Figures

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Contents

This file includes the R code used to create Figures in Beier et al. 2022 **(doi:XXX)**. The input files are either online available or were created as detailed in scripts_genomic.traits.md and scripts_phylogenetic.signal.md.

1 Packages

```
rm(list = ls())
library(tidyverse)
library(vegan)
library(PCAtest)
library("PerformanceAnalytics")
library(treemap)
```

```r
library(data.tree)
library(networkD3)
```

2 Functions

```r
# panel.smooth.mod
## The function panel.smooth.mod is delineated from the
## function panel.smooth {graphics} with the setting 'lwd=3' added
panel.smooth.mod <- function (x, y, col = par("col"), bg = NA, pch = par("pch"),
                          cex = 1, col.smooth = 2, span = 2/3, iter = 3, ...)
{
  points(x, y, pch = pch, col = col, bg = bg, cex = cex)
  ok <- is.finite(x) & is.finite(y)
  if (any(ok))
    lines(stats::lowess(x[ok], y[ok], f = span, iter = iter),
          col = col.smooth, lwd=3, ...) #add lwd=3
}

# chart.Correlation.mod
## The function chart.Correlation.mod is delineated from the
## function chart.Correlation {PerformanceAnalytics}
## with some graphical modifications.
## Lines in the original chart.Correlation that were ignored
## were deactivated with '#'.
## Comments indicate lines that were added or changed.
chart.Correlation.mod <-function (R, histogram = TRUE, method = c("pearson", "kendall",
                                                                  "spearman"), ...)
{
  x = checkData(R, method = "matrix")
  if (missing(method))
    method = method[1]
  cormeth <- method
  panel.cor <- function(x, y, digits = 2, prefix = "", use = "pairwise.complete.obs",
                        method = cormeth, cex.cor, col='red',...) {
    usr <- par("usr")
    on.exit(par(usr))
    par(usr = c(0, 1, 0, 1))
    r <- cor(x, y, use = use, method = method)
    txt <- format(c(r, 0.123456789), digits = digits)[1]
    txt <- paste(prefix, txt, sep = "")
    if (missing(cex.cor))
      cex <- 0.8/strwidth(txt)
    test <- cor.test(as.numeric(x), as.numeric(y), method = method)
    #Signif <- symnum(test$p.value, corr = FALSE, na = FALSE,
    #                 cutpoints = c(0, 0.001, 0.01, 0.05, 0.1, 1),
    #                 symbols = c("***","**", "*", ".", " "))
    text(0.5, 0.5, txt, cex = cex * (abs(r) + 0.3)/1.3)
    #text(0.8, 0.8, Signif, cex = cex, col = 2)
  }
  f <- function(t) {
    dnorm(t, mean = mean(x), sd = sd.xts(x))
  }
```

```
  dotargs <- list(...)
  dotargs$method <- NULL
  rm(method)
  hist.panel = function(x, ... = NULL) {
    par(new = TRUE)
    #hist(x, col = "light gray", probability = TRUE, axes = FALSE,
    #     main = "", breaks = "FD", col.main="red")

    #added line: replace original histogram
    hist(x, col = "light gray", probability = TRUE, axes = FALSE,
         main = "", breaks = "FD")
    #lines(density(x, na.rm = TRUE), col = "red", lwd = 1)
    #rug(x)
  }
  if (histogram)
    pairs(x, gap = 0, lower.panel = panel.smooth.mod, upper.panel = panel.cor,
          diag.panel = hist.panel,cex.axis=2) #add cex.axis
  #use panel.smooth.mod function with bolder trend line
  else pairs(x, gap = 0, lower.panel = panel.smooth.mod, upper.panel = panel.cor)
}

#unsigned.range
## The unsigned.range determines graphical limits (xlim, ylim) for PCA biplots
unsigned.range <- function(x) c(-abs(min(x, na.rm = TRUE)),abs(max(x, na.rm = TRUE)))
```

3 Load and format input data

Input files are Table S1, RRN values available via the Ribosomal RNA Operon Copy Number Database (rrnDB) as well as mantel correlogram output files created as detailed in scripts_phylogenetic.signal.md

```
# Load habitat information, GTDB taxonomy and trait values from Table S1
setwd("/Users/sara/Documents/R-scripts/GenomicTraits_Figures/")
gtraits <- tibble(read.table ("TableS1.tsv", header=T, sep='\t', fill=T)) %>%
  select(IMG.Genome.ID,Habitat,
         GTDB_division,GTDB_phylum,GTDB_class,GTDB_order,GTDB_family,GTDB_genus,
         GTDB_species, FASTANI_species,
         Genome.size,X.GC,RRN_IMG,RRN_rrnDB,X.HGT,CUB.F.,Generation.time..Vieira.Silva.,
         Generation.time..gRodon.,Gene.duplication,Gene.richness,X.TF,Prophages,
         Genome.size_read.based, RRN_read.based) %>% #

  #Genome.size values displayed as Mbp
  mutate(Genome.size= Genome.size/1000000)  %>%
  mutate(Genome.size_read.based= Genome.size_read.based/1000000)  %>% #

  #create column with  %HGT values larger 65% removed
  mutate(per.HGT.corr = ifelse(X.HGT < 65, X.HGT, NA))  %>% #

  #create column with Prophages >20 removed
  mutate(Prophages.corr = ifelse(Prophages < 20, Prophages, NA)) #
gtraits
```

```
## # A tibble: 17,856 x 26
##    IMG.Genome.ID Habitat         GTDB_division GTDB_phylum GTDB_class GTDB_order
```

```
##            <dbl> <chr>           <chr>         <chr>       <chr>      <chr>
##  1    2740891980 ""              Archaea       Altarchaeo~ Altarchae~ IMC4
##  2    2740891986 ""              Archaea       Asgardarch~ Lokiarcha~ Odinarcha~
##  3    2740891981 ""              Archaea       Asgardarch~ Thorarcha~ Thorarcha~
##  4    2528311132 "Aquatic, Mari~ Archaea       Halobacter~ Archaeogl~ Archaeogl~
##  5    2522125074 "Aquatic, Mari~ Archaea       Halobacter~ Archaeogl~ Archaeogl~
##  6     646311906 "Aquatic, Deep~ Archaea       Halobacter~ Archaeogl~ Archaeogl~
##  7    2504136002 "Aquatic, Deep~ Archaea       Halobacter~ Archaeogl~ Archaeogl~
##  8     646564534 "Aquatic, Fres~ Archaea       Halobacter~ Archaeogl~ Archaeogl~
##  9    2634166507 ""              Archaea       Halobacter~ Archaeogl~ Archaeogl~
## 10    2728369302 ""              Archaea       Halobacter~ Archaeogl~ JdFR-21
## # ... with 17,846 more rows, and 20 more variables: GTDB_family <chr>,
## #   GTDB_genus <chr>, GTDB_species <chr>, FASTANI_species <chr>,
## #   Genome.size <dbl>, X.GC <dbl>, RRN_IMG <int>, RRN_rrnDB <dbl>, X.HGT <dbl>,
## #   CUB.F. <dbl>, Generation.time..Vieira.Silva. <dbl>,
## #   Generation.time..gRodon. <dbl>, Gene.duplication <dbl>,
## #   Gene.richness <int>, X.TF <dbl>, Prophages <int>,
## #   Genome.size_read.based <dbl>, RRN_read.based <int>, per.HGT.corr <dbl>, ...
```

```
# Load rrnDB RRN trait data
rrnDB <- tibble(read.csv ("rrnDB-5.7.tsv", sep='\t', header=T, fill=T)[,c(1,7,12)])
rrnDB
```

```
## # A tibble: 20,642 x 3
##    Data.source.record.id RDP.taxa X16S.gene.count
##    <chr>                 <chr>              <int>
##  1 rrnDBv3-1403          ""                     4
##  2 rrnDBv3-1404          ""                     4
##  3 rrnDBv3-1405          ""                     4
##  4 rrnDBv3-1407          ""                     2
##  5 rrnDBv3-1408          ""                     2
##  6 rrnDBv3-1409          ""                    NA
##  7 rrnDBv3-1410          ""                     7
##  8 rrnDBv3-1411          ""                     7
##  9 rrnDBv3-1412          ""                     7
## 10 rrnDBv3-1413          ""                     7
## # ... with 20,632 more rows
```

```
# Load read based RRN trait data
#rrn_reads2 <- tibble(read.table ("TableS2_old.tsv", header=T, sep='\t', fill=T))
#rrn_reads2

#rrn_reads <- tibble(read.table ("TableS1.tsv", header=T, sep='\t', fill=T)) %>%
#  select(IMG.Genome.ID,runID,runID,
#         RRN_IMG,RRN_rrnDB,RRN_read.based,Genome.size,Genome.size_read.based,Genome.equivalents_read.b
#  filter(!is.na(runID))

# Load output data from mantel correlogram analyses (scripts_phylogenetic.signal.md)
mpm.2 <- read.table("mpm.2.tab", sep= '\t', header=T)
mpm.3 <- read.table("mpm.3.tab", sep= '\t', header=T)
mpm.4 <- read.table("mpm.rrn.tab", sep= '\t', header=T)
mpm.5 <- read.table("mpm.5.tab", sep= '\t', header=T)
mpm.6 <- read.table("mpm.6.tab", sep= '\t', header=T)
mpm.7 <- read.table("mpm.7.tab", sep= '\t', header=T)
mpm.8 <- read.table("mpm.8.tab", sep= '\t', header=T)
```

```
mpm.9 <- read.table("mpm.9.tab", sep= '\t', header=T)
mpm.10 <- read.table("mpm.10.tab", sep= '\t', header=T)
mpm.11<- read.table("mpm.11.tab", sep= '\t', header=T)
```

