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,]
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),]
```

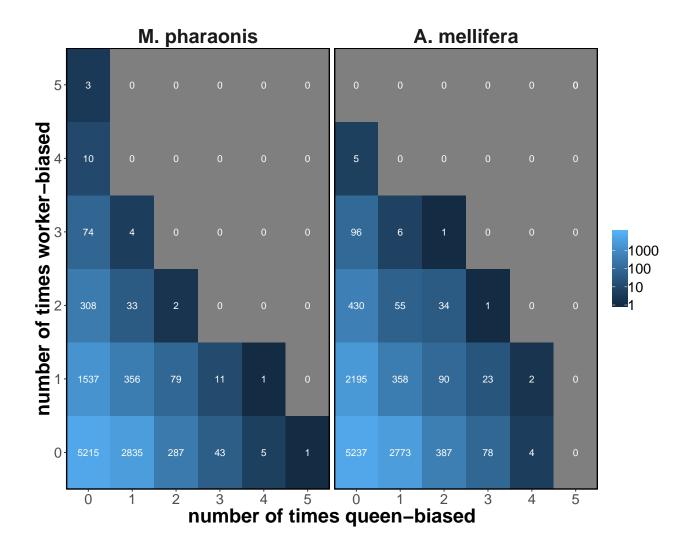
0.1 Number of times a gene is queen- and worker-biased across development

```
#Summarize number of times DE
sumDE <- function(dfDE,type1,type2){
  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)
}</pre>
```

```
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))
```

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

Warning: Transformation introduced infinite values in discrete y-axis

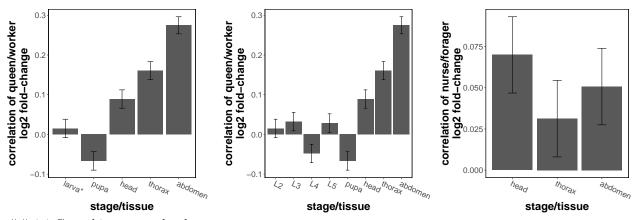


0.2 Correlation of log fold-change across stages

```
#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

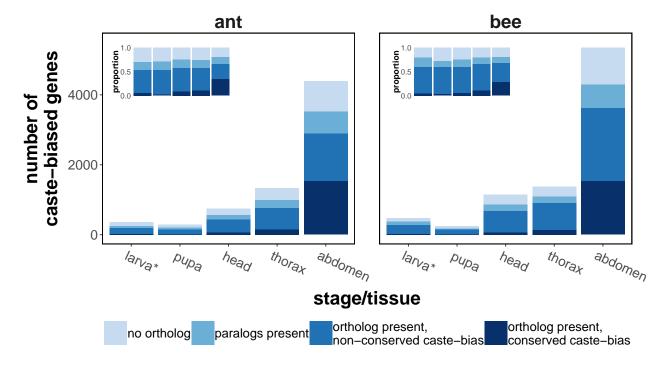
```
compDef <- function(antR,beeR){

#Define whether or not orthologs exist
antR$ortholog_found = antR$0GG_found = FALSE
antR$ortholog_found[antR$Gene %in% AllPS$Gene.x] = TRUE
antR$0GG_found[antR$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",],
             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

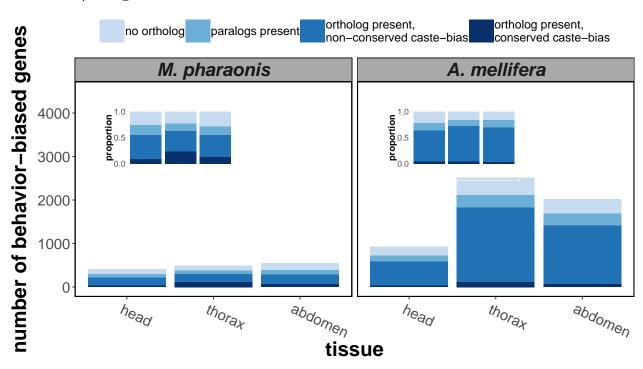


1.2 Nurse/forager bias

