

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")
beePlaid <- read.csv("results/beePlaidGenes.csv")
tau <- read.csv("results/bee_tau.csv")
sexGenes <- read.csv("results/dmel_sexGenes.csv")
antConn <- read.csv("results/antConnectivity.csv")
beeConn <- read.csv("results/beeConnectivity.csv")

beeT <- read.table("data/bees.tpm.txt",header=TRUE)
antT <- read.table("data/ants.tpm.txt",header=TRUE)
modifyDF <- function(data){
  rownames(data)=data[,1]
  return(data[!grepl("ERCC",rownames(data)),-c(1)])
}
beeT <- modifyDF(beeT)
antT <- modifyDF(antT)
antT = antT[rowSums(antT) > 0,]
beeT = beeT[rowSums(beeT) > 0,]

TGmap <- read.table("phylostratigraphy/out/TGmap_Amel.txt")
TNmap <- as.data.frame(fread("data/AmelTranName.txt",sep="~",header=FALSE))

AmelName <- 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){
  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)

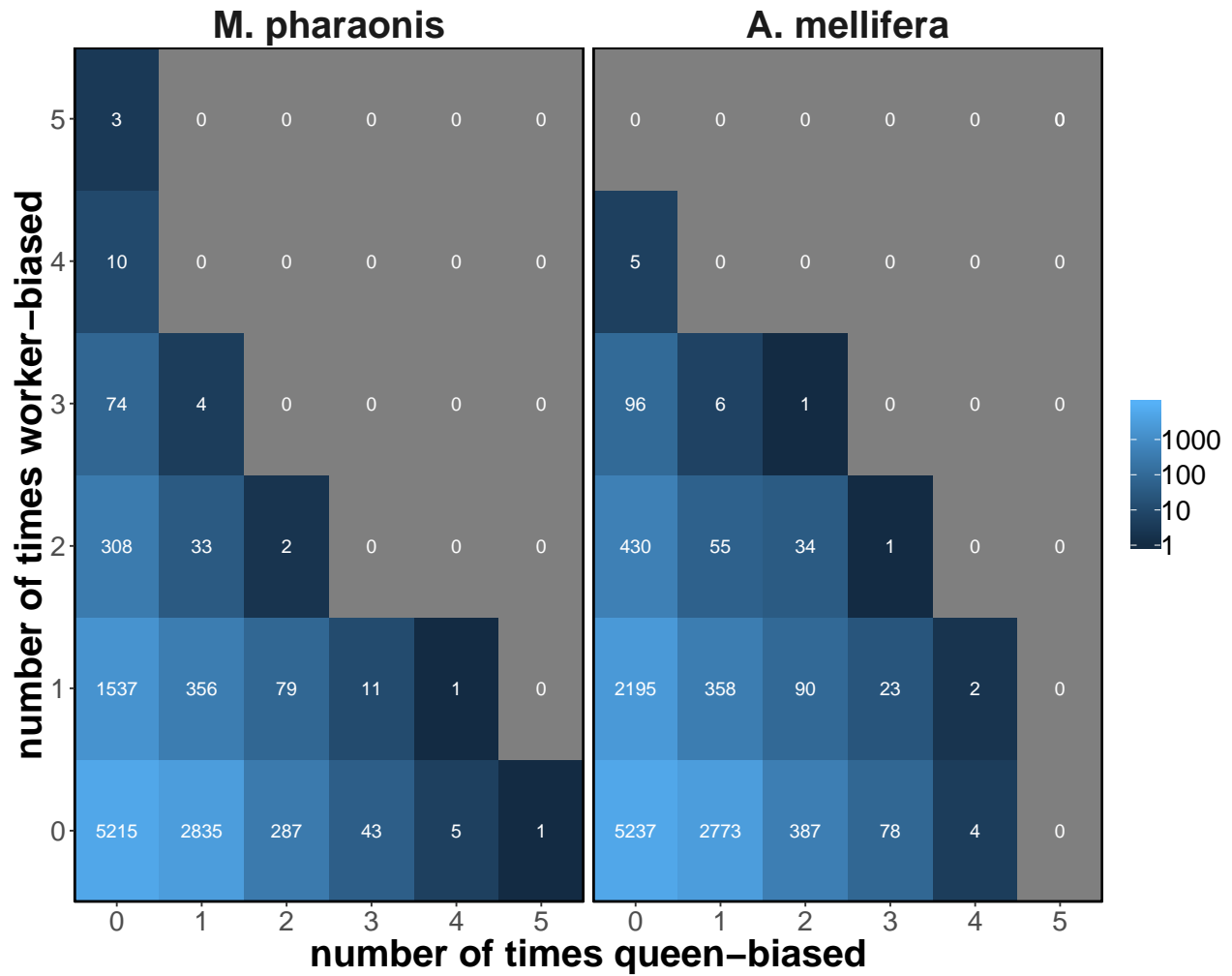
m1$species = "M. pharaonis"
m2$species = "A. mellifera"
mA = rbind(m1,m2)
mA$species = factor(mA$species,levels= c("M. pharaonis","A. mellifera"))