4 Schematic phylogentic tree (Figure S1)

```
phylo <- gtraits %>%
    select(GTDB_division, GTDB_phylum, GTDB_class, GTDB_order) %>%
    replace(is.na(.), "unclassified") %>%
    mutate(pathString = paste("Prokaryotes", GTDB_division, GTDB_phylum,
        GTDB_class, GTDB_order, sep = "/"))

Node <- as.Node(phylo)
useRtreeList <- ToListExplicit(Node, unname = TRUE)
radialNetwork(useRtreeList, fontSize = 5)
```

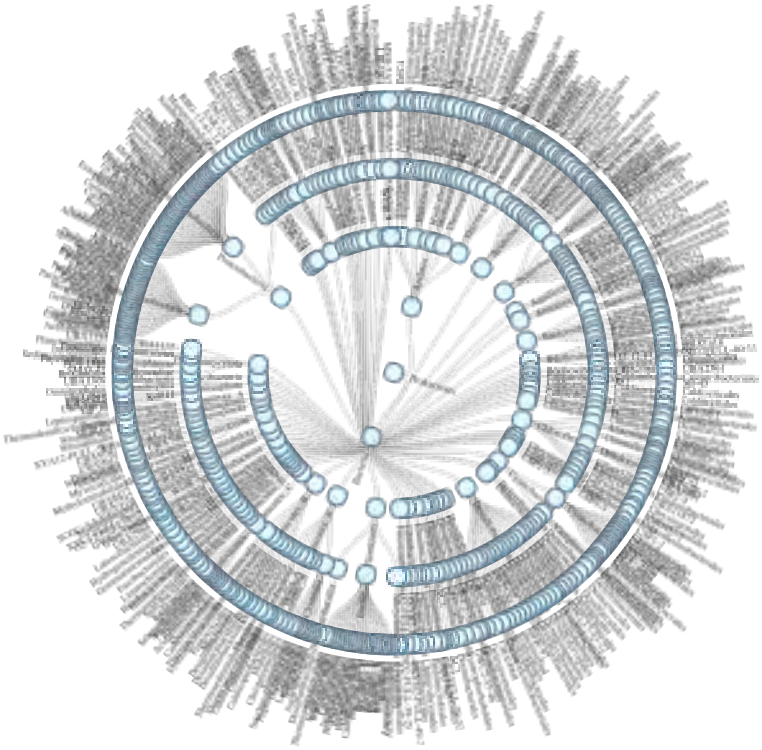


Figure S1 Schematic phylogenetic tree based on the GTDB taxonomy to visualize the phylogenetic coverage of the genome database used for our analyses. The tree displays from the outer to inner circles the order, class, phylum and division information, respectively.

5 Scatterplott RRN_IMG versus RRN_rrnDB and within genus variance of RRN_rrnDB (Figure S4)

```
# Prepare data set for RRN_IMG versus RRN_rrnDB scatterplot
dat <- gtraits %>%
  filter(RRN_IMG<20)%>% #remove rows with RRN_IMG < 20
  filter(!is.na(RRN_IMG)& !is.na(RRN_rrnDB)) #remove rows with NA in either RRN_IMG or RRN_rrnDB
dat
```