```
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



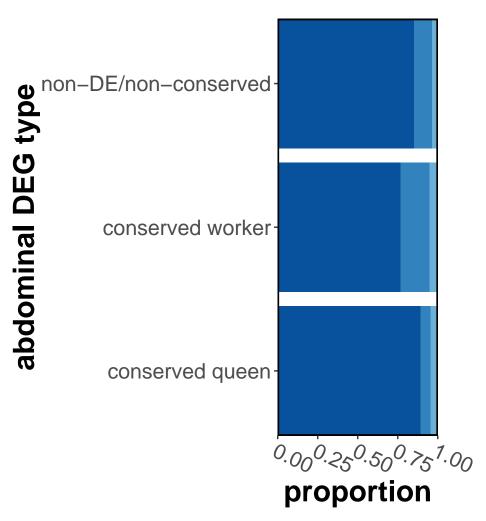
1.3 Phylostrata of genes with conserved abdominal caste bias

```
ps0 = merge(AllPS_sum, ACUogg, by="OGGacu")
ps0$a2 = "non-DE/non-conserved"
#Find genes with conserved queen or worker bias
ps0$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



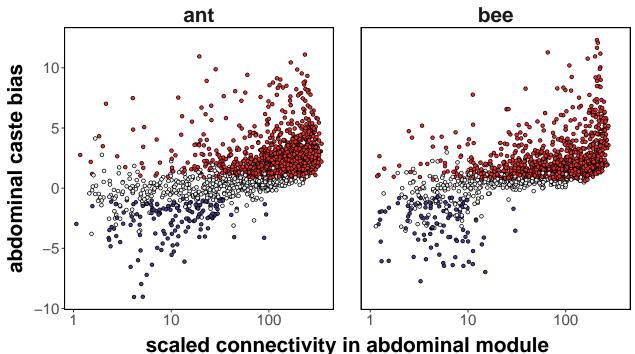


2.1 Bicluster associated with queen abdominal expression

-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))
р1
```



2.2 Specific genes within the bicluster

• holding off plotting specific genes for now

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] Phosphatidylinositide 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
```

- ## [145] N-acetyltransferase 10 ## [146] G2/mitotic-specific cyclin-B3
- ## [147] N6-adenosine-methyltransferase subunit METTL3

[142] G2/M phase-specific E3 ubiquitin-protein ligase

[144] Eukaryotic translation initiation factor 3 subunit C

[143] Putative tRNA (cytidine(32)/guanosine(34)-2'-0)-methyltransferase

```
## [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 TRAIP-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)</pre>
```

Identify genes with conserved bias for plotting

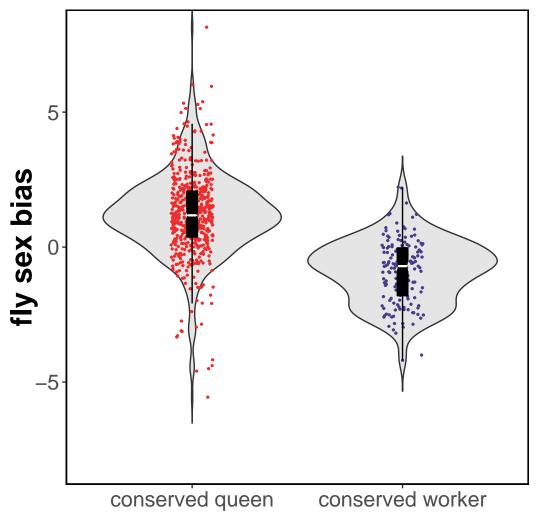
```
ogg11 = ACUogg
ogg11$abdDE = "non-conserved/non-DE"
ogg11$abdDE[(ogg11$gene_Amel %in% BcasteRes[[3]][[2]] & ogg11$gene_Mphar %in% AcasteRes[[3]][[2]])] = "
ogg11$abdDE[(ogg11$gene_Amel %in% BcasteRes[[3]][[3]] & ogg11$gene_Mphar %in% AcasteRes[[3]][[3]])] = "
```

3.1 Construct sex bias vs caste bias plots