#Create heatmap of differential expression (number of times DE for queens and workers)
p <- ggplot(mA,aes(x=queen,y=worker))+
  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))

```

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){
  nStage = ncol(antD) - 1
  antD <- merge(antD,ACUogg,by.x = "Gene",by.y = "gene_Mphar")
  beeD <- merge(beeD,ACUogg,by.x = "Gene",by.y = "gene_Amel")
  antD = antD[antD$OGG %in% beeD$OGG,]
  beeD = beeD[beeD$OGG %in% antD$OGG,]
  antD = antD[order(antD$OGG),]
  beeD = beeD[order(beeD$OGG),]
  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] = t$estimate
    d[i,3] = t$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
```

```

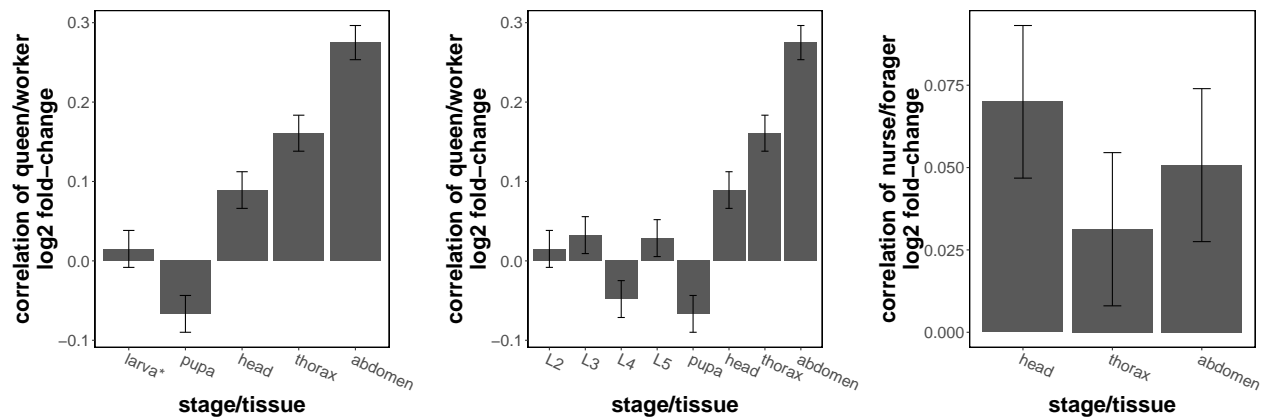
dAbs[i,3] = t$conf.int[1]
dAbs[i,4] = t$conf.int[2]
}
colnames(d) = colnames(dAbs) = c("Stage","cor","c1","c2")
return(list(d,dAbs))
}
CasteCor <- lfcCor(antRes[[1]],beeRes[[1]])
CasteCor_allStage <- lfcCor(antRes_allstage[[1]],beeRes_allstage[[1]])
BehavCor <- lfcCor(antSocRes[[1]],beeSocRes[[1]])

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]]$Stage)
BehavCor[[1]]$Stage = factor(BehavCor[[1]]$Stage,levels = BehavCor[[1]]$Stage)

p1 <- lapply(list(CasteCor[[1]],CasteCor_allStage[[1]],BehavCor[[1]]), function(x){
  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))
})
p1[[3]] = p1[[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

```

```

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", "non-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", ],
  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)),

```

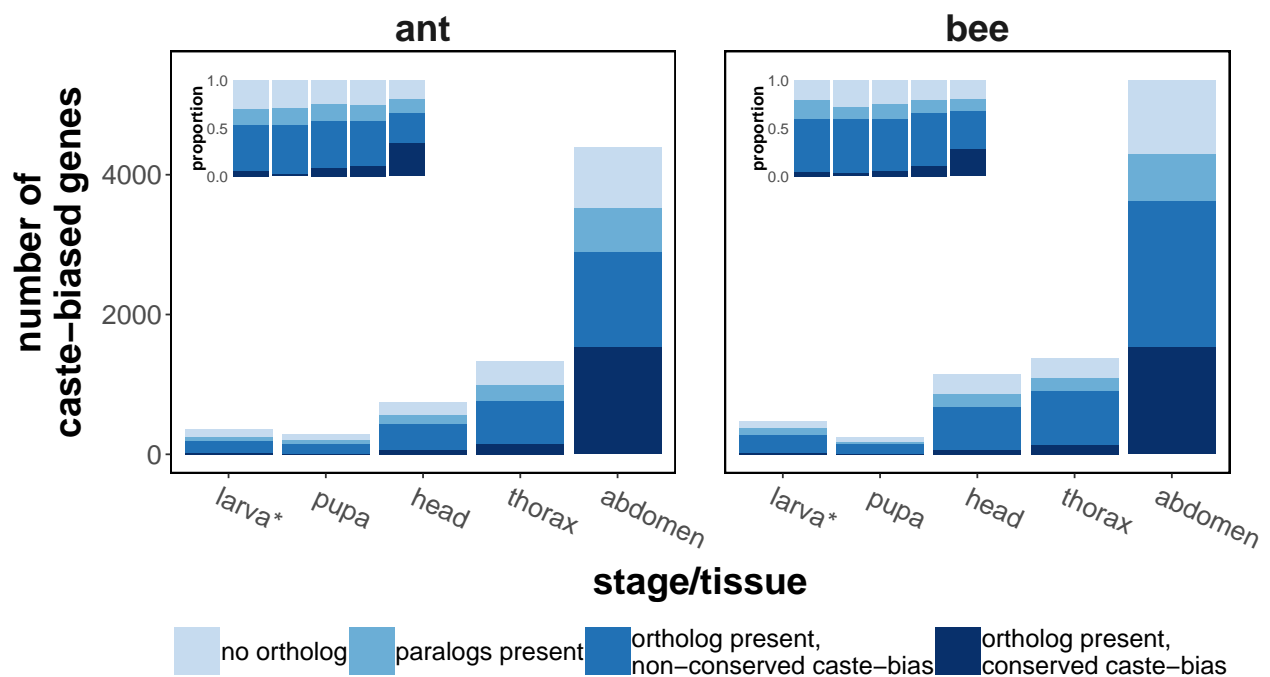
```

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"))

pQ <- ggdraw()+
  draw_plot(p1m+
    theme(legend.text = element_text(size=15),
          legend.key.width = unit(1,"cm"),
          legend.position = "bottom"))+
  draw_plot(p2,x=0.15,y=0.69,height=0.18,width=0.18)+
  draw_plot(p3,x=0.57,y=0.69,height=0.18,width=0.18)

```

1.1 Caste bias across development



1.2 Nurse/forager bias

```

d = compDef(antSocRes[[2]],beeSocRes[[2]])

p1 <- ggplot(d[d$DEtype!="NDE",],
  aes(x = stage, y = value, fill = DEtype))+
  geom_bar(stat="identity")+
  main_theme+
  xlab("tissue")+
  ylim(0,4500)+
  facet_grid(. ~ species)+
  scale_fill_manual(values = DE_palette[c(3,5,7,9)))+
  ylab("number of behavior-biased genes")+
  theme(axis.text.x = element_text(angle=-25,hjust=0.1),
        legend.position = "top",

