Genetic Effects of a Foundation Tree on Networks of Leaf Modifying Arthropods

Species/Functional Groups

- PB = P. betae
- pb.pred = parasitic fly
- pb.abort =
- edge.fold = sawfly
- pinch =
- mid.miner = lep
- edge.miner = lep
- tip.miner = lep
- fish.eye = ??
- tier = lep
- thrips = Thysanura
- chew.holes = ??
- chomp = large herbivore
- scrape = radula
- chew.edge = lep?

Pre-processing Data

Load datasets

```
pit <- read.csv('../data/acn/arth_cooc_PIT_Lau.csv')
pit[is.na(pit)] <- 0</pre>
```

Restrict main analysis to live leaves only

```
### separating out "fungal" necrosis
### necrosis <- pit$fungal
pit <- pit[,colnames(pit) != 'fungal']</pre>
```

Remove Genotype 1007

```
pit <- pit[pit[, "geno"] != "1007", ]</pre>
```

Remove mite

```
pit <- pit[, colnames(pit) != "mite"]</pre>
```

Combine gall variants

```
pb <- pit$pb.upper + pit$pb.lower + pit$pb.woody
pb.pred <- pit$pb.pred + pit$pb.woody.pred + pit$pb.hole
pb.abort <- pit$pb.abort
pit <- pit[,!(grepl('pb',colnames(pit)))]
pit <- data.frame(pit,pb.abort,pb.pred,pb)
pit <- data.frame(pit[,1:6],pit[,ncol(pit):7])
tree.info <- paste(pit[, "tree"], pit[, "geno"], pit[, "leaf.type"])
tree.arth <- pit[, 7:ncol(pit)]
tree.arth <- split(tree.arth, tree.info)</pre>
```

Create the community matrix

```
com.acn <- do.call(rbind, lapply(tree.arth, function(x) apply(x, 2, sum)))</pre>
```

Tree level network models

```
cn.acn <- lapply(tree.arth, coNet, ci.p = 95, cond = TRUE)</pre>
```

Tree network distances

```
d.cn.acn <- netDist(cn.acn, method = "euclidean")</pre>
```

Calculate network metrics

Tree info compilation

```
acn.dat <- data.frame(do.call(rbind, strsplit(names(cn.acn), split = " ")))
names(acn.dat) <- c("tree", "geno", "leaf.type")</pre>
```

Limit senscent trees with greater than 20 leaves

```
n.sen <- unlist(lapply(tree.arth[acn.dat[, "leaf.type"] == "sen"], nrow))
n.live <- unlist(lapply(tree.arth[acn.dat[, "leaf.type"] == "live"], nrow))
sen.trees <- tree.arth[names(tree.arth) %in% names(n.sen)[n.sen >= 20]]
sen.dat <- data.frame(do.call(rbind, strsplit(names(sen.trees), split = " ")))
colnames(sen.dat) <- c("tree", "geno", "leaf.type")
com.sen <- do.call(rbind, lapply(sen.trees, function(x) apply(x, 2 ,sum)))</pre>
```

Network ordination

Senescent network models

```
cn.sen <- lapply(sen.trees, coNet, ci.p = 95, cond = TRUE)</pre>
```

Leaf sample size relativization

```
lsr <- unlist(lapply(tree.arth, nrow))
com.acn.lsr <- com.acn / lsr</pre>
```