```
biasPlot <- function(d1,d2,spec){</pre>
  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).
   10
                                                      10
                                                   bee caste bias (queen/worker)
ant caste bias (queen/worker)
  -10
                                                     -10
      -10
                <u> –</u>5
                                             10
                                                         -10
                                                                                                10
          ant sex bias (queen/male)
                                                              bee sex bias (queen/male)
```

3.2 Comparison to D. melanogaster



abdominal caste bias

Comparison

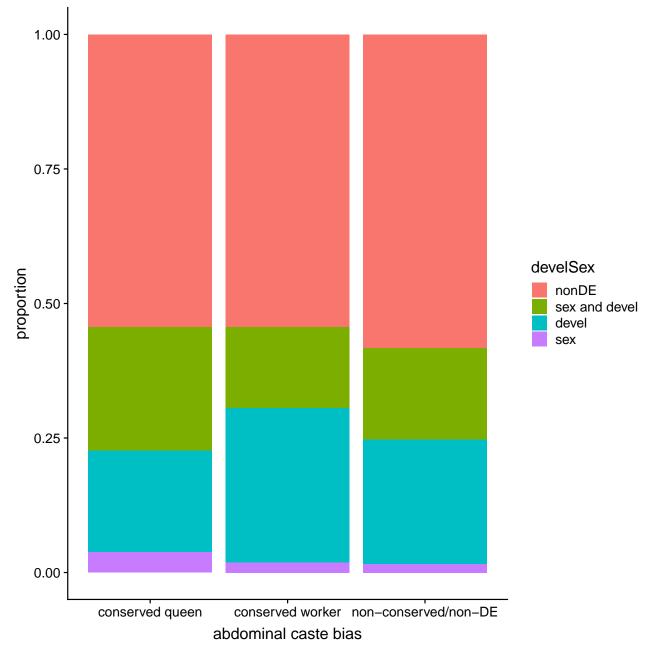
to development

```
DmelSC = merge(sexGenes,ogg11,by="gene_Amel")
key <- read.table("data/DmelKey.txt") #Generated from Drosophila melanogaster gff file
key2 <- read.table("data/Dmel_CDStoGene_key.txt")
devel <- read.table("data/Dmel_Devel_IDs.txt")
sex <- read.table("data/Dmel_Sex_IDs.txt")

key3 = merge(key,key2,by="V1")
DmelSC = merge(DmelSC,key3,by.x="Gene",by.y="V2.y")
DmelSC$develSex = "nonDE"</pre>
```

```
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"))
ggplot(DmelSC[!grepl("ant",DmelSC$abdDE),],aes(x = abdDE,fill=develSex))+
    geom_bar(stat="count",position="fill")+
    ylab("proportion")+
    xlab("abdominal caste bias")
```



```
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()+
    ggtitle("devel measured in ant")

beeDevel2$Gene=rownames(beeDevel2)
bD = merge(ogg11,beeDevel2,by.x="gene_Amel",by.y="Gene")

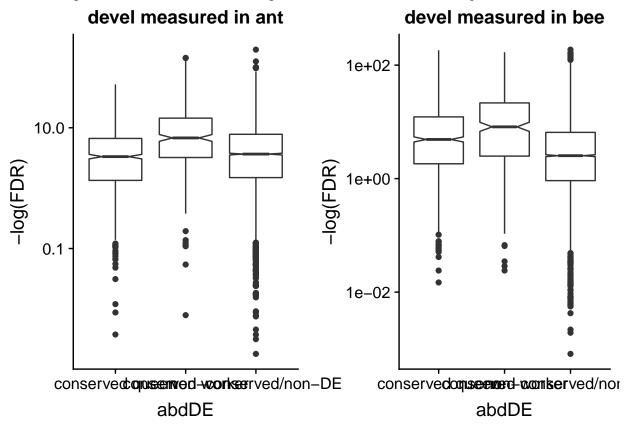
p2 <- ggplot(bD[!grepl("ant",bD$abdDE),],aes(x = abdDE,y=-log(FDR)))+
    geom_boxplot(notch = T)+
    scale_y_log10()+
    ggtitle("devel measured in bee")

