

# 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)

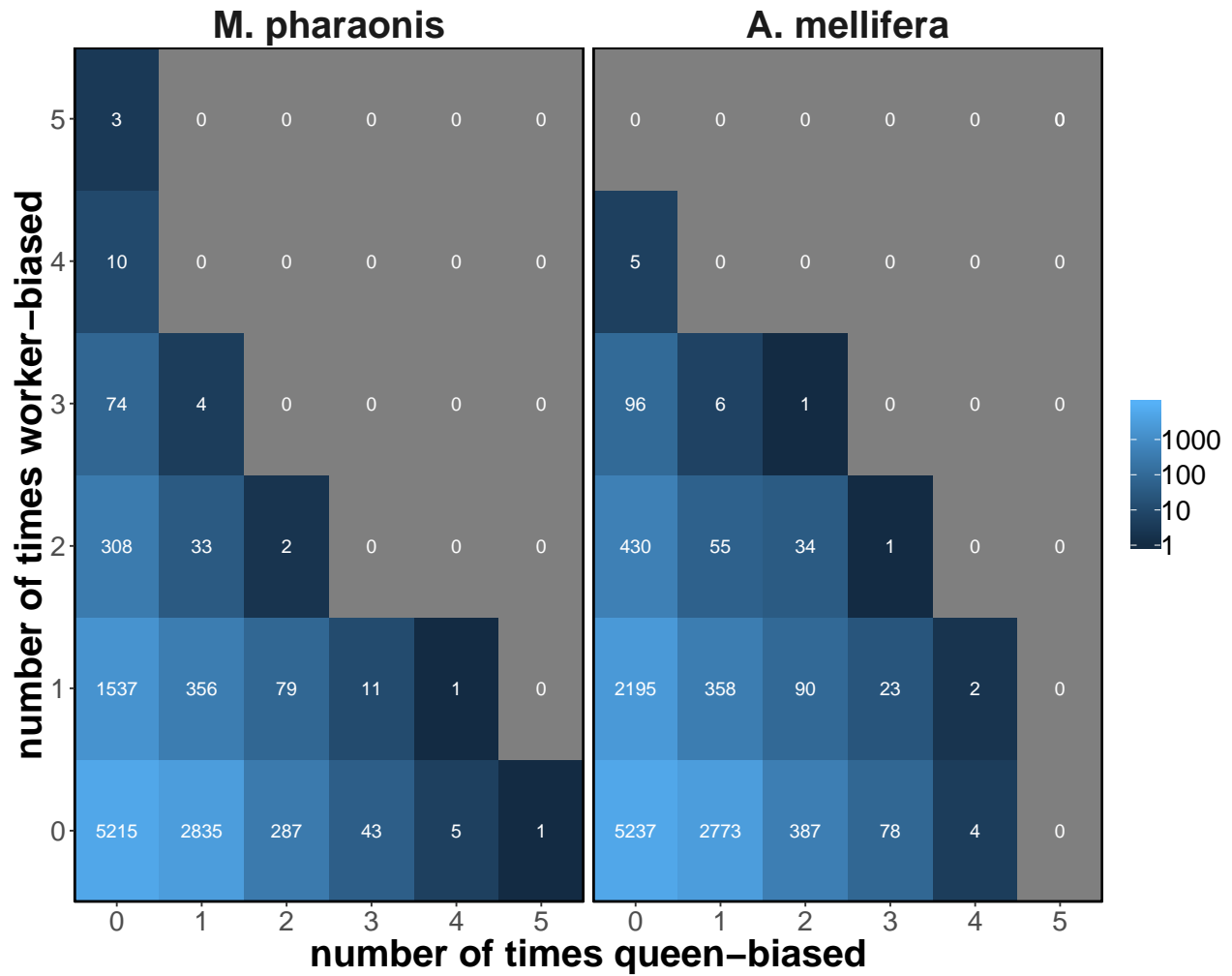
m1$species = "M. pharaonis"
m2$species = "A. mellifera"
mA = rbind(m1,m2)
mA$species = factor(mA$species,levels= c("M. pharaonis","A. mellifera"))

#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
```

```

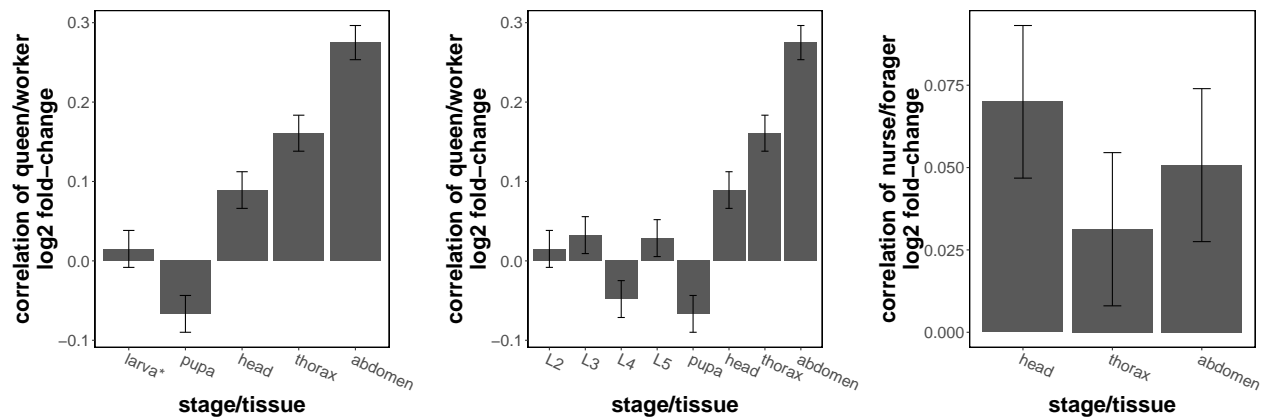
dAbs[i,3] = t$conf.int[1]
dAbs[i,4] = t$conf.int[2]
}
colnames(d) = colnames(dAbs) = c("Stage","cor","c1","c2")
return(list(d,dAbs))
}
CasteCor <- lfcCor(antRes[[1]],beeRes[[1]])
CasteCor_allStage <- lfcCor(antRes_allstage[[1]],beeRes_allstage[[1]])
BehavCor <- lfcCor(antSocRes[[1]],beeSocRes[[1]])

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)),

```

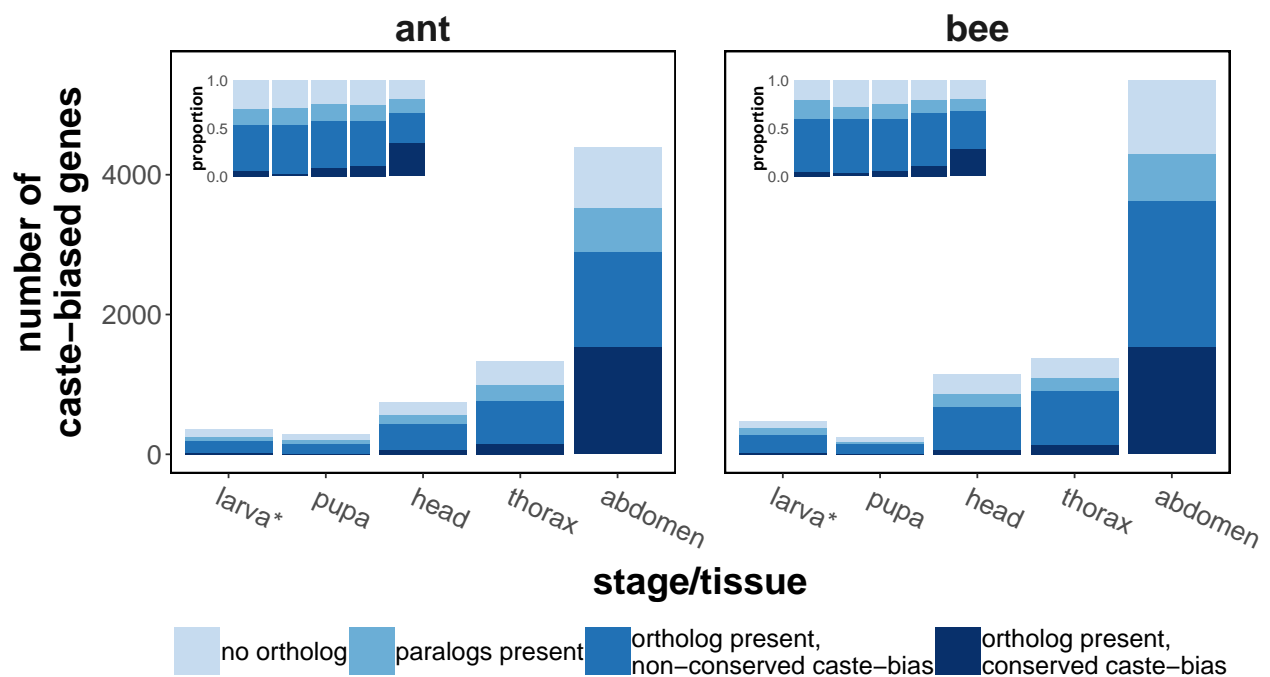
```