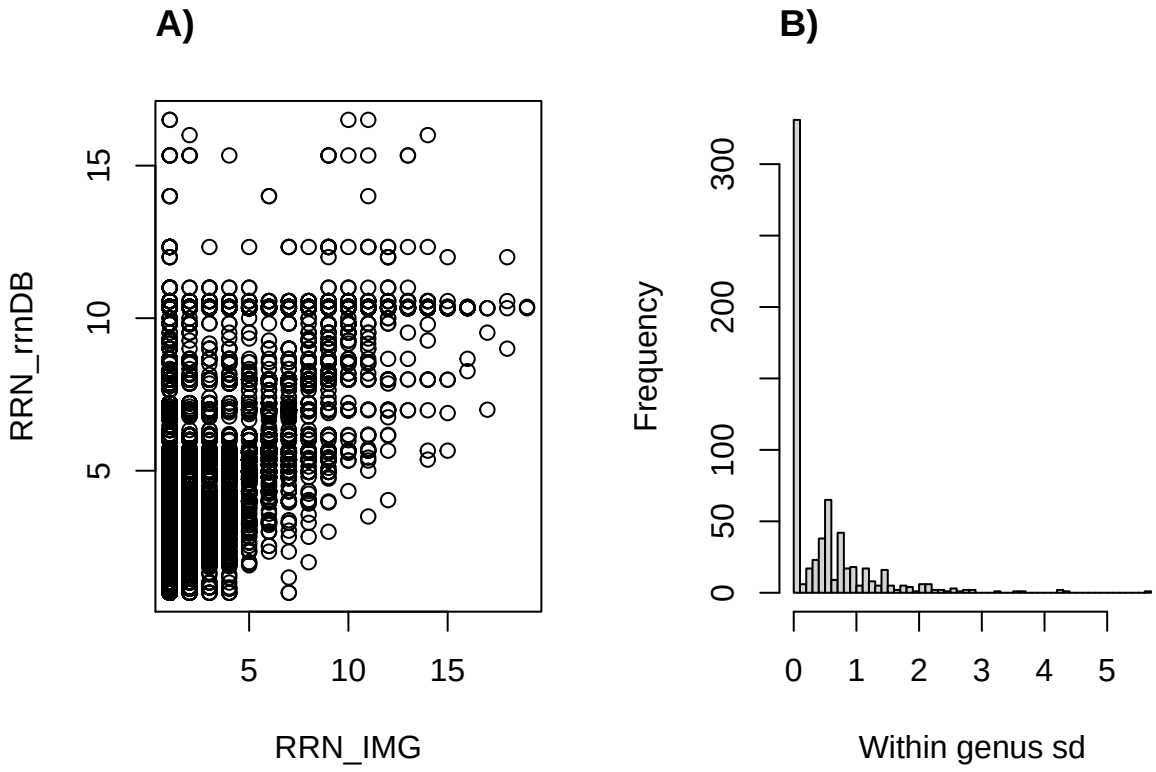
```
## # A tibble: 12,870 x 26
##    IMG.Genome.ID Habitat         GTDB_division GTDB_phylum GTDB_class GTDB_order
##            <dbl> <chr>           <chr>         <chr>       <chr>      <chr>
##  1    2528311132 "Aquatic, Mari~ Archaea       Halobacter~ Archaeogl~ Archaeogl~
##  2    2522125074 "Aquatic, Mari~ Archaea       Halobacter~ Archaeogl~ Archaeogl~
##  3     646311906 "Aquatic, Deep~ Archaea       Halobacter~ Archaeogl~ Archaeogl~
##  4    2504136002 "Aquatic, Deep~ Archaea       Halobacter~ Archaeogl~ Archaeogl~
##  5     646564534 "Aquatic, Fres~ Archaea       Halobacter~ Archaeogl~ Archaeogl~
##  6    2634166507 ""              Archaea       Halobacter~ Archaeogl~ Archaeogl~
##  7     648028029 "Fermented foo~ Archaea       Halobacter~ Halobacte~ Halobacte~
##  8    2528311097 "Fermented foo~ Archaea       Halobacter~ Halobacte~ Halobacte~
##  9    2684622629 ""              Archaea       Halobacter~ Halobacte~ Halobacte~
## 10    2630968753 ""              Archaea       Halobacter~ Halobacte~ Halobacte~
## # ... with 12,860 more rows, and 20 more variables: GTDB_family <chr>,
## #   GTDB_genus <chr>, GTDB_species <chr>, FASTANI_species <chr>,
## #   Genome.size <dbl>, X.GC <dbl>, RRN_IMG <int>, RRN_rrnDB <dbl>, X.HGT <dbl>,
## #   CUB.F. <dbl>, Generation.time..Vieira.Silva. <dbl>,
## #   Generation.time..gRodon. <dbl>, Gene.duplication <dbl>,
## #   Gene.richness <int>, X.TF <dbl>, Prophages <int>,
## #   Genome.size_read.based <dbl>, RRN_read.based <int>, per.HGT.corr <dbl>, ...
```

```
# Create data set containing within genus sd of rrnDB RRN values
rrnDB.genus.sd <- tibble(aggregate(. ~ RDP.taxa, data=rrnDB[grepl("(genus)",rrnDB$RDP.taxa),
                                                            c(2:3)], FUN=sd))
rrnDB.genus.sd
```

```
## # A tibble: 1,199 x 2
##    RDP.taxa                                      X16S.gene.count
##    <chr>                                                   <dbl>
##  1 "\"Candidatus Korarchaeum\" (genus)"                       NA
##  2 "Abiotrophia (genus)"                                      NA
##  3 "Acetoanaerobium (genus)"                                  NA
##  4 "Acetobacter (genus)"                                       0.575
##  5 "Acetobacterium (genus)"                                   NA
##  6 "Acetohalobium (genus)"                                    NA
##  7 "Acetothermia_genera_incertae_sedis (genus)"               NA
##  8 "Acholeplasma (genus)"                                      0.599
##  9 "Achromobacter (genus)"                                     0.502
## 10 "Acidaminococcus (genus)"                                   2.12
## # ... with 1,189 more rows
```

```
# Figure S1
par(mfrow=c(1,2))
plot(dat$RRN_IMG, dat$RRN_rrnDB, xlab='RRN_IMG',ylab='RRN_rrnDB')
title('A)', adj=0)
hist(rrnDB.genus.sd$X16S.gene.count, breaks=50, xlab="Within genus sd", main="")
```

```
title('B)', adj=0)
```




```
## pdf
##   2
```

Figure S4 A) The scatter plot of RRN_IMG versus RRN_rrnDB indicates that RRN values available via the JGI/IMG platform are often biased towards an underestimation of RRN counts that are available via the rrnDB. B) The histogram of withing genus standard deviations (sd) of RRN values from the rrnDB demonstrates a large majority of genera with sd<1.

6 Scatterplott RRN_IMG, RRN_rrnDB and IMG genome size against the corresponding read based values (Figure S5)

```
# Pearson correlation
c1 <- cor.test(dat$RRN_IMG, dat$RRN_read.based)
c2 <- cor.test(dat$RRN_rrnDB, dat$RRN_read.based)
c3 <- cor.test(dat$Genome.size, dat$Genome.size_read.based)

# Figure S5
par(mfrow = c(1, 3))
par(mgp = c(1.5, 0.4, 0))

plot(dat$RRN_IMG, dat$RRN_read.based, xlab = "RRN_IMG", ylab = "RRN_read.based")
title("A)", adj = 0, line = 0.7)
legend("topright", paste("r=", round(c1[[4]], 2), " p=", round(c1[[3]],
```

```
    2)), bty = "n")

plot(dat[, 14, drop = T], dat[, 24, drop = T], xlab = "RRN_rrnDB", ylab = "RRN_read.based")
title("B)", adj = 0, line = 0.7)
legend("topright", paste("r=", round(c2[[4]], 2), " p=", round(c2[[3]],
    3)), bty = "n")

plot(dat$Genome.size, dat$Genome.size_read.based, xlab = "Genome.size_IMG",
    ylab = "Genome.size_read.based", xlim = (c(0, max(na.omit(dat[, c(11,
        23)]))[, 2, drop = T])))
title("C)", adj = 0, line = 0.7)
legend("topright", paste("r=", round(c3[[4]], 2), " p=", round(c3[[3]],
    3)), bty = "n")
```