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

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

 $\hbox{\tt\#\# Warning: Transformation introduced infinite values in continuous y-axis}$

Warning: Removed 12 rows containing non-finite values (stat_boxplot).



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))
   \label{eq:cb_abd} 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)
```

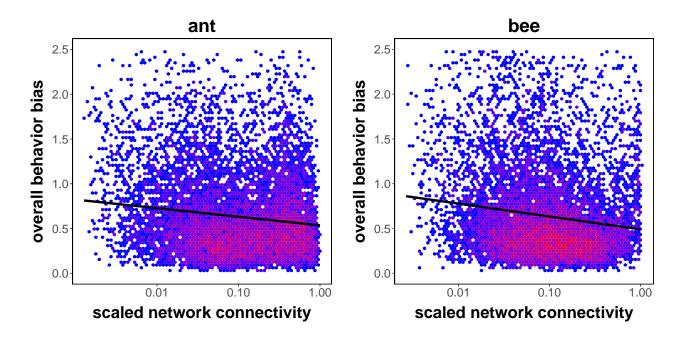
4.1 Caste/Behavior bias vs connectivity

```
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+
  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")</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). ant bee 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 0.01 0.10 1.00 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).

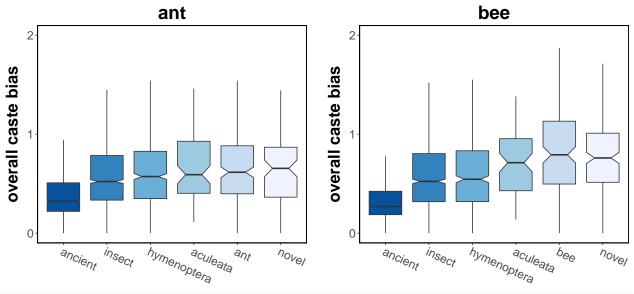
Warning: Removed 156 rows containing non-finite values (stat_binhex).
Warning: Removed 156 rows containing non-finite values (stat_smooth).

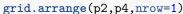
Warning: Removed 37 rows containing missing values (geom_hex).