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"))

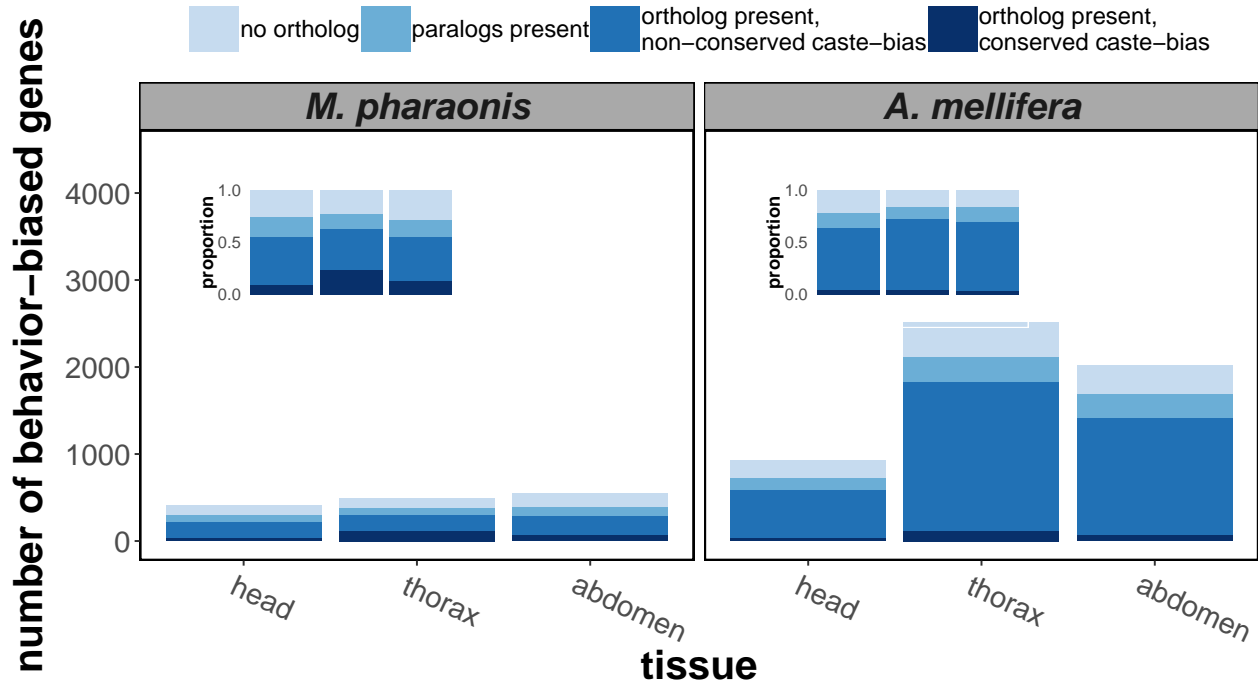
pSoc <- ggdraw()+
  draw_plot(p1+
    theme(legend.text = element_text(size=13),
          legend.key.width = unit(1,"cm")))+

```



```
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"

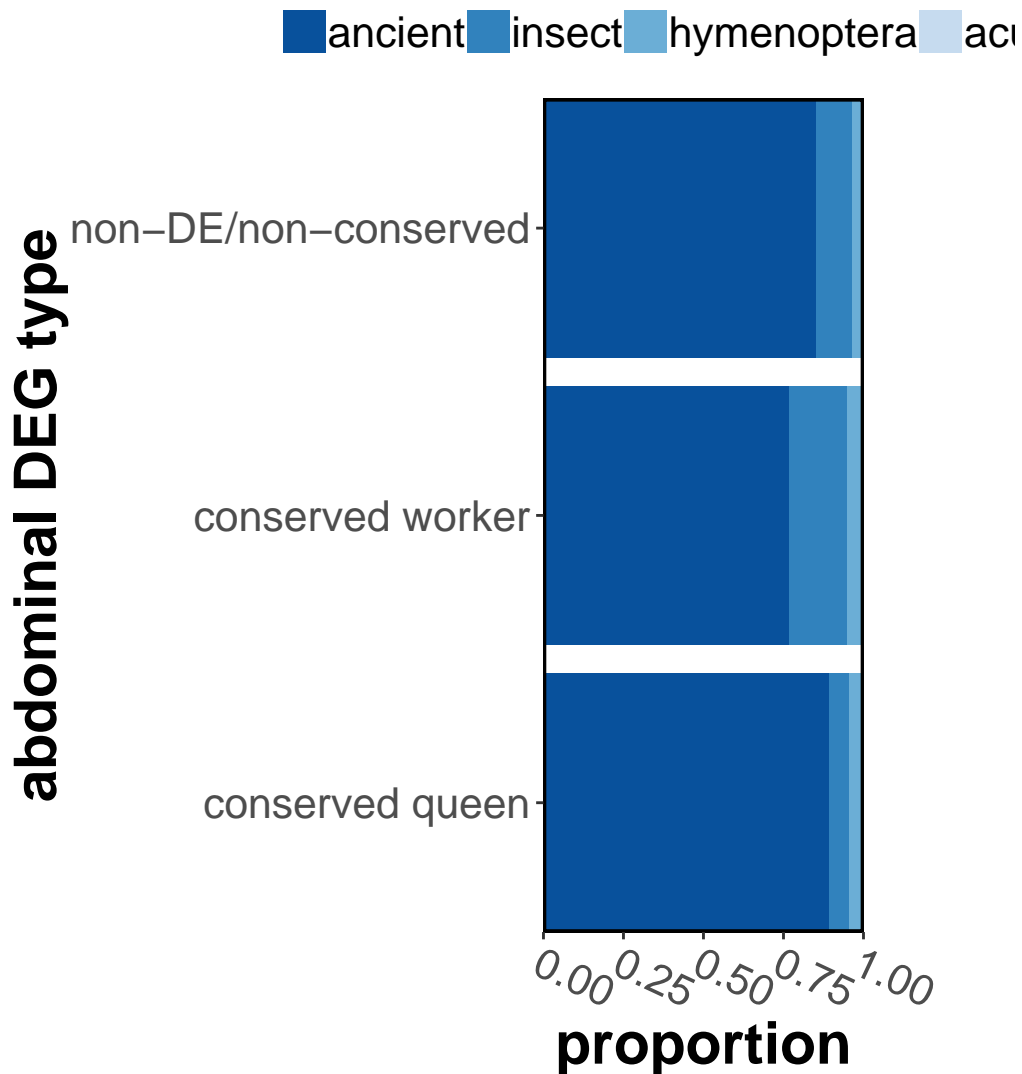
#Find genes with conserved queen or worker bias
ps0$a2[ps0$gene_Amel %in% beeRes[[2]]$Gene[beeRes[[2]]$abdomen=="worker"] &
  ps0$gene_Mphar %in% antRes[[2]]$Gene[antRes[[2]]$abdomen=="worker"]] = "conserved worker"
ps0$a2[ps0$gene_Amel %in% beeRes[[2]]$Gene[beeRes[[2]]$abdomen=="queen"] &
  ps0$gene_Mphar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