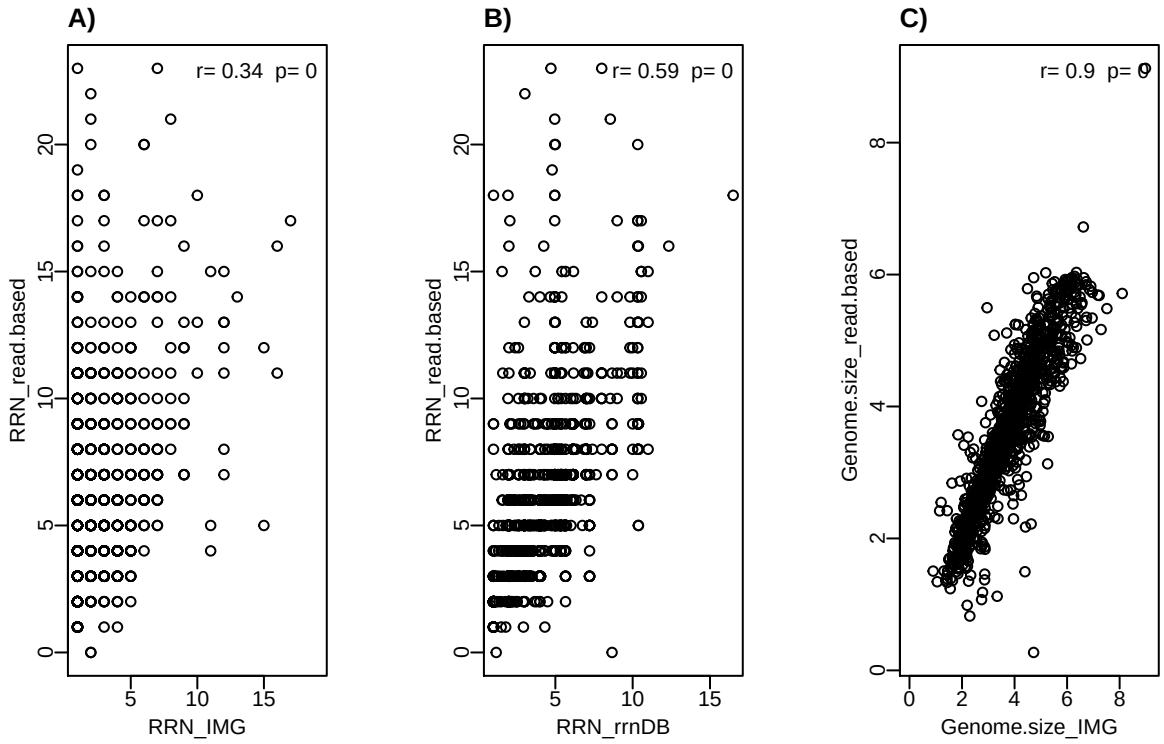


Figure S5: . A) Pearson correlation between the read based RRN estimate and the JGI RRN estimate. B) Pearson correlation between the read based RRN estimate and the rrnDB RRN estimate as given in Table S1. C) Pearson correlation between the read based genome size estimate and the genome size given in the JGI database.

```
## pdf
##   2
```

7 PCAs (global dataset)

7.1 PCA including 9 genomic traits as variables (Figure 3)

Trait data were averaged at the species level via the mean function to remove phylogentic redundancy in our data set (e.g. due to numerous close relatives affiliating with individual clinically relevant strains)

```
#Aggregate trait table at the species level
gtraits.sp <- gtraits %>%
  select(GTDB_division,GTDB_phylum,GTDB_class,GTDB_order,GTDB_family,GTDB_genus,
         GTDB_species, FASTANI_species,
         Genome.size,X.TF,Gene.duplication,Gene.richness,CUB.F.,RRN_rrnDB,Prophages.corr,
         per.HGT.corr,X.GC,Generation.time..Vieira.Silva.,Generation.time..gRodon.) %>%
  group_by(GTDB_division,GTDB_phylum, GTDB_class, GTDB_order, GTDB_family, GTDB_genus,
           GTDB_species , FASTANI_species) %>%
  summarise_at(vars(Genome.size:Generation.time..gRodon.), mean, na.rm = TRUE) %>%
  ungroup %>%
  mutate(log.HGT_per= log(per.HGT.corr+0.01))  %>% #log(x+0.01) data transformation
  mutate(log.d= log(Generation.time..Vieira.Silva.))  %>% #log(x) data transformation
  mutate(log.d.gRodon = log(Generation.time..gRodon.)) #log(x) data transformation
gtraits.sp
```

```
## # A tibble: 8,847 x 22
##    GTDB_division GTDB_phylum     GTDB_class    GTDB_order GTDB_family GTDB_genus
##    <chr>         <chr>           <chr>         <chr>      <chr>       <chr>
##  1 Archaea       Altarchaeota    Altarchaeia   IMC4       WOR-SM1-SCG WOR-SM1-S~
##  2 Archaea       Asgardarchaeota Lokiarchaeia~ Odinarcha~ LCB-4       LCB-4
##  3 Archaea       Asgardarchaeota Thorarchaeia  Thorarcha~ Thorarchae~ SMTZ1-83
##  4 Archaea       Halobacteriota  Archaeoglobi  Archaeogl~ Archaeoglo~ Archaeogl~
##  5 Archaea       Halobacteriota  Archaeoglobi  Archaeogl~ Archaeoglo~ Archaeogl~
##  6 Archaea       Halobacteriota  Archaeoglobi  Archaeogl~ Archaeoglo~ Archaeogl~
##  7 Archaea       Halobacteriota  Archaeoglobi  Archaeogl~ Archaeoglo~ Archaeogl~
##  8 Archaea       Halobacteriota  Archaeoglobi  Archaeogl~ Archaeoglo~ Ferroglob~
##  9 Archaea       Halobacteriota  Archaeoglobi  Archaeogl~ Archaeoglo~ Geoglobus
## 10 Archaea       Halobacteriota  Archaeoglobi  JdFR-21    JdFR-21     JdFR-21
## # ... with 8,837 more rows, and 16 more variables: GTDB_species <chr>,
## #   FASTANI_species <chr>, Genome.size <dbl>, X.TF <dbl>,
## #   Gene.duplication <dbl>, Gene.richness <dbl>, CUB.F. <dbl>, RRN_rrnDB <dbl>,
## #   Prophages.corr <dbl>, per.HGT.corr <dbl>, X.GC <dbl>,
## #   Generation.time..Vieira.Silva. <dbl>, Generation.time..gRodon. <dbl>,
## #   log.HGT_per <dbl>, log.d <dbl>, log.d.gRodon <dbl>
```

```
print(paste("#Nr phyla: ", length(unique(gtraits.sp$GTDB_phylum))))
```

```
## [1] "#Nr phyla:  73"
```

```
print(paste("#Nr classes: ", length(unique(gtraits.sp$GTDB_class))))
```

```
## [1] "#Nr classes:  172"
```

```
print(paste("#Nr orders: ", length(unique(gtraits.sp$GTDB_order))))
```

```
## [1] "#Nr orders:  382"
```

```
print(paste("#Nr families: ", length(unique(gtraits.sp$GTDB_family))))
```

```
## [1] "#Nr families:  762"
```

```r
print(paste("#Nr genera: ", length(unique(gtraits.sp$GTDB_genus))))
```

