Coevolution with a seed bank

26 August, 2022

Analyze composition of mutations from pooled population sequencing

Setup Work Environment

```
# Load dependencies
library(here)
```

```
## here() starts at C:/Users/danschw/GitHub/coevolution/coevo-seedbank-seq
```

```
library(tidyverse)
```

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.1 --

## v ggplot2 3.3.5     v purrr   0.3.4
## v tibble  3.1.6     v dplyr   1.0.8
## v tidyr   1.2.0     v stringr 1.4.0
## v readr   2.1.2     v forcats 0.5.1

## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
library(vegan)
```

```
## Loading required package: permute

## Loading required package: lattice

## This is vegan 2.6-2
```

```
library(BiodiversityR)
```

```
## Loading required package: tcltk

## BiodiversityR 2.14-3: Use command BiodiversityRGUI() to launch the Graphical User Interface;
## to see changes use BiodiversityRGUI(changeLog=TRUE, backward.compatibility.messages=TRUE)
```

Load data

Matrix of multiplicity data organized as population X gene.

```
mutdat <- read_csv(here("data/mult_phage.csv")) %>%
  rename(trt = 1)
```

```
## New names:
## * `` -> ...1
```

```
## Rows: 6 Columns: 21
## -- Column specification --------------------------------------------------------
## Delimiter: ","
## chr  (1): ...1
## dbl (20): SPO1_6, SPO1_8, SPO1_15, SPO1_30, SPO1_39, SPO1_53, SPO1_74, SPO1_...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

PCoA procedures

```
# Define treatments and data
seed <- str_detect(mutdat$trt, "long")

# multiplicity data only
mut <- mutdat %>% select(-trt)


# Calculate pairwise distances
mut.dist <- vegdist(mut, method = "bray", binary = "FALSE")


# Principal Coordinates Analysis (PCoA)
pc <- cmdscale(mut.dist, eig = TRUE, k = nrow(mut)-1)
```

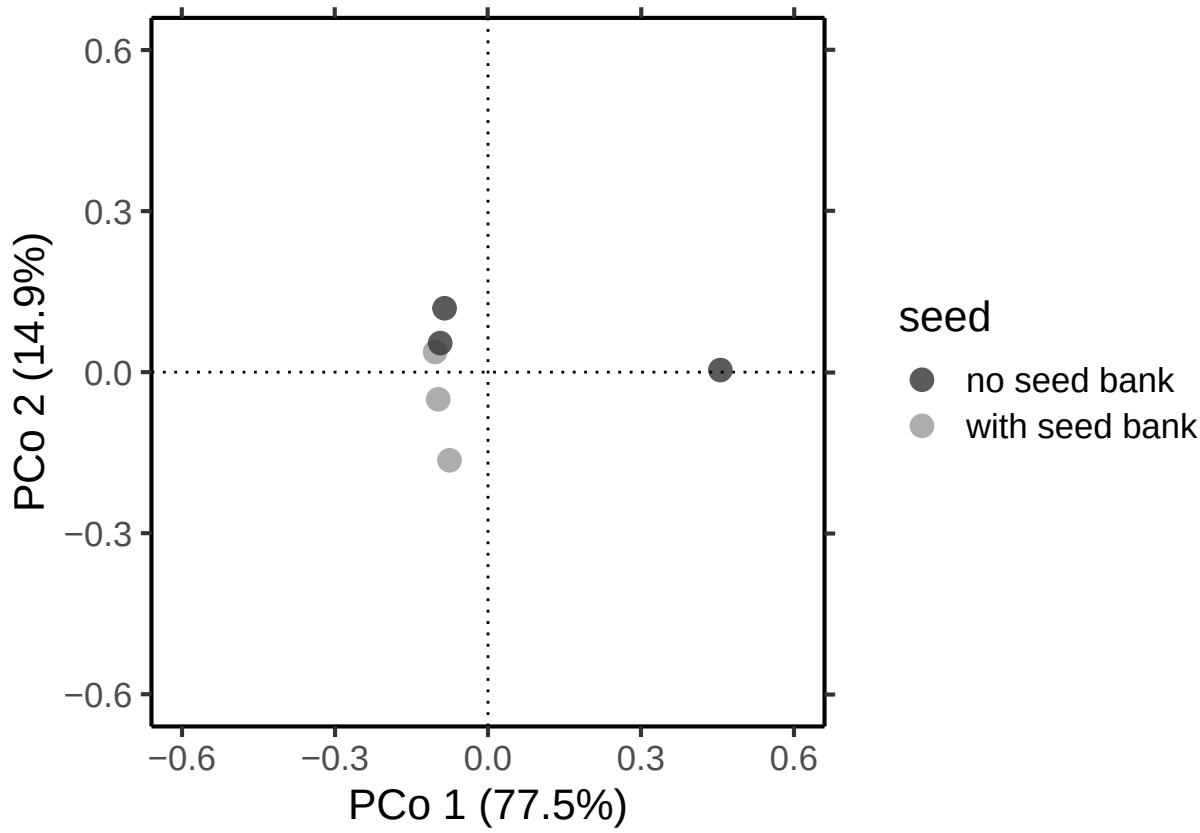
```
## Warning in cmdscale(mut.dist, eig = TRUE, k = nrow(mut) - 1): only 4 of the
## first 5 eigenvalues are > 0
```

```
explainvar1 <- round(pc$eig[1] / sum(pc$eig), 3) * 100
explainvar2 <- round(pc$eig[2] / sum(pc$eig), 3) * 100
explainvar3 <- round(pc$eig[3] / sum(pc$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)



p <-
  as_tibble(pc$points, .name_repair = "universal" ) %>%
  rename_with(~gsub("...", "PCoA", .x, fixed = TRUE)) %>%
  mutate(seed = seed) %>%
```

```
  relocate(seed,.before = 1) %>%
    mutate(seed = if_else(seed==1, "with seed bank", "no seed bank") ) %>%
  ggplot(aes(x=PCoA1,y=PCoA2)) +
  geom_point(aes(color = seed, fill = seed), size=3, stroke=1,alpha=0.8)+
  # geom_polygon(data = dl, linetype = 3 ,fill="transparent",
  #              aes(x=x, y =y, group = interaction(seed, phage),color = seed))+
  theme_bw(base_size=32) +
  labs(x=paste0("PCo 1 (",round(explainvar1,1),"%)"),
       y=paste0("PCo 2 (",round(explainvar2,1),"%)")) +
  geom_hline(yintercept = 0, linetype = 3)+
  geom_vline(xintercept = 0, linetype = 3)+
  scale_shape_manual(values = c(21,24))+
  scale_fill_grey(end = 0.8)+
  scale_color_grey(end = 0.6)+
  scale_x_continuous(sec.axis = dup_axis(name = NULL, labels = NULL),
                     limits = c(-0.6,0.6)) +
  scale_y_continuous(sec.axis = dup_axis(name = NULL, labels = NULL),
                     limits = c(-0.6,0.6))+
  theme_classic(base_size = 16)
```