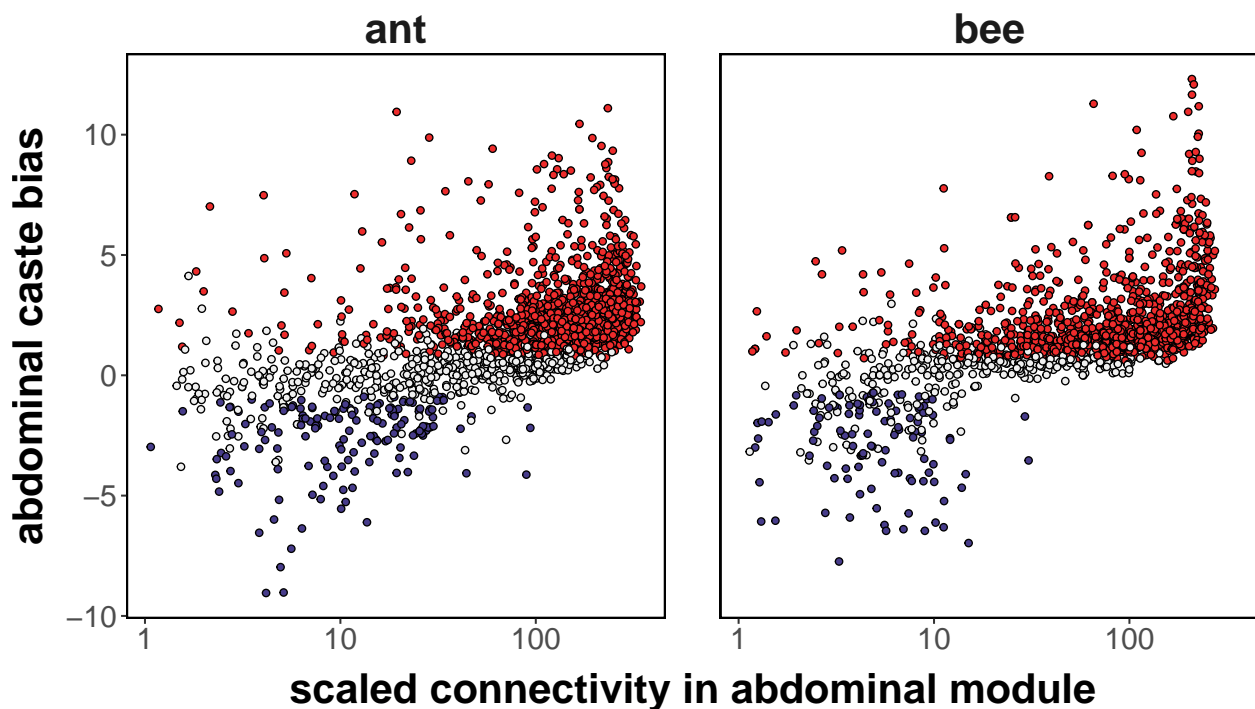
```

#Make general plot
allC = rbind(antGrC,beeGrC)
allC$DEcat = factor(allC$DEcat,levels = c("queen","worker","non-DE"))

#Note -abdomen because we want queen genes to be upregulated
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



## 2.2 Specific genes within the bicluster

- holding off plotting specific genes for now

```

beeAnn <- read.csv("data/Amel_Gene_Names.csv")
ann <- read.csv("data/MpharAnn.csv")

#Pick genes with connectivity in the top 90% that are four-fold upregulated in queens
antGenes = antGrC$Gene[antGrC$conn > quantile(antGrC$conn,0.9) &
  antGrC$abdomen < -2]
beeGenes = beeGrC$Gene[beeGrC$conn > quantile(beeGrC$conn,0.9) &
  beeGrC$abdomen < -2]
annSwiss = ann$SwissProt[ann$Gene %in% antGenes]
beeSwiss = beeAnn$GeneName[beeAnn$Gene %in% beeGenes]
beeSwiss[!grepl("ncharacterized",beeSwiss) & beeSwiss!="-"]

```

```

## [1] ATP-dependent RNA helicase vasa
## [2] PREDICTED: kinesin-like protein KIF18A
## [3] PREDICTED: prion-like-(Q/N-rich) domain-bearing protein 25
## [4] PREDICTED: disks large-associated protein 5-like
## [5] PREDICTED: E3 ubiquitin-protein ligase TRIM71
## [6] PREDICTED: zinc finger protein 470-like
## [7] PREDICTED: CDC42 small effector protein homolog
## [8] PREDICTED: NF-kappa-B inhibitor cactus-like
## [9] PREDICTED: hyaluronan mediated motility receptor
## [10] PREDICTED: ADP-ribosylation factor-like protein 1
## [11] PREDICTED: facilitated trehalose transporter Tret1-like
## [12] PREDICTED: histone H2B-like
## [13] PREDICTED: protein claret segregational
## [14] PREDICTED: dual specificity protein kinase TTK
## [15] PREDICTED: prolyl 3-hydroxylase OGFOD1
## [16] PREDICTED: targeting protein for Xklp2 homolog
## [17] PREDICTED: zinc finger protein 480
## [18] PREDICTED: kinetochore protein ndc80
## [19] PREDICTED: SET and MYND domain-containing protein 4-like
## [20] PREDICTED: rhoGEF domain-containing protein gxcJ-like
## [21] PREDICTED: helix-loop-helix protein delilah-like
## [22] PREDICTED: nucleoredoxin-like
## [23] PREDICTED: interaptin-like
## [24] PREDICTED: polycomb protein Asx
## [25] PREDICTED: F-BAR domain only protein 2
## [26] PREDICTED: glucose-6-phosphate 1-epimerase
## [27] PREDICTED: kanadaptin
## [28] PREDICTED: S-phase kinase-associated protein 2
## [29] PREDICTED: protein kintoun
## [30] PREDICTED: WW domain-containing adapter protein with coiled-coil
## [31] PREDICTED: citron Rho-interacting kinase-like
## [32] PREDICTED: maternal embryonic leucine zipper kinase-like
## [33] PREDICTED: spindle and kinetochore-associated protein 1-like, partial
## [34] PREDICTED: protein LSM14 homolog A
## [35] PREDICTED: geminin
## [36] PREDICTED: protein kinase 4-like
## [37] PREDICTED: ankyrin repeat domain-containing protein 54
## [38] PREDICTED: protein Smaug homolog 1
## [39] PREDICTED: transformation/transcription domain-associated protein
## [40] PREDICTED: serine protease gd
## [41] PREDICTED: golgin subfamily A member 6-like protein 22
## [42] PREDICTED: RWD domain-containing protein 1

```

## [43] PREDICTED: PERQ amino acid-rich with GYF domain-containing protein CG11148  
 ## [44] PREDICTED: N-acetylglucosamine-1-phosphotransferase subunits alpha/beta  
 ## [45] PREDICTED: cyclin-A2  
 ## [46] PREDICTED: protein groucho  
 ## [47] PREDICTED: CCR4-NOT transcription complex subunit 1  
 ## [48] PREDICTED: enhancer of mRNA-decapping protein 3  
 ## [49] PREDICTED: mitotic spindle assembly checkpoint protein MAD2A  
 ## [50] PREDICTED: G2/mitotic-specific cyclin-B3  
 ## [51] PREDICTED: micronuclear linker histone polyprotein-like  
 ## [52] PREDICTED: polycomb protein Pcl  
 ## [53] PREDICTED: KAT8 regulatory NSL complex subunit 1  
 ## [54] PREDICTED: UBX domain-containing protein 7  
 ## [55] PREDICTED: ankyrin repeat domain-containing protein 17  
 ## [56] PREDICTED: MAU2 chromatid cohesion factor homolog  
 ## [57] PREDICTED: macoilin  
 ## [58] PREDICTED: probable helicase with zinc finger domain  
 ## [59] PREDICTED: DNA replication factor Cdt1  
 ## [60] PREDICTED: DNA repair protein complementing XP-G cells  
 ## [61] PREDICTED: ras GTPase-activating-like protein IQGAP1  
 ## [62] PREDICTED: histone H1.2-like  
 ## [63] PREDICTED: protein maelstrom 2  
 ## [64] PREDICTED: LOW QUALITY PROTEIN: protein lin-9 homolog  
 ## [65] PREDICTED: lachesin-like  
 ## [66] PREDICTED: protein Daple  
 ## [67] PREDICTED: protein split ends-like  
 ## [68] PREDICTED: LOW QUALITY PROTEIN: bromodomain-containing protein 4  
 ## [69] PREDICTED: SPX and EXS domain-containing protein 4-like  
 ## [70] PREDICTED: zinc finger CCHC domain-containing protein 10-like  
 ## [71] PREDICTED: protein distal antenna  
 ## [72] PREDICTED: vitellogenin receptor  
 ## [73] PREDICTED: homeobox protein OTX1 A  
 ## [74] PREDICTED: eukaryotic translation initiation factor 4E transporter  
 ## [75] PREDICTED: E3 ubiquitin-protein ligase TRAP1-like  
 ## [76] PREDICTED: poly(A) RNA polymerase gld-2 homolog A  
 ## [77] PREDICTED: serine/threonine-protein kinase polo  
 ## [78] PREDICTED: importin subunit alpha  
 ## [79] PREDICTED: centrosomal protein of 162 kDa-like  
 ## [80] PREDICTED: LOW QUALITY PROTEIN: meiosis arrest female protein 1  
 ## [81] PREDICTED: protein ovo  
 ## [82] PREDICTED: P protein-like  
 ## [83] PREDICTED: suppressor protein SRP40  
 ## [84] PREDICTED: male-specific lethal 1 homolog  
 ## [85] PREDICTED: GRB2-associated-binding protein 2  
 ## [86] PREDICTED: enolase-phosphatase E1  
 ## [87] PREDICTED: G kinase-anchoring protein 1-like  
 ## [88] PREDICTED: histone-lysine N-methyltransferase pr-set7  
 ## [89] PREDICTED: myotubularin-related protein 14  
 ## [90] PREDICTED: TATA-box-binding protein  
 ## [91] PREDICTED: coiled-coil domain-containing protein 43  
 ## [92] PREDICTED: serine/threonine-protein kinase VRK1-like  
 ## [93] PREDICTED: putative ATP-dependent RNA helicase me31b  
 ## 9976 Levels: 18-wheeler precursor ... zinc finger protein 629-like

ant Genes

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

