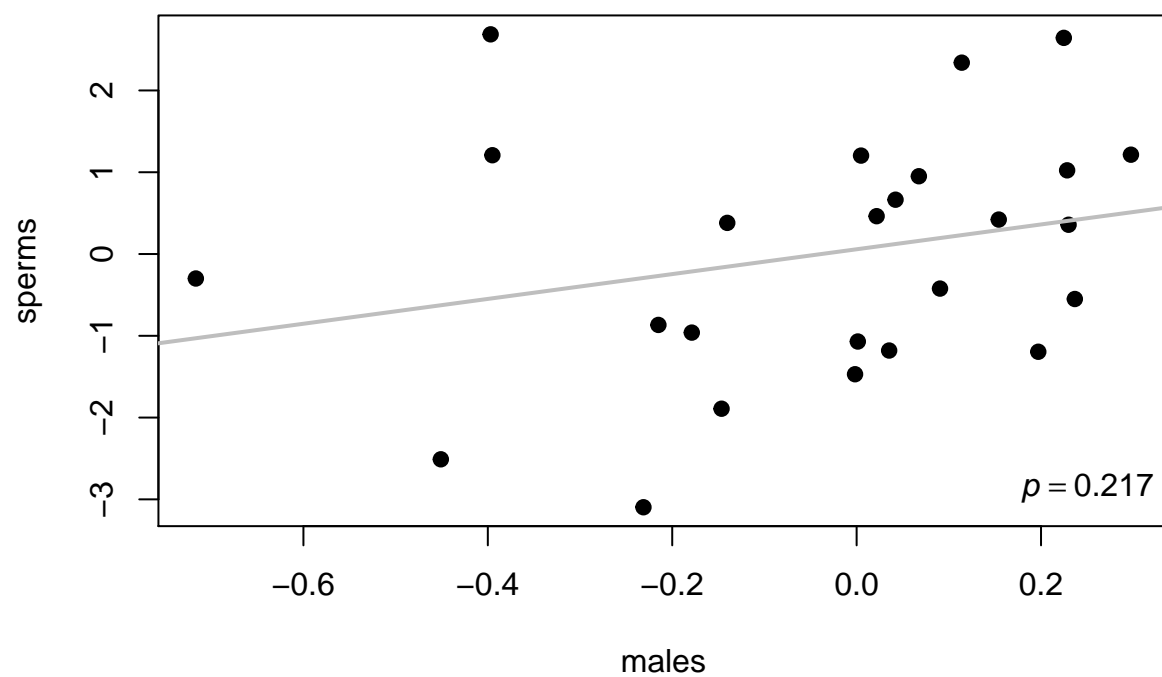
Body length male vs female



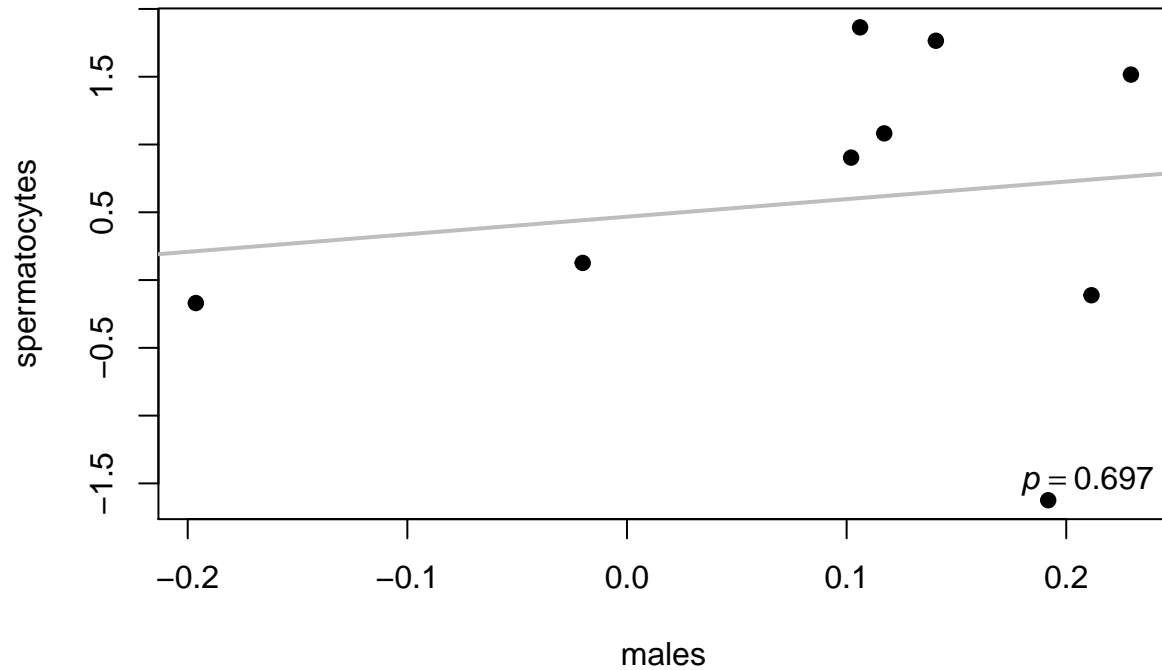
### Body length female vs oocytesize



Body length male vs spermsize



## Body length male vs spermatocytesize



## PGLS - testing for correlation with evolutionary models

Output are summary tables from fits. The OU model won't fit the spermatocyte fits due to overparameterisation.

```
##
## Call:
## pgls(formula = meansperm ~ meanooarea, data = compsperm, lambda = "ML")
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.9643 -0.5988  0.4323  0.6861  1.8263
##
## Branch length transformations:
##
## kappa  [Fix]  : 1.000
## lambda [ML]  : 0.000
##   lower bound : 0.000, p = 1
##   upper bound : 1.000, p = 0.11287
##   95.0% CI    : (NA, NA)
## delta  [Fix]  : 1.000
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) -6.09037    6.57159  -0.9268  0.3812
```

```

## meanooarea  1.46171    0.94975  1.5390   0.1624
##
## Residual standard error: 1.057 on 8 degrees of freedom
## Multiple R-squared:  0.2284,   Adjusted R-squared:  0.132
## F-statistic: 2.369 on 1 and 8 DF,  p-value: 0.1624

##
## Call:
## pgls(formula = meansperm ~ meanoo, data = compsperm, lambda = "ML")
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.4586 -1.1960  0.6374  1.0989  1.4686
##
## Branch length transformations:
##
## kappa  [Fix]   : 1.000
## lambda [ ML]   : 0.895
##   lower bound : 0.000, p = 1
##   upper bound : 1.000, p = 0.79696
##   95.0% CI    : (NA, NA)
## delta  [Fix]   : 1.000
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) -11.31303     6.94067  -1.6300  0.14176
## meanoo       1.50685     0.65983   2.2837  0.05177 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.315 on 8 degrees of freedom
## Multiple R-squared:  0.3946,   Adjusted R-squared:  0.319
## F-statistic: 5.215 on 1 and 8 DF,  p-value: 0.05177

##
## Call:
## pgls(formula = meansperm ~ meanprimsp, data = compsperm, lambda = "ML")
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.8244 -0.3185 -0.1789  0.1091  0.5614
##
## Branch length transformations:
##
## kappa  [Fix]   : 1.000
## lambda [ ML]   : 0.000
##   lower bound : 0.000, p = 1
##   upper bound : 1.000, p = 0.0066141
##   95.0% CI    : (NA, 0.708)
## delta  [Fix]   : 1.000
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) -2.63590     0.94589  -2.7867  0.0236793 *