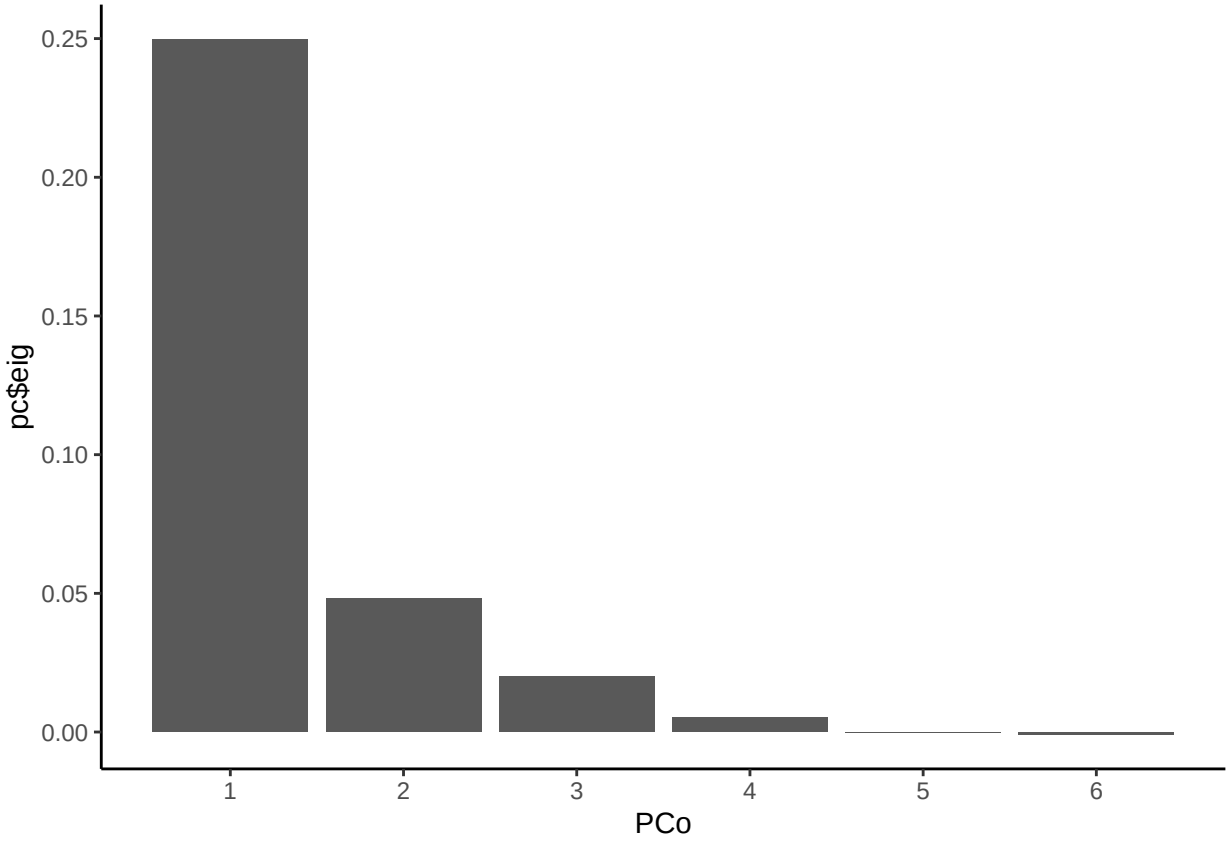
```
## New names:
## * `` -> ...1
## * `` -> ...2
## * `` -> ...3
## * `` -> ...4
```

```
ggsave(here("analysis","PCoA_mult_phage.png"),p, width = 5, height = 3)

p
```



