Mphar Amel

Loading libraries, themes

Loading previous results

```
setwd("~/GitHub/devnetwork/")
load("results/DEtests.RData")
load("results/collectedPhylo.RData")
antPlaid <- read.csv("results/antPlaidGenes.csv")</pre>
beePlaid <- read.csv("results/beePlaidGenes.csv")</pre>
tau <- read.csv("results/bee tau.csv")</pre>
sexGenes <- read.csv("results/dmel sexGenes.csv")</pre>
antConn <- read.csv("results/antConnectivity.csv")</pre>
beeConn <- read.csv("results/beeConnectivity.csv")</pre>
beeT <- read.table("data/bees.tpm.txt",header=TRUE)</pre>
antT <- read.table("data/ants.tpm.txt",header=TRUE)</pre>
modifyDF <- function(data){</pre>
  rownames (data) = data[,1]
  return(data[!grepl("ERCC",rownames(data)),-c(1)])
beeT <- modifyDF(beeT)</pre>
antT <- modifyDF(antT)</pre>
antT = antT[rowSums(antT) > 0,]
beeT = beeT[rowSums(beeT) > 0,]
genFactor <- function(counts){</pre>
  factors <- data.frame(sample=colnames(counts))</pre>
  factors$stage = 8
  factors$stage[grepl("_E",factors$sample)]=1
  factors$stage[grepl("L1",factors$sample)]=2
  factors$stage[grep1("L2",factors$sample)]=3
  factors$stage[grepl("L3",factors$sample)]=4
  factors$stage[grepl("L4",factors$sample)]=5
  factors$stage[grepl("L5",factors$sample)]=6
  factors$stage[grepl("P",factors$sample)]=7
  factors$tissue="larva"
  factors$tissue[grepl("P",factors$sample)]="pupa"
  factors$tissue[grepl("_E",factors$sample)]="egg"
  factors$tissue[grepl("G\\.",factors$sample)]="gaster"
  factors$tissue[grepl("H\\.",factors$sample)]="head"
  factors$tissue[grepl("M\\.",factors$sample)]="mesosoma"
  factors$NF=NA
  factors$NF[grepl("_N",factors$sample)]="nurse"
  factors$NF[grepl("_F",factors$sample)]="forager"
  factors$caste="worker"
  factors$caste[grepl("_S|_V|_AQ|_G",factors$sample)]="queen"
  factors$caste[grepl("_M",factors$sample)]="male"
  factors$VM=NA
```

```
factors$VM[grepl("_V",factors$sample)]="virgin"
  factors$VM[grepl("_AQ",factors$sample)]="mated"
  factors$colony=1
  factors$colony[grep1(".2",factors$sample)]=2
  factors$colony[grepl(".3",factors$sample)]=3
  for (i in 2:7){
    factors[,i]=as.factor(factors[,i])
  rownames(factors)=factors$sample
  factors$caste = factor(factors$caste,levels = c("queen","male","worker")) #Queen genes will always be
  factors$tissue = factor(factors$tissue,levels = c("egg","larva","pupa","head","mesosoma","gaster"))
  factors$NF = factor(factors$NF,levels = c("nurse", "forager")) #Make nurse genes down-regulated becaus
  return(factors)
factorA <- genFactor(antT)</pre>
factorB <- genFactor(beeT)</pre>
TGmap <- read.table("phylostratigraphy/out/TGmap_Amel.txt")</pre>
TNmap <- as.data.frame(fread("data/AmelTranName.txt",sep="~",header=FALSE))</pre>
AmelName \leftarrow merge(TGmap,TNmap,by.x = "V2",by.y = "V1")[,c(2,3)]
## Warning in merge.data.frame(TGmap, TNmap, by.x = "V2", by.y = "V1"): column
## name 'V2' is duplicated in the result
colnames(AmelName) = c("Gene", "GeneName")
AmelName$GeneName = gsub(" isoform X[0-9]","", AmelName$GeneName)
aName = AmelName[!duplicated(AmelName$Gene),]
```

0.1 Number of times a gene is queen- and worker-biased across development

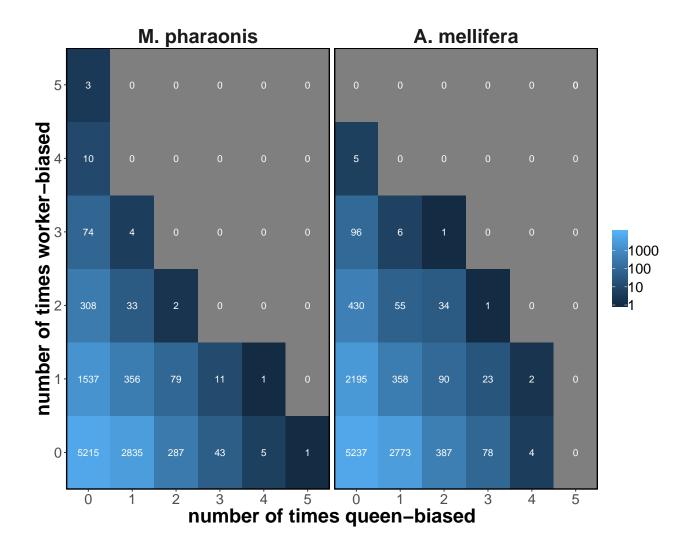
```
#Summarize number of times DE
sumDE <- function(dfDE,type1,type2){</pre>
  dfDE$numQueen = apply(dfDE[,c(2:ncol(dfDE))],1,function(x) sum(x == type1))
  dfDE$numWorker = apply(dfDE[,c(2:ncol(dfDE))],1,function(x) sum(x == type2))
  d = table(dfDE$numQueen,dfDE$numWorker)
  m = melt(d)
  colnames(m)[c(1,2)] = c(type1,type2)
  return(m)
}
m1 = sumDE(antRes[[2]], "queen", "worker")
m2 = sumDE(beeRes[[2]], "queen", "worker")
#Add these since there are no genes DE all five times in Apis
m2E = t(sapply(seq(0,5),function(i) c(queen=i,worker=5,value=0)))
m2Eb = t(sapply(seq(0,5),function(i) c(queen=5,worker=i,value=0)))
m2E = rbind(m2Eb, m2E)
m2 = rbind(m2, m2E)
m1$species = "M. pharaonis"
```

```
m2$species = "A. mellifera"
mA = rbind(m1, m2)
mA$species = factor(mA$species,levels= c("M. pharaonis", "A. mellifera"))
#Create heatmap of differential expression (number of times DE for queens and workers)
p <- ggplot(mA,aes(x=queen,y=worker))+</pre>
 geom tile(aes(fill = value))+
 facet_grid(. ~ species)+
  scale_fill_gradient(name = "number of genes",trans = "log",
                      breaks = c(1,10,100,1000),
                      limits = c(1,10000),
                      labels = c(1,10,100,1000))+
  geom_text(aes(x = queen,y = worker,label = value),color="white")+
  main_theme+
  scale_y_continuous(name = "number of times worker-biased",
                     breaks = seq(0,5),
                     expand = c(0,0)+
  scale_x_continuous(name = "number of times queen-biased",
                     breaks = seq(0,5),
                     expand = c(0,0)+
  theme(legend.position = "right",
        axis.line=element_line(color="black"),
        axis.text = element_text(size=16),
        axis.title = element_text(size = 22,face="bold"),
        strip.text = element_text(size=22,face="bold"),
        legend.title = element_blank(),
        strip.background = element_rect(color=NA,fill=NA),
        plot.title = element_text(hjust = 0.5,size=25,face = "bold"),
        panel.border = element_rect(size = 1, color = "black",fill = NA))
ggsave(p,file = "figures/p2.pdf",height=8,width=10)
```