Main Results

Note: genotype 1007 is removed because it only has one rep

Genotype replication

Total abundance

```
abund <- apply(com.acn, 1, sum)
abund <- apply(com.acn[acn.dat[, "tree"] %in% sen.dat[, "tree"], ], 1, sum)
reml.abund.acn <- lme4::lmer(I(abund^(1/2)) ~ (1 | geno),</pre>
```

```
data = acn.dat[acn.dat[, "tree"] %in% sen.dat[, "tree"],],
                            REML = TRUE)
p.reml.abund.acn <- RLRsim::exactRLRT(reml.abund.acn)</pre>
p.reml.abund.acn
##
##
    simulated finite sample distribution of RLRT.
##
##
    (p-value based on 10000 simulated values)
##
## data:
## RLRT = 6.9127, p-value = 0.0019
                                         # live trees
RLRsim::exactRLRT(
    lme4::lmer(I(abund[acn.dat[, "leaf.type"] == "live"]^(1/2)) ~ (1 | geno),
               data = acn.dat[acn.dat[, "leaf.type"] == "live", ],
               REML = TRUE)
)
## singular fit
##
##
   simulated finite sample distribution of RLRT.
##
    (p-value based on 10000 simulated values)
##
##
## data:
## RLRT = 0, p-value = 1
                                         # sen trees
sen.A <- apply(com.sen, 1, sum)</pre>
RLRsim::exactRLRT(
    lme4::lmer(I(sen.A) ~ (1 | geno),
               data = sen.dat,
               REML = TRUE)
##
   simulated finite sample distribution of RLRT.
## (p-value based on 10000 simulated values)
##
## data:
## RLRT = 4.102, p-value = 0.0176
```

Richness

```
##
##
   simulated finite sample distribution of RLRT.
##
##
    (p-value based on 10000 simulated values)
##
## data:
## RLRT = 7.1066, p-value = 0.0019
                                         # live trees
RLRsim::exactRLRT(
    lme4::lmer(I(rich[acn.dat[, "leaf.type"] == "live"]^(1/2)) ~ (1 | geno),
               data = acn.dat[acn.dat[, "leaf.type"] == "live", ],
               REML = TRUE)
)
##
##
   simulated finite sample distribution of RLRT.
##
##
    (p-value based on 10000 simulated values)
##
## data:
## RLRT = 0.49745, p-value = 0.2055
sen.R <- apply(com.sen, 1, function(x) sum(sign(x)))</pre>
RLRsim::exactRLRT(
    lme4::lmer(I(sen.R) ~ (1 | geno),
               data = sen.dat,
               REML = TRUE)
##
##
   simulated finite sample distribution of RLRT.
##
##
  (p-value based on 10000 simulated values)
##
## data:
## RLRT = 4.7246, p-value = 0.0119
```

Community Similarity

Terms added sequentially (first to last)

```
## Permutation: free
## Number of permutations: 10000
## vegan::adonis2(formula = rel.com.acn ~ geno * leaf.type, data = acn.dat[acn.dat[, "tree"] %in% sen.d
##
                 Df SumOfSqs
                                  R2
                                               Pr(>F)
                      2.4295 0.14588 1.3928
                                               0.0043 **
## geno
                  1 1.3471 0.08089 4.6336 9.999e-05 ***
## leaf.type
## geno:leaf.type 6 1.8302 0.10990 1.0492
                                               0.3271
## Residual
                 38 11.0475 0.66334
## Total
                 51 16.6544 1.00000
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
                                       # live
set.seed(12234)
vegan::adonis2(rel.com.acn[acn.dat[acn.dat[, "tree"] %in% sen.dat[, "tree"],
                                  "leaf.type"] == "live", ] ~ geno,
              data = acn.dat[acn.dat[, "leaf.type"] == "live" &
                                 acn.dat[, "tree"] %in% sen.dat[, "tree"], ],
              perm = 10000, sqrt.dist = FALSE, mrank = TRUE)
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 10000
## vegan::adonis2(formula = rel.com.acn[acn.dat[acn.dat[, "tree"] %in% sen.dat[, "tree"], "leaf.type"]
           Df SumOfSqs
                                    F Pr(>F)
                            R2
                1.3372 0.32436 1.5202 0.0461 *
## Residual 19
                2.7855 0.67564
## Total
           25
                4.1227 1.00000
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
                                       # sen
set.seed(12234)
vegan::adonis2(com.sen ~ geno,
              data = sen.dat,
              perm = 10000, sqrt.dist = TRUE, mrank = TRUE)
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 10000
## vegan::adonis2(formula = com.sen ~ geno, data = sen.dat, permutations = 10000, sqrt.dist = TRUE, mra
##
           Df SumOfSqs
                            R2
                                    F
                                       Pr(>F)
                2.3265 0.33532 1.5976 0.009899 **
## geno
            6
## Residual 19 4.6115 0.66468
                6.9380 1.00000
## Total
           25
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

Network similarity