There are negative eigenvalues

```
qplot(1:6,pc$eig, geom = "col")+
  theme_classic()+
  scale_x_continuous(breaks = 1:6)+
  xlab("PCo")
```

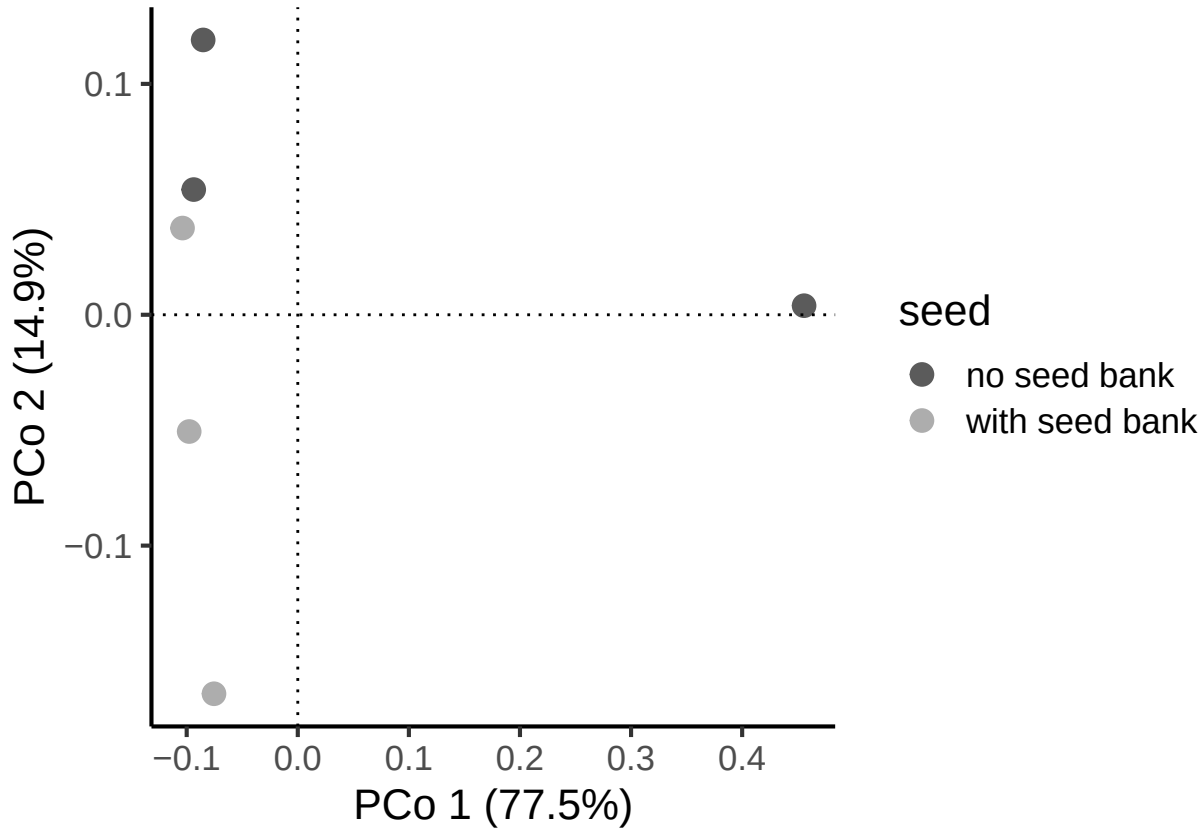



```
library(ape)


# Principal Coordinates Analysis (PCoA)
pc.ape <-  pcoa(mut.dist, correction = "none")
pc.ape.corct <-  pcoa(mut.dist, correction = "lingoes")

tibble(PCo = 1:6,
       cmdscale = pc$eig,
       ape = pc.ape$values$Eigenvalues,
       ape_corrected = pc.ape.corct$values$Eigenvalues)
```

```
## # A tibble: 6 x 4
##     PCo  cmdscale       ape ape_corrected
##   <int>     <dbl>     <dbl>         <dbl>
## 1     1  2.50e- 1  0.250         0.250
## 2     2  4.80e- 2  0.0480        0.0480
## 3     3  2.00e- 2  0.0200        0.0200
## 4     4  5.22e- 3  0.00522       0.00522
## 5     5 -4.55e-18  0             0
## 6     6 -7.67e- 4 -0.000767     -0.000767
```

```
# ape pcoa has only one single negative, and small value
```