```

```

## meanprimsp    1.21417    0.17131    7.0873 0.0001033 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.446 on 8 degrees of freedom
## Multiple R-squared:  0.8626, Adjusted R-squared:  0.8454
## F-statistic: 50.23 on 1 and 8 DF,  p-value: 0.0001033

## Generalized least squares fit by REML
##   Model: meansperm ~ meanooarea
##   Data: fullpglsframe
##       AIC      BIC    logLik
##  54.95884 59.67106 -23.47942
##
## Correlation Structure: corMartins
## Formula: ~1
## Parameter estimate(s):
##   alpha
## 6.841132
##
## Coefficients:
##              Value Std.Error   t-value p-value
## (Intercept) -3.044212  3.398811 -0.8956698  0.3793
## meanooarea   1.005070  0.498035  2.0180725  0.0549
##
## Correlation:
##      (Intr)
## meanooarea -0.999
##
## Standardized residuals:
##      Min      Q1      Med      Q3      Max
## -1.7775727 -0.6837636 -0.2886598  0.4237510  2.2862305
##
## Residual standard error: 0.6334179
## Degrees of freedom: 26 total; 24 residual

## Generalized least squares fit by REML
##   Model: meansperm ~ meanoo
##   Data: fullpglsframe
##       AIC      BIC    logLik
##  50.30285 55.01506 -21.15142
##
## Correlation Structure: corMartins
## Formula: ~1
## Parameter estimate(s):
##   alpha
## 5.469326
##
## Coefficients:
##              Value Std.Error   t-value p-value
## (Intercept) -7.068653  3.346328 -2.112361  0.0452
## meanoo       1.074887  0.329803  3.259179  0.0033
##
## Correlation:

```



```
##      (Intr)
## meanoo -0.999
##
## Standardized residuals:
##      Min      Q1      Med      Q3      Max
## -1.9084685 -0.7013875 -0.3474741  0.5038124  2.4034645
##
## Residual standard error: 0.5843052
## Degrees of freedom: 26 total; 24 residual
```

Some phylogenetic signal methods - lambda

```
## Loading required package: parallel
```

```
##      Length Class      Mode
## lik   1      bm      function
## bnd   2    data.frame list
## res 400    -none-    numeric
## opt   8    -none-    list
```

```
##      Length Class      Mode
## lik   1      bm      function
## bnd   2    data.frame list
## res 400    -none-    numeric
## opt   8    -none-    list
```

```
##      Length Class      Mode
## lik   1      bm      function
## bnd   2    data.frame list
## res 400    -none-    numeric
## opt   8    -none-    list
```

```
##      Length Class      Mode
## lik   1      bm      function
## bnd   2    data.frame list
## res 400    -none-    numeric
## opt   8    -none-    list
```

Blombergs K

```
##      K PIC.variance.obs PIC.variance.rnd.mean PIC.variance.P
## 1 0.3738911      2.289904      3.920744      0.044
##      PIC.variance.Z
## 1      -1.403023
```

```
##      K PIC.variance.obs PIC.variance.rnd.mean PIC.variance.P
## 1 0.670685      0.4572932      1.224798      0.001
##      PIC.variance.Z
## 1      -1.948734
```

```
## [1] "Dropping tips from the tree because they are not present in the data:"
## [1] "C. sp. 1"      "C. portoensis"  "C. virilis"
## [4] "C. sp. 8"      "C. angaria"     "C. castelli"
## [7] "C. sp. 2"      "C. kamaaina"    "C. imperialis"
## [10] "C. afra"       "C. nouraguensis" "C. doughertyi"
## [13] "C. wallacei"   "C. tropicalis"  "C. sp. 5"
## [16] "C. nigoni"
```

```
##          K PIC.variance.obs PIC.variance.rnd.mean PIC.variance.P
## 1 1.218587      1.696502      2.569825      0.035
## PIC.variance.Z
## 1      -1.818388
```

```
##          K PIC.variance.obs PIC.variance.rnd.mean PIC.variance.P
## 1 0.5877787      0.2622437      0.6166013      0.001
## PIC.variance.Z
## 1      -1.785553
```