Warning: Transformation introduced infinite values in discrete y-axis

0.1 Number of times a gene is queen- and worker-biased across development

Warning: Transformation introduced infinite values in discrete y-axis

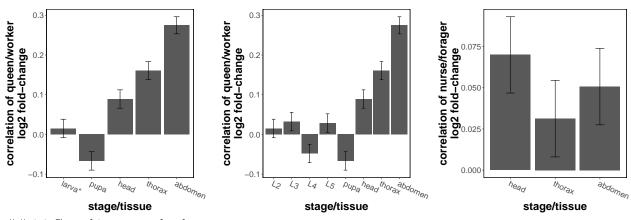


0.2 Correlation of log fold-change across stages

```
#Correlation of log fold change across development
lfcCor <- function(antD,beeD){</pre>
  nStage = ncol(antD) - 1
  antD <- merge(antD, ACUogg, by.x = "Gene", by.y = "gene_Mphar")</pre>
  beeD <- merge(beeD,ACUogg,by.x = "Gene",by.y = "gene_Amel")</pre>
  antD = antD[antD$0GG %in% beeD$0GG,]
  beeD = beeD[beeD$0GG %in% antD$0GG,]
  antD = antD[order(antD$0GG),]
  beeD = beeD[order(beeD$0GG),]
  d = data.frame(stage = colnames(antD)[c(2:(nStage+1))])
  dAbs = data.frame(stage = colnames(antD)[c(2:(nStage+1))])
  for (i in 1:nStage){
    t = cor.test(antD[,i+1],beeD[,i+1])
    d[i,2] = tsestimate
    d[i,3] = t\$conf.int[1]
    d[i,4] = t\$conf.int[2]
    t = cor.test(abs(antD[,i+1]),abs(beeD[,i+1]))
    dAbs[i,2] = t$estimate
```

```
dAbs[i,3] = t$conf.int[1]
    dAbs[i,4] = t$conf.int[2]
  colnames(d) = colnames(dAbs) = c("Stage", "cor", "c1", "c2")
  return(list(d,dAbs))
}
CasteCor <- lfcCor(antRes[[1]],beeRes[[1]])</pre>
CasteCor allStage <- lfcCor(antRes allstage[[1]],beeRes allstage[[1]])</pre>
BehavCor <- lfcCor(antSocRes[[1]],beeSocRes[[1]])</pre>
CasteCor[[1]]$Stage = as.character(CasteCor[[1]]$Stage)
CasteCor[[1]]$Stage[1] = "larva*"
CasteCor_allStage[[1]]$Stage = c("L2","L3","L4","L5","pupa","head","thorax","abdomen")
BehavCor[[1]]$Stage = c("head", "thorax", "abdomen")
CasteCor[[1]]$Stage = factor(CasteCor[[1]]$Stage,levels = CasteCor[[1]]$Stage)
CasteCor_allStage[[1]]$Stage = factor(CasteCor_allStage[[1]]$Stage,levels = CasteCor_allStage[[1]]$Stag
BehavCor[[1]]$Stage = factor(BehavCor[[1]]$Stage,levels = BehavCor[[1]]$Stage)
pl <- lapply(list(CasteCor[[1]],CasteCor_allStage[[1]],BehavCor[[1]]), function(x){</pre>
  ggplot(x,aes(x = Stage,y=cor))+
    geom_bar(stat="identity")+main_theme+
    xlab("stage/tissue")+
    geom_errorbar(aes(ymin=c1,ymax=c2),width=0.2)+
    ylab("correlation of queen/worker\nlog2 fold-change")+
    theme(plot.margin=unit(c(0.5,1.5,0.5,0.5),"cm"),
          axis.text.x = element_text(hjust=0,angle=-25))
})
pl[[3]] = pl[[3]] + ylab("correlation of nurse/forager\nlog2 fold-change")
```

0.2 Correlation of log fold-change across stages



1.1 Caste-bias across development

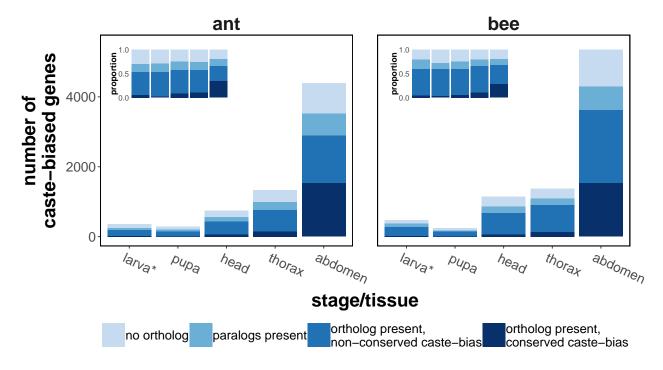
```
compDef <- function(antR,beeR){

#Define whether or not orthologs exist
antR$ortholog_found = antR$OGG_found = FALSE
antR$ortholog_found[antR$Gene %in% AllPS$Gene.x] = TRUE
antR$OGG_found[antR$Gene %in% ACUogg$gene_Mphar] = TRUE</pre>
```

```
aM = melt(antR,id.vars = c("Gene","ortholog_found","OGG_found"))
  aD = ddply(aM,~variable,summarize,
             NDE = sum(value=="nonDE"),
             no_ortholog = sum(value!="nonDE" & !ortholog_found),
             dup = sum(value!="nonDE" & ortholog_found & !OGG_found),
             OGG = sum(value!="nonDE" & OGG_found))
  #Do same thing for apis
  beeR$ortholog_found = beeR$OGG_found = FALSE
  beeR$ortholog_found[beeR$Gene %in% AllPS$Gene.y] = TRUE
  beeR$OGG_found[beeR$Gene %in% ACUogg$gene_Amel] = TRUE
  bM = melt(beeR,id.vars = c("Gene","ortholog_found","OGG_found"))
  bD = ddply(bM,~variable,summarize,
             NDE = sum(value=="nonDE"),
             no_ortholog = sum(value!="nonDE" & !ortholog_found),
             dup = sum(value!="nonDE" & ortholog_found & !OGG_found),
             OGG = sum(value!="nonDE" & OGG_found))
  colnames(bM)[5] = "value_apis"
  #Getting all results together, tabulating
  aM = merge(aM[,-c(2,3)], ACUogg,by.x="Gene",by.y="gene_Mphar")
  bM = merge(bM[,-c(2,3)], ACUogg,by.x="Gene",by.y="gene_Amel")
  allM = merge(aM,bM,by=c("OGGacu","variable"))
  allD = ddply(allM,~variable,summarize,
               DEboth = sum(value apis!="nonDE" & value != "nonDE"))
  #Calculate number of genes which are DE, have ortholog, and aren't commonly DEG
  aD$DEboth = bD$DEboth = allD$DEboth
  aD\$OGG = aD\$OGG - aD\$DEboth
  bD$OGG = bD$OGG - bD$DEboth
  aDM = melt(aD,id.vars = "variable")
  bDM = melt(bD,id.vars = "variable")
  colnames(aDM) = colnames(bDM) = c("stage", "DEtype", "value")
  aDM$species = "ant"
  bDM$species = "bee"
  #Get data back together
  d = rbind(aDM,bDM)
  d$species=as.factor(d$species)
  levels(d$species) = c("M. pharaonis","A. mellifera")
  levels(d$DEtype) = c("NDE", "no ortholog", "paralogs present", "ortholog present, \nnon-conserved caste-b
  return(d)
d = compDef(antRes[[2]],beeRes[[2]])
levels(d$stage)[1] = "larva*"
levels(d$species) = c("ant", "bee")
p1m <- ggplot(d[d$DEtype!="NDE",],</pre>
             aes(x = stage, y = value, fill = DEtype))+
  geom_bar(stat="identity")+
```

```
plot2theme+
  ylim(0,5500) +
  facet_grid(. ~ species)+
  xlab("stage/tissue")+
  scale_fill_manual(values = DE_palette[c(3,5,7,9)])+
  ylab("number of\ncaste-biased genes")+
  theme(axis.text.x = element_text(angle=-25,hjust=0.1),
        legend.position = "top",
        strip.text = element_text(size=22,face="bold"),
        legend.title = element_blank(),
        strip.background = element_rect(color=NA,fill=NA),
        plot.margin = margin(0.5, 2, 0.5, 0.5, "cm"))+
  theme(panel.spacing = unit(2, "lines"))
p2 <- ggplot(d[d$DEtype!="NDE" & d$species=="ant",],
             aes(x = stage, y = value, fill = DEtype))+
  geom_bar(stat="identity",position = "fill")+
  main theme+
  scale_fill_manual(values = DE_palette[c(3,5,7,9)])+
  theme(axis.text = element_text(size = 8.5),axis.ticks = element_blank())+
  xlab("")+
  ylab("proportion")+
  scale_y_continuous(breaks = c(0,0.5,1))+
  theme(axis.text.x = element_blank(),
        axis.ticks.x = element_blank(),
        rect = element rect(fill="transparent"),
        legend.position = "none",
        panel.border = element_blank(),
        panel.background = element_blank(),
        legend.title = element_blank(),
        axis.title.y = element_text(margin=margin(t=0,r=0,l=0,b=0)),
        axis.text.y = element_text(margin=margin(t=0,r=-3,l=0,b=0)),
        axis.title = element_text(size=10),
        plot.margin = margin(0,0,0,0,"cm"))
p3 <- ggplot(d[d$DEtype!="NDE" & d$species=="bee",],
             aes(x = stage, y = value, fill = DEtype))+
  geom_bar(stat="identity",position = "fill")+
  main theme+
  theme(axis.text = element_text(size = 8.5),axis.ticks = element_blank())+
  xlab("")+
  scale_fill_manual(values = DE_palette[c(3,5,7,9)])+
  ylab("proportion")+
  scale_y_continuous(breaks = c(0,0.5,1)) +
  theme(axis.text.x = element_blank(),
        axis.ticks.x = element_blank(),
        rect = element_rect(fill="transparent"),
        legend.position = "none",
        panel.border = element_blank(),
        panel.background = element_blank(),
        legend.title = element blank(),
        axis.title.y = element_text(margin=margin(t=0,r=0,l=0,b=0)),
```