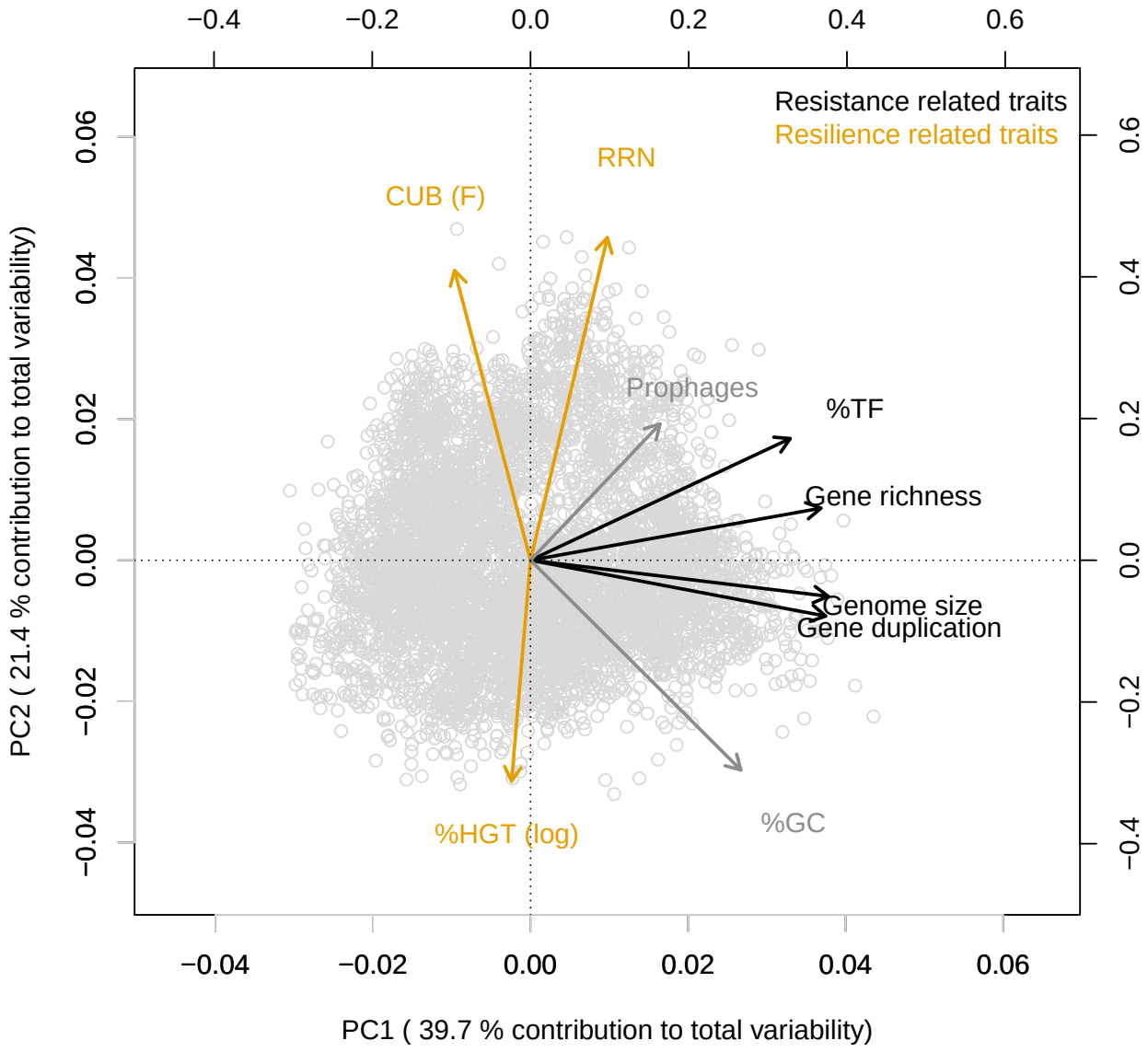
```
## [1] "#Nr genera:  2669"
```

```r
print(paste("#Nr species: ", dim(gtraits.sp)[1]))
```

```
## [1] "#Nr species:  8847"
```

```r
# Select columns relevant for Figure 3 and Figure 4
vars <- gtraits.sp %>%
  select(Genome.size,X.TF,Gene.duplication,Gene.richness,CUB.F.,RRN_rrnDB,Prophages.corr,
         log.HGT_per,X.GC)

# Change column names for Figure 3 and Figure 4
colnames(vars) <- c('Genome size', '%TF', 'Gene duplication', 'Gene richness',
                    'CUB (F)','RRN', 'Prophages', '%HGT (log)','%GC')

# Principle component analysis
pca<-prcomp(na.omit(vars),center=TRUE, scale=TRUE)

# Proportion variance explained in component 1 and 2
prop.1<-summary(pca)$importance[2]*100
prop.2<-summary(pca)$importance[5]*100

# Extract the eigenvectors and the loading factors
pca.scores<-pca$x[,1:2]
pca.loadings<-pca$rotation[,1:2]

# Normalize PC axes to unit length
pca.scores[,1]<-pca.scores[,1]*(1/sqrt(sum(pca.scores[,1]^2)))
pca.scores[,2]<-pca.scores[,2]*(1/sqrt(sum(pca.scores[,2]^2)))

# Set scaling parameters for biplot
sc<-1.5
xlim <- unsigned.range(pca.scores[,1])*sc
ylim <- unsigned.range(pca.scores[,1])*sc
ratio <-max(abs(unsigned.range(pca.loadings[,1])[1]
                -unsigned.range(pca.loadings[,1])[2])/abs(xlim[1]-xlim[2]),
            abs(unsigned.range(pca.loadings[,2])[1]
                -unsigned.range(pca.loadings[,2])[2])/abs(ylim[1]-ylim[2]))*sc/1.3

# Set colors
col=c(rep("black",4), rep("#E69F00",2), c("gray55","#E69F00","gray55"))

# PCA biplot
plot(pca.scores,xlim=xlim,ylim=ylim,
     ylab=paste("PC2 (",round(prop.2,1),"% contribution to total variability)"),
     xlab=paste("PC1 (",round(prop.1,1),"% contribution to total variability)"),
     col='gray85',cex=1)
axis(1, col = "gray85")
axis(2, col = "gray85")

par(new=TRUE)
plot(pca.loadings,axes=FALSE,type="n",xlim=xlim*ratio,ylim=ylim*ratio,xlab = "", ylab = "")
axis(3, col = "black")
```

```
axis(4, col = "black")
text(pca.loadings, labels = rownames(pca.loadings), cex = 1, col = col)
arrows(0, 0, pca.loadings[, 1] * 0.8, pca.loadings[, 2] *0.8, col = col,length = 0.1,lwd =2)
abline(v=0,h=0,lty="dotted")
legend('topright',c("Resistance related traits","Resilience related traits"),
       text.col=c('black','#E69F00'),bty="n")
```




```
## pdf
##   2
```

Figure 3: Principal Component Analyses of trait values averaged at the species level. (The positions of some text labels were later adjusted with an external graphic program)

7.2 PCA statistical evaluation (Table 1, Figure S2 A-C)

```
# Loadings PC1
pca$rotation[, 1]
```

```
##      Genome size              %TF Gene duplication    Gene richness
##        0.4704307        0.4103073        0.4663164        0.4588267
##          CUB (F)              RRN        Prophages       %HGT (log)
##       -0.1199248        0.1213981        0.2045905       -0.0298933
##              %GC
##        0.3324148
```

```
# Loadings PC2
pca$rotation[, 2]
```

```
##      Genome size              %TF Gene duplication    Gene richness
##      -0.06361299       0.21464280      -0.09846166       0.09182169
##          CUB (F)              RRN        Prophages       %HGT (log)
##       0.51118688       0.56912131       0.24035658      -0.38976980
##              %GC
##      -0.36993656
```