Morans I

```
## class: krandtest
## Monte-Carlo tests
## Call: as.krandtest(sim = matrix(res$result, ncol = nvar, byrow = TRUE),
##      obs = res$obs, alter = alter, names = test.names)
##
## Number of tests: 1
##
## Adjustment method for multiple comparisons: none
## Permutation number: 999
## Test      Obs Std.Obs Alter Pvalue
## 1 x -0.09396389 -1.384882 greater 0.943
##
## other elements: adj.method call
```

```
## class: krandtest
## Monte-Carlo tests
## Call: as.krandtest(sim = matrix(res$result, ncol = nvar, byrow = TRUE),
##      obs = res$obs, alter = alter, names = test.names)
##
## Number of tests: 1
##
## Adjustment method for multiple comparisons: none
## Permutation number: 999
## Test      Obs Std.Obs Alter Pvalue
## 1 x -0.01730539 0.6379105 greater 0.219
##
## other elements: adj.method call
```

```
## class: krandtest
## Monte-Carlo tests
## Call: as.krandtest(sim = matrix(res$result, ncol = nvar, byrow = TRUE),
##      obs = res$obs, alter = alter, names = test.names)
```

```

##
## Number of tests: 1
##
## Adjustment method for multiple comparisons: none
## Permutation number: 999
## Test      Obs      Std.Obs  Alter Pvalue
## 1      x -0.1482137 -0.8806813 greater 0.805
##
## other elements: adj.method call

## class: krandtest
## Monte-Carlo tests
## Call: as.krandtest(sim = matrix(res$result, ncol = nvar, byrow = TRUE),
##      obs = res$obs, alter = alter, names = test.names)
##
## Number of tests: 1
##
## Adjustment method for multiple comparisons: none
## Permutation number: 999
## Test      Obs      Std.Obs  Alter Pvalue
## 1      x -0.03082416 0.2856757 greater 0.358
##
## other elements: adj.method call

abouheifs cmean