```

```

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",],
            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"))

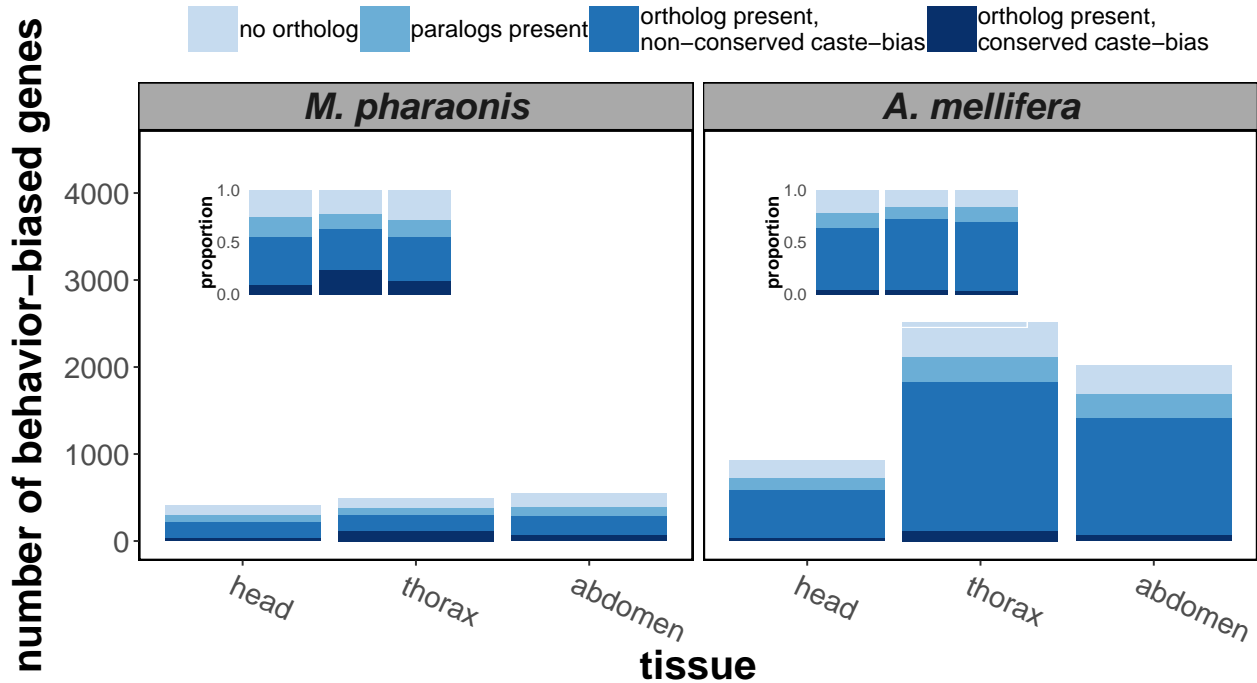
pSoc <- ggdraw()+
  draw_plot(p1+
    theme(legend.text = element_text(size=13),
          legend.key.width = unit(1,"cm")))+

```



```
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"

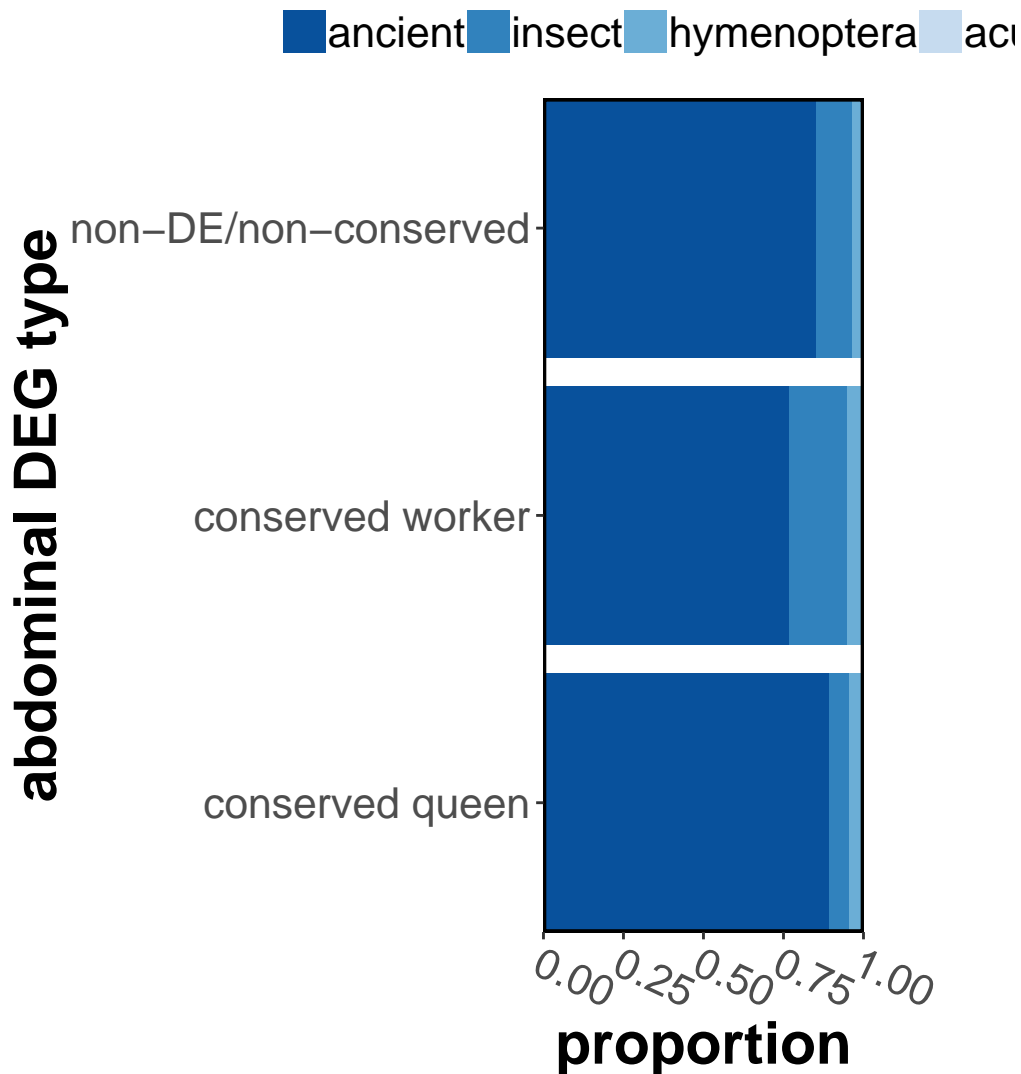
#Find genes with conserved queen or worker bias
ps0$a2[ps0$gene_Amel %in% beeRes[[2]]$Gene[beeRes[[2]]$abdomen=="worker"] &
  ps0$gene_Mphar %in% antRes[[2]]$Gene[antRes[[2]]$abdomen=="worker"]] = "conserved worker"
ps0$a2[ps0$gene_Amel %in% beeRes[[2]]$Gene[beeRes[[2]]$abdomen=="queen"] &
  ps0$gene_Mphar %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)))+
  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



2.1 Bicluster associated with queen abdominal expression

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

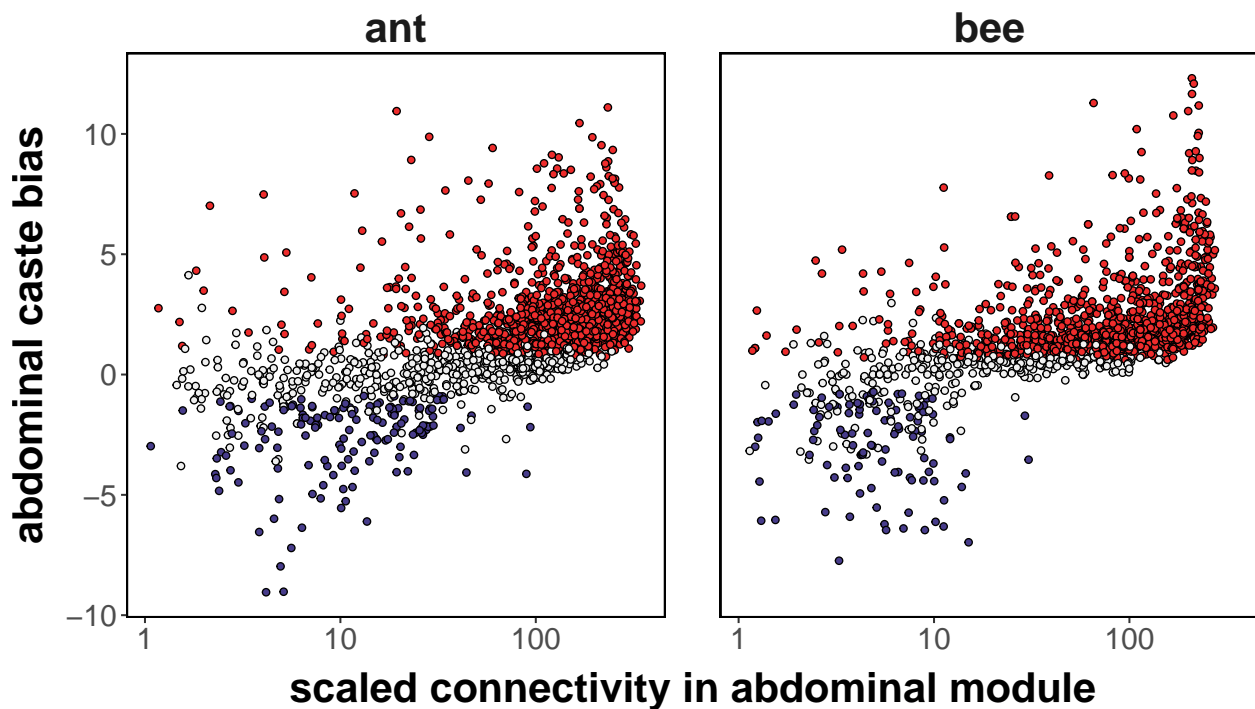
```
#Find out what the genes are
antGrC = merge(antPlaid,Aps,by="Gene")
beeGrC = merge(beePlaid,Bps,by="Gene")
antGrC$species = "ant"
beeGrC$species = "bee"
```