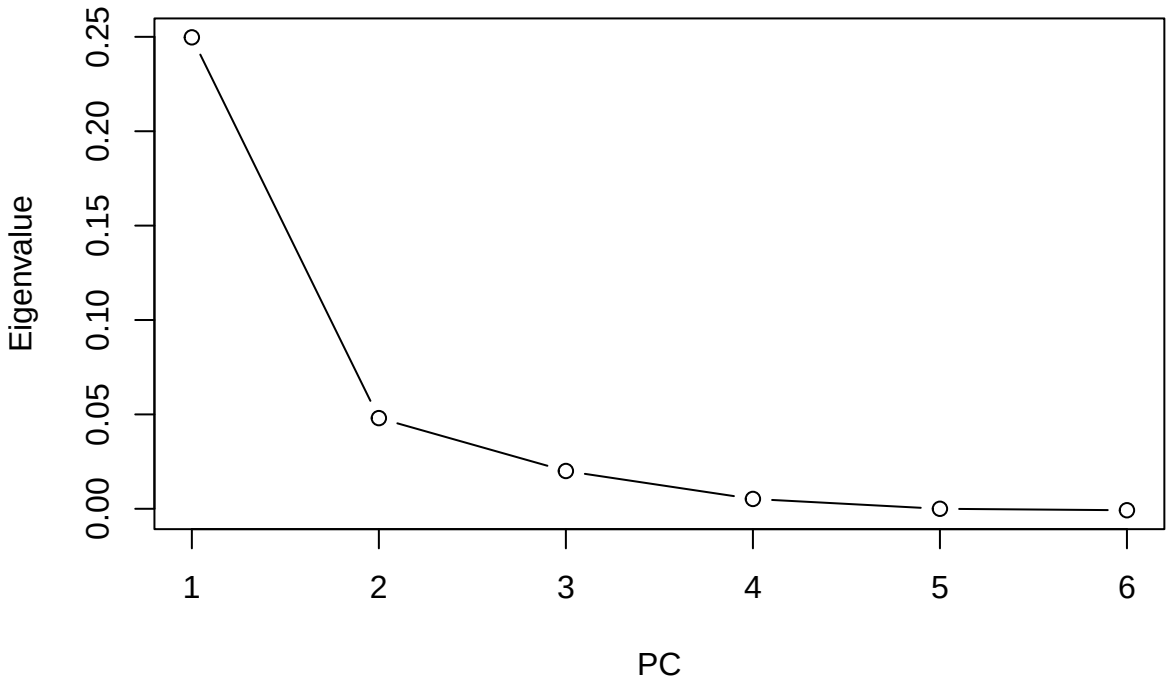
```r
explainvar1.ape <-
  round(pc.ape.corct$values$Eigenvalues[1] / sum(pc.ape.corct$values$Eigenvalues), 3) * 100
explainvar2.ape <-
  round(pc.ape.corct$values$Eigenvalues[2] / sum(pc.ape.corct$values$Eigenvalues), 3) * 100
explainvar3.ape <-
  round(pc.ape.corct$values$Eigenvalues[3] / sum(pc.ape.corct$values$Eigenvalues), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)

p <-
  as_tibble(pc.ape.corct$vectors, .name_repair = "universal" ) %>%
  rename_with(~gsub("Axis.", "PCoA", .x, fixed = TRUE)) %>%
  mutate(seed = seed) %>%
  relocate(seed,.before = 1) %>%
    mutate(seed = if_else(seed==1, "with seed bank", "no seed bank") ) %>%
  ggplot(aes(x=PCoA1,y=PCoA2)) +
  geom_point(aes(color = seed, fill = seed), size=3, stroke=1,alpha=0.8)+
  # geom_polygon(data = dl, linetype = 3 ,fill="transparent",
  #              aes(x=x, y =y, group = interaction(seed, phage),color = seed))+
  theme_bw(base_size=32) +
  labs(x=paste0("PCo 1 (",round(explainvar1,1),"%)"),
       y=paste0("PCo 2 (",round(explainvar2,1),"%)")) +
  geom_hline(yintercept = 0, linetype = 3)+
  geom_vline(xintercept = 0, linetype = 3)+
  scale_shape_manual(values = c(21,24))+
  scale_fill_grey(end = 0.8)+
  scale_color_grey(end = 0.6)+
  # scale_x_continuous(sec.axis = dup_axis(name = NULL, labels = NULL),
  #                    limits = c(-0.4,0.4)) +
  # scale_y_continuous(sec.axis = dup_axis(name = NULL, labels = NULL),
  #                    limits = c(-0.4,0.4))+
  theme_classic(base_size = 16)

# ggsave(here("analysis","PCoA_mult_phage.png"),p, width = 5, height = 3)

p
```