## class: krandtest
## Monte-Carlo tests
## Call: as.krandtest(sim = matrix(res$result, ncol = nvar, byrow = TRUE),
##      obs = res$obs, alter = alter, names = test.names)
##
## Number of tests: 1
##
## Adjustment method for multiple comparisons: none
## Permutation number: 999
## Test      Obs      Std.Obs  Alter Pvalue
## 1      x -0.09396389 -1.374005 greater 0.94
##
## other elements: adj.method call

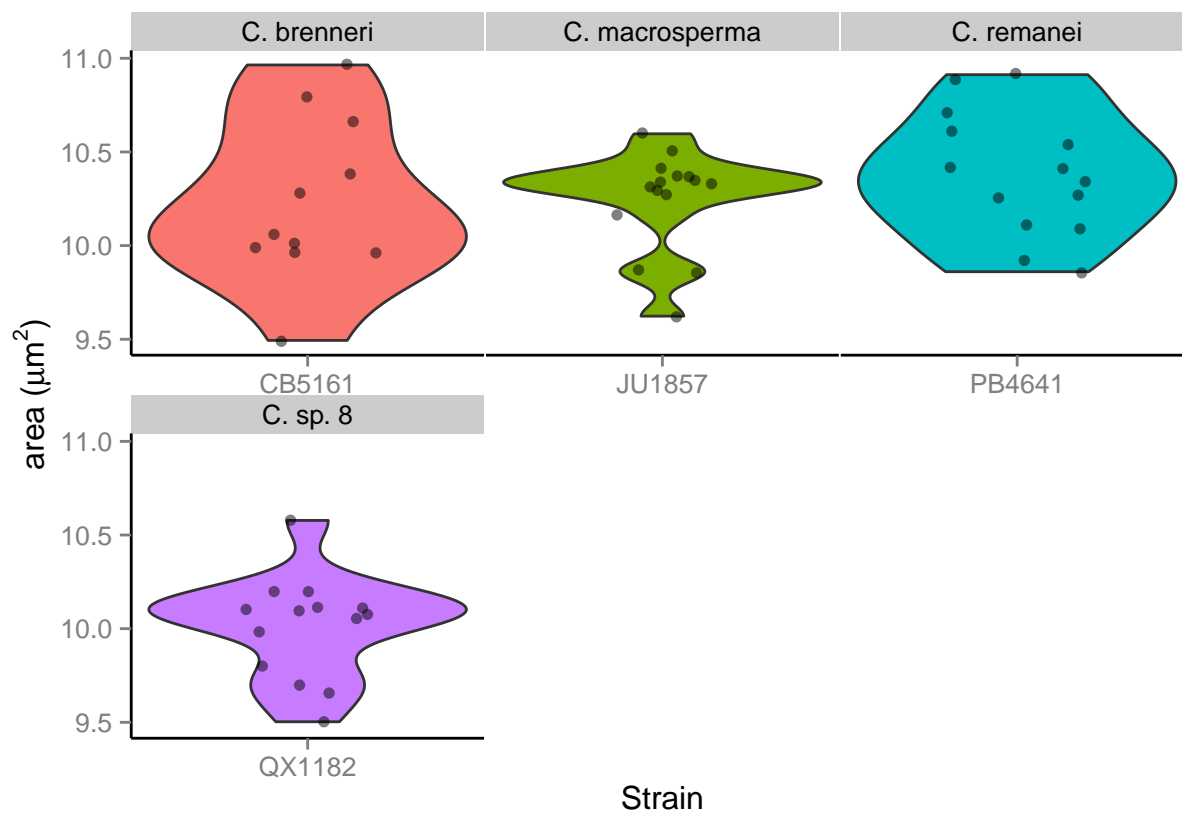
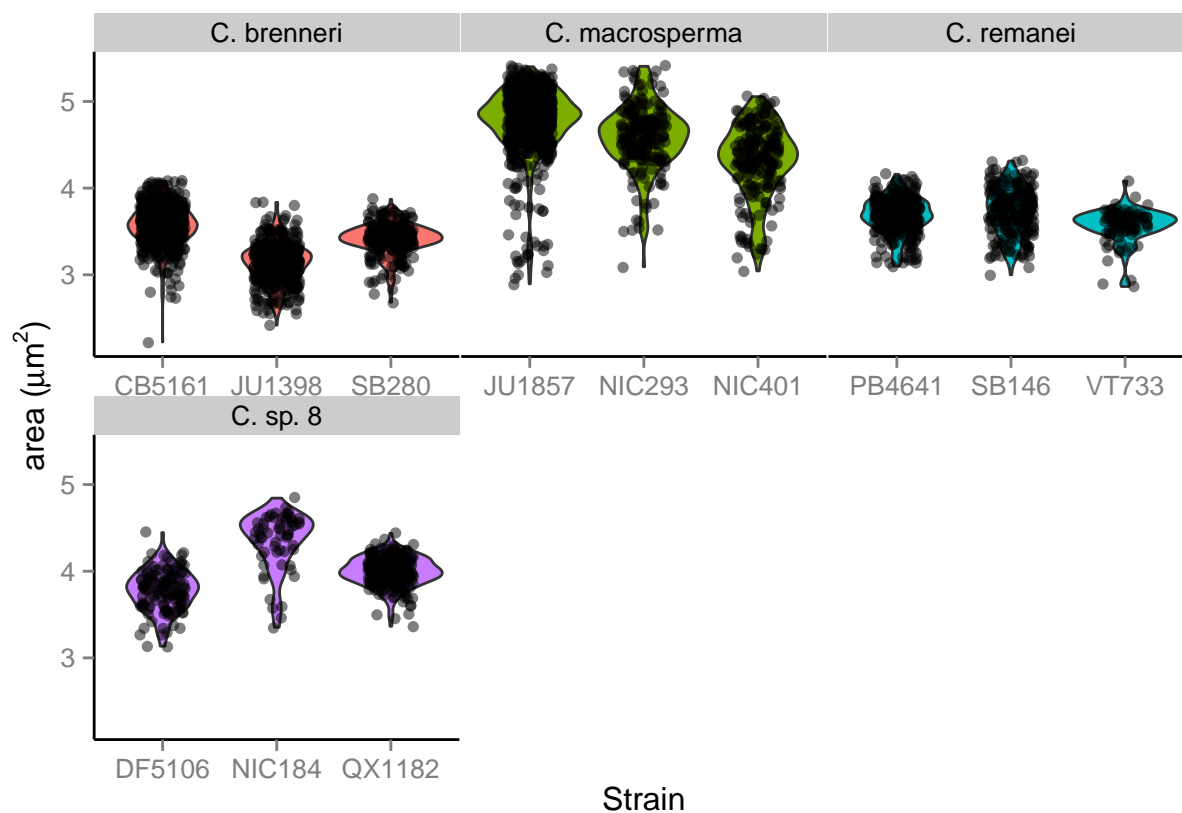
## class: krandtest
## Monte-Carlo tests
## Call: as.krandtest(sim = matrix(res$result, ncol = nvar, byrow = TRUE),
##      obs = res$obs, alter = alter, names = test.names)
##
## Number of tests: 1
##
## Adjustment method for multiple comparisons: none
## Permutation number: 999
## Test      Obs      Std.Obs  Alter Pvalue
## 1      x -0.01730539 0.632356 greater 0.227
##
## other elements: adj.method call

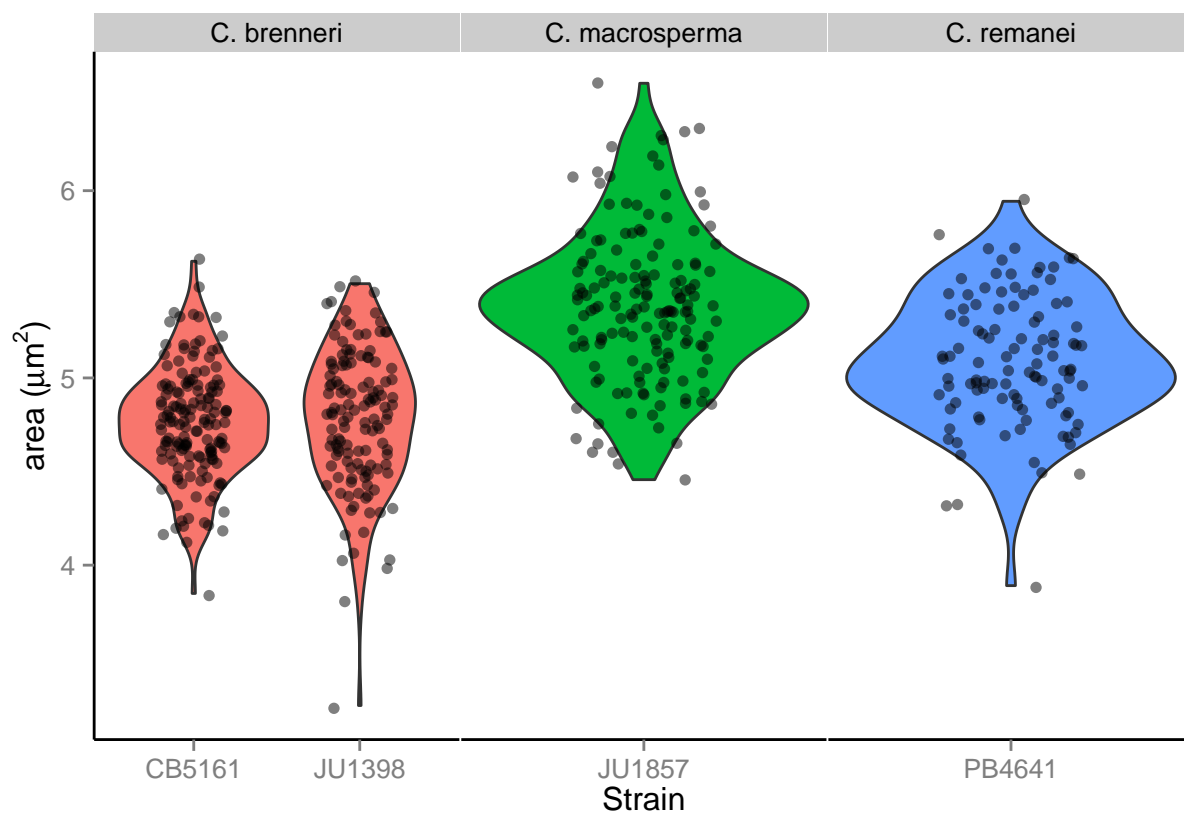
```

```
## class: krandtest
## Monte-Carlo tests
## Call: as.krandtest(sim = matrix(res$result, ncol = nvar, byrow = TRUE),
##      obs = res$obs, alter = alter, names = test.names)
##
## Number of tests: 1
##
## Adjustment method for multiple comparisons: none
## Permutation number: 999
## Test      Obs      Std.Obs  Alter Pvalue
## 1      x -0.1482137 -0.8759883 greater 0.801
##
## other elements: adj.method call

## class: krandtest
## Monte-Carlo tests
## Call: as.krandtest(sim = matrix(res$result, ncol = nvar, byrow = TRUE),
##      obs = res$obs, alter = alter, names = test.names)
##
## Number of tests: 1
##
## Adjustment method for multiple comparisons: none
## Permutation number: 999
## Test      Obs      Std.Obs  Alter Pvalue
## 1      x -0.03082416 0.2717917 greater 0.37
##
## other elements: adj.method call
```