```
set.seed(12234)
vegan::adonis2(netDist(cn.acn[acn.dat[, "tree"] %in% sen.dat[, "tree"]]) ~
                  leaf.type * geno,
              data = acn.dat[acn.dat[, "tree"] %in% sen.dat[, "tree"], ],
              strata = acn.dat[acn.dat[, "tree"] %in% sen.dat[, "tree"], "tree"],
              sqrt.dist = FALSE, mrank = TRUE)
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## vegan::adonis2(formula = netDist(cn.acn[acn.dat[, "tree"] %in% sen.dat[, "tree"]]) ~ leaf.type * gen
                 Df SumOfSqs
                                  R2
                                          F Pr(>F)
                        2.75 0.00360 0.2207 0.718
## leaf.type
                  1
                     200.43 0.26273 2.6821 0.028 *
## geno
                  6
                     86.43 0.11330 1.1566 0.349
## leaf.type:geno 6
## Residual
                 38 473.26 0.62037
## Total
                 51
                      762.87 1.00000
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
                                       # Live
set.seed(12234)
vegan::adonis2(netDist(cn.acn[acn.dat[, "leaf.type"] == "live"]) ~ geno,
              data = acn.dat[acn.dat[, "leaf.type"] == "live", ],
              sqrt.dist = FALSE, mrank = TRUE)
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
## vegan::adonis2(formula = netDist(cn.acn[acn.dat[, "leaf.type"] == "live"]) ~ geno, data = acn.dat[ac
##
           Df SumOfSqs
                            R2
                                    F Pr(>F)
            6 127.79 0.40888 3.2279
## geno
                                       0.01 **
## Residual 28 184.75 0.59112
## Total
           34 312.54 1.00000
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
                                       # Senescence
set.seed(12234)
vegan::adonis2(netDist(cn.sen) ~ geno, data = sen.dat,
              sqrt.dist = FALSE, mrank = TRUE)
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
## vegan::adonis2(formula = netDist(cn.sen) ~ geno, data = sen.dat, sqrt.dist = FALSE, mrank = TRUE)
##
           Df SumOfSqs
                            R2
                                    F Pr(>F)
```

```
## geno 6 57.311 0.28482 1.2611 0.311
## Residual 19 143.911 0.71518
## Total 25 201.222 1.00000
```

Network metrics

```
# number of links
reml.l.acn <- lme4::lmer(I(l.cn.acn^(1/1)) ~ (1 | geno),
                            data = acn.dat,
                            REML = TRUE)
p.reml.l.acn <- RLRsim::exactRLRT(reml.l.acn)</pre>
p.reml.l.acn
##
    simulated finite sample distribution of RLRT.
##
   (p-value based on 10000 simulated values)
##
## data:
## RLRT = 5.8921, p-value = 0.0047
RLRsim::exactRLRT(
    lme4::lmer(I(1.cn.acn[acn.dat[, "leaf.type"] == "live"]^(1/1)) ~ (1 | geno),
               data = acn.dat[acn.dat[, "leaf.type"] == "live", ],
               REML = TRUE
)
## simulated finite sample distribution of RLRT.
##
##
  (p-value based on 10000 simulated values)
## data:
## RLRT = 7.197, p-value = 0.0032
                                         # centralization
reml.cen.acn \leftarrow lme4::lmer(I(cen.cn.acn^(1/2)) ~ (1 | geno),
                            data = acn.dat,
                            REML = TRUE)
p.reml.cen.acn <- RLRsim::exactRLRT(reml.cen.acn)</pre>
p.reml.cen.acn
##
##
  simulated finite sample distribution of RLRT.
##
##
  (p-value based on 10000 simulated values)
##
## data:
## RLRT = 2.6561, p-value = 0.0409
RLRsim::exactRLRT(
    lme4::lmer(I(cen.cn.acn[acn.dat[, "leaf.type"] == "live"]^(1/2)) ~ (1 | geno),
               data = acn.dat[acn.dat[, "leaf.type"] == "live", ],
               REML = TRUE)
```