1.1 Caste bias across development

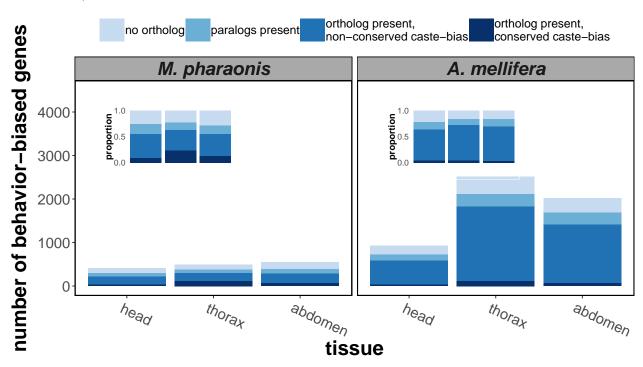


1.2 Nurse/forager bias

```
strip.text = element_text(size=20,face="bold.italic"),
        axis.title.y=element_text(margin=margin(t=0,l=0,r=10,b=0)),
        legend.title = element_blank(),
        strip.background = element_rect(color="black",fill="darkgrey"),
        plot.margin = margin(0,2,2,2,"cm"))
p2 <- ggplot(d[d$DEtype!="NDE" & d$species=="M. pharaonis",],</pre>
             aes(x = stage, y = value, fill = DEtype))+
  geom_bar(stat="identity",position = "fill")+
  main theme+
  scale_fill_manual(values = DE_palette[c(3,5,7,9)])+
  theme(axis.text = element_text(size = 8.5),axis.ticks = element_blank())+
  xlab("")+
  ylab("proportion")+
  scale_y_continuous(breaks = c(0,0.5,1)) +
  theme(axis.text.x = element_blank(),
        axis.ticks.x = element_blank(),
        rect = element_rect(fill="transparent"),
        legend.position = "none",
        panel.border = element_blank(),
        panel.background = element_blank(),
        legend.title = element_blank(),
        axis.title.y = element_text(margin=margin(t=0,r=0,l=0,b=0)),
        axis.text.y = element_text(margin=margin(t=0,r=-3,l=0,b=0)),
        axis.title = element_text(size=10),
        plot.margin = margin(0,0,0,0,"cm"))
p3 <- ggplot(d[d$DEtype!="NDE" & d$species=="A. mellifera",],
             aes(x = stage, y = value, fill = DEtype))+
  geom_bar(stat="identity",position = "fill")+
  main theme+
  theme(axis.text = element_text(size = 8.5),axis.ticks = element_blank())+
  xlab("")+
  scale_fill_manual(values = DE_palette[c(3,5,7,9)])+
  ylab("proportion")+
  scale_y_continuous(breaks = c(0,0.5,1))+
  theme(axis.text.x = element_blank(),
        axis.ticks.x = element_blank(),
        rect = element_rect(fill="transparent"),
        legend.position = "none",
        panel.border = element_blank(),
        panel.background = element blank(),
        legend.title = element blank(),
        axis.title.y = element_text(margin=margin(t=0,r=0,l=0,b=0)),
        axis.text.y = element_text(margin=margin(t=0,r=-3,l=0,b=0)),
        axis.title = element_text(size=10),
       plot.margin = margin(0,0,0,0,"cm"))
pSoc <- ggdraw()+
  draw_plot(p1+
              theme(legend.text = element_text(size=13),
                    legend.key.width = unit(1,"cm")))+
```

```
draw_plot(p2,x=0.2,y=0.58,height=0.18,width=0.18)+
draw_plot(p3,x=0.59,y=0.58,height=0.18,width=0.18)
```

1.2 nurse/forager bias



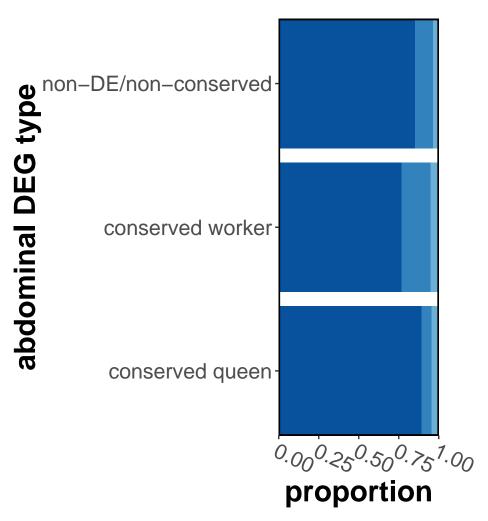
1.3 Phylostrata of genes with conserved abdominal caste bias

```
ps0 = merge(AllPS_sum, ACUogg, by="OGGacu")
ps0$a2 = "non-DE/non-conserved"
#Find genes with conserved gueen or worker bias
ps0$a2[ps0$gene Amel %in% beeRes[[2]]$Gene[beeRes[[2]]$abdomen=="worker"] &
         psO$gene_Mphar %in% antRes[[2]]$Gene[antRes[[2]]$abdomen=="worker"]] = "conserved worker"
ps0$a2[ps0$gene_Ame1 %in% beeRes[[2]]$Gene[beeRes[[2]]$abdomen=="queen"] &
         ps0$gene_Mphar %in% antRes[[2]]$Gene[antRes[[2]]$abdomen=="queen"]] = "conserved queen"
levels(ps0$psName)[1] = "ancient"
p <- ggplot(ps0,aes(x = a2,fill = forcats::fct_rev(psName)))+</pre>
  ylab("proportion")+
  geom_bar(stat = "count", position = "fill")+
  scale_fill_manual(values = mypalette2[c(2,4,5,6)],name = "phylostrata")+
  main theme+
  coord flip()+
  xlab("abdominal DEG type")+
  scale x discrete(expand=c(0,0))+
  guides(fill = guide_legend(reverse=T))+
  scale_y_continuous(expand=c(0,0))+
```

```
theme(axis.text.x = element_text(angle=-25,hjust=0),
    legend.position = "top",
    legend.title = element_blank(),
    plot.margin = unit(c(0.5,2,0.5,2),"cm"))
```