figure 3A with violin plots





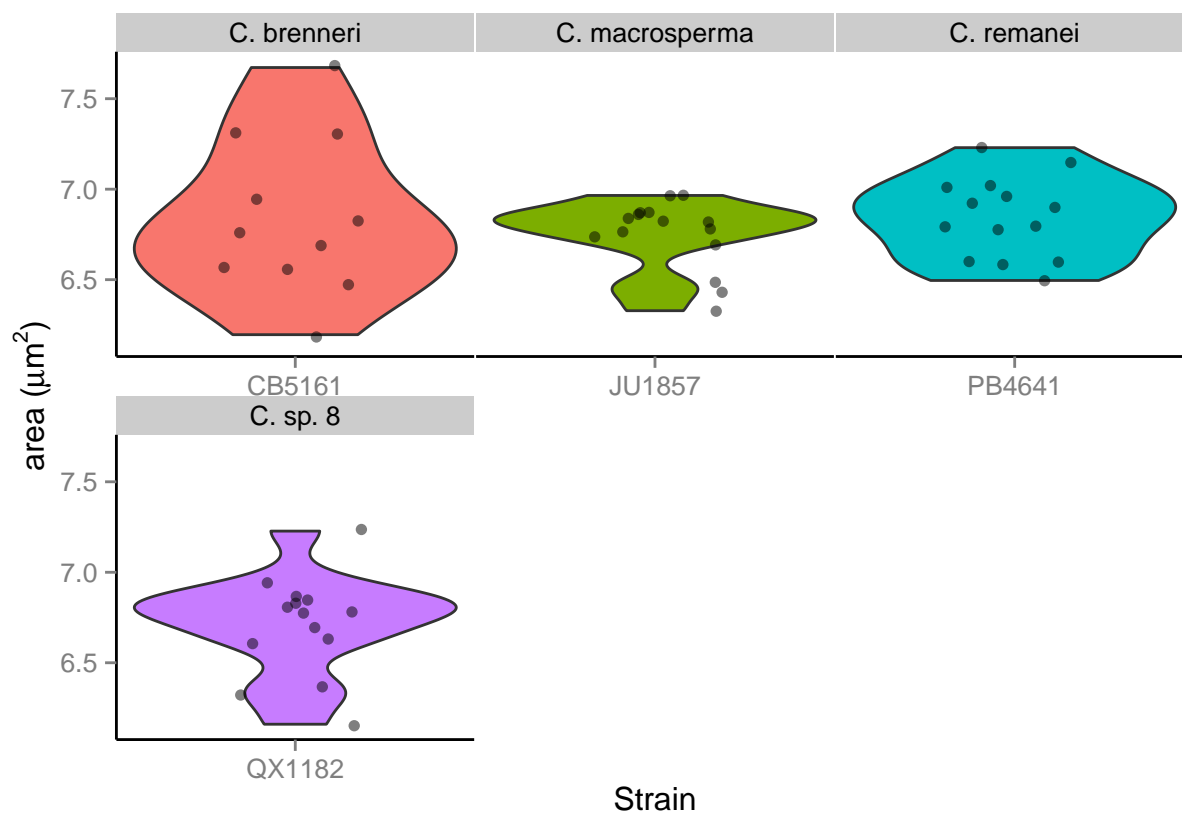
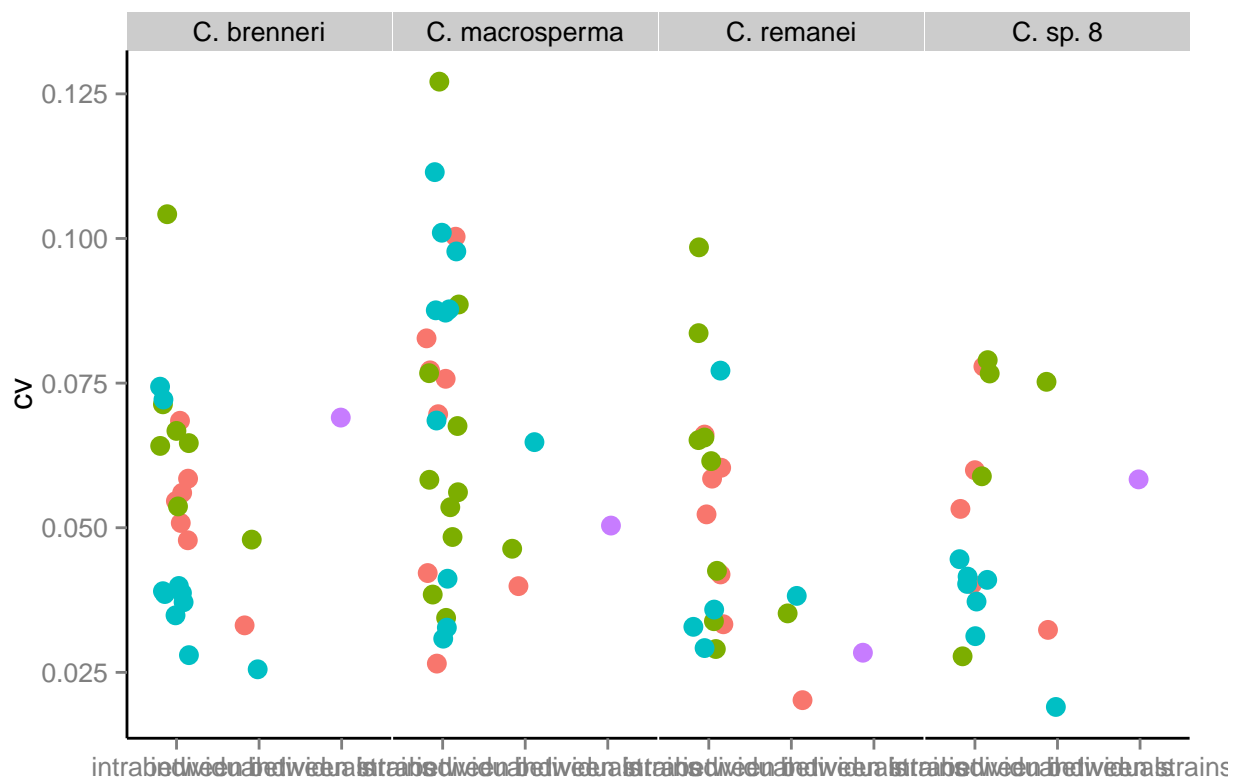
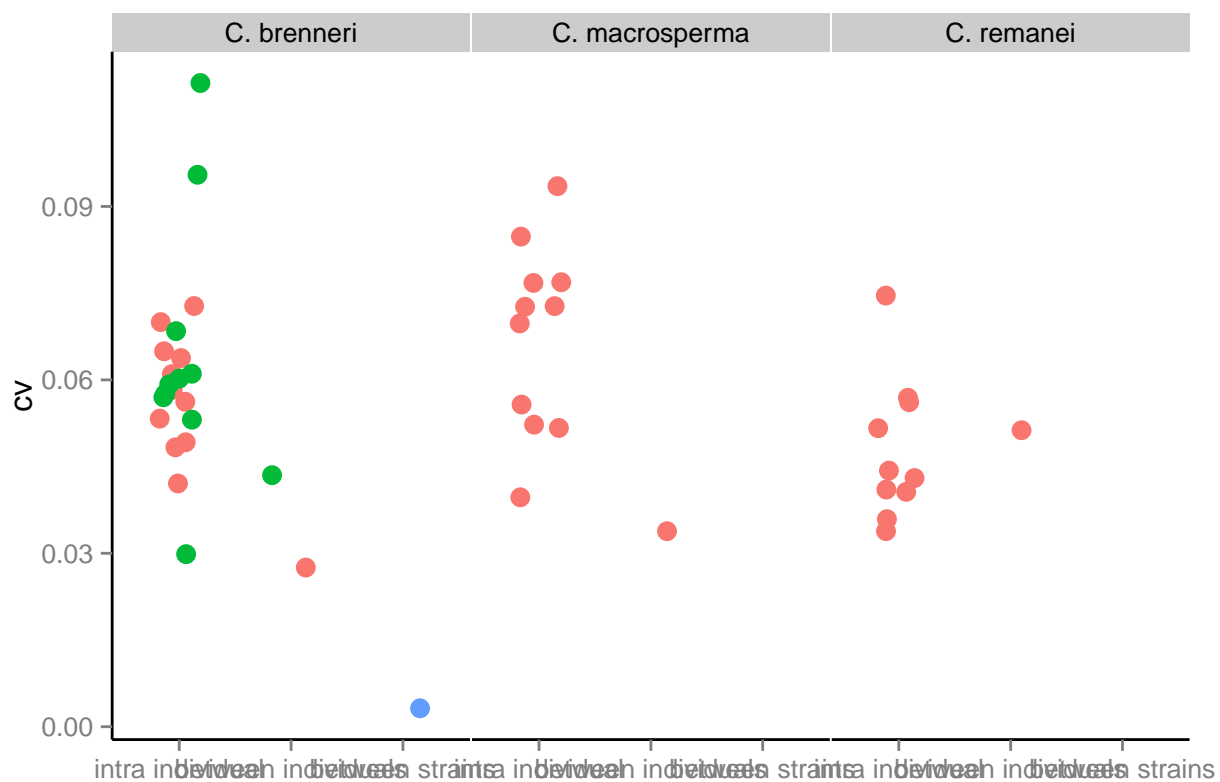
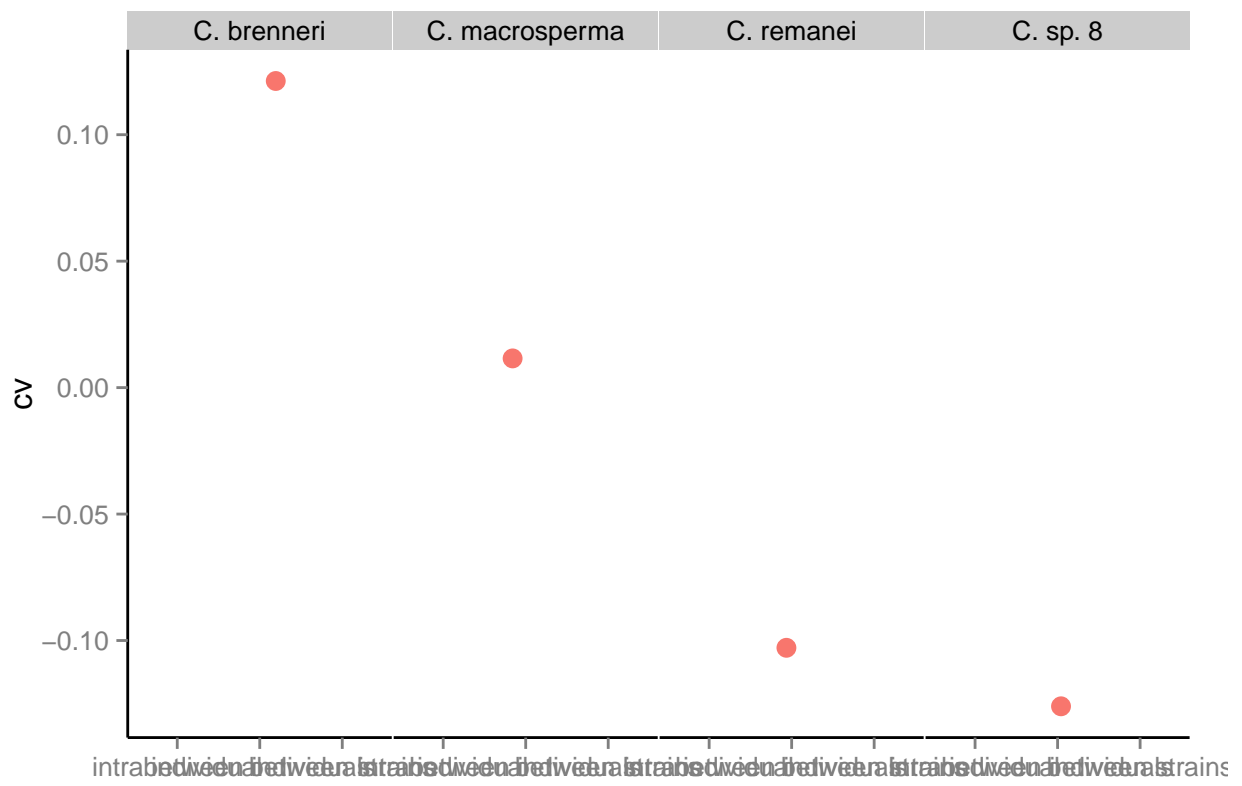