```
##
## simulated finite sample distribution of RLRT.
##
## (p-value based on 10000 simulated values)
##
## data:
## RLRT = 2.3608, p-value = 0.0468
```

Proportion of PB singles, doubles and triples

```
n.leaf <- unlist(lapply(tree.arth, nrow))</pre>
pb1 <- unlist(lapply(tree.arth, function(x) sum(sign(x[, "pb"] == 1))))</pre>
pb2 <- unlist(lapply(tree.arth, function(x) sum(sign(x[, "pb"] == 2))))</pre>
pb3 <- unlist(lapply(tree.arth, function(x) sum(sign(x[, "pb"] == 3))))</pre>
pb4 <- unlist(lapply(tree.arth, function(x) sum(sign(x[, "pb"] == 4))))
pb.1 <- unlist(lapply(tree.arth, function(x) sum(sign(x[, "pb"] == 1))))</pre>
pb1.d <- pb1[grep1("live", names(pb1))]/n.leaf[grep1("live", names(n.leaf))] -</pre>
    pb1[grepl("sen", names(pb1))]/n.leaf[grepl("sen", names(n.leaf))]
pb2.d <- pb2[grep1("live", names(pb2))]/n.leaf[grep1("live", names(n.leaf))] -</pre>
    pb2[grepl("sen", names(pb2))]/n.leaf[grepl("sen", names(n.leaf))]
pb3.d <- pb3[grepl("live", names(pb3))]/n.leaf[grepl("live", names(n.leaf))] -
    pb3[grepl("sen", names(pb3))]/n.leaf[grepl("sen", names(n.leaf))]
                                          # pb1
RLRsim::exactRLRT(
    lme4::lmer(I(pb1.d[acn.dat[, "tree"] %in% sen.dat[, "tree"]]) ~ (1 | geno),
               data = acn.dat[acn.dat[, "tree"] %in% sen.dat[, "tree"], ],
               REML = TRUE)
)
##
##
    simulated finite sample distribution of RLRT.
##
##
    (p-value based on 10000 simulated values)
##
## data:
## RLRT = 0.00011911, p-value = 0.4459
                                          # pb2
RLRsim::exactRLRT(
    lme4::lmer(I(pb2.d[acn.dat[, "tree"] %in% sen.dat[, "tree"]]) ~ (1 | geno),
               data = acn.dat[acn.dat[, "tree"] %in% sen.dat[, "tree"], ],
               REML = TRUE)
)
##
    simulated finite sample distribution of RLRT.
##
##
##
    (p-value based on 10000 simulated values)
##
## data:
## RLRT = 0.00015571, p-value = 0.4383
```

```
RLRsim::exactRLRT(
    lme4::lmer(I(pb3.d[acn.dat[, "tree"] %in% sen.dat[, "tree"]]) ~ (1 | geno),
               data = acn.dat[acn.dat[, "tree"] %in% sen.dat[, "tree"], ],
               REML = TRUE)
)
##
##
   simulated finite sample distribution of RLRT.
##
   (p-value based on 10000 simulated values)
##
##
## data:
## RLRT = 0.048602, p-value = 0.3615
No genotype effect, so all trees are tested together
t.test(pb1.d)
##
   One Sample t-test
##
## data: pb1.d
## t = -5.5738, df = 34, p-value = 3.084e-06
## alternative hypothesis: true mean is not equal to 0
## 95 percent confidence interval:
## -0.3001851 -0.1397729
## sample estimates:
## mean of x
## -0.219979
t.test(pb2.d)
##
   One Sample t-test
##
## data: pb2.d
## t = -3.4302, df = 34, p-value = 0.001599
## alternative hypothesis: true mean is not equal to 0
## 95 percent confidence interval:
## -0.06366719 -0.01629347
## sample estimates:
    mean of x
## -0.03998033
t.test(pb3.d)
##
## One Sample t-test
##
## data: pb3.d
## t = -0.44204, df = 34, p-value = 0.6613
## alternative hypothesis: true mean is not equal to 0
```

pb3

95 percent confidence interval:

```
## -0.003198507 0.002055650
## sample estimates:
## mean of x
## -0.0005714286
```

Leaf type indicator species

```
isa.type <- labdsv::indval(com.acn.lsr, acn.dat[, "leaf.type"])</pre>
summary(isa.type)
##
          cluster indicator_value probability
## scrape
                1
                            0.5969
                                         0.004
## chomp
                1
                            0.5197
                                         0.009
                            0.2222
## pinch
                1
                                          0.027
## pb
                2
                            0.7153
                                         0.001
## tier
                            0.3405
                                         0.007
##
## Sum of probabilities
                                             6.229
## Sum of Indicator Values
                                             4.39
##
## Sum of Significant Indicator Values =
##
## Number of Significant Indicators
                                            5
## Significant Indicator Distribution
##
## 1 2
## 3 2
```

Genotype indicator species

```
isa.geno <- labdsv::indval(com.acn.lsr, acn.dat[, "geno"])</pre>
summary(isa.geno)
##
             cluster indicator_value probability
## chew.edge
                               0.2358
                   4
                                            0.048
                   5
## pb.pred
                               0.2900
                                            0.007
                               0.2664
                                            0.033
## pb.abort
##
## Sum of probabilities
                                            5.228
## Sum of Indicator Values
                                            2.53
##
## Sum of Significant Indicator Values =
                                            0.79
## Number of Significant Indicators
                                            3
## Significant Indicator Distribution
## 4 5 6
## 1 1 1
```

Genotype-species clusters

```
gsc <- computeModules(com.acn.lsr)
slot(gsc, "likelihood")
## [1] 0.271276</pre>
```

Cluster based on PB similarity

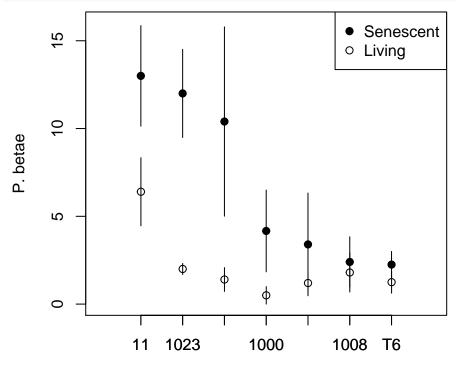
```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 10000
## adonis2(formula = vegdist(ls.pb) ~ ls.com.geno, permutations = 10000, sqrt.dist = TRUE, mrank = FALS
              Df SumOfSqs
                                      F Pr(>F)
                              R2
## ls.com.geno 6
                   2.5723 0.25429 1.5913 0.05549 .
## Residual
              28 7.5434 0.74571
## Total
              34 10.1158 1.00000
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

Modularity of bipartite networks

Plots

Main Results

- Genotypes differ in network structure on living leaves
- Response to PB is linked to senescence, more PB higher prob(senscence)