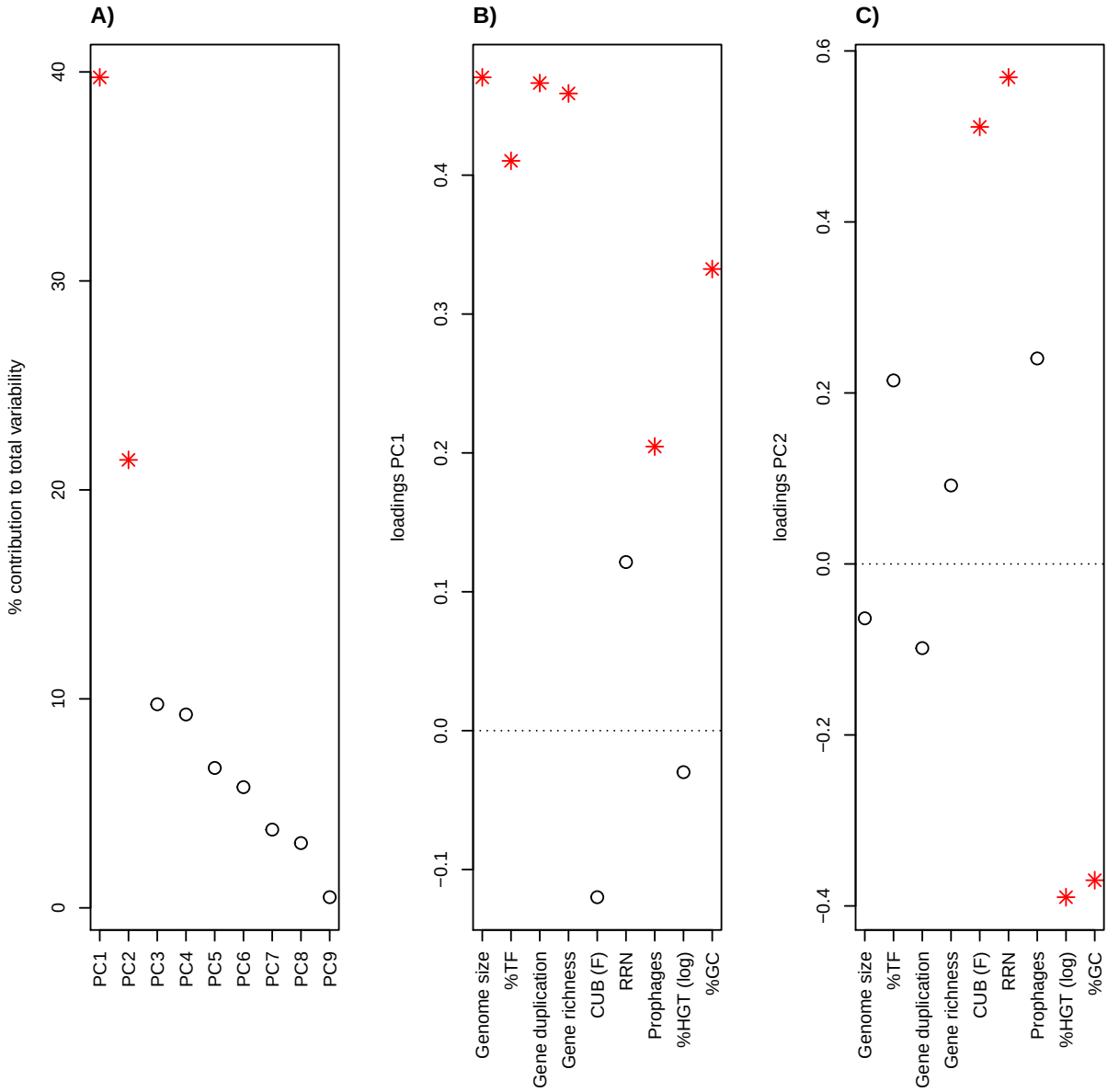
```
# Permutation based testing of statistical significance
PCAstats.glob <- PCAtest(na.omit(vars), 100, 100, plot = F)
```

```
##
## Sampling bootstrap replicates... Please wait
##  1 of 100 bootstrap replicates 2 of 100 bootstrap replicates 3 of 100 bootstrap replicates 4 of 100 b
## Calculating confidence intervals of empirical statistics... Please wait
##
## Sampling random permutations... Please wait
##  1 of 100 random permutations                                                    2 of 100 random permu
## Comparing empirical statistics with their null distributions... Please wait
##
## ============================================================
## Test of PCA significance: 9 variables, 5823 observations
## 100 bootstrap replicates, 100 random permutations
## ============================================================
##
## Empirical Psi = 9.8039, Max null Psi = 0.0215, Min null Psi = 0.0070, p-value = 0
## Empirical Phi = 0.3690, Max null Phi = 0.0173, Min null Phi = 0.0099, p-value = 0
##
## Empirical eigenvalue #1 = 3.57668, Max null eigenvalue = 1.09872, p-value = 0
## Empirical eigenvalue #2 = 1.92919, Max null eigenvalue = 1.06016, p-value = 0
## Empirical eigenvalue #3 = 0.87689, Max null eigenvalue = 1.04256, p-value = 1
## Empirical eigenvalue #4 = 0.8329, Max null eigenvalue = 1.02385, p-value = 1
## Empirical eigenvalue #5 = 0.60252, Max null eigenvalue = 1.01437, p-value = 1
## Empirical eigenvalue #6 = 0.51983, Max null eigenvalue = 0.99725, p-value = 1
## Empirical eigenvalue #7 = 0.33701, Max null eigenvalue = 0.98657, p-value = 1
## Empirical eigenvalue #8 = 0.27904, Max null eigenvalue = 0.97761, p-value = 1
## Empirical eigenvalue #9 = 0.04594, Max null eigenvalue = 0.95962, p-value = 1
##
## PC 1 is significant and accounts for 39.7% (95%-CI:39.3-40.2) of the total variation
## PC 2 is significant and accounts for 21.4% (95%-CI:21-22) of the total variation
##
## The first 2 PC axes are significant and account for 61.2% of the total variation
```

```
##
## Variables 1, 2, 3, 4, 7, and 9 have significant loadings on PC 1
## Variables 5, 6, 8, and 9 have significant loadings on PC 2
```

```r
# default color and symbol vectors
color = c(rep("black", 9))
sym = c(rep(1, 9))

# replace elements in color and symbol vectors based on PCAstats.glob
# results
color1.1 <- replace(color, c(1, 2), "red")
sym1.1 <- replace(sym, c(1, 2), 8)
color1.2 <- replace(color, c(1:4, 7, 9), "red")
sym1.2 <- replace(sym, c(1:4, 7, 9), 8)
color1.3 <- replace(color, c(5, 6, 8, 9), "red")
sym1.3 <- replace(sym, c(5, 6, 8, 9), 8)

# Figure S2 A-C
par(mfrow = c(1, 3))
# par(mar=c(5.1, 4.1, 4.1, 2.1)) default
par(mar = c(8, 4.1, 4.1, 2.1))
plot(PCAstats.glob[[5]], ylab = "% contribution to total variability",
    xlab = "", xaxt = "n", col = color1.1, pch = sym1.1, cex = 1.5)
axis(1, at = 1:9, labels = colnames(summary(pca)$importance), las = 2)
title("PCA statistics for Figure 3: all genomes", line = 3, adj = 0)
title("A)", adj = 0, line = 1)
plot(pca$rotation[, 1], ylab = "loadings PC1", xlab = "", xaxt = "n", col = color1.2,
    pch = sym1.2, cex = 1.5)
abline(v = 0, h = 0, lty = "dotted")
axis(1, at = 1:9, labels = colnames(PCAstats.glob[[8]]), las = 2)
title("B)", adj = 0, line = 1)
plot(pca$rotation[, 2], ylab = "loadings PC2", xlab = "", xaxt = "n", col = color1.3,
    pch = sym1.3, cex = 1.5)
abline(v = 0, h = 0, lty = "dotted")
axis(1, at = 1:9, labels = colnames(PCAstats.glob[[8]]), las = 2)
title("C)", adj = 0, line = 1)
```

PCA statistics for Figure 3:



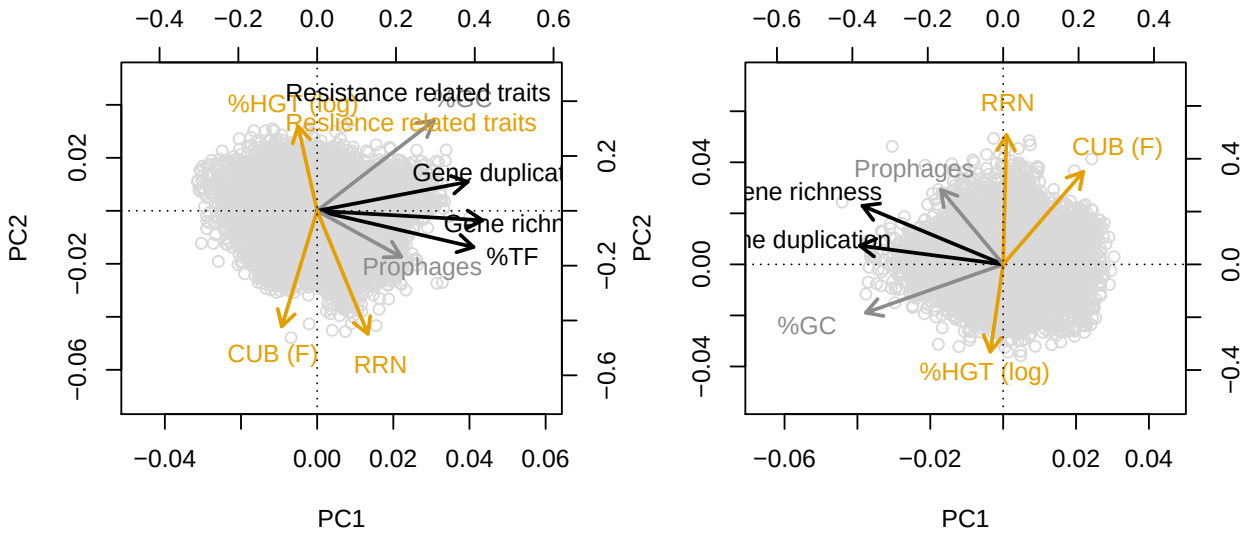
7.3 PCAs after random removal of 1,2,3 or for of the variables (Figure S3)