```

#Make general plot
allC = rbind(antGrC,beeGrC)
allC$DEcat = factor(allC$DEcat,levels = c("queen","worker","non-DE"))
p1 <- ggplot(allC,aes(x = conn,y=-abdomen))+
  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))

```

p1



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)

```

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

```

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)))+
    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)")

```

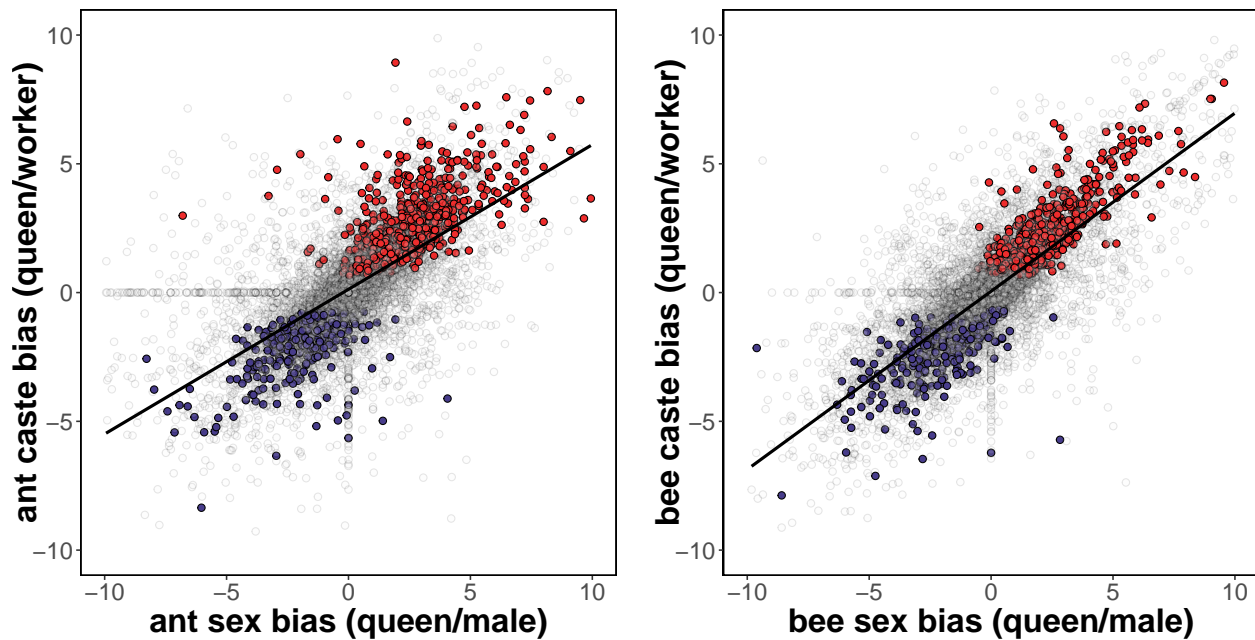
```
xlab("bee sex bias (queen/male)")
grid.arrange(p1,p2,nrow=1)
```

```
## 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).
```