1.3 Estimated evolutionary age of genes with abdominal caste bias





Phylostrata across all comparisons

```
ant_PS = merge(antRes[[1]],Aps,by="Gene")
aPM = melt(ant_PS,id.vars = c("Gene","OGGacu","ps","psName","ODBgene"))
aPM$species = "ant"
bee_PS = merge(beeRes[[1]],Bps,by="Gene")
bPM = melt(bee_PS,id.vars = c("Gene","OGGacu","ps","psName","ODBgene"))
bPM$species = "bee"
PM = rbind(aPM,bPM)
```

```
PM$psName = factor(PM$psName,levels = levels(PM$psName)[c(1:5,7,6)])
p <- ggplot(PM[!is.na(PM$psName),],aes(x = variable,y=-value,fill=psName))+</pre>
  geom_boxplot(outlier.shape = NA, notch=T)+
  facet_wrap(. ~ species)+
  ylab("log2 fold-change (queen/worker)")+
  coord_cartesian(ylim=c(-5,5))+
  theme(legend.position="bottom")+
  xlab("stage/tissue")+
  geom_hline(yintercept = 0,linetype="dashed")
ggsave(p,file = "figures/p5.pdf",height=6,width=12)
ant_PS = merge(antSocRes[[1]],Aps,by="Gene")
aPM = melt(ant_PS,id.vars = c("Gene","OGGacu","ps","psName","ODBgene"))
aPM$species = "ant"
bee_PS = merge(beeSocRes[[1]],Bps,by="Gene")
bPM = melt(bee_PS,id.vars = c("Gene","OGGacu","ps","psName","ODBgene"))
bPM$species = "bee"
PM = rbind(aPM,bPM)
PM$psName = factor(PM$psName,levels = levels(PM$psName)[c(1:5,7,6)])
p <- ggplot(PM[!is.na(PM$psName),],aes(x = variable,y=-value,fill=psName))+</pre>
  geom_boxplot(outlier.shape = NA, notch=T)+
  facet_wrap(. ~ species)+
 ylab("log2 fold-change (nurse/forager)")+
  coord_cartesian(ylim=c(-5,5))+
  theme(legend.position="bottom")+
  xlab("tissue")+
  geom_hline(yintercept = 0,linetype="dashed")
ggsave(p,file = "figures/p18.pdf",height=6,width=12)
## notch went outside hinges. Try setting notch=FALSE.
```

2.1 Bicluster associated with queen abdominal expression

-Previously performed plaid clustering and extracted genes commonly found in a queen abdomen-specific bicluster.

```
load("results/PlaidResults.RData")
freq_set <- function(data,maxMod,factor,tpm,type){
  res <- lapply(data,function(x){
    checkMod = seq(1:nrow(x@NumberxCol))[rowSums(x@NumberxCol) <= maxMod]
  r = list()
  modI = 0
  for (mod in checkMod){
    qp = x@NumberxCol
    samp = factor$sample[x@NumberxCol[mod,]]
    if (sum(grepl(type,samp))==3){
        modI = modI+1
        genes = rownames(tpm)[x@RowxNumber[,mod]]
        r[[modI]]=list(samples=samp,genes=genes)
    }
}</pre>
```

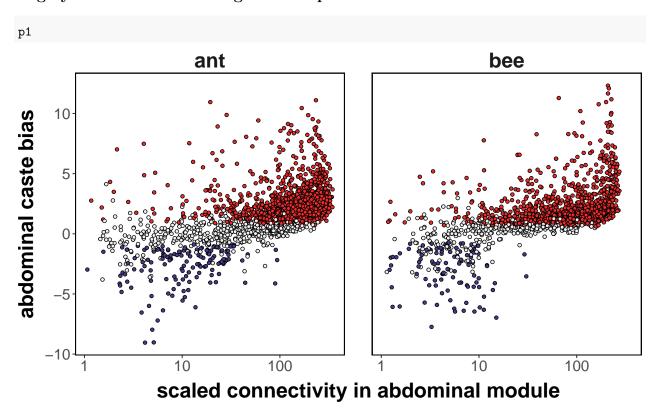
```
if (length(r) > 1){
      rnew = r[[1]]
      rnew$genes = unique(c(r[[2]]$genes,r[[1]]$genes)) #combine genes from queen abdomen module
      r = rnew
    return(r)
  })
 return(res)
#Calculate number of significant biclusters for a given set
num_set <- function(set){</pre>
 nums = lapply(names(set),function(x){
    length(set[[x]][lapply(set[[x]],length) > 0])
 })
 numDF = data.frame(Type = names(set),Freq=unlist(nums))
commonGenes <- function(QGres,tpm){</pre>
  df = data.frame(Gene=rownames(tpm), KeepNum=0)
  num = 0
  for (res in QGres){
    if (length(res) == 1){
      num = num+1
      df$KeepNum[df$Gene %in% res[[1]]$genes] = df$KeepNum[df$Gene %in% res[[1]]$genes] + 1
    }
  }
  df$KeepNum = df$KeepNum/num
 return(df)
samp_types = sapply(factorA$sample,function(x) gsub(".*_","",x) %>% gsub("\\.[0-9]","",.)) %>%
  unique()
#Output significant biclusters for each sample type
type_bicAmel <- lapply(samp_types,function(x) freq_set(beePl,6,factorB,beeT,x))</pre>
type_bicMphar <- lapply(samp_types,function(x) freq_set(antPl,6,factorA,antT,x))</pre>
names(type_bicAmel) = names(type_bicMphar) = samp_types
typeNum_Amel = num_set(type_bicAmel)
typeNum_Mphar = num_set(type_bicMphar)
antQG <- type_bicMphar[["AQG"]]</pre>
beeQG <- type_bicAmel[["AQG"]]</pre>
antQG = antQG[lapply(antQG,length) > 0]
beeQG = beeQG[lapply(beeQG,length) > 0]
aG <- commonGenes(antQG,antT)
bG <- commonGenes(beeQG,beeT)
pdf("figures/p6.pdf")
hist(aG$KeepNum)
dev.off()
```

```
## pdf
##
pdf("figures/p7.pdf")
hist(bG$KeepNum)
dev.off()
## pdf
##
#Find out what the genes are
antGrC = merge(antPlaid, Aps, by="Gene")
beeGrC = merge(beePlaid, Bps, by="Gene")
antGrC$species = "ant"
beeGrC$species = "bee"
\#Add - log(p)
antGrC = merge(antGrC,antRes[[3]],by="Gene")
beeGrC = merge(beeGrC,beeRes[[3]],by="Gene")
#Make general plot
allC = rbind(antGrC,beeGrC)
allC$DEcat = factor(allC$DEcat,levels = c("queen","worker","non-DE"))
#Note -abdomen because we want queen genes to be upregulated
p1 <- ggplot(allC,aes(x = conn,y=-abdomen.x))+
  geom_point(aes(fill = DEcat),pch=21,color="black")+
  scale fill manual(values = SexPal[c(1,2,5)],name="DEG type")+
  scale_alpha_continuous(guide="none")+
  ylab("abdominal caste bias")+
  xlab("scaled connectivity in abdominal module")+
 plot2theme+
  facet_grid(. ~ species)+
  theme(panel.spacing = unit(2, "lines"))+
  guides(fill = guide_legend(override.aes = list(size=4)))+
  scale_x_log10()+
  theme(legend.position ="none",
        legend.text = element_text(size=15),
        strip.text = element_text(size=22,face="bold"),
        axis.title.y=element_text(margin=margin(t=0,l=0,r=10,b=0)),
        legend.title = element_blank(),
        plot.margin = unit(rep(1,4), "cm"),
        strip.background = element_rect(color=NA,fill=NA))
ggsave(p1,file = "figures/p8.pdf",height=6,width=10)
p2 <- ggplot(allC,aes(x = conn,y=-log(abdomen.y)))+</pre>
  geom_point(aes(fill = DEcat),pch=21,color="black")+
  scale fill manual(values = SexPal[c(1,2,5)],name="DEG type")+
  scale_alpha_continuous(guide="none")+
  ylab("-log(P) of queen/worker\ndifferential expression")+
  xlab("scaled connectivity in abdominal module")+
  plot2theme+
  facet_grid(. ~ species)+
```

```
theme(panel.spacing = unit(2, "lines"))+
guides(fill = guide_legend(override.aes = list(size=4)))+
scale_x_log10()+
theme(legend.position ="none",
    legend.text = element_text(size=15),
    strip.text = element_text(size=22,face="bold"),
    axis.title.y=element_text(margin=margin(t=0,l=0,r=10,b=0)),
    legend.title = element_blank(),
    plot.margin = unit(rep(1,4),"cm"),
    strip.background = element_rect(color=NA,fill=NA))

ggsave(p2,file = "figures/p9.pdf",height=6,width=10)
```