```
## [1] JNK1/MAPK8-associated membrane protein
## [2] Protein halfway
## [3] Protein aubergine
## [4] DnaJ homolog subfamily C member 2
## [5] REST corepressor 3
## [6] Nucleoporin GLE1
## [7] Peroxisomal N(1)-acetyl-spermine/spermidine oxidase
## [8] PRKCA-binding protein
## [9] Double-strand-break repair protein rad21 homolog
## [10] Importin subunit alpha-7
## [11] Histone RNA hairpin-binding protein
## [12] Protein abnormal spindle
## [13] RNA exonuclease 4
## [14] Enhancer of mRNA-decapping protein 3
## [15] Probable phenylalanine--tRNA ligase alpha subunit
## [16] N-alpha-acetyltransferase 25 NatB auxiliary subunit
## [17] Protein CNPPD1
## [18] Gephyrin
## [19] FACT complex subunit spt16
## [20] Rho GTPase-activating protein 11B
## [21] Protein Smaug homolog 1
## [22] Coiled-coil domain-containing protein 22 homolog
## [23] ATP-binding cassette sub-family G member 4
## [24] Maternal embryonic leucine zipper kinase
## [25] Histone H2B
## [26] Nuclear pore complex protein Nup160 homolog
## [27] Dystrophin isoform B
## [28] E3 ubiquitin-protein ligase UHRF1
## [29] Apoptosis inhibitor IAP
## [30] CCR4-NOT transcription complex subunit 1
## [31] Polynucleotide 5'-hydroxyl-kinase NOL9
## [32] S-phase kinase-associated protein 2
## [33] WD repeat-containing protein 44
## [34] Transcriptional regulator ATRX
## [35] Male-specific lethal 3 homolog
## [36] U4/U6 small nuclear ribonucleoprotein Prp4
## [37] WD repeat-containing protein 3
## [38] Pre-mRNA 3'-end-processing factor FIP1
## [39] Importin subunit alpha-1
## [40] Probable DNA mismatch repair protein Msh6
## [41] CSC1-like protein 2
## [42] NCK-interacting protein with SH3 domain
## [43] snRNA-activating protein complex subunit 4
## [44] Protein dispatched
## [45] Glycylpeptide N-tetradecanoyltransferase 1
## [46] Probable nucleolar GTP-binding protein 1
## [47] E3 ubiquitin-protein ligase SIAH1
## [48] Protein TSSC4
## [49] DNA-directed RNA polymerase I subunit RPA1
## [50] Zinc finger protein 800
## [51] Activated CDC42 kinase 1
## [52] Symplekin
```

## [53] Probable histone-binding protein Caf1  
 ## [54] Lipase 3  
 ## [55] Probable nucleoporin Nup58  
 ## [56] Ribonucleoside-diphosphate reductase large subunit  
 ## [57] Chromosome-associated kinesin KIF4  
 ## [58] COP9 signalosome complex subunit 1  
 ## [59] Squamous cell carcinoma antigen recognized by T-cells 3  
 ## [60] Integrator complex subunit 11  
 ## [61] Structural maintenance of chromosomes protein 5  
 ## [62] Macoilin  
 ## [63] Pogo transposable element with ZNF domain  
 ## [64] Zinc finger protein 346  
 ## [65] DNA replication licensing factor Mcm2  
 ## [66] Piwi-like protein 1  
 ## [67] HEAT repeat-containing protein 1  
 ## [68] Gephyrin  
 ## [69] Protein LSM14 homolog B  
 ## [70] WD repeat-containing protein 89  
 ## [71] Nuclear RNA export factor 1  
 ## [72] Cell division control protein 6 homolog  
 ## [73] Fanconi-associated nuclease 1  
 ## [74] Putative bifunctional UDP-N-acetylglucosamine transferase and deubiquitinase ALG13  
 ## [75] Putative ATP-dependent RNA helicase me31b  
 ## [76] Erythroid differentiation-related factor 1  
 ## [77] Mediator of RNA polymerase II transcription subunit 8  
 ## [78] CCR4-NOT transcription complex subunit 2  
 ## [79] Probable malonyl-CoA-acyl carrier protein transacylase mitochondrial  
 ## [80] MKL/myocardin-like protein 1  
 ## [81] Phosphatidylinositol phosphatase SAC1  
 ## [82] STIP1 homology and U box-containing protein 1  
 ## [83] Ubiquitin-like-conjugating enzyme ATG3  
 ## [84] Glucose-fructose oxidoreductase domain-containing protein 1  
 ## [85] Protein misato  
 ## [86] Serine/threonine-protein kinase 3  
 ## [87] Zinc finger protein-like 1  
 ## [88] Nuclear RNA export factor 1  
 ## [89] Methionine--tRNA ligase cytoplasmic  
 ## [90] Cytoplasmic phosphatidylinositol transfer protein 1  
 ## [91] Glyoxylate reductase  
 ## [92] Histone H2B  
 ## [93] LisH domain and HEAT repeat-containing protein KIAA1468 homolog  
 ## [94] RCC1 and BTB domain-containing protein 1  
 ## [95] BRCA1-associated protein  
 ## [96] Rho GTPase-activating protein 19  
 ## [97] Digestive organ expansion factor homolog  
 ## [98] tRNA (adenine(58)-N(1))-methyltransferase non-catalytic subunit TRM6  
 ## [99] Transcription factor SPT20 homolog  
 ## [100] Serine protease nudel  
 ## [101] Ubiquitin-conjugating enzyme E2 5A  
 ## [102] Rab5 GDP/GTP exchange factor  
 ## [103] Serine/threonine-protein phosphatase 2A regulatory subunit B'' subunit gamma  
 ## [104] Protein brambleberry  
 ## [105] Cell division cycle protein 27 homolog  
 ## [106] Probable E3 ubiquitin-protein ligase makorin-1

## [107] Segment polarity protein dishevelled homolog DVL-3  
 ## [108] DNA replication complex GINS protein PSF2  
 ## [109] GPI mannosyltransferase 4  
 ## [110] Protein FAM60A  
 ## [111] ATP-dependent RNA helicase Ddx1  
 ## [112] Leukocyte elastase inhibitor A  
 ## [113] Rac GTPase-activating protein 1  
 ## [114] DNA repair and recombination protein RAD54-like (Fragment)  
 ## [115] DNA mismatch repair protein Msh2  
 ## [116] Sentrin-specific protease 1  
 ## [117] Maternal protein tudor  
 ## [118] Nucleoporin NDC1  
 ## [119] Ankyrin-2  
 ## [120] Metallophosphoesterase 1  
 ## [121] Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX16  
 ## [122] Serine/threonine-protein phosphatase 6 catalytic subunit  
 ## [123] UPF0586 protein C9orf41 homolog  
 ## [124] Ubiquitin carboxyl-terminal hydrolase 36  
 ## [125] Nucleoporin NUP53  
 ## [126] Macrophage erythroblast attacher  
 ## [127] Multiple PDZ domain protein  
 ## [128] Structural maintenance of chromosomes protein 6  
 ## [129] U1 small nuclear ribonucleoprotein 70 kDa  
 ## [130] Cullin-associated NEDD8-dissociated protein 1  
 ## [131] Microspherule protein 1  
 ## [132] Transcription factor SOX-14  
 ## [133] Zinc finger protein 184  
 ## [134] Pyridine nucleotide-disulfide oxidoreductase domain-containing protein 1  
 ## [135] Cell division cycle 5-like protein  
 ## [136] Spondin-1  
 ## [137] Brahma-associated protein of 60 kDa  
 ## [138] Broad-complex core protein isoform 6  
 ## [139] DNA polymerase alpha catalytic subunit  
 ## [140] Probable ATP-dependent RNA helicase DDX43  
 ## [141] Tudor domain-containing protein 5  
 ## [142] G2/M phase-specific E3 ubiquitin-protein ligase  
 ## [143] Putative tRNA (cytidine(32)/guanosine(34)-2'-O)-methyltransferase  
 ## [144] Eukaryotic translation initiation factor 3 subunit C  
 ## [145] N-acetyltransferase 10  
 ## [146] G2/mitotic-specific cyclin-B3  
 ## [147] N6-adenosine-methyltransferase subunit METTL3  
 ## [148] Rho guanine nucleotide exchange factor 3  
 ## [149] Importin-4  
 ## [150] Rho GTPase-activating protein 18  
 ## [151] ATP-dependent RNA helicase vasa isoform A  
 ## [152] Checkpoint protein HUS1  
 ## [153] Pre-mRNA-splicing factor RBM22  
 ## [154] Transmembrane protein 209  
 ## [155] Translation initiation factor eIF-2B subunit delta  
 ## [156] Protein ariadne-2  
 ## [157] Acidic repeat-containing protein  
 ## [158] ATPase family AAA domain-containing protein 5  
 ## 7725 Levels: Pyruvate dehydrogenase acetyl-transferring -phosphatase 1 mitochondrial ...



## bee Genes