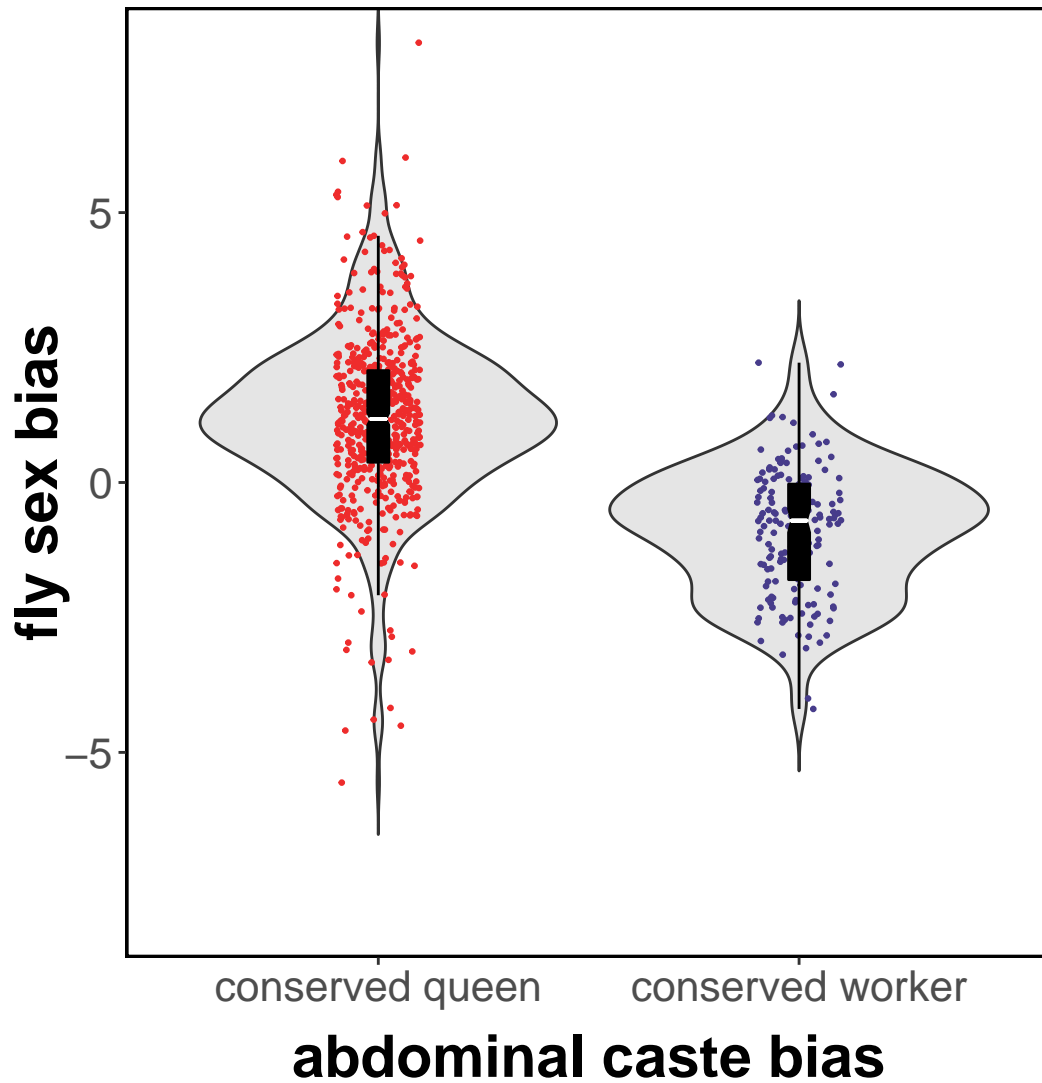
```
## Warning: Removed 47 rows containing missing values (geom_point).
```



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-DE/inconsistent"))

p4 <- ggplot(DmelSC[grepl("conserved",DmelSC$abdDE),],aes(x = abdDE,y=-logFC))+
  geom_violin(fill="grey90",trim=FALSE)+
  geom_jitter(width = 0.1,size=0.5,aes(color=abdDE))+
  geom_boxplot(width=0.05,outlier.shape = NA,fill="black",color="black",notch=TRUE,notchwidth = 0.7)+
  plot2theme+
  ylab("fly sex bias")+
  ylim(-10,10)+
  theme(axis.title.y=element_text(margin = margin(t=0,l=15,r=0,b=0)))+
  xlab("abdominal caste bias")+
  scale_fill_manual(values=SexPal)+
  scale_color_manual(values=SexPal)+
  theme(legend.position="none")+
  stat_summary(geom = "crossbar", width=0.035, fatten=0, size=0.7,color="white",
    fun.data = function(x){c(y=median(x), ymin=median(x), ymax=median(x))})+
  coord_cartesian(ylim = c(-8,8))
p4
```



4.1 Calculation of overall caste bias

```
#Calculate euclidean distance
euclDist <- function(res){
  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){
  p1C <- ggplot(data[data$type==type,],aes(x = kTotal,y=cb))+
    geom_hex(bins=70)+
    scale_fill_gradient(low = "blue",high="red")+
    plot2theme+
    ylim(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")
p2 <- cbPlot(cbAps,"behavior","ant")
p3 <- cbPlot(cbBps,"caste","bee")
p4 <- cbPlot(cbBps,"behavior","bee")

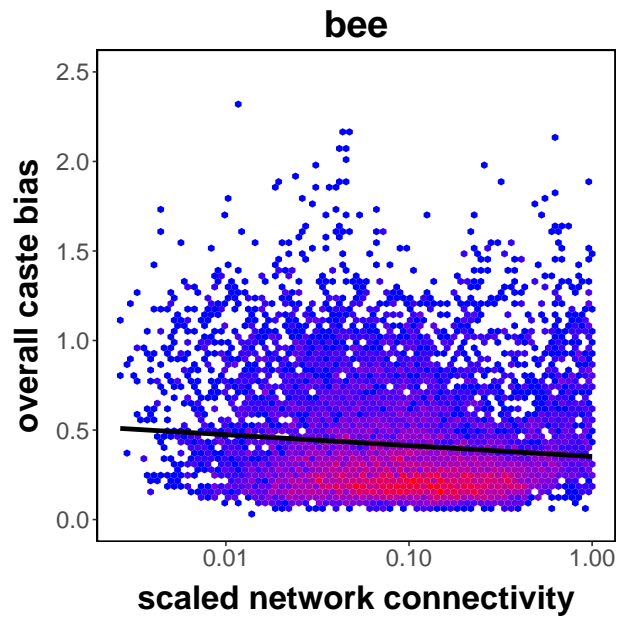
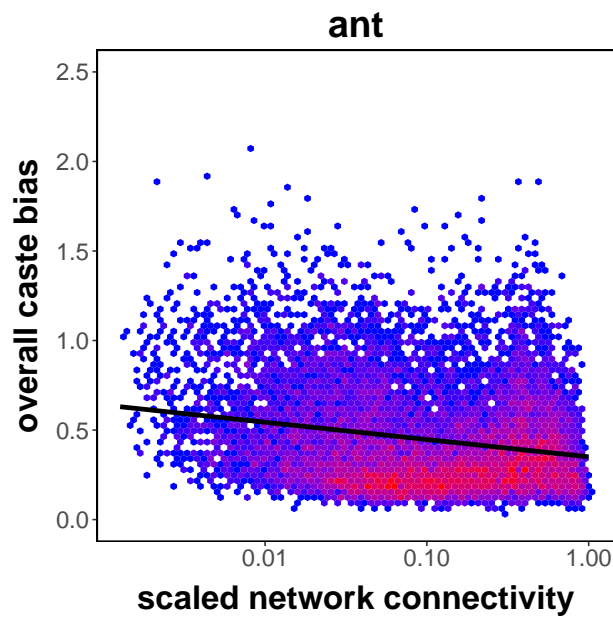
grid.arrange(p1,p3,nrow=1)

```