figure 3B







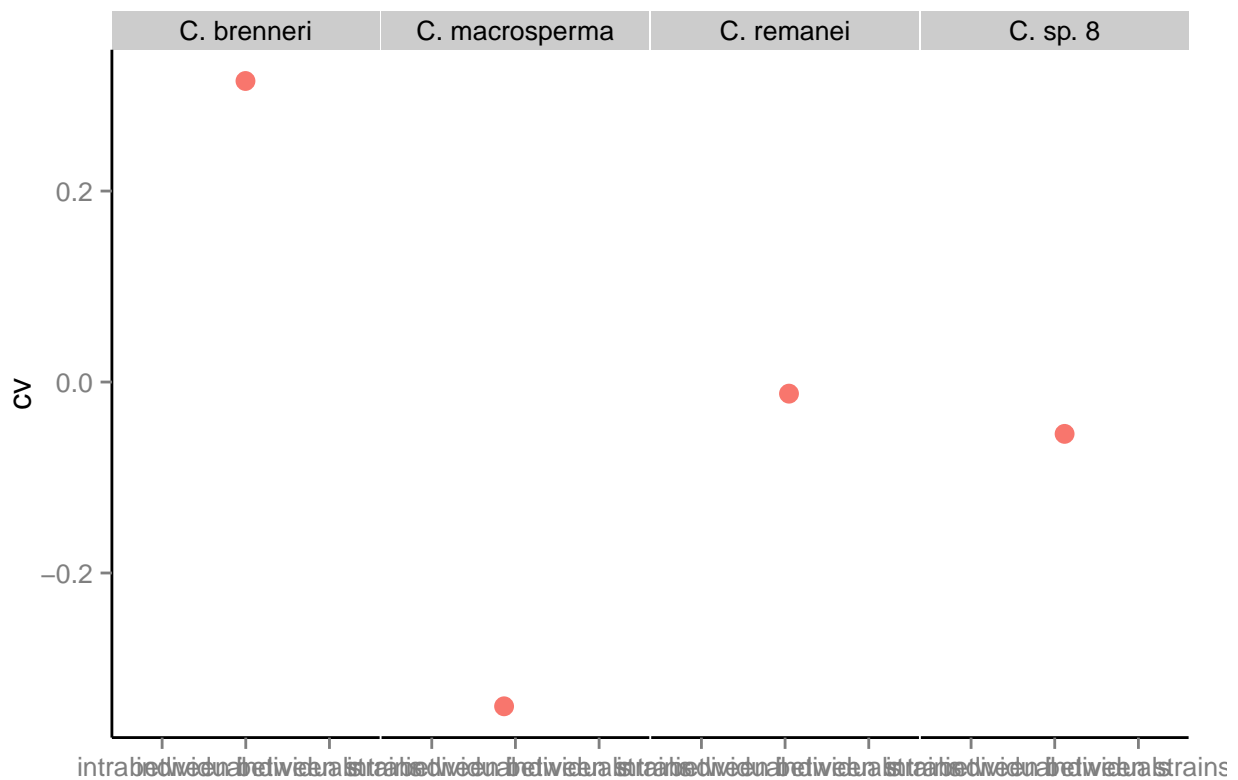


figure mockup a we are starting agin here, as I've modified some of the variables etc above Easier to start with a clean slate Clean and read in data:

mean and sd

```
groupedmeans=ff %>% group_by(Species,sex) %>% summarise(mean=mean(area,na.rm=T),n=n(),sd=sd(area,na.rm=T))
limits <- aes(ymax = mean + 1.96*sd/sqrt(n), ymin=mean - 1.96*sd/sqrt(n))
dodge <- position_dodge(width=0.9)
ggplot(data=groupedmeans, aes(x=Species, y=mean, fill=sex)) + geom_bar(stat="identity", position=position_dodge()) +
  scale_fill_manual(values=c("gray","white"))+ylab( expression(paste("area (", mu, m^{2},")")))+xlab("Species")
theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
  panel.background = element_blank(),axis.line = element_line(colour = "black"),legend.position = "none")
```