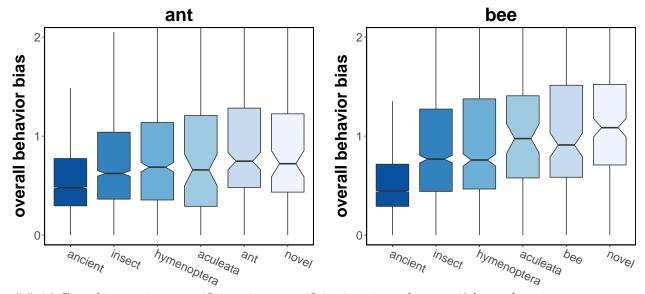


4.2 Caste/Behavior bias vs phylostrata

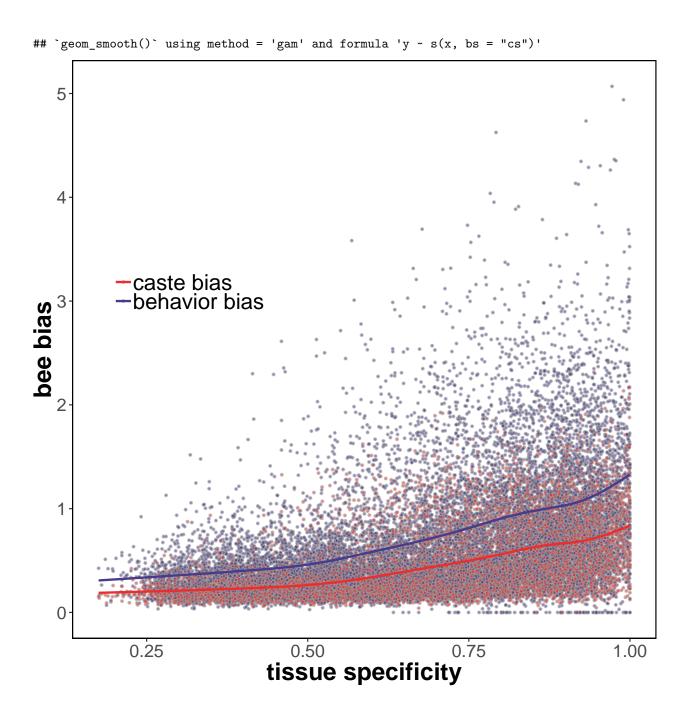
```
phPlot <- function(data, type, spec){</pre>
  pA <- ggplot(data[data$type==type & !is.na(data$psName),],aes(x = psName,y=cb,fill=psName))+
  geom_boxplot(notch=TRUE, outlier.shape=NA)+
  plot2theme+
  ggtitle(spec)+
  xlab("")+
  coord_cartesian(ylim=c(0,2))+
  scale_y_continuous(breaks = c(0,1,2,3))+
  scale_fill_manual(values = rev(mypalette2), name = "phylostrata", guide=guide_legend(title.position="to")
  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")</pre>
p2 <- phPlot(cbAps, "behavior", "ant")</pre>
p3 <- phPlot(cbBps,"caste","bee")</pre>
p4 <- phPlot(cbBps, "behavior", "bee")
grid.arrange(p1,p3,nrow=1)
```







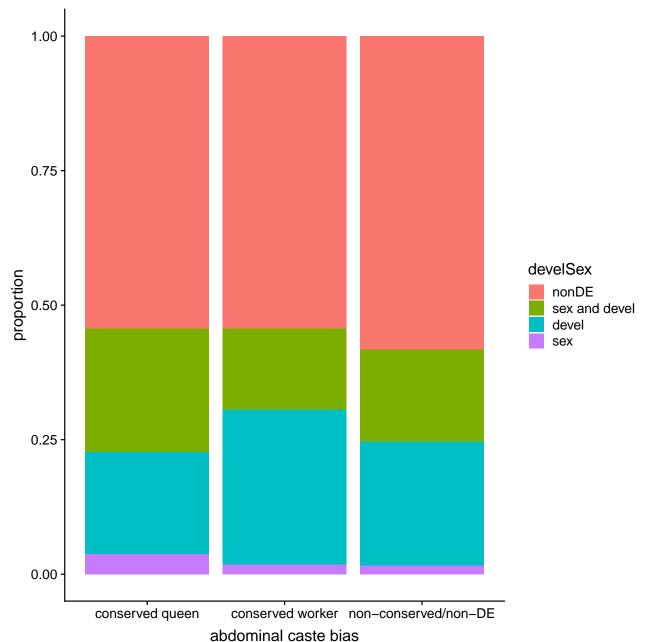
4.3 Caste bias vs tissue specificity -tissue specificity is estimated across 12 honey bee tissues.



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/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
key3 = merge(key,key2,by="V1")</pre>
```



-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)))+</pre>
  geom_boxplot(notch = T)+
  scale_y_log10()+
  theme(axis.text.x = element_text(angle=90))+
  ggtitle("devel measured in ant")
beeDevel2$Gene=rownames(beeDevel2)
bD = merge(ogg11,beeDevel2,by.x="gene Amel",by.y="Gene")
p2 <- ggplot(bD[!grep1("ant",bD$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).
```