Tree Genotype

```
coord <- ch.plot(nmds.min(pb.ord), g = ls.com.geno, mu.pch = 19, pt.col = "white", cex = 4)
## Minimum stress for given dimensionality: 0.05143438
## r^2 for minimum stress configuration: 0.9805986

text(coord, labels = rownames(coord))
plot(envfit(nmds.min(pb.ord), ls.pb))

## Minimum stress for given dimensionality: 0.05143438
## r^2 for minimum stress configuration: 0.9805986

## Warning in cor(H, Pw): the standard deviation is zero

## Warning in cor(Hperm, take): the standard deviation is zero

## Warning in cor(Hperm, take): the standard deviation is zero

## Warning in cor(Hperm, take): the standard deviation is zero

## Warning in cor(Hperm, take): the standard deviation is zero

## Warning in cor(Hperm, take): the standard deviation is zero

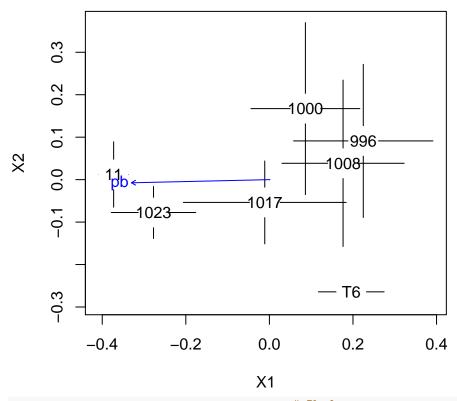
## Warning in cor(Hperm, take): the standard deviation is zero

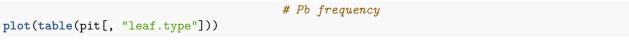
## Warning in cor(Hperm, take): the standard deviation is zero

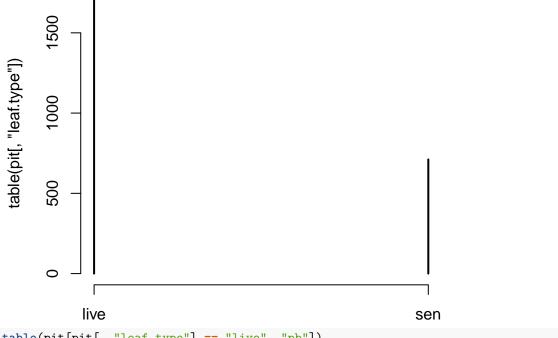
## Warning in cor(Hperm, take): the standard deviation is zero

## Warning in cor(Hperm, take): the standard deviation is zero</pre>
```

```
## Warning in cor(Hperm, take): the standard deviation is zero
## Warning in cor(Hperm, take): the standard deviation is zero
## Warning in cor(Hperm, take): the standard deviation is zero
## Warning in cor(Hperm, take): the standard deviation is zero
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## Warning in cor(Hperm, take): the standard deviation is zero
## Warning in cor(Hperm, take): the standard deviation is zero
## Warning in cor(Hperm, take): the standard deviation is zero
## Warning in cor(Hperm, take): the standard deviation is zero
## Warning in cor(Hperm, take): the standard deviation is zero
## Warning in cor(Hperm, take): the standard deviation is zero
## Warning in arrows(at[1], at[2], vect[, 1], vect[, 2], len = 0.05, col =
## col): zero-length arrow is of indeterminate angle and so skipped
```







```
table(pit[pit[, "leaf.type"] == "live", "pb"])
##
```

0 1 2 3 ## 1682 65 2 1

Need to fix the following:

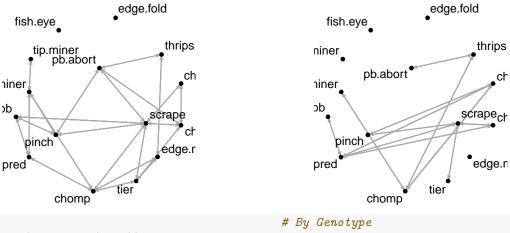
Need to fix the following:

Network Plots

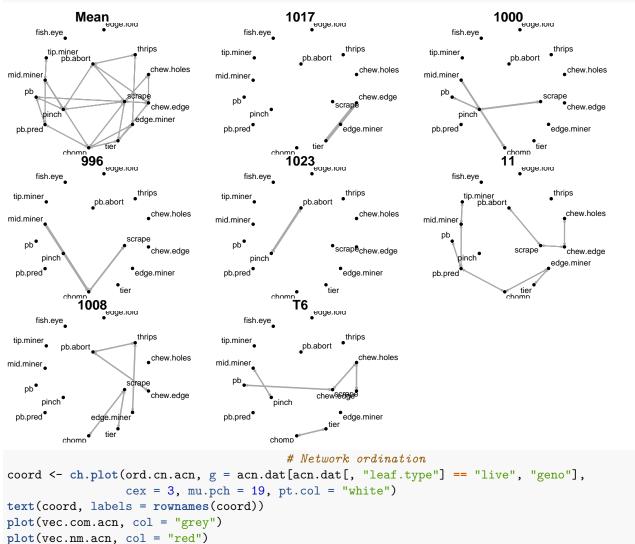
```
# Live versus Sen
par(mfrow = c(1, 2))
set.seed(12234)
net.col <- sign(netMean(cn.acn))</pre>
net.col[net.col == -1] <- "red"</pre>
net.col[net.col == "1"] <- "darkgrey"</pre>
coord <- gplot(abs(netMean(cn.acn[acn.dat[, "leaf.type"] == "live"])),</pre>
                gmode = "digraph",
                displaylabels = TRUE,
                edge.lwd = (abs(netMean(cn.acn[acn.dat[, "leaf.type"] == "live"]))) * 10,
                edge.col = net.col,
                vertex.col = "black",
                vertex.cex = 0.5,
                arrowhead.cex = 0.5,
                label.cex = 0.75,
                main = "Live")
```