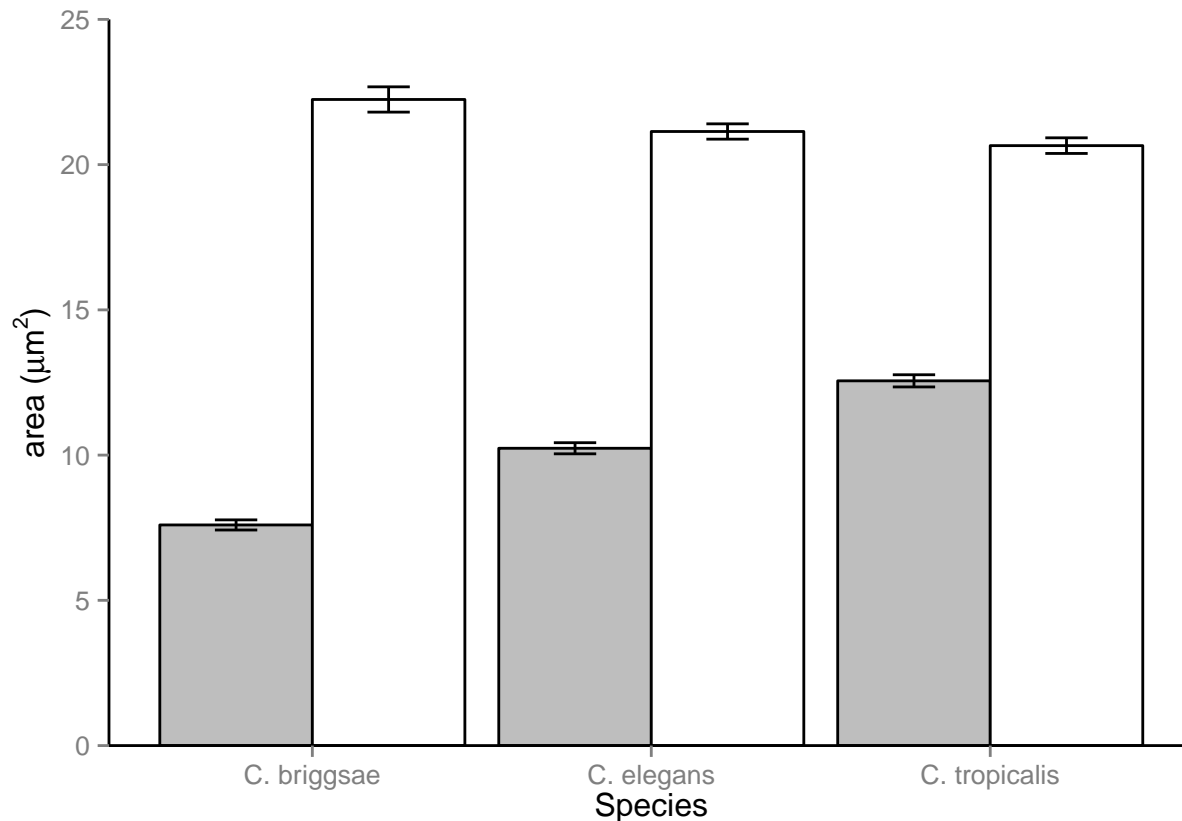


Figure mockup b group to get the data I want it's ugly, but it works

```
groupedstrains=ff %>% group_by(Species,Strain) %>%
  do(data.frame(meanM=mean(. [which(. $sex=="M"),]$area,na.rm=T),meanH=mean(. [which(. $sex=="H"),]$area,
    sdM=sd(. [which(. $sex=="M"),]$area,na.rm=T),sdH=sd(. [which(. $sex=="H"),]$area,na.rm=T))
groupedstrains$sem=1.96*groupedstrains$sdM/sqrt(groupedstrains$nM)
groupedstrains$seh=1.96*groupedstrains$sdH/sqrt(groupedstrains$nH)
p=ggplot(data=groupedstrains, aes(x=meanM, y=meanH, colour=Species, ymin = meanH - seh,ymax=meanH + seh))
p+geom_point(size=5)+coord_cartesian(ylim=c(0,15),xlim=c(0,33))+theme(panel.grid.major = element_blank(),
  panel.background = element_blank(),axis.line = element_line(colour = "black"),legend.position = "none")
p+ylab(expression(paste("Hermaphrodite Sperm area (", mu, m^{2},")")))+xlab(expression(paste("Male Sperm area (", mu, m^{2},")")))
p+geom_smooth(aes(group=Species), method="lm",size=1.5,colour="black")
```