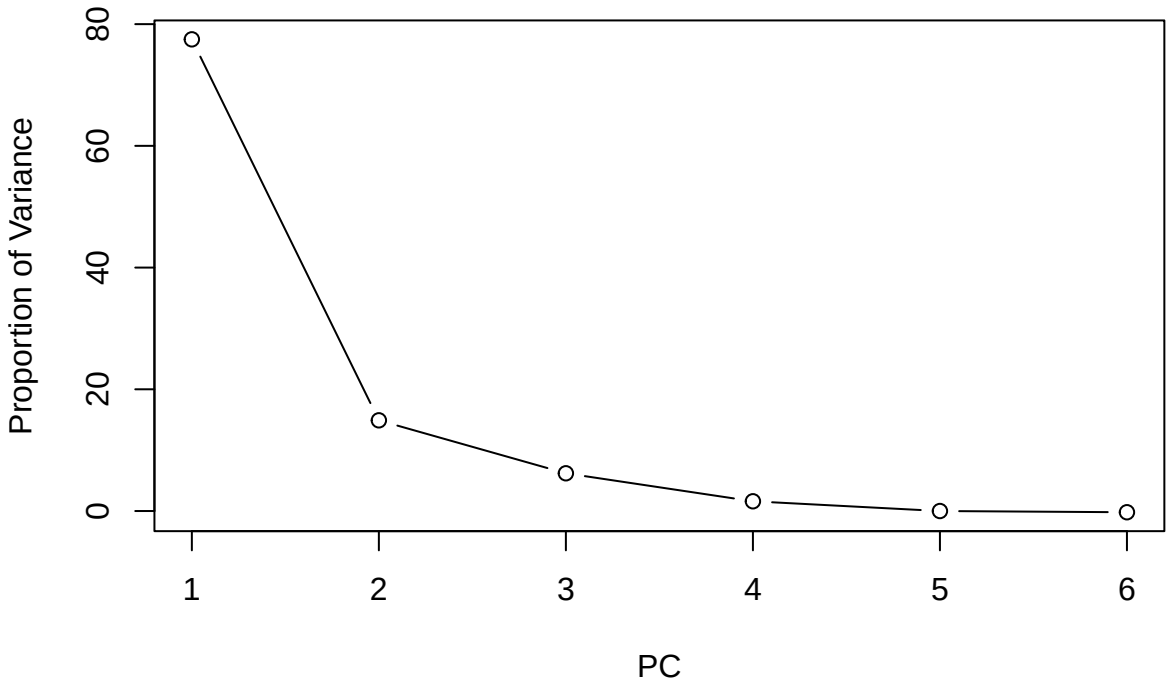
Using APE pcoa with correction does not change anything. Defaulting back to the same procedure used for host.

How many PCs to include in stats?

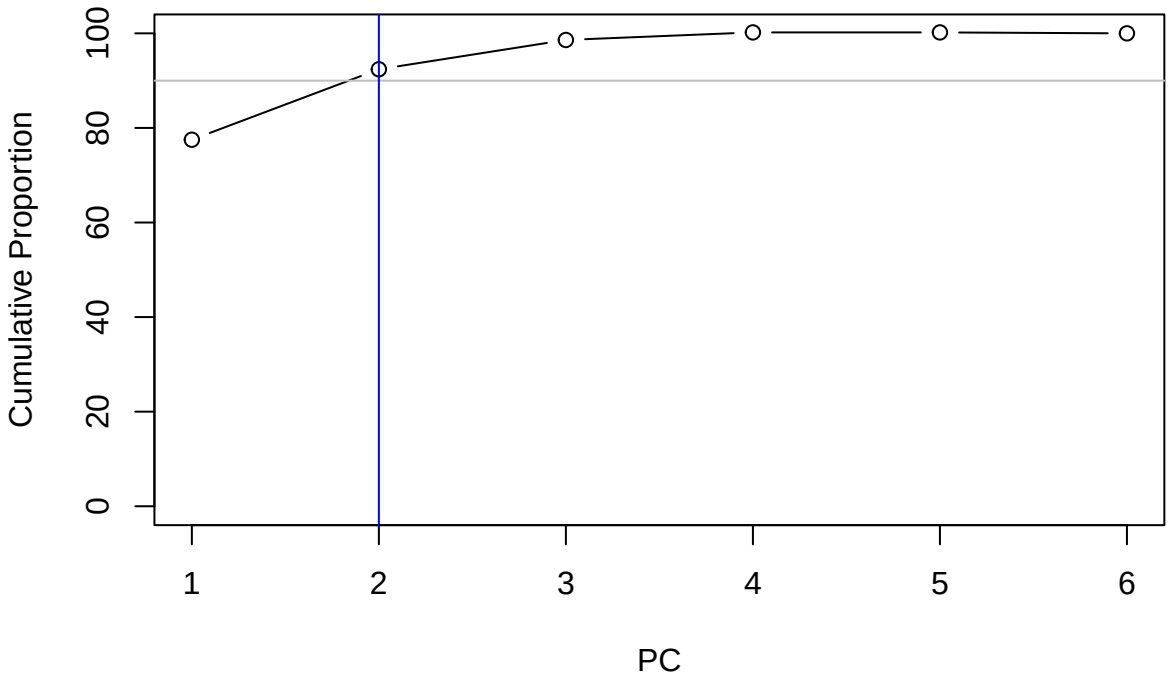
```
plot(1:length(pc$eig),pc$eig,type = "b",
     xlab = "PC", ylab = "Eigenvalue")
```




```
prop_var <- round(pc$eig / sum(pc$eig), 3) * 100
plot(1:length(pc$eig),prop_var, type = "b",
     xlab = "PC", ylab = "Proportion of Variance")
```




```
# PCs explaining 90% variation
pc_var90 <- min(which(cumsum(prop_var)>90))
plot(1:length(pc$eig),cumsum(prop_var), type = "b",
     xlab = "PC", ylab = "Cumulative Proportion", ylim = c(0,100))
abline(v=pc_var90,h=90, col = c("grey", "blue"))
```