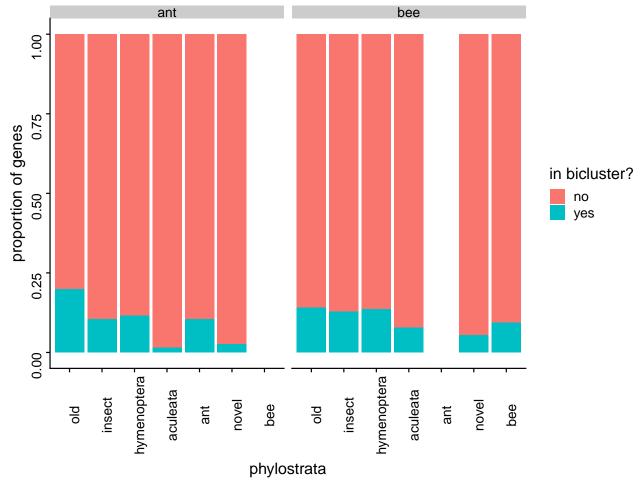
Highly connected bicluster genes are queen-biased



Age of genes in bicluster

```
Aps$bic = Bps$bic = "no"
Aps$bic[Aps$Gene %in% antGrC$Gene] = "yes"
Bps$bic[Bps$Gene %in% beeGrC$Gene] = "yes"
Aps$species = "ant"
Bps$species = "bee"
psBic = rbind(Aps,Bps)
p <- ggplot(psBic[!is.na(psBic$psName),],aes(x = psName,fill=bic))+
    geom_bar(stat="count",position="fill")+</pre>
```

```
facet_wrap(. ~ species)+
  xlab("phylostrata")+
  ylab("proportion of genes")+
  theme(axis.text = element_text(angle=90))+
  scale_fill_discrete(name = "in bicluster?")
p
```



ggsave(p,file = "figures/p10.pdf",height=6,width=8)

2.2 Specific genes within the bicluster

• holding off plotting specific genes for now

ant Genes

```
annSwiss[!grep1("ncharacterized",annSwiss) & annSwiss!="-"]

## factor(0)
## 7725 Levels: Pyruvate dehydrogenase acetyl-transferring -phosphatase 1 mitochondrial ...
bee Genes
beeSwiss[!grep1("ncharacterized",beeSwiss) & beeSwiss!="-"]

## factor(0)
## 9976 Levels: 18-wheeler precursor ... zinc finger protein 629-like
```

3.1 Comparing caste bias to sex bias

```
extractBias <- function(DEres){
    sexQ <- rownames(DEres) [DEres$FDR < 0.1 & DEres$logFC < 0]
    sexM <- rownames(DEres) [DEres$FDR < 0.1 & DEres$logFC > 0]
    sexFC <- data.frame(Gene = rownames(DEres), FC = DEres$logFC)
    return(list(FC = sexFC,Queen = sexQ,nonQueen = sexM))
}

AsexRes <- lapply(ant_sexDE,extractBias)
AcasteRes <- lapply(antTests_oneLarv[c(3:5)],extractBias)
BsexRes <- lapply(bee_sexDE,extractBias)
BcasteRes <- lapply(beeTests_oneLarv[c(3:5)],extractBias)</pre>
```

Identify genes with conserved bias for plotting

```
ogg11 = ACUogg
ogg11$abdDE = "non-conserved/non-DE"
ogg11$abdDE[(ogg11$gene_Amel %in% BcasteRes[[3]][[2]] & ogg11$gene_Mphar %in% AcasteRes[[3]][[2]])] = "
ogg11$abdDE[(ogg11$gene_Amel %in% BcasteRes[[3]][[3]] & ogg11$gene_Mphar %in% AcasteRes[[3]][[3]])] = "
```

3.1 Construct sex bias vs caste bias plots

```
biasPlot <- function(d1,d2,spec){

FC = merge(d1,d2,by = "Gene")
 FC = merge(FC,ogg11,by.x = "Gene",by.y=spec,all.x=TRUE)
 FC$abdDE[is.na(FC$abdDE)] = "non-conserved/non-DE"
 FC$abdDE = factor(FC$abdDE,levels = c("conserved queen","conserved worker","non-conserved/non-DE"))
 FC$alpha = 0.2
 FC$alpha[FC$abdDE!="non-conserved/non-DE"]=0.8

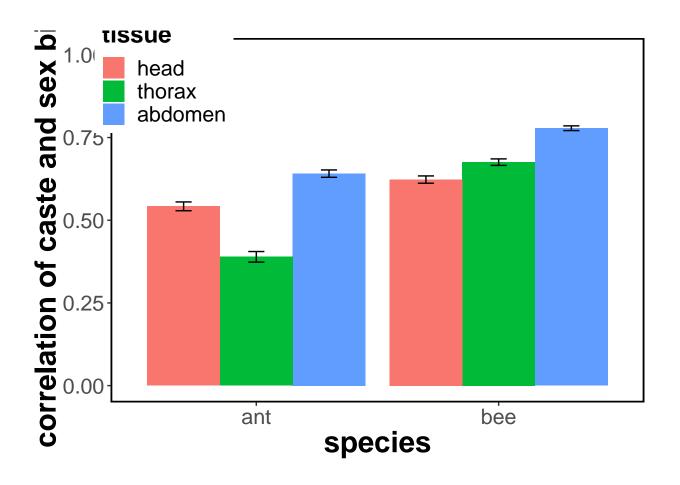
p1 <- ggplot(FC,aes(x = -FC.x,y = -FC.y))+ #Queen-up will be positive
    geom_point(aes(fill = abdDE,alpha=alpha),pch=21,color="black",size=2)+
    geom_smooth(method="lm",se=FALSE,color="black")+
    scale_fill_manual(values = SexPal[c(1,2,5)],name = "abdominal caste bias")+
    guides(fill = guide_legend(override.aes = list(size=4)))+</pre>
```

```
scale_alpha_continuous(guide="none")+
    main_theme+
    ylab("caste bias (queen/worker)")+
    xlab("sex bias (queen/male)")+
    ylim(-10,10)+xlim(-10,10)+
    theme(axis.title.y=element_text(margin = margin(t=0,l=15,r=-5,b=0)))+
    theme(legend.position="none",
          legend.text = element text(size=17),
          legend.title = element_text(size=19,face="bold"))
  return(p1)
}
p1 = biasPlot(AsexRes[[3]][[1]], AcasteRes[[3]][[1]], "gene_Mphar")+
  ylab("ant caste bias (queen/worker)")+
  xlab("ant sex bias (queen/male)")
p2 = biasPlot(BsexRes[[3]][[1]],BcasteRes[[3]][[1]],"gene_Amel")+
  ylab("bee caste bias (queen/worker)")+
  xlab("bee sex bias (queen/male)")
grid.arrange(p1,p2,nrow=1)
## Warning: Removed 54 rows containing non-finite values (stat_smooth).
## Warning: Removed 54 rows containing missing values (geom_point).
## Warning: Removed 47 rows containing non-finite values (stat_smooth).
## Warning: Removed 47 rows containing missing values (geom_point).
  10
                                                    10
ant caste bias (queen/worker)
                                                 bee caste bias (queen/worker)
                                                    -5
 -10
                                           10
     -i0
                                                       -<del>1</del>0
                                                                                             10
                                                           bee sex bias (queen/male)
          ant sex bias (queen/male)
p =arrangeGrob(p1,p2,nrow=1)
## Warning: Removed 54 rows containing non-finite values (stat_smooth).
## Warning: Removed 54 rows containing missing values (geom_point).
## Warning: Removed 47 rows containing non-finite values (stat_smooth).
```

```
## Warning: Removed 47 rows containing missing values (geom_point).
ggsave(p,file = "figures/p11.pdf",height=6,width=12)
```