```

## 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).

```



```
grid.arrange(p2,p4,nrow=1)
```

```
## Warning: Removed 56 rows containing non-finite values (stat_binhex).
```

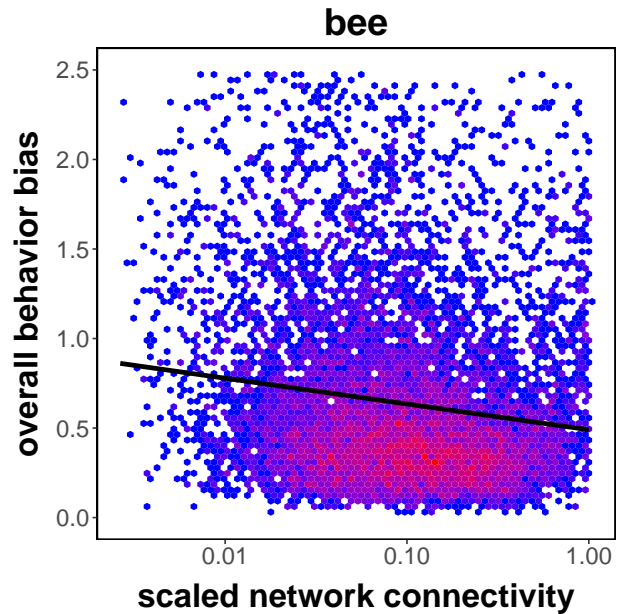
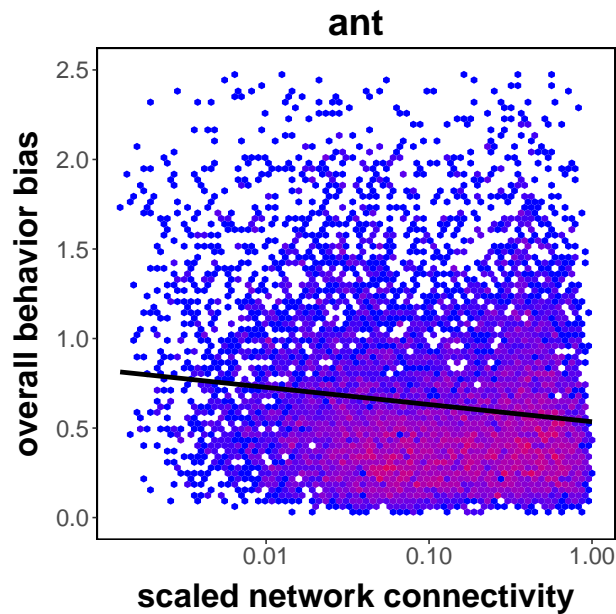
```
## Warning: Removed 56 rows containing non-finite values (stat_smooth).
```