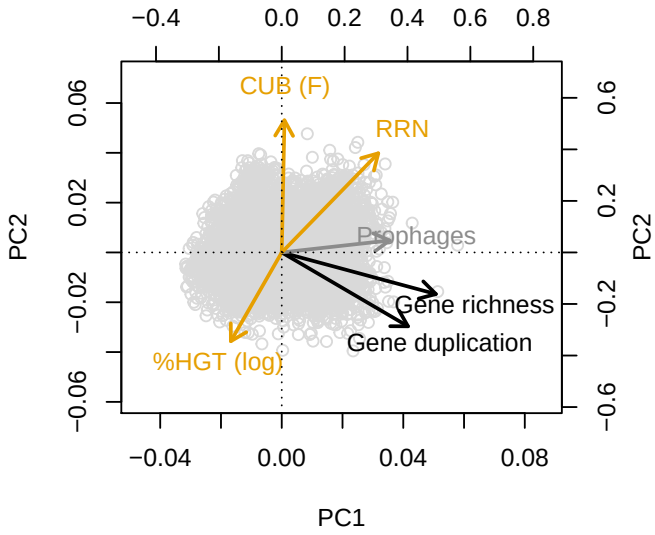
```
# Randomly remove 1,2,3,4 parameters

set.seed(2022)
vars8 <- sample(vars, 8)
vars7 <- sample(vars8, 7)
vars6 <- sample(vars7, 6)
vars5 <- sample(vars6, 5)

# Principal component analyses
pca8 <- prcomp(na.omit(vars8), center = TRUE, scale = TRUE)
pca7 <- prcomp(na.omit(vars7), center = TRUE, scale = TRUE)
```

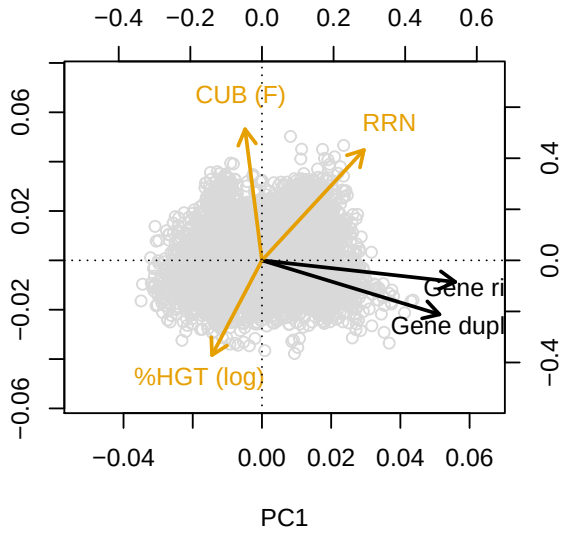
```
pca6 <- prcomp(na.omit(vars6), center = TRUE, scale = TRUE)
pca5 <- prcomp(na.omit(vars5), center = TRUE, scale = TRUE)

# Extract the eigenvectors and the loading factors and normalize PC
# axes to unit length
pca8.scores <- pca8$x[, 1:2]
pca8.loadings <- pca8$rotation[, 1:2][order(row.names(pca8$rotation[, 1:2])),
    ]
pca8.scores[, 1] <- pca8.scores[, 1] * (1/sqrt(sum(pca8.scores[, 1]^2)))
pca8.scores[, 2] <- pca8.scores[, 2] * (1/sqrt(sum(pca8.scores[, 2]^2)))

pca7.scores <- pca7$x[, 1:2]
pca7.loadings <- pca7$rotation[, 1:2][order(row.names(pca7$rotation[, 1:2])),
    ]
pca7.scores[, 1] <- pca7.scores[, 1] * (1/sqrt(sum(pca7.scores[, 1]^2)))
pca7.scores[, 2] <- pca7.scores[, 2] * (1/sqrt(sum(pca7.scores[, 2]^2)))

pca6.scores <- pca6$x[, 1:2]
pca6.loadings <- pca6$rotation[, 1:2][order(row.names(pca6$rotation[, 1:2])),
    ]
pca6.scores[, 1] <- pca6.scores[, 1] * (1/sqrt(sum(pca6.scores[, 1]^2)))
pca6.scores[, 2] <- pca6.scores[, 2] * (1/sqrt(sum(pca6.scores[, 2]^2)))

pca5.scores <- pca5$x[, 1:2]
pca5.loadings <- pca5$rotation[, 1:2][order(row.names(pca5$rotation[, 1:2])),
    ]
pca5.scores[, 1] <- pca5.scores[, 1] * (1/sqrt(sum(pca5.scores[, 1]^2)))
pca5.scores[, 2] <- pca5.scores[, 2] * (1/sqrt(sum(pca5.scores[, 2]^2)))

# Set scaling parameters for biplots
sc <- 1.5
xlim8 <- unsigned.range(pca8.scores[, 1]) * sc
ylim8 <- unsigned.range(pca8.scores[, 2]) * sc
ratio <- max(abs(unsigned.range(pca8.loadings[, 1])[1] - unsigned.range(pca8.loadings[,
    1])[2])/abs(xlim8[1] - xlim8[2]), abs(unsigned.range(pca8.loadings[,
    2])[1] - unsigned.range(pca8.loadings[, 2])[2])/abs(ylim8[1] - ylim8[2])) *
    sc/1.3

xlim7 <- unsigned.range(pca7.scores[, 1]) * sc
ylim7 <- unsigned.range(pca7.scores[, 2]) * sc
ratio <- max(abs(unsigned.range(pca7.loadings[, 1])[1] - unsigned.range(pca7.loadings[,
    1])[2])/abs(xlim7[1] - xlim7[2]), abs(unsigned.range(pca7.loadings[,
    2])[1] - unsigned.range(pca7.loadings[, 2])[2])/abs(ylim7[1] - ylim7[2])) *
    sc/1.3

xlim6 <- unsigned.range(pca6.scores[, 1]) * sc
ylim6 <- unsigned.range(pca6.scores[, 2]) * sc
ratio <- max(abs(unsigned.range(pca6.loadings[, 1])[1] - unsigned.range(pca6.loadings[,
    1])[2])/abs(xlim6[1] - xlim6[2]), abs(unsigned.range(pca6.loadings[,
    2])[1] - unsigned.range(pca6.loadings[, 2])[2])/abs(ylim6[1] - ylim6[2])) *
    sc/1.3

xlim5 <- unsigned.range(pca5.scores[, 1]) * sc
```

```
ylim5 <- unsigned.range(pca5.scores[, 2]) * sc
ratio <- max(abs(unsigned.range(pca5.loadings[, 1])[1] - unsigned.range(pca5.loadings[,
    1])[2])/abs(xlim5[1] - xlim5[2]), abs(unsigned.range(pca5.loadings[,
    2])[1] - unsigned.range(pca5.loadings[, 2])[2])/abs(ylim5[1] - ylim5[2])) *
    sc/1.3

# Colors
col = c(rep("black", 4), rep("#E69F00", 2), c("gray55", "#E69F00", "gray55"))
color.schema <- data.frame(names(vars), col)

# Figure S3
par(mfrow = c(2, 2))
col = merge(pca8.loadings, color.schema, by.x = 0, by.y = "names.vars.")[,
    4]
plot(pca8.scores, xlim = xlim8, ylim = ylim8, col = "gray85", cex = 1)
par(new = TRUE)
plot(pca8.loadings, axes = FALSE, type = "n", xlim = xlim8 * ratio, ylim = ylim8 *
    ratio, xlab = "", ylab = "")
axis(3, col = "black")
axis(4, col = "black")
text(pca8.loadings, labels = rownames(pca8.loadings), cex = 1, col = col)
arrows(0, 0, pca8.loadings[, 1] * 0.8, pca8.loadings[, 2] * 0.8, col = col,
    length = 0.1, lwd = 2)
abline(v = 0, h = 0, lty = "dotted")
legend("topright", c("Resistance related traits", "Reslience related traits"),
    text.col = c("black", "#E69F00"), bty = "n")

col = merge(pca7.loadings, color.schema, by.x = 0, by.y = "names.vars.")[,
    4]
plot(pca7.scores, xlim = xlim7, ylim = ylim7, col = "gray85", cex = 1)
par(new = TRUE)
plot(pca7.loadings, axes = FALSE, type = "n", xlim = xlim7 * ratio, ylim = ylim7 *
    ratio, xlab = "", ylab = "")
axis(3, col = "black")
axis(4, col = "black")
text(pca7.loadings, labels = rownames(pca7.loadings), cex = 1, col = col)
arrows(0, 0, pca7.loadings[, 1] * 0.7, pca7.loadings[, 2] * 0.8, col = col,
    length = 0.1, lwd = 2)
abline(v = 0, h = 0, lty = "dotted")

col = merge(pca6.loadings, color.schema, by.x = 0, by.y = "names.vars.")[,
    4]
plot(pca6.scores, xlim = xlim6, ylim = ylim6, col = "gray85", cex = 1)
par(new = TRUE)
plot(pca6.loadings, axes = FALSE, type = "n", xlim = xlim6 * ratio, ylim = ylim6 *
    ratio, xlab = "", ylab = "")
axis(3, col = "black")
axis(4, col = "black")
text(pca6.loadings, labels = rownames(pca6.loadings), cex = 1, col = col)
arrows(0, 0, pca6.loadings[, 1] * 0.8, pca6.loadings[, 2] * 0.8, col = col,
    length = 0.1, lwd = 2)
abline(v = 0, h = 0, lty = "dotted")
```

```r
col = merge(pca5.loadings, color.schema, by.x = 0, by.y = "names.vars.")[,
    4]
plot(pca5.scores, xlim = xlim5, ylim = ylim5, col = "gray85", cex = 1)
par(new = TRUE)
plot(pca5.loadings, axes = FALSE, type = "n", xlim = xlim5 * ratio, ylim = ylim5 *
    ratio, xlab = "", ylab = "")
axis(3, col = "black")
axis(4, col = "black")
text(pca5.loadings, labels = rownames(pca5.loadings), cex = 1, col = col)
arrows(0, 0, pca5.loadings[, 1] * 0.8, pca5.loadings[, 2] * 0.8, col = col,
    length = 0.1, lwd = 2)
abline(v = 0, h = 0, lty = "dotted")
```