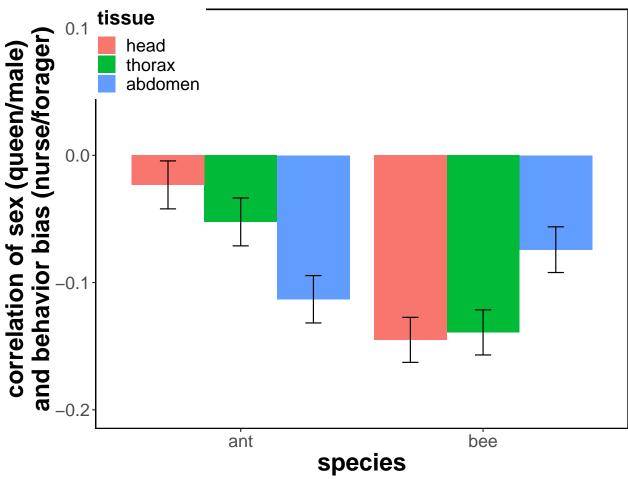
Sex bias vs caste bias in all tissues

```
FCcor <- function(test1,test2){</pre>
 FC = merge(test1[[1]],test2[[1]],by = "Gene")
  r = cor.test(FC$FC.x,FC$FC.y)
 return(c(r$estimate,c1=r$conf.int[1],c2=r$conf.int[2]))
Acor <- ldply(lapply(seq(1,3),function(i){</pre>
  FCcor(AcasteRes[[i]],AsexRes[[i]])
}))
Bcor <- ldply(lapply(seq(1,3),function(i){</pre>
  FCcor(BcasteRes[[i]],BsexRes[[i]])
}))
Acor$tissue = Bcor$tissue = c("head","thorax","abdomen")
Acor$species = "ant"
Bcor$species = "bee"
AllCor = rbind(Acor,Bcor)
AllCor$tissue=factor(AllCor$tissue,levels = c("head","thorax","abdomen"))
p <- ggplot(AllCor,aes(x=species,y=cor,fill=tissue))+</pre>
  geom_bar(stat="identity",position=position_dodge())+
  geom_errorbar(aes(ymin=c1,ymax=c2),position = position_dodge(.9),width=0.2)+
  main_theme+
  ylim(0,1)+
  ylab("correlation of caste and sex bias")+
  theme(legend.title = element_text(size=18,face="bold"),
        legend.position = c(0.1,0.9))
ggsave(p,file = "figures/p12.pdf",height=6,width=8)
p
```



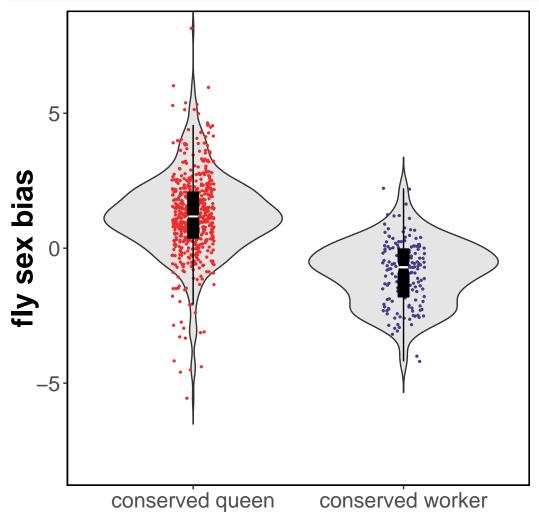
Sex bias vs caste bias for nurses and foragers

```
AsocRes <- lapply(antSocial,extractBias)</pre>
BsocRes <- lapply(beeSocial,extractBias)</pre>
Acor <- ldply(lapply(seq(1,3),function(i){
  FCcor(AsocRes[[i]],AsexRes[[i]])
}))
Bcor <- ldply(lapply(seq(1,3),function(i){</pre>
  FCcor(BsocRes[[i]],BsexRes[[i]])
}))
Acor$tissue = c("head", "thorax", "abdomen")
Bcor$tissue = c("head","thorax","abdomen")
Acor$species = "ant"
Bcor$species = "bee"
AllCor = rbind(Acor,Bcor)
AllCor$tissue=factor(AllCor$tissue,levels = c("head","thorax","abdomen"))
p <- ggplot(AllCor,aes(x=species,y=cor,fill=tissue))+</pre>
  geom_bar(stat="identity",position=position_dodge())+
  geom_errorbar(aes(ymin=c1,ymax=c2),position = position_dodge(.9),width=0.2)+
  main_theme+
```



3.2 Comparison to D. melanogaster

```
DmelSC = merge(sexGenes,ogg11,by="gene_Amel")
DmelSC$abdDE = factor(DmelSC$abdDE,levels = c("conserved queen","conserved worker","non-conserved/non-Defended for the second formula of the second
```