First 2 PCs explain >90% of the variartion

PERMANOVA

```
# # on multiplicity data
# perm <-
#   adonis2(mut.dist ~ seed * phage,
#           binary = FALSE, permutations = 9999)

# on PCs explaining >90% var
perm <-
  adonis2(pc$points[,1:pc_var90] ~ seed ,method = "euclidean",
          binary = FALSE, permutations = 9999)
```

```
## 'nperm' >= set of all permutations: complete enumeration.
```

```
## Set of permutations < 'minperm'. Generating entire set.
```

```
perm
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
```

```
## Permutation: free
## Number of permutations: 719
##
## adonis2(formula = pc$points[, 1:pc_var90] ~ seed, permutations = 9999, method = "euclidean", binary =
##          Df SumOfSqs      R2      F Pr(>F)
## seed      1 0.072048 0.24196 1.2768    0.2
## Residual  4 0.225718 0.75804
## Total     5 0.297766 1.00000
```

Gene correlations

```
# Phage annotation ------------------------------------------------------
d.spo1 <-
  read_delim(here("data/SPO1-ANC_no_fasta.gff3"),
             col_names = c(letters[1:9]),
             trim_ws = T,
             comment = "#") %>%
  select(seqname =a, feature =c, start = d, end = e,
         strand = g, frame = h, attribute =i)
```

```
## Rows: 425 Columns: 9
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr (7): a, b, c, f, g, h, i
## dbl (2): d, e
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
d.spo1_genes <-
  d.spo1 %>%
  filter(feature == "gene") %>%
  separate(attribute, into = c("Alias", "ID", "Name", "Pseudo"), sep = ";") %>%
  mutate(Alias = str_remove(Alias, ".*="),
         ID = str_remove(ID, ".*="),
         Name = str_remove(Name, ".*="),
         Pseudo = str_remove(Pseudo, ".*="))
```

```
## Warning: Expected 4 pieces. Missing pieces filled with `NA` in 208 rows [1, 2,
## 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, ...].
```

```
# Test correlation to PCoA axes ----------------------------------------
gene.corr <- add.spec.scores(pc, mut, method = "cor.scores")$cproj
gene.corr <-
  tibble(gene = rownames(gene.corr)) %>%
  bind_cols(as_tibble(gene.corr ))

# Genes correlated with PCo1 -------------------------------

fit <- envfit(pc, mut, choices=1, perm = 999)
```

```
## 'nperm' >= set of all permutations: complete enumeration.
## Set of permutations < 'minperm'. Generating entire set.
## 'nperm' >= set of all permutations: complete enumeration.
## Set of permutations < 'minperm'. Generating entire set.
```

```
d.fit1 <- tibble(gene = names(fit$vectors$r),
       r = fit$vectors$r,
       pvals = fit$vectors$pvals)

# combine with Correlation for significant genes
sig_genes1 <- d.fit1 %>%
  filter(pvals<0.05) %>%
  left_join(., gene.corr %>% select(gene, cor = Dim1))
```

```
## Joining, by = "gene"
```

```
# add annotations
sig_genes1 <- d.spo1_genes %>%
  select(Alias, Name, strand) %>%
  left_join(sig_genes1, ., by = c("gene" = "Alias"))

# export positively correlated
sig_genes1 %>%
  filter(cor > 0) %>%
  arrange(desc(abs(cor))) %>%
  rename(P_value = pvals) %>%   write_csv(here("data", "significant_PHAGE_genes_pc1_positive.csv"))

# export negatively correlated
sig_genes1 %>%
  filter(cor < 0) %>%
  arrange(desc(abs(cor))) %>%
    rename(P_value = pvals) %>%
  write_csv(here("data", "significant_PHAGE_genes_pc1_negative.csv"))


# Genes correlated wit PCo2 ------------------------------------------
fit <- envfit(pc, mut, choices=2, perm = 999)
```

```
## 'nperm' >= set of all permutations: complete enumeration.
## Set of permutations < 'minperm'. Generating entire set.
## 'nperm' >= set of all permutations: complete enumeration.
## Set of permutations < 'minperm'. Generating entire set.
```

```
d.fit2 <- tibble(gene = names(fit$vectors$r),
       r = fit$vectors$r,
       pvals = fit$vectors$pvals)

# Correlation of significant genes
sig_genes2 <- d.fit2 %>%
  filter(pvals<0.05) %>%
  left_join(., gene.corr %>% select(gene, cor = Dim2))
```

```
## Joining, by = "gene"
```

```
# none significant
```