```
beeSwiss[!grepl("ncharacterized",beeSwiss) & beeSwiss!="-"]
```

```
## [1] ATP-dependent RNA helicase vasa
## [2] PREDICTED: kinesin-like protein KIF18A
## [3] PREDICTED: prion-like-(Q/N-rich) domain-bearing protein 25
## [4] PREDICTED: disks large-associated protein 5-like
## [5] PREDICTED: E3 ubiquitin-protein ligase TRIM71
## [6] PREDICTED: zinc finger protein 470-like
## [7] PREDICTED: CDC42 small effector protein homolog
## [8] PREDICTED: NF-kappa-B inhibitor cactus-like
## [9] PREDICTED: hyaluronan mediated motility receptor
## [10] PREDICTED: ADP-ribosylation factor-like protein 1
## [11] PREDICTED: facilitated trehalose transporter Tret1-like
## [12] PREDICTED: histone H2B-like
## [13] PREDICTED: protein claret segregational
## [14] PREDICTED: dual specificity protein kinase TTK
## [15] PREDICTED: prolyl 3-hydroxylase OGFOD1
## [16] PREDICTED: targeting protein for Xklp2 homolog
## [17] PREDICTED: zinc finger protein 480
## [18] PREDICTED: kinetochore protein ndc80
## [19] PREDICTED: SET and MYND domain-containing protein 4-like
## [20] PREDICTED: rhoGEF domain-containing protein gxcJ-like
## [21] PREDICTED: helix-loop-helix protein delilah-like
## [22] PREDICTED: nucleoredoxin-like
## [23] PREDICTED: interaptin-like
## [24] PREDICTED: polycomb protein Asx
## [25] PREDICTED: F-BAR domain only protein 2
## [26] PREDICTED: glucose-6-phosphate 1-epimerase
## [27] PREDICTED: kanadaptin
## [28] PREDICTED: S-phase kinase-associated protein 2
## [29] PREDICTED: protein kintoun
## [30] PREDICTED: WW domain-containing adapter protein with coiled-coil
## [31] PREDICTED: citron Rho-interacting kinase-like
## [32] PREDICTED: maternal embryonic leucine zipper kinase-like
## [33] PREDICTED: spindle and kinetochore-associated protein 1-like, partial
## [34] PREDICTED: protein LSM14 homolog A
## [35] PREDICTED: geminin
## [36] PREDICTED: protein kinase 4-like
## [37] PREDICTED: ankyrin repeat domain-containing protein 54
## [38] PREDICTED: protein Smaug homolog 1
## [39] PREDICTED: transformation/transcription domain-associated protein
## [40] PREDICTED: serine protease gd
## [41] PREDICTED: golgin subfamily A member 6-like protein 22
## [42] PREDICTED: RWD domain-containing protein 1
## [43] PREDICTED: PERQ amino acid-rich with GYF domain-containing protein CG11148
## [44] PREDICTED: N-acetylglucosamine-1-phosphotransferase subunits alpha/beta
## [45] PREDICTED: cyclin-A2
## [46] PREDICTED: protein groucho
## [47] PREDICTED: CCR4-NOT transcription complex subunit 1
## [48] PREDICTED: enhancer of mRNA-decapping protein 3
## [49] PREDICTED: mitotic spindle assembly checkpoint protein MAD2A
## [50] PREDICTED: G2/mitotic-specific cyclin-B3
## [51] PREDICTED: micronuclear linker histone polyprotein-like
```

```

## [52] PREDICTED: polycomb protein Pcl
## [53] PREDICTED: KAT8 regulatory NSL complex subunit 1
## [54] PREDICTED: UBX domain-containing protein 7
## [55] PREDICTED: ankyrin repeat domain-containing protein 17
## [56] PREDICTED: MAU2 chromatid cohesion factor homolog
## [57] PREDICTED: macoilin
## [58] PREDICTED: probable helicase with zinc finger domain
## [59] PREDICTED: DNA replication factor Cdt1
## [60] PREDICTED: DNA repair protein complementing XP-G cells
## [61] PREDICTED: ras GTPase-activating-like protein IQGAP1
## [62] PREDICTED: histone H1.2-like
## [63] PREDICTED: protein maelstrom 2
## [64] PREDICTED: LOW QUALITY PROTEIN: protein lin-9 homolog
## [65] PREDICTED: lachesin-like
## [66] PREDICTED: protein Daple
## [67] PREDICTED: protein split ends-like
## [68] PREDICTED: LOW QUALITY PROTEIN: bromodomain-containing protein 4
## [69] PREDICTED: SPX and EXS domain-containing protein 4-like
## [70] PREDICTED: zinc finger CCHC domain-containing protein 10-like
## [71] PREDICTED: protein distal antenna
## [72] PREDICTED: vitellogenin receptor
## [73] PREDICTED: homeobox protein OTX1 A
## [74] PREDICTED: eukaryotic translation initiation factor 4E transporter
## [75] PREDICTED: E3 ubiquitin-protein ligase TRAIIP-like
## [76] PREDICTED: poly(A) RNA polymerase gld-2 homolog A
## [77] PREDICTED: serine/threonine-protein kinase polo
## [78] PREDICTED: importin subunit alpha
## [79] PREDICTED: centrosomal protein of 162 kDa-like
## [80] PREDICTED: LOW QUALITY PROTEIN: meiosis arrest female protein 1
## [81] PREDICTED: protein ovo
## [82] PREDICTED: P protein-like
## [83] PREDICTED: suppressor protein SRP40
## [84] PREDICTED: male-specific lethal 1 homolog
## [85] PREDICTED: GRB2-associated-binding protein 2
## [86] PREDICTED: enolase-phosphatase E1
## [87] PREDICTED: G kinase-anchoring protein 1-like
## [88] PREDICTED: histone-lysine N-methyltransferase pr-set7
## [89] PREDICTED: myotubularin-related protein 14
## [90] PREDICTED: TATA-box-binding protein
## [91] PREDICTED: coiled-coil domain-containing protein 43
## [92] PREDICTED: serine/threonine-protein kinase VRK1-like
## [93] PREDICTED: putative ATP-dependent RNA helicase me31b
## 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)

```

## 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]])] = "conserved queen"
ogg11$abdDE[(ogg11$gene_Amel %in% BcasteRes[[3]][[3]] & ogg11$gene_Mphar %in% AcasteRes[[3]][[3]])] = "conserved worker"