abdominal caste bias

```
ggsave(p4,file = "figures/p13.pdf",height=6,width=6)

Dmel_male = DmelSC$gene_Amel[DmelSC$FDR < 0.05 & DmelSC$logFC > 0]

Dmel_female = DmelSC$gene_Amel[DmelSC$FDR < 0.05 & DmelSC$logFC < 0]

cQ = DmelSC$gene_Amel[DmelSC$abdDE=="conserved queen"]

cW = DmelSC$gene_Amel[DmelSC$abdDE=="conserved worker"]

Gtype = list(Dmel_m=Dmel_male,Dmel_f=Dmel_female,conQ=cQ,conW=cW)

p <- venn.diagram(Gtype,filename=NULL)

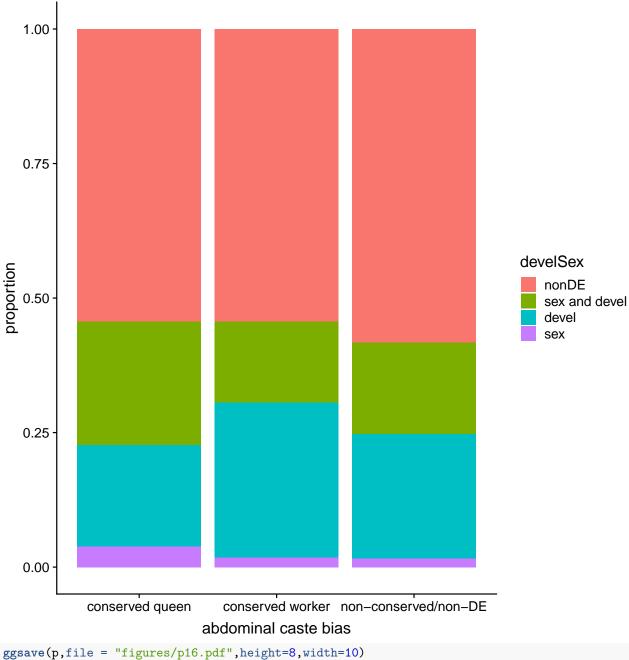
ggsave(p,file="figures/p14.pdf")</pre>
```

Saving 6 x 6 in image

Development section

Comparison to pre-defined sex and development genes

```
DmelSC = merge(sexGenes,ogg11,by="gene_Amel")
key <- read.table("data/DmelKey.txt") #protein-CDS</pre>
key2 <- read.table("data/Dmel_CDStoGene_key.txt") #Gene-CDS</pre>
devel <- read.table("data/Dmel_Devel_IDs.txt") #Downloaded from flybase, genes with developmental terms
sex <- read.table("data/Dmel_Sex_IDs.txt") #Downloaded from flybase, genes with sex terms</pre>
key3 = merge(key,key2,by="V1")
DmelSC = merge(DmelSC, key3, by.x="Gene", by.y="V2.y")
DmelSC$develSex = "nonDE"
DmelSC$develSex[DmelSC$V2.x %in% devel$V1] = "devel"
DmelSC$develSex[DmelSC$V2.x %in% sex$V1] = "sex"
DmelSC$develSex[DmelSC$V2.x %in% sex$V1 & DmelSC$V2.x %in% devel$V1] = "sex and devel"
DmelSC$develSex = factor(DmelSC$develSex,levels = c("nonDE", "sex and devel", "devel", "sex"))
p <- ggplot(DmelSC[!grep1("ant",DmelSC$abdDE),],aes(x = abdDE,fill=develSex))+</pre>
  geom_bar(stat="count",position="fill")+
  ylab("proportion")+
  xlab("abdominal caste bias")
р
```



-log(P-value) for genes with conserved abdominal differential expression

```
#using DE results
antDevel2$Gene=rownames(antDevel2)
aD = merge(ogg11,antDevel2,by.x="gene_Mphar",by.y="Gene")
p1 <- ggplot(aD[!grepl("ant",aD$abdDE),],aes(x = abdDE,y=-log(PValue)))+</pre>
  geom_boxplot(notch = T)+
  scale_y_log10()+
  theme(axis.text.x = element_text(angle=90))+
```

```
ggtitle("devel measured in ant")
beeDevel2$Gene=rownames(beeDevel2)
bD = merge(ogg11,beeDevel2,by.x="gene_Amel",by.y="Gene")

p2 <- ggplot(bD[!grepl("ant",bD$abdDE),],aes(x = abdDE,y=-log(PValue)))+
    geom_boxplot(notch = T)+
    scale_y_log10()+
    theme(axis.text.x = element_text(angle=90))+
    ggtitle("devel measured in bee")

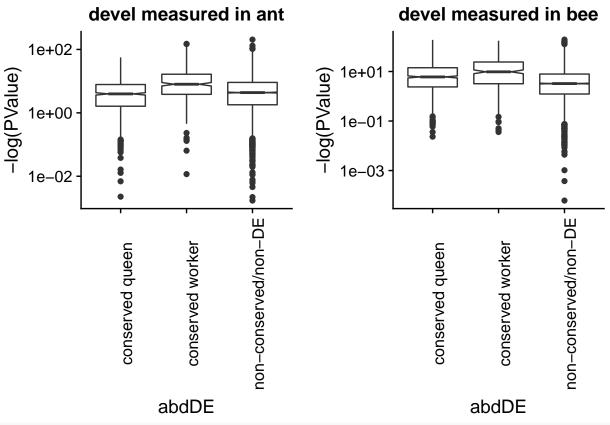
grid.arrange(p1,p2,nrow=1)</pre>
```

Warning: Transformation introduced infinite values in continuous y-axis

Warning: Removed 11 rows containing non-finite values (stat_boxplot).

Warning: Transformation introduced infinite values in continuous y-axis

Warning: Removed 9 rows containing non-finite values (stat_boxplot).



p = arrangeGrob(p1,p2,nrow=1)

Warning: Transformation introduced infinite values in continuous y-axis

Warning: Removed 11 rows containing non-finite values (stat_boxplot).

Warning: Transformation introduced infinite values in continuous y-axis

Warning: Removed 9 rows containing non-finite values (stat_boxplot).

```
ggsave(p,file = "figures/p15.pdf",height=8,width=10)
```

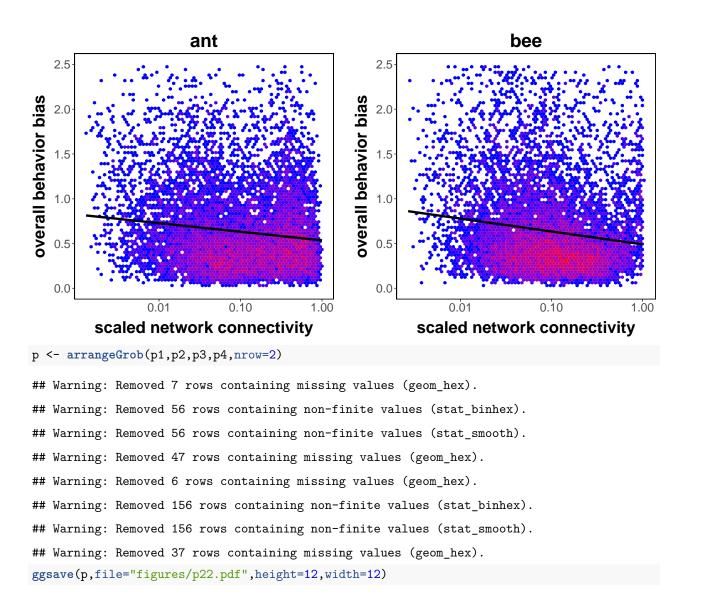
4.1 Calculation of overall caste bias

```
#Calculate euclidean distance
euclDist <- function(res){</pre>
  cb = apply(res[,-c(1)],1,function(x) sqrt(sum(x^2))/length(x))
  cb_noAbd = apply(res[,-c(1,ncol(res))],1,function(x) sqrt(sum(x^2))/length(x))
  cb_noAdult = apply(res[,-c(1,(ncol(res) - 2):ncol(res))],1,function(x) sqrt(sum(x^2))/length(x))
  cb_larva = apply(res[,-c(1,(ncol(res) - 3):ncol(res))],1,function(x) sqrt(sum(x^2))/length(x))
  cb_adult = apply(res[,-c(1:(ncol(res) - 3))],1,function(x) sqrt(sum(x^2))/length(x))
  cb_abd = apply(as.data.frame(res[,-c(1:(ncol(res) - 1))]),1,function(x) sqrt(sum(x^2))/length(x))
  results = data.frame(Gene = res$Gene,cb=cb,cb_noAbd=cb_noAbd,cb_noAdult=cb_noAdult,cb_larva=cb_larva,
  return(results)
}
#For this analysis, we include all larval stages
antCB = euclDist(antRes allstage[[1]])
beeCB = euclDist(beeRes_allstage[[1]])
antSB = euclDist(antSocRes[[1]])
beeSB = euclDist(beeSocRes[[1]])
antSB$type=beeSB$type="behavior"
antCB$type=beeCB$type="caste"
antBias2 = rbind(antCB,antSB)
beeBias2 = rbind(beeCB, beeSB)
```