Ellipses

The ggplot function of stat_ellipse does not allow CI ellipses on less than 4 data points. We have three points per treatment. However three points should be alowed "because your CI depends on the variance, which takes two degrees of freedom".

According to stat_ellipses help "The method for calculating the ellipses has been modified from car::dataEllipse (Fox and Weisberg, 2011)". The limit on 3 points does not exist in the original function.

```
library(car)
```

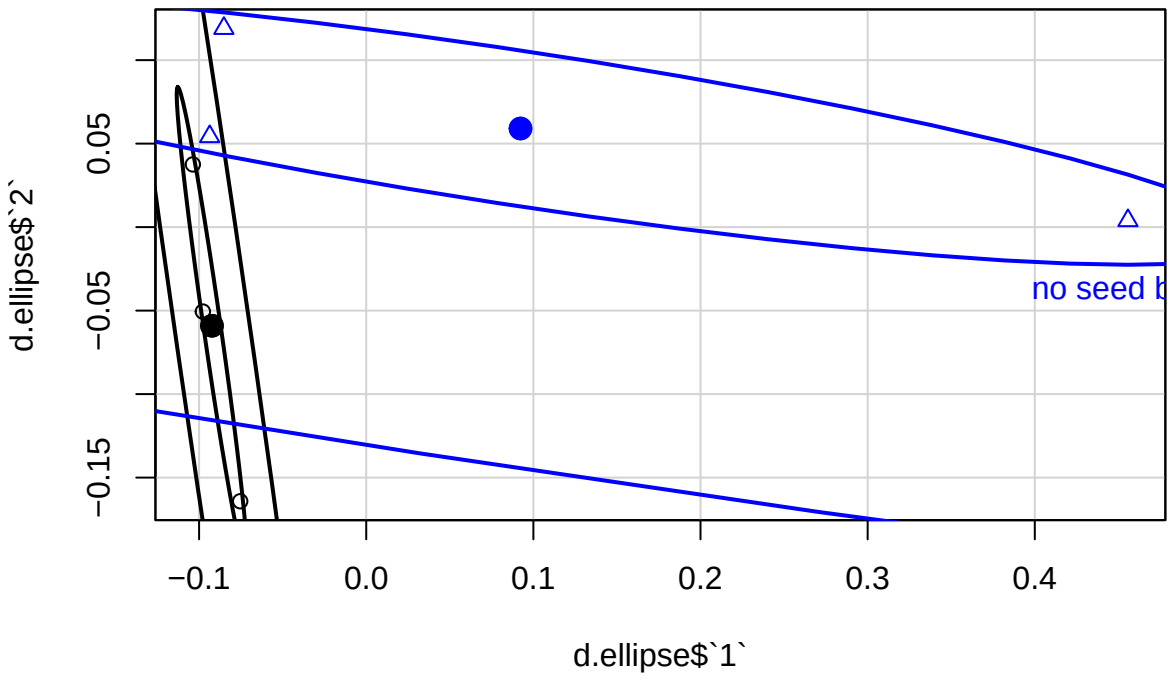
```
## Loading required package: carData
```

```
##
## Attaching package: 'car'
```

```
## The following object is masked from 'package:dplyr':
##
##     recode
```

```
## The following object is masked from 'package:purrr':
##
##     some
```

```
d.ellipse <-  cbind(mut[,1:2],pc$points) %>%
  as.data.frame %>%
  mutate(seed = if_else(seed, "with seed bank", "no seed bank") %>% as_factor())

el <- dataEllipse(d.ellipse$`1`, d.ellipse$`2`, groups = d.ellipse$seed)
```




```
# unpack list
dl <- rbind(
cbind("with seed bank",el$`with seed bank`$`0.95`),
cbind("no seed bank",el$`no seed bank`$`0.95`)
)


dl <- dl %>%
  as_tibble() %>%
  mutate(x= as.numeric(x), y=as.numeric(y)) %>%
  rename(seed = V1)
```

```
## Warning: The `x` argument of `as_tibble.matrix()` must have unique column names if `.name_repair` is
## Using compatibility `.name_repair`.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was generated.
```