```

## 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

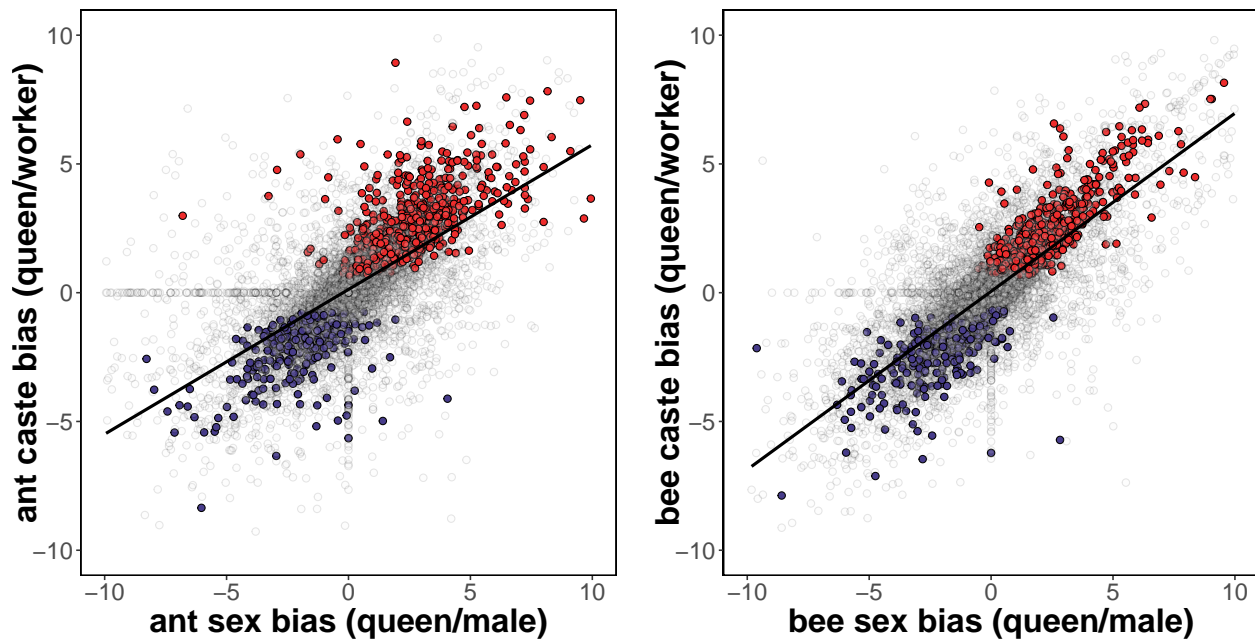
  p1 <- ggplot(FC,aes(x = -FC.x,y = -FC.y))+ #Queen-up will be positive
    geom_point(aes(fill = abdDE,alpha=alpha),pch=21,color="black",size=2)+
    geom_smooth(method="lm",se=FALSE,color="black")+
    scale_fill_manual(values = SexPal[c(1,2,5)],name = "abdominal caste bias")+
    guides(fill = guide_legend(override.aes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

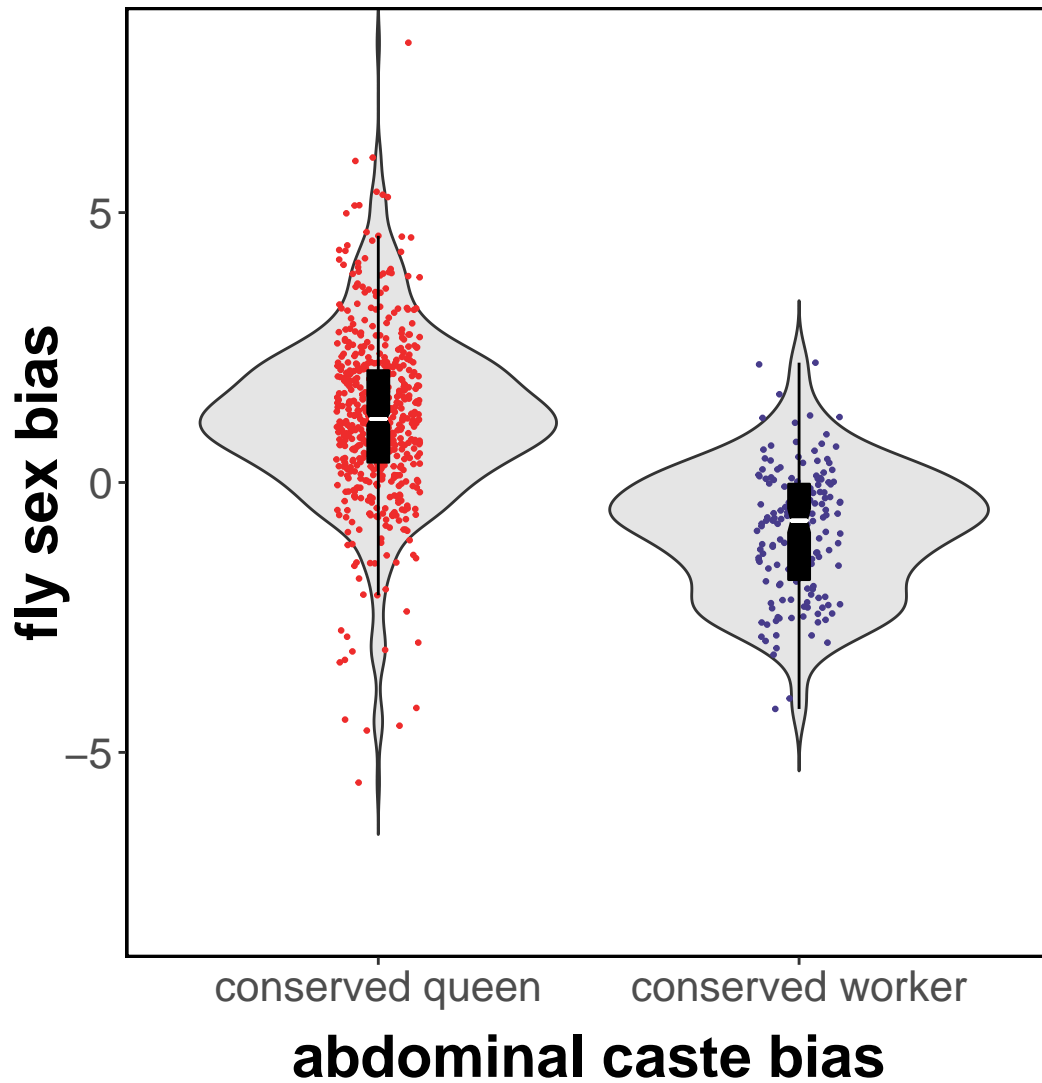
```
## Warning: Removed 54 rows containing missing values (geom_point).
## Warning: Removed 47 rows containing non-finite values (stat_smooth).
## Warning: Removed 47 rows containing missing values (geom_point).
```



### 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")

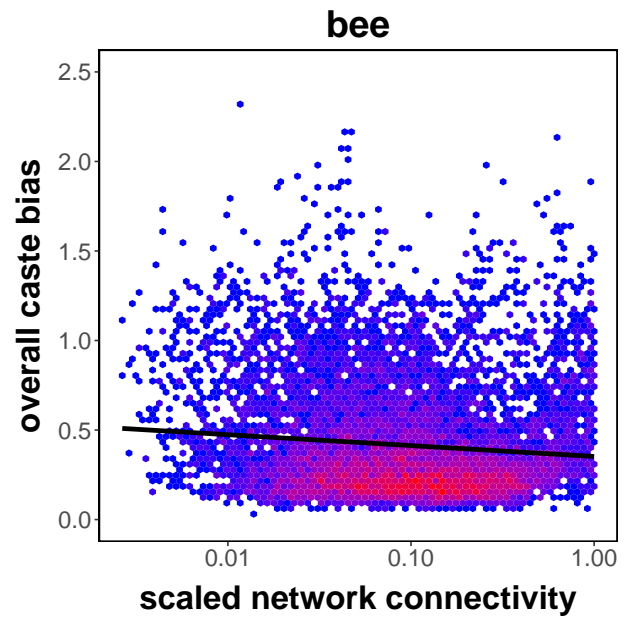
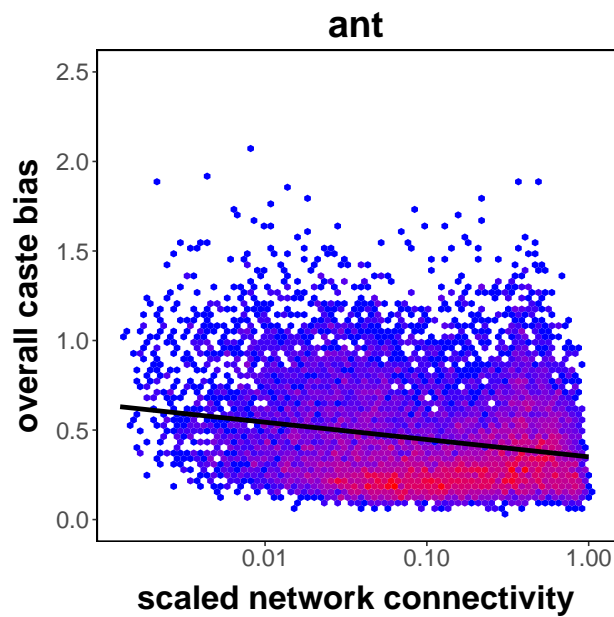
grid.arrange(p1,p3,nrow=1)

```