4.1 Caste/Behavior bias vs connectivity

```
cbAps = merge(antBias2,Aps,by="Gene")
cbBps = merge(beeBias2,Bps,by="Gene")
cbAps = merge(antConn,cbAps,by="Gene")
cbBps = merge(beeConn,cbBps,by="Gene")
cbBps$kTotal = cbBps$kTotal/max(cbBps$kTotal)
cbAps$kTotal = cbAps$kTotal/max(cbAps$kTotal)
levels(cbAps$psName)[1] = levels(cbBps$psName)[1] = "ancient"
cbPlot <- function(data, type, spec){</pre>
 p1C <- ggplot(data[data$type==type,],aes(x = kTotal,y=cb))+
  geom hex(bins=70)+
  scale_fill_gradient(low = "blue",high="red")+
  plot2theme+
  vlim(0,2.5)+
  geom_smooth(method="lm",size=1.5,se=FALSE,color="black")+
  xlab("scaled network connectivity")+
  ggtitle(spec)+
  ylab(paste("overall", type, "bias", sep = " "))+
  scale_x_{log10}(breaks = c(0.01,0.1,1))+
```

```
theme(legend.position="none")
  return(p1C)
p1 <- cbPlot(cbAps, "caste", "ant")</pre>
p2 <- cbPlot(cbAps,"behavior","ant")</pre>
p3 <- cbPlot(cbBps, "caste", "bee")
p4 <- cbPlot(cbBps, "behavior", "bee")
grid.arrange(p1,p3,nrow=1)
## Warning: package 'hexbin' was built under R version 3.4.3
## Warning: Removed 7 rows containing missing values (geom_hex).
## Warning: Removed 6 rows containing missing values (geom_hex).
                                                                          bee
                        ant
   2.5
                                                     2.5
   2.0
                                                     2.0
overall caste bias
                                                  overall caste bias
   1.5
                                                     1.5
                                                     1.0
   0.5
                                                     0.5
                                                     0.0
   0.0
                 0.01
                              0.10
                                           1.00
                                                                0.01
                                                                              0.10
                                                                                             1.00
         scaled network connectivity
                                                           scaled network connectivity
grid.arrange(p2,p4,nrow=1)
## Warning: Removed 56 rows containing non-finite values (stat_binhex).
## Warning: Removed 56 rows containing non-finite values (stat_smooth).
## Warning: Removed 47 rows containing missing values (geom_hex).
## Warning: Removed 156 rows containing non-finite values (stat_binhex).
## Warning: Removed 156 rows containing non-finite values (stat_smooth).
## Warning: Removed 37 rows containing missing values (geom_hex).
```



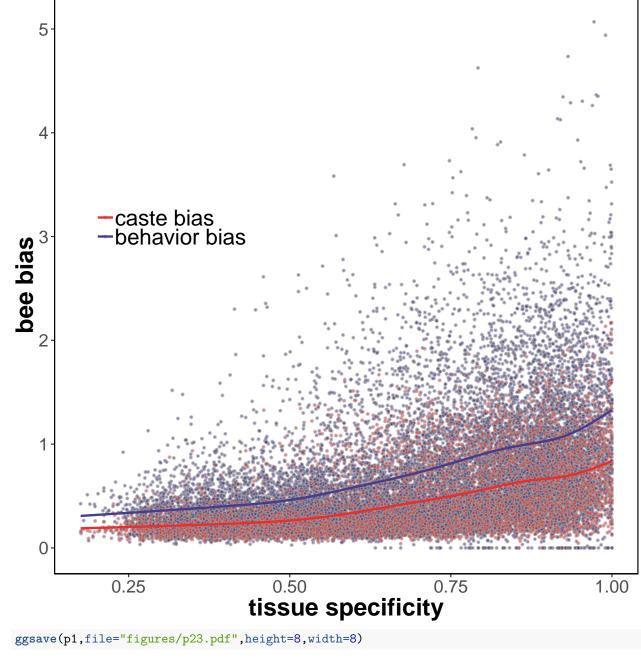
4.2 Caste/Behavior bias vs phylostrata

```
p1 <- phPlot(cbAps,"caste","ant")</pre>
p2 <- phPlot(cbAps, "behavior", "ant")
p3 <- phPlot(cbBps,"caste","bee")
p4 <- phPlot(cbBps, "behavior", "bee")
grid.arrange(p1,p3,nrow=1)
                                                                                                  bee
                               ant
    2
                                                                       2
overall caste bias
                                                                   overall caste bias
    0
                                                                       0
                             hymenoptera
                                                                                                hymenoptera
                                      aculeata
                                                                                       insect
           an<sub>cient</sub>
                    in<sub>Sect</sub>
                                                        novel
                                                                              an<sub>cient</sub>
                                                                                                         aculeata
                                                                                                                           no<sub>ve/</sub>
                                                                                                                  6<sub>6</sub>
                                               ant
grid.arrange(p2,p4,nrow=1)
                                ant
                                                                                                  bee
    2
                                                                       2
overall behavior bias
                                                                   overall behavior bias
                                                                       0
                            hymenoptera
                                                                                                hymenoptera
           an<sub>cient</sub>
                    insect
                                      aculeata
                                                                                       insect
                                                                                                         aculeata
                                                        no<sub>ve/</sub>
                                                                              an<sub>cient</sub>
                                                                                                                  bee
                                                                                                                           novel
                                               ant
p <- arrangeGrob(p1,p2,p3,p4,nrow=2)</pre>
ggsave(p,file="figures/p21.pdf",height=12,width=12)
```

4.3 Caste bias vs tissue specificity

-tissue specificity is estimated across 12 honey bee tissues.

$'geom_smooth()$ using method = 'gam' and formula $'y \sim s(x, bs = "cs")'$



```
## `geom_smooth()` using method = 'gam' and formula 'y ~ s(x, bs = "cs")'
```

Comparison of caste and behavior bias between species

```
cbBias = merge(beeCB,ACUogg,by.x="Gene",by.y="gene_Amel")
cbBias = merge(cbBias,antCB,by.x="gene_Mphar",by.y="Gene")
cbBias = cbBias[!is.na(cbBias$OGGacu),]
socBias = merge(beeSB,ACUogg,by.x="Gene",by.y="gene_Amel")
socBias = merge(socBias,antSB,by.x="gene_Mphar",by.y="Gene")
socBias = socBias[!is.na(socBias$OGGacu),]
p3 <- ggplot(cbBias,aes(x=cb_noAdult.x,y=cb_noAdult.y))+
```

```
geom_point(size=2,alpha=0.3)+
geom_smooth(method="lm",se=FALSE,color="red")+
main_theme+xlab("bee caste bias")+ylab("ant caste bias")

p4 <- ggplot(socBias,aes(x=cb.x,y=cb.y))+
geom_point(size=2,alpha=0.3)+
geom_smooth(method="lm",se=FALSE,color="red")+
scale_x_continuous(breaks = c(0,1,2))+
main_theme+xlab("bee behavioral bias")+ylab("ant behavior bias")

p <- arrangeGrob(p3,p4,nrow=1)
ggsave(p,file="figures/p20.pdf",width=12,height=7)</pre>
```