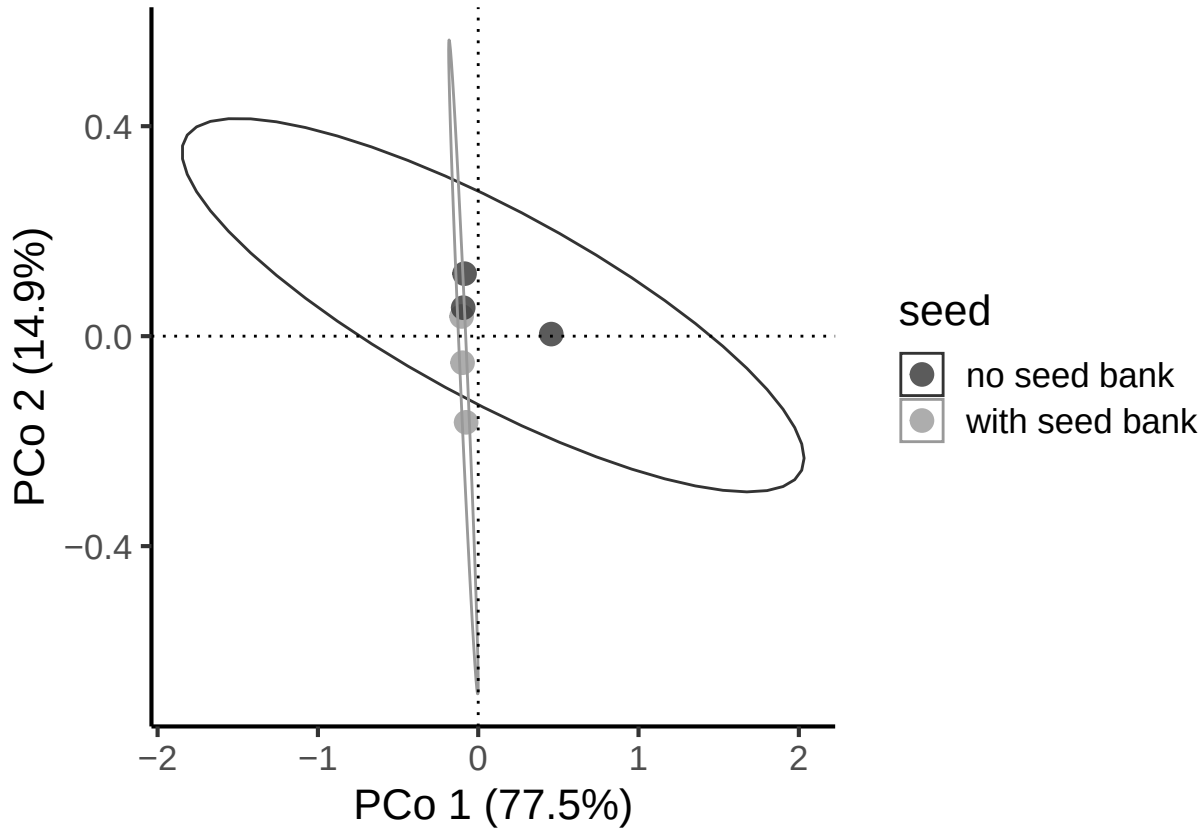
PCA with ellipses

```
p <-
  as_tibble(pc$points, .name_repair = "universal" ) %>%
  rename_with(~gsub("...", "PCoA", .x, fixed = TRUE)) %>%
  mutate(seed = seed) %>%
```

```
  relocate(seed,.before = 1) %>%
    mutate(seed = if_else(seed==1, "with seed bank", "no seed bank") ) %>%
  ggplot(aes(x=PCoA1,y=PCoA2)) +
  geom_point(aes(color = seed, fill = seed), size=3, stroke=1,alpha=0.8)+
  geom_polygon(data = dl, linetype = 1 ,fill="transparent",
               aes(x=x, y =y,color = seed))+
  theme_bw(base_size=32) +
  labs(x=paste0("PCo 1 (",round(explainvar1,1),"%)"),
       y=paste0("PCo 2 (",round(explainvar2,1),"%)")) +
  geom_hline(yintercept = 0, linetype = 3)+
  geom_vline(xintercept = 0, linetype = 3)+
  scale_shape_manual(values = c(21,24))+
  scale_fill_grey(end = 0.8)+
  scale_color_grey(end = 0.6)+
  # scale_x_continuous(sec.axis = dup_axis(name = NULL, labels = NULL),
  #                    limits = c(-0.4,0.4)) +
  # scale_y_continuous(sec.axis = dup_axis(name = NULL, labels = NULL),
  #                    limits = c(-0.4,0.4))+
  theme_classic(base_size = 16)
```

```
## New names:
## * `` -> ...1
## * `` -> ...2
## * `` -> ...3
## * `` -> ...4
```

```
p
```


0.4
0.0
−0.4
PCo 2 (14.9%)
−2
−1
0
1
2
PCo 1 (77.5%)
seed
no seed bank
with seed bank