```

## 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).
```

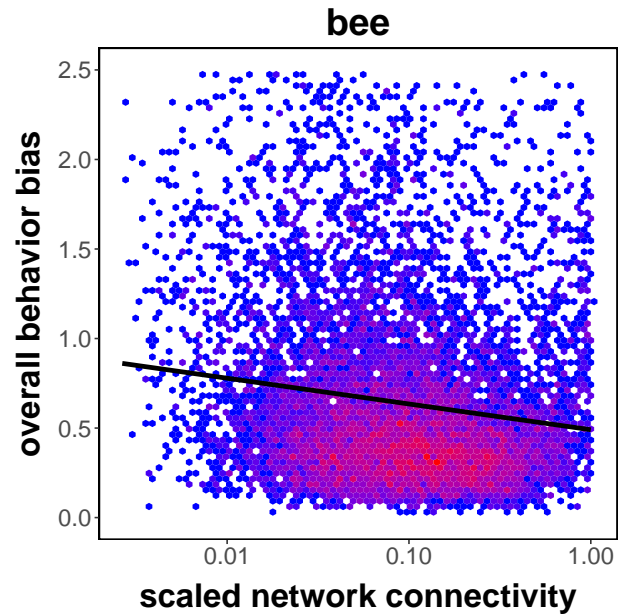
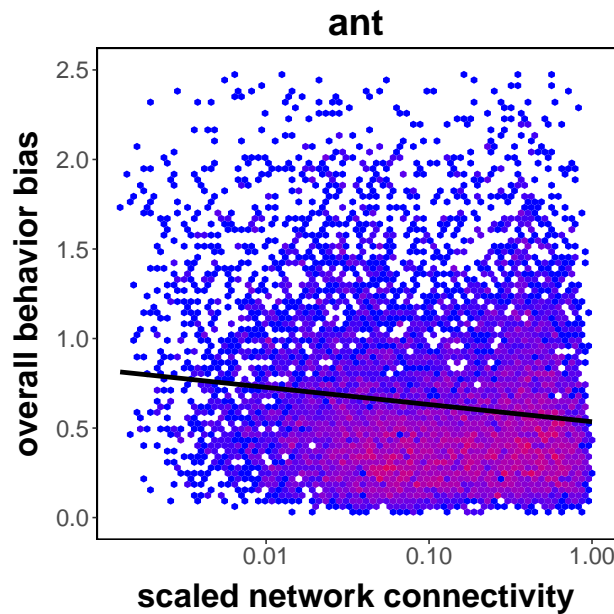
```
## Warning: Removed 56 rows containing non-finite values (stat_smooth).
```

```
## 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).
```



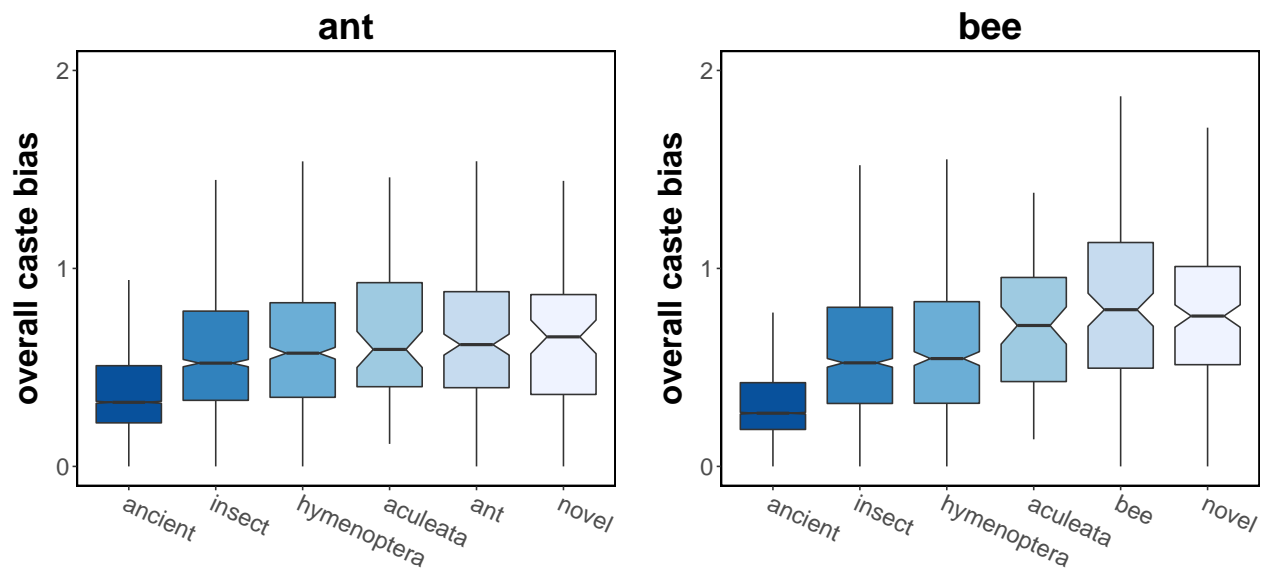


## 4.2 Caste/Behavior bias vs phylostrata

```
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)
}

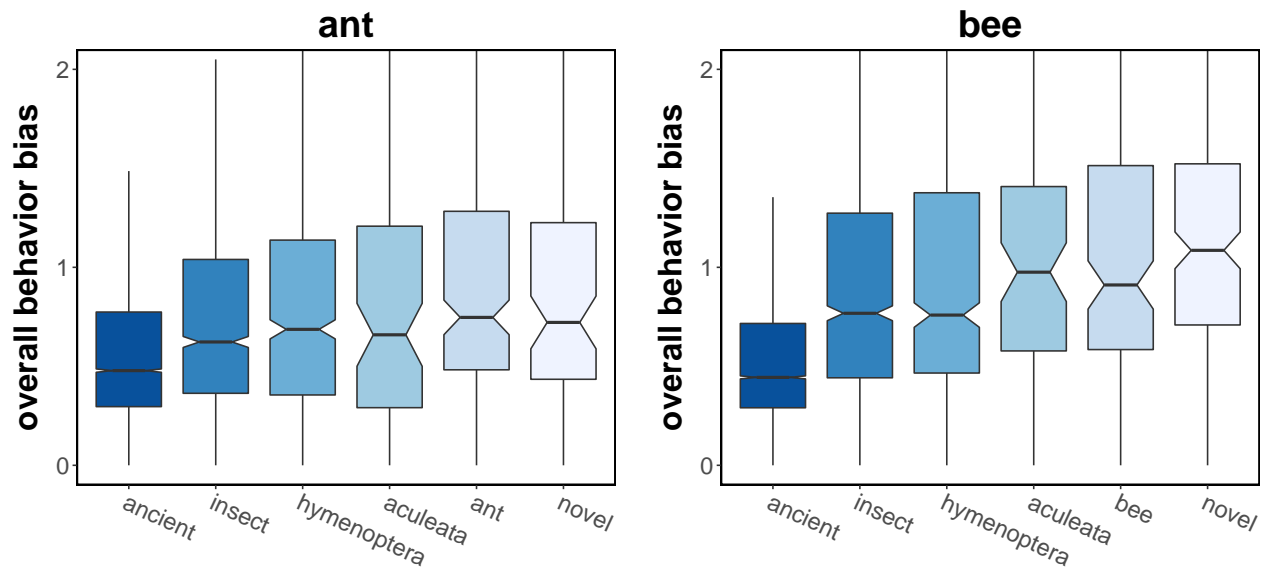
p1 <- phPlot(cbAps,"caste","ant")
p2 <- phPlot(cbAps,"behavior","ant")
p3 <- phPlot(cbBps,"caste","bee")
p4 <- phPlot(cbBps,"behavior","bee")