```
## 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).
```

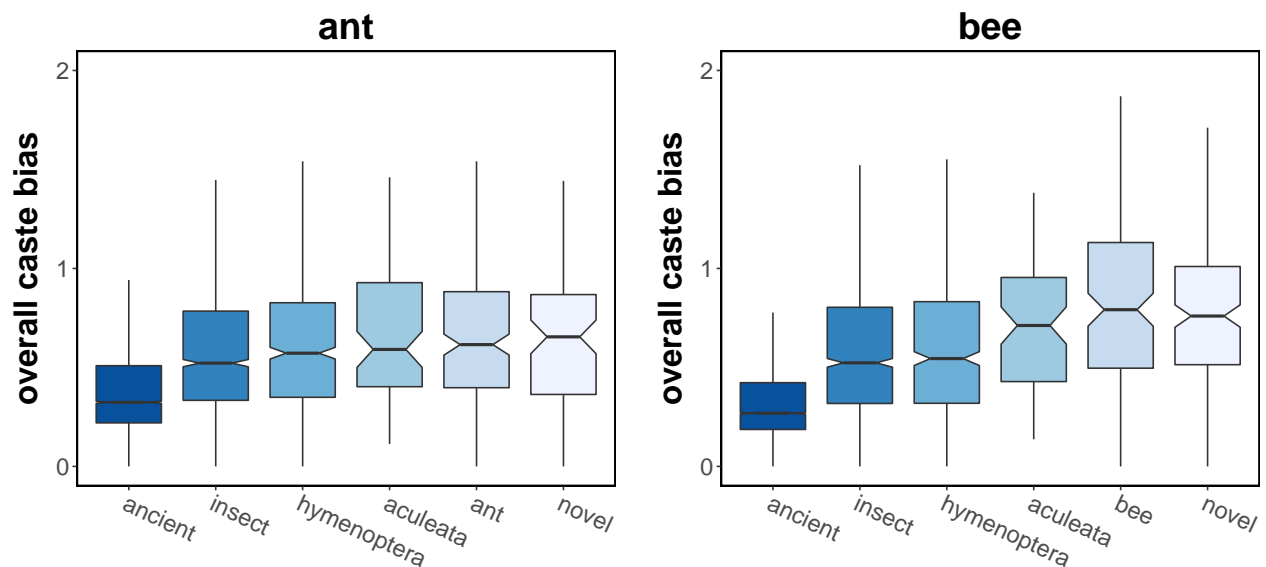


4.2 Caste/Behavior bias vs phylostrata

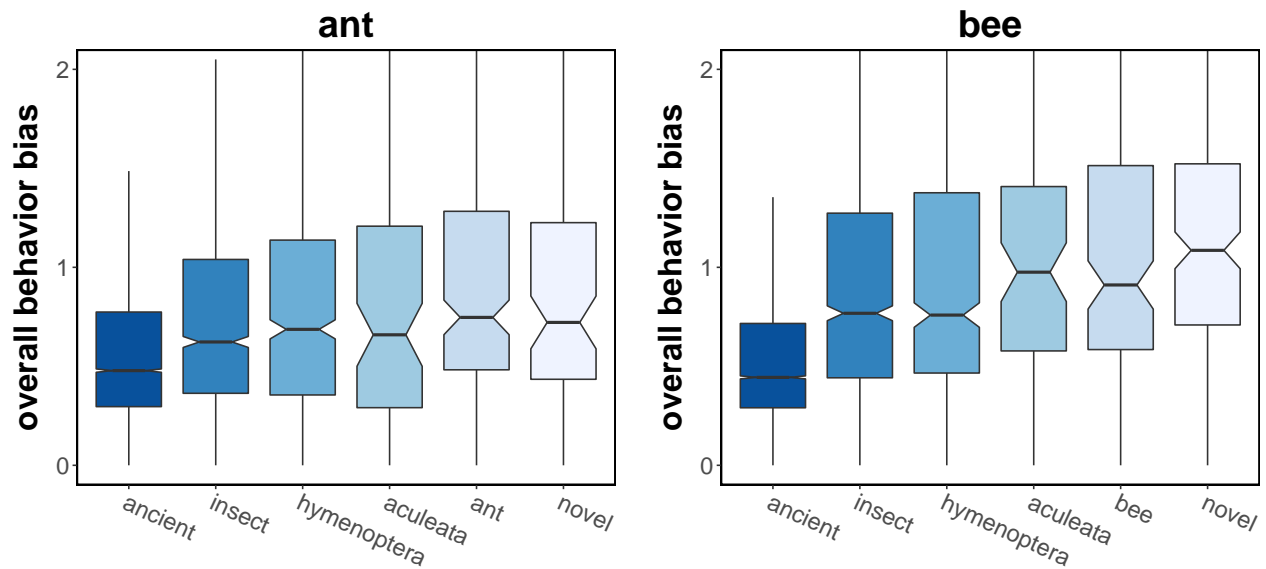
```
phPlot <- function(data,type,spec){
  pA <- ggplot(data[data$type==type & !is.na(data$psName),],aes(x = psName,y=cb,fill=psName))+
    geom_boxplot(notch=TRUE,outlier.shape=NA)+
    plot2theme+
    ggtitle(spec)+
    xlab("")+
    coord_cartesian(ylim=c(0,2))+
    scale_y_continuous(breaks = c(0,1,2,3))+
    scale_fill_manual(values = rev(mypalette2),name = "phylostrata",guide=guide_legend(title.position="top")+
    ylab(paste("overall",type,"bias",sep = " "))+
    theme(axis.text.x = element_text(angle = -25,hjust=0),
          legend.position="none")
  return(pA)
}

p1 <- phPlot(cbAps,"caste","ant")
p2 <- phPlot(cbAps,"behavior","ant")
p3 <- phPlot(cbBps,"caste","bee")
p4 <- phPlot(cbBps,"behavior","bee")

grid.arrange(p1,p3,nrow=1)
```



```
grid.arrange(p2,p4,nrow=1)
```



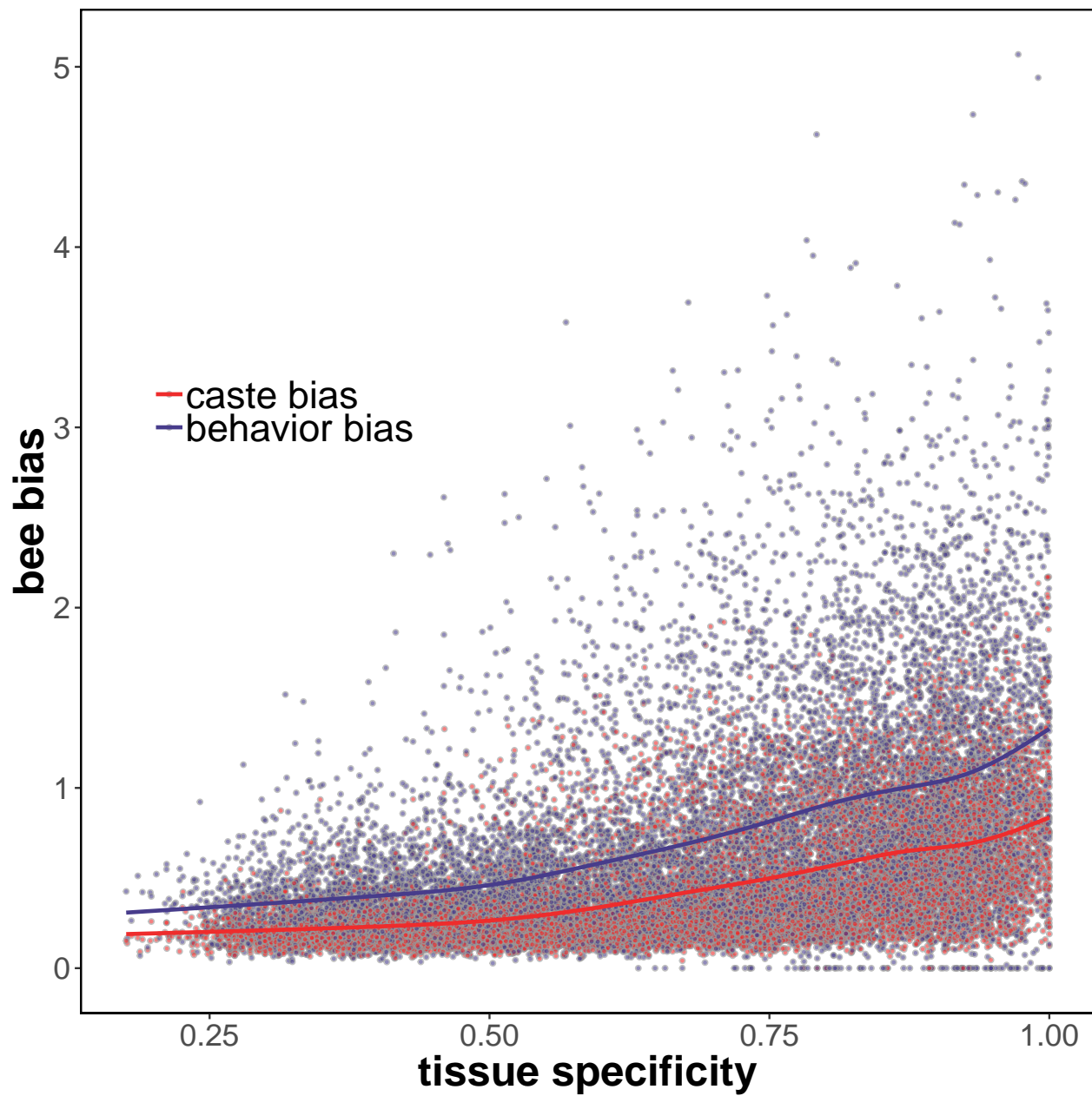
4.3 Caste bias vs tissue specificity -tissue specificity is estimated across 12 honey bee tissues.