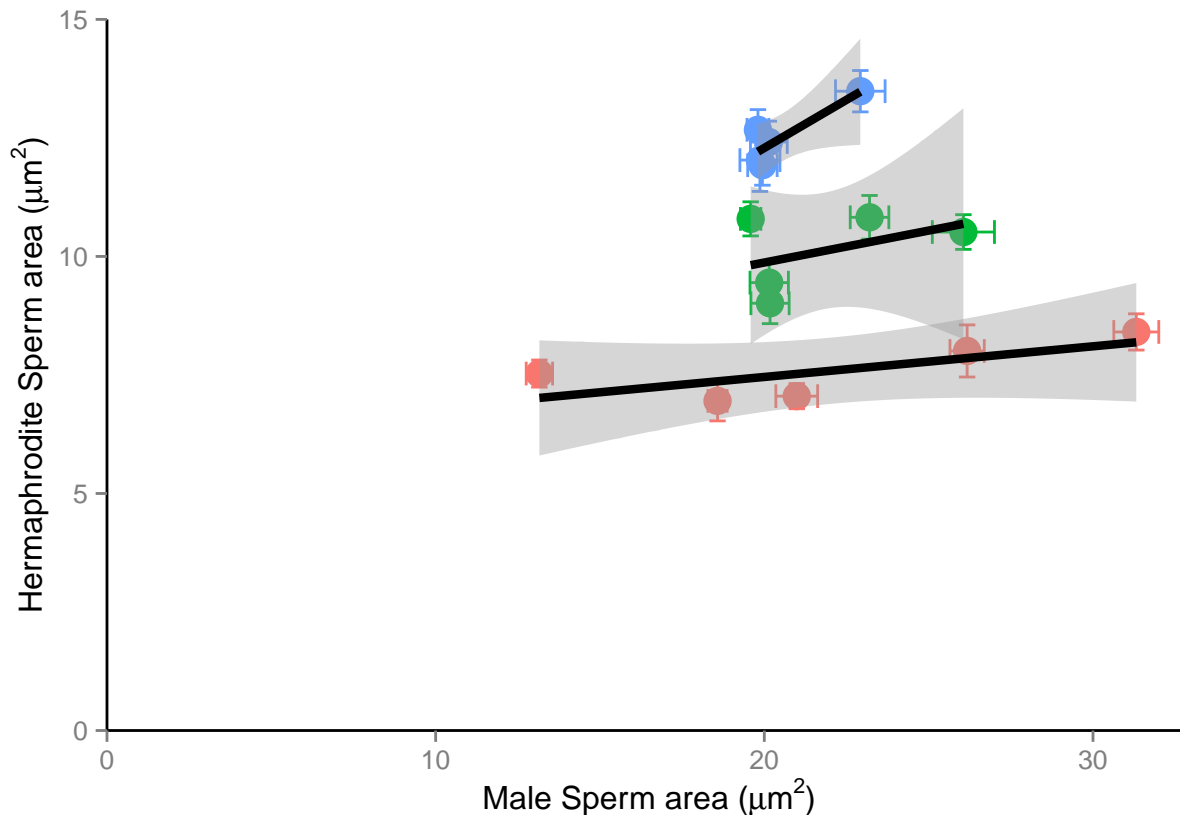


figure1a - bars need to rerun the above

```
ff<-spermsizes<-read.table("C:/Users/jeremy/Desktop/geiger/compileddata.csv",header=T,sep=",")
ff<-renameworms(ff)
spermsize2<-ff %>% group_by(Species,Strain) %>% summarise(means=mean(area,na.rm=T),cv=sd(area,na.rm=T)/
countspec=function(dataframe){
  holding=c()
  for(i in 1:nrow(dataframe)){
    holding=c(holding,sum(dataframe$Species==dataframe[i,]$Species))
  }
  return(holding)
}
spermsize2$Species<-ordered(spermsize2$Species,levels=c(tr$tip.label))
spermsize2<-spermsize2[order(spermsize2$Species),]
spermsize2$Strain<-factor(spermsize2$Strain, levels = spermsize2$Strain)
spermsize2$specnum=countspec(spermsize2)
spermsize2$cols=cut(spermsize2$means,3)
findchange<-function(df){
  holding=c()
  for(i in 1:length(df$Species)){
    if(i==1){
      holding=c(holding,0)
    } else if(df$Species[i]==df$Species[i-1]){
      holding=c(holding,0)
    } else {
      holding=c(holding,1)
    }
  }
}
```

```

    }
  }
  return(holding)
}

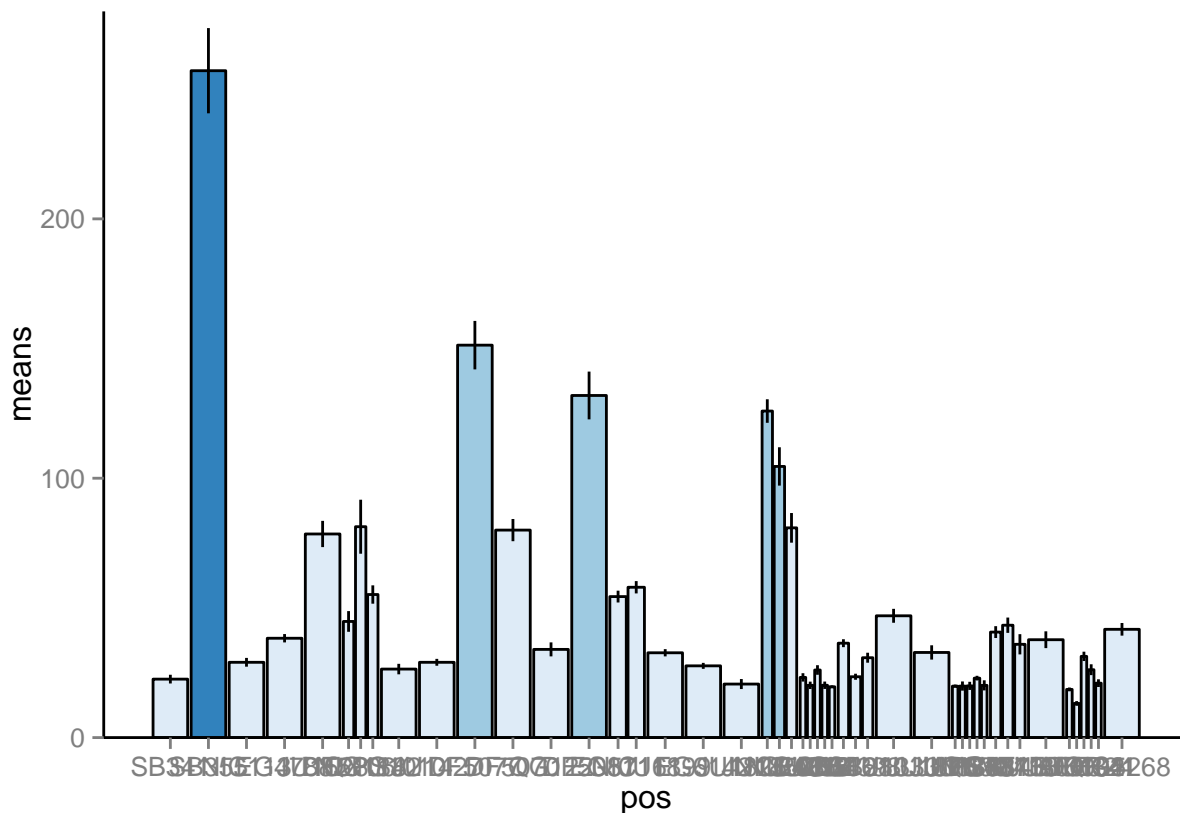
spermsize2$change<-findchange(spermsize2)
w<-(1-(spermsize2$specnum-1)*0.05)/spermsize2$specnum
pos <- 0.5 * (cumsum(w) + cumsum(c(0, w[-length(w)])))

gapsizes<-function(df,pos){
  pos1<-pos
  for(i in 1:length(df$specnum)){
    if(df$specnum[i]==1){
      pos1[i:length(pos1)]<-pos1[i:length(pos1)]+0.1
    } else if(df$change[i]==1){
      pos1[i:length(pos1)]<-pos1[i:length(pos1)]+0.1
    } else {
      pos1[i:length(pos1)]<-pos1[i:length(pos1)]+0.05
    }
  }
  return(pos1)
}

pos<-gapsizes(spermsize2,pos)
ggplot(data=spermsize2,aes(x = pos, width = w, y = means, fill=cols,ymin=means-sem,ymax=means+sem)) +
  geom_bar(stat = "identity",colour="black") + scale_x_continuous(labels = spermsize2$Strain, breaks =
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
    panel.background = element_blank(),axis.line = element_line(colour = "black"),legend.position = "n

```

## Warning: position\_stack requires constant width: output may be incorrect



large tree first group all the data

```
spermsize1<-ff %>% group_by(Species) %>% summarise(means=mean(area,na.rm=T))
spermsize2<-ff %>% group_by(Species,Strain) %>% summarise(means=mean(area,na.rm=T))
spermsize3<-ff %>% group_by(Species,Strain,individual) %>% summarise(means=mean(area,na.rm=T))
```

function to fix the format of spermsizes, and fit tree

```
fitace <- function(spermsizes,tree){
  holding<-spermsizes$means
  names(holding)<-spermsizes$Species
  return(fastAnc(tree,holding,CI=T))
}
```

function to choose a subset - works on any level, just chooses one from each species

```
subsetter<-function(spermsizes){
  x=lapply(split(spermsizes, spermsizes$Species),function(subdf) subdf[sample(1:nrow(subdf), 1),])
  do.call("rbind",x)
}
```

example usage fitace(subsetter(spermsizes2),tr) now need to loop and keep the values

```

looper<-function(spermsizes, reps, tree){
  outputdata=data.frame()
  outputCI=data.frame()
  for(i in 1:reps){
    outputdata=rbind(outputdata, fitace(subsetter(spermsizes), tree)$ace)
    outputCI=rbind(outputCI, fitace(subsetter(spermsizes), tree)$CI95)
  }
  names(outputdata)=seq(from=27, to=51)
  return(c(outputdata, outputCI))
}

```

ok we can call eg `x=looper(spermsize3, 10, tr)` then mean and sd, etc. etc.

```

x<-fitace(spermsize1, tr)
AncSperm=x$ace
#or AncSperm=apply(x, 2, mean) if it's bootstrapped
spermsize1$Species <- factor(spermsize1$Species, levels = c("C. sp. 1", "C. plicata", "C. guadeloupensis"))
spermsize=spermsize1[order(spermsize1$Species),]$means
names(spermsize)<-spermsize1[order(spermsize1$Species),]$Species
ggtree(tr)+geom_text(aes(label=label), hjust=-0.19, fontface="italic")+geom_point(subset=.(!isTip),
  color="#1b9e77", size=sqrt(AncSperm/pi))+
  geom_point(subset=.(isTip), color="#7570b3", size=sqrt(spermsize/pi))+
  geom_text(subset=.(isTip), aes(label=round(spermsize, 1)), hjust=1, vjust=-0.4, size=3)+
  geom_text(subset=.(!isTip), aes(label=round(AncSperm, 1)), hjust=1.5, vjust=-0.4, size=3)+
  scale_x_continuous(expand = c(0.1, 0.1))

```

## Warning: Removed 25 rows containing missing values (geom\_text).