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



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





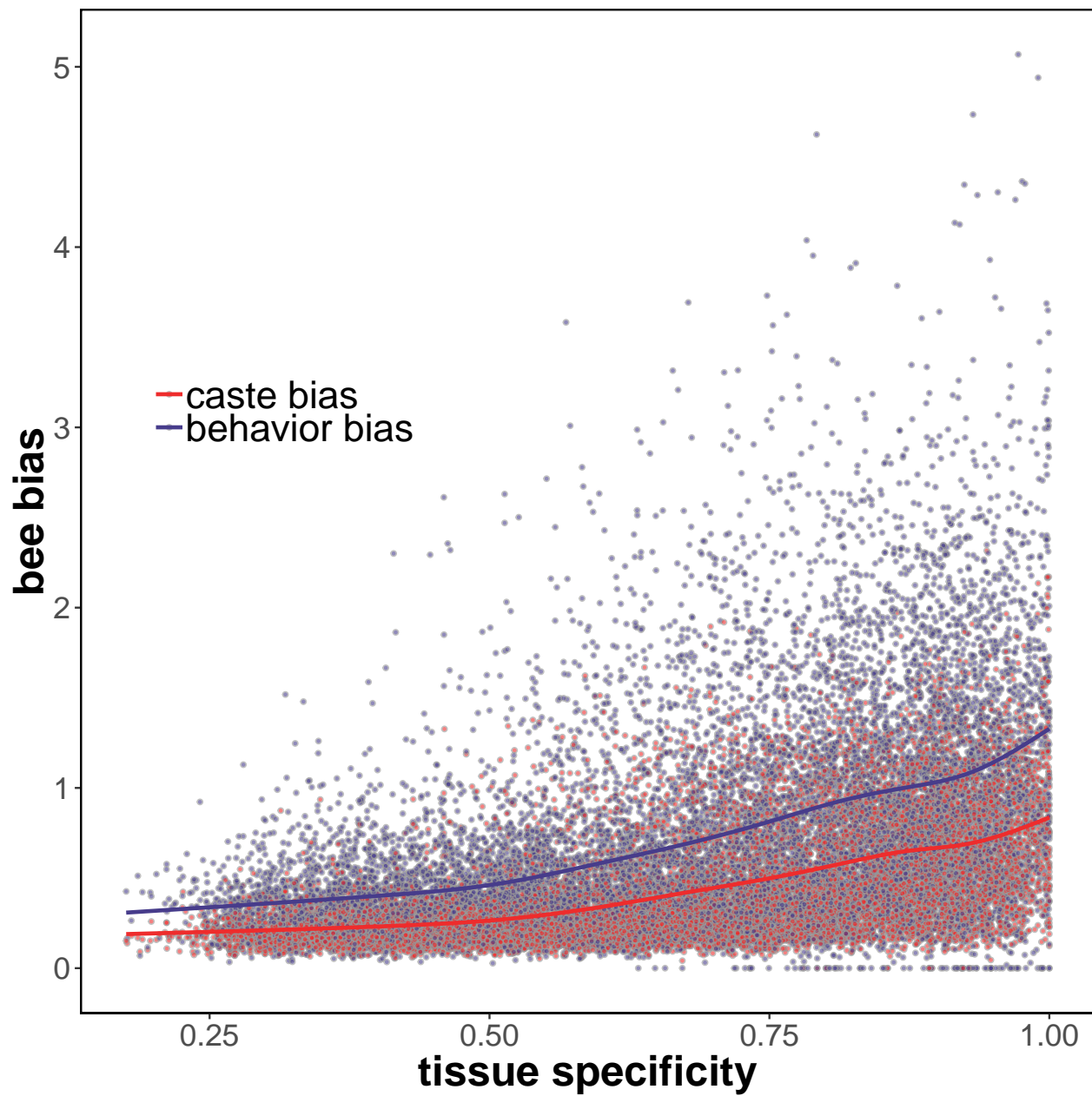
## 4.3 Caste bias vs tissue specificity -tissue specificity is estimated across 12 honey bee tissues.

```
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

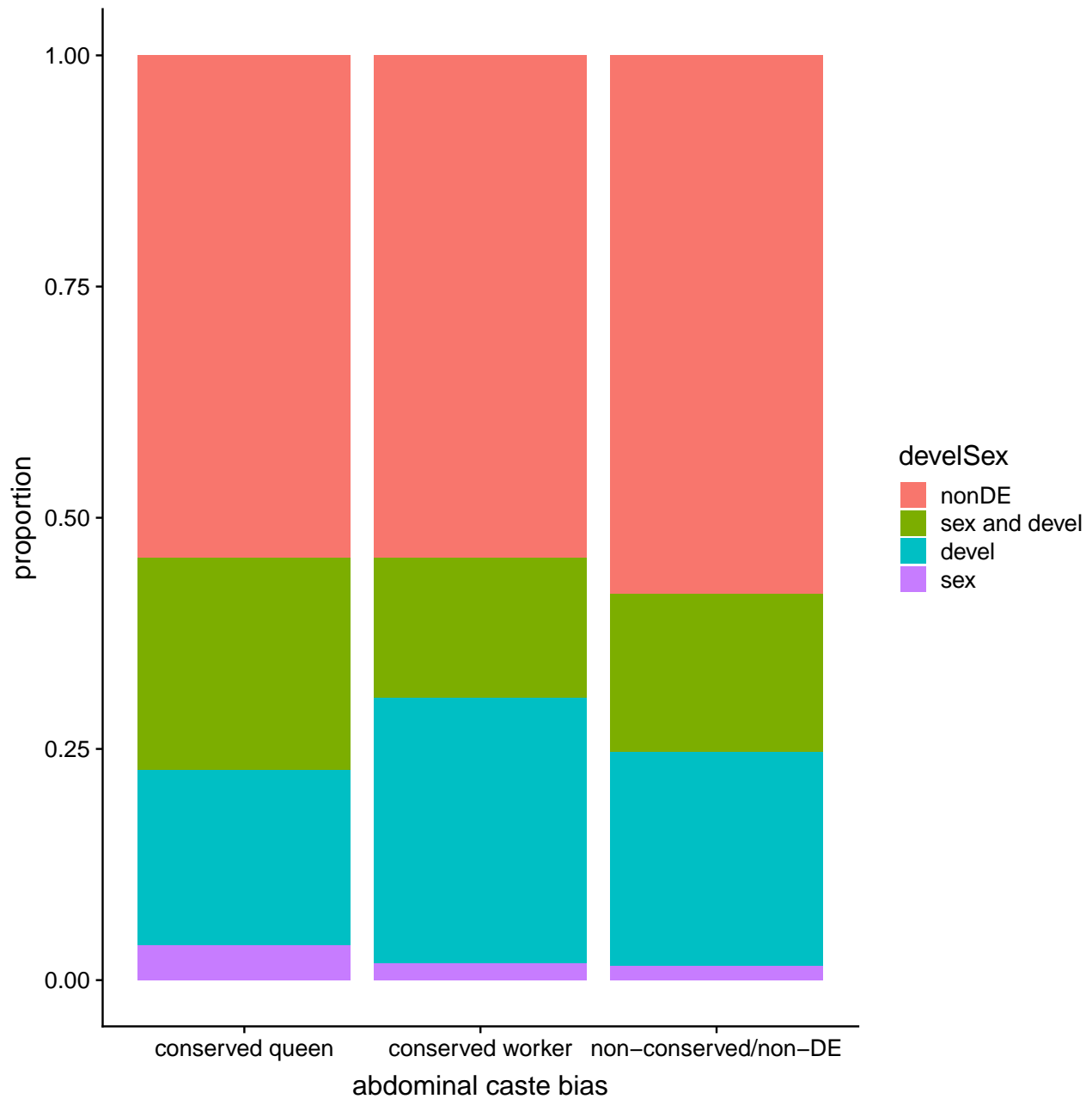
```
DmelSC = merge(sexGenes,ogg11,by="gene_Amel")
key <- read.table("data/DmelKey.txt") #protein-CDS
key2 <- read.table("data/Dmel_CDStoGene_key.txt") #Gene-CDS
devel <- read.table("data/Devel_IDS.txt") #Downloaded from flybase, genes with developmental terms
sex <- read.table("data/Sex_IDS.txt") #Downloaded 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).
```