```
bT = merge(tau,beeBias2,by="Gene")
bT$species = "bee"
bT$type = factor(bT$type,levels = c("caste","behavior"))
levels(bT$type) = c("caste bias","behavior bias")

p1 <- ggplot(bT,aes(x=tau,y=cb))+
  geom_point(size=1,alpha=0.6,pch=21,aes(fill=type),color="grey60")+
  geom_smooth(se=FALSE,aes(color=type))+
  scale_color_manual(values = SexPal)+
  scale_fill_manual(values = SexPal)+
  main_theme+xlabs("tissue specificity")+ylabs("bee bias")+
  theme(legend.position=c(0.2,0.6),
        legend.text = element_text(size=20),
        legend.title=element_blank())

p1
```

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



Development section

Comparison to pre-defined sex and development genes

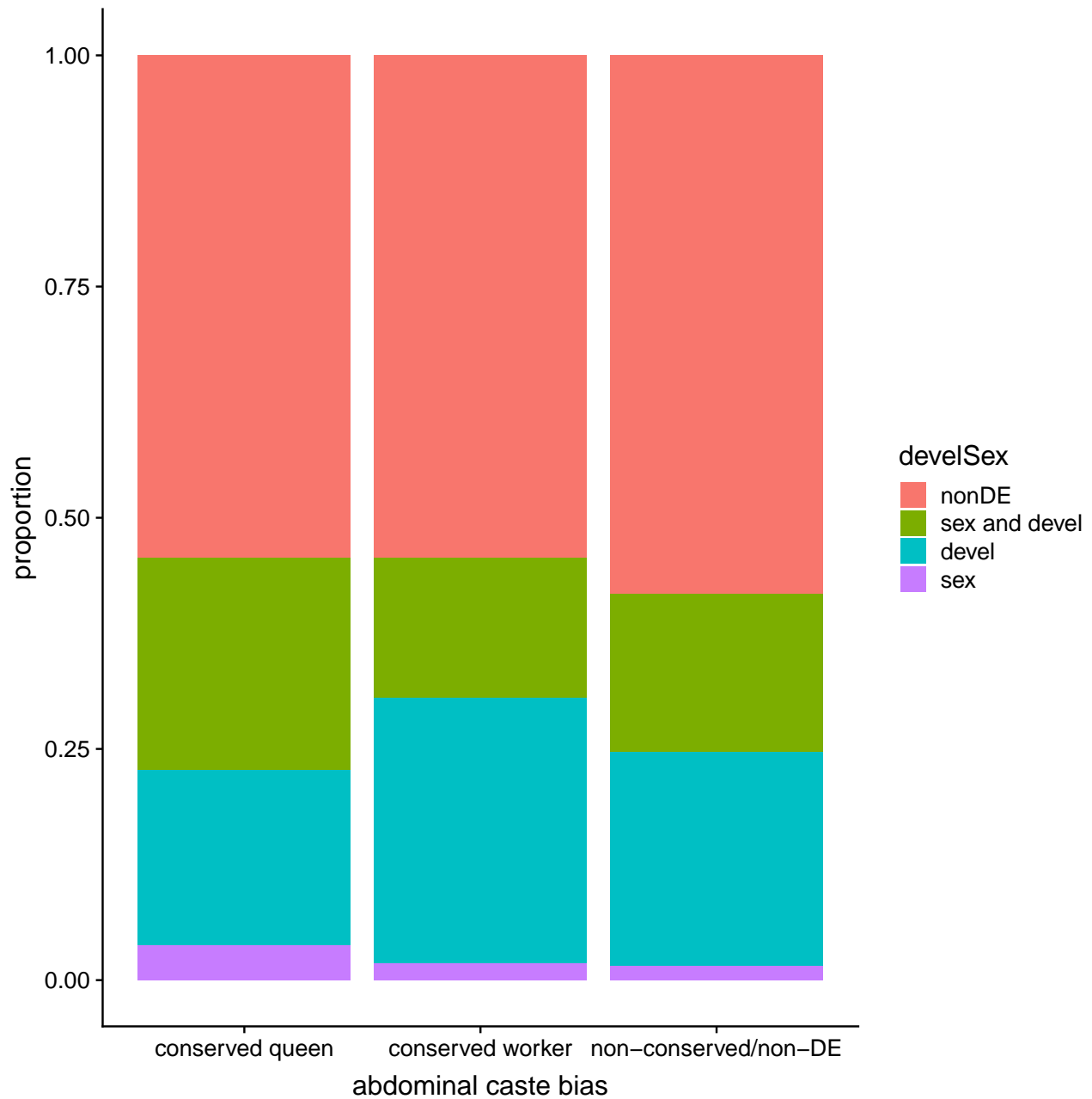
```
DmelSC = merge(sexGenes,ogg11,by="gene_Amel")
key <- read.table("data/DmelKey.txt") #protein-CDS
key2 <- read.table("data/Dmel_CDStoGene_key.txt") #Gene-CDS
devel <- read.table("data/Devel_IDS.txt") #Downloaded from flybase, genes with developmental terms
sex <- read.table("data/Sex_IDS.txt") #Downloaded from flybase, genes with sex terms
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[!grepl("ant", DmelSC$abdDE), ], aes(x = abdDE, fill = develSex)) +
  geom_bar(stat = "count", position = "fill") +
  ylab("proportion") +
  xlab("abdominal caste bias")
p

```



-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(FDR)))+
  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(FDR)))+
  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)

## Warning: Transformation introduced infinite values in continuous y-axis
## Warning: Removed 14 rows containing non-finite values (stat_boxplot).
## Warning: Transformation introduced infinite values in continuous y-axis
## Warning: Removed 12 rows containing non-finite values (stat_boxplot).
```