Live

Senescent



```
par(mfrow = c(1, 2))
set.seed(12234)
net.col <- sign(netMean(cn.acn))</pre>
net.col[net.col == -1] <- 2
net.col[net.col == 1] <- "darkgrey"</pre>
# pdf(file = "../results/acn_live_nets.pdf", width = 9, height = 9)
par(mfrow = c(3, 3), mar = c(0, 0, 1, 0))
coord <- gplot(abs(netMean(cn.acn[acn.dat[, "leaf.type"] == "live"])),</pre>
               gmode = "digraph",
               displaylabels = TRUE,
               edge.lwd = (abs(netMean(cn.acn[acn.dat[, "leaf.type"] == "live"]))) * 10,
               edge.col = net.col,
               vertex.col = "black",
               vertex.cex = 0.5,
               arrowhead.cex = 0.5,
               label.cex = 0.75,
               main = "Mean")
for (i in unique(acn.dat[, "geno"])){
    gplot(abs(netMean(cn.acn[acn.dat[, "geno"] == i &
                                  acn.dat[, "leaf.type"] == "live"])),
```



Bipartite representation

```
plotModuleWeb(gsc)
```

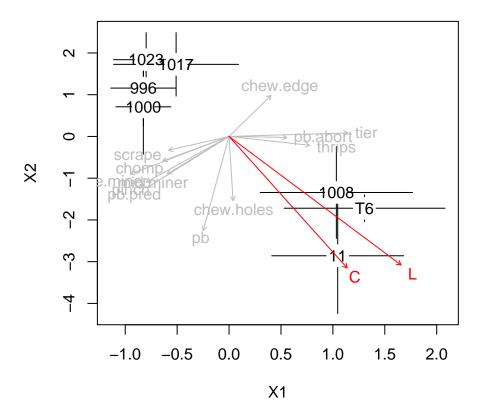
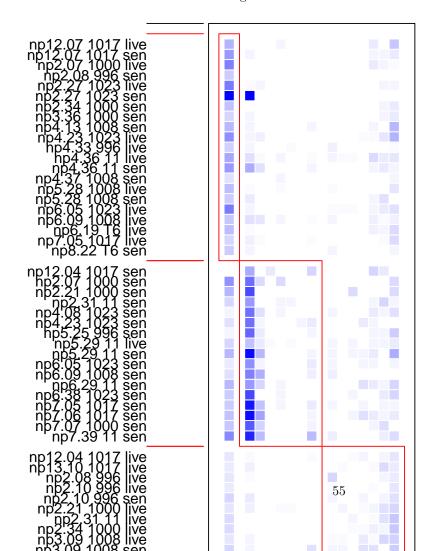


Figure 1: Network distance ordination



Library call script

```
libs <- c("ComGenR", "coNet")
if (!(all(libs %in% installed.packages()[, 1]))){
    devtools::install_github("ECGen/ComGenR")
    devtools::install_github("ECGen/coNet", ref = "master")
}
sapply(libs, require, character.only = TRUE, quietly = TRUE)</pre>
```

Tables

%latex table generated in R3.5.2 by x
table 1.8-2 package % Fri Feb2214:15:24
 2019

	r	pvals
pb	0.374	0.001
chew.holes	0.172	0.060
pb.pred	0.151	0.079
pinch	0.133	0.105
edge.miner	0.125	0.125
tier	0.096	0.200
mid.miner	0.084	0.232
chew.edge	0.082	0.246
chomp	0.055	0.410
thrips	0.047	0.482
scrape	0.032	0.575
pb.abort	0.022	0.721

Table 1: Table of leaf modifier vector analyses.