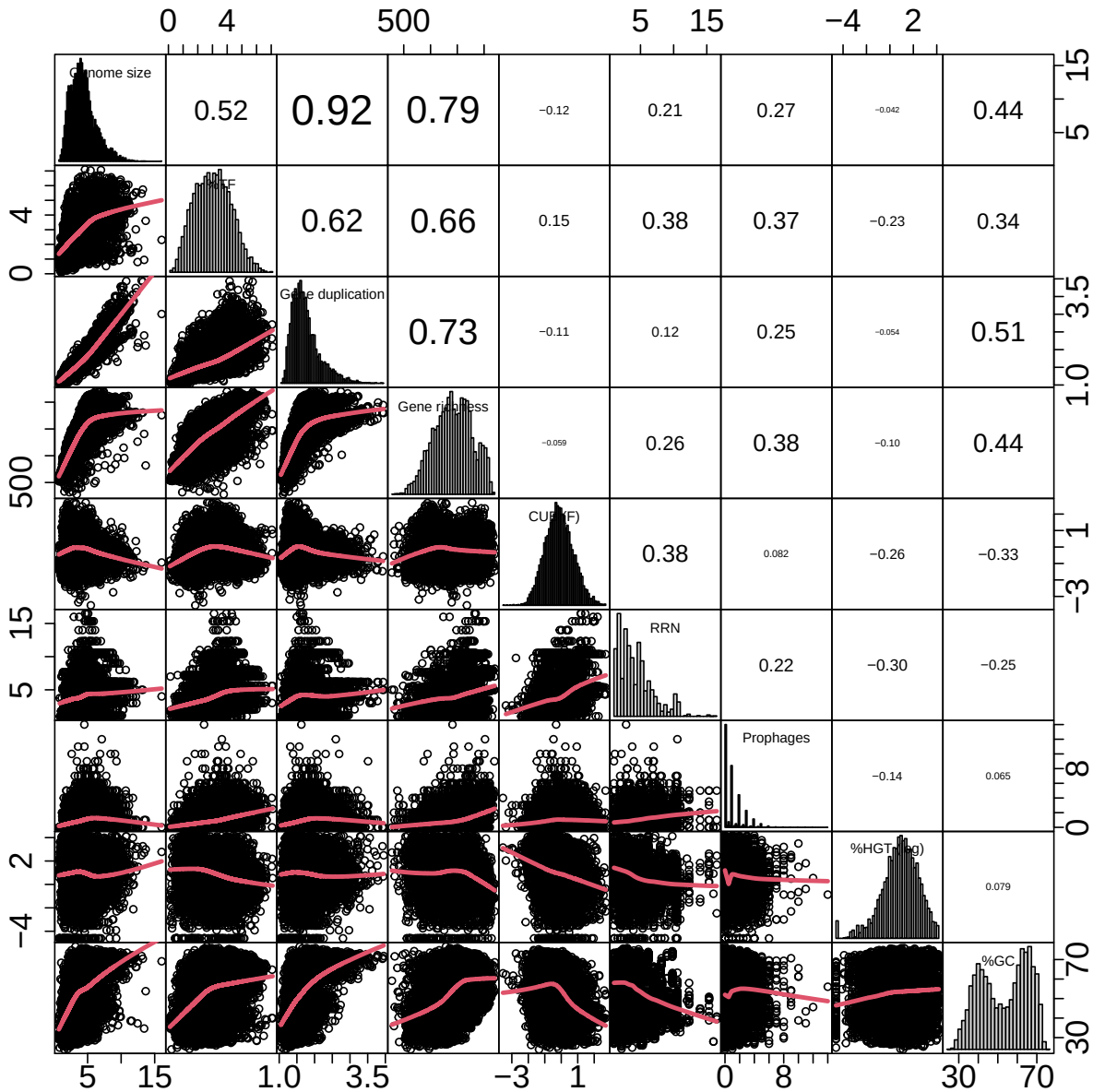
```
## pdf
##   2
```

Figure S4: The random removal of 1,2,3 or 4 variable from the principal component analyses presented in Figure 3 demonstrates that the spatial patterns of resistance versus resilience related genomic traits are robust against the removal of individual variables from the dataset. (The positions of some text labels were later adjusted with an external graphic program)

8 Pairwise correlations (traits avereged at the species level)

8.1 Pairwise scatterplots among 9 genomic traits (Figure 4)

```
# Figure 4
chart.Correlation.mod(vars, histogram = TRUE, pch = 19, method = c("spearman"))
```



```
## [1] "Genome size"      "%TF"              "Gene duplication" "Gene richness"
## [5] "CUB (F)"          "RRN"              "Prophages"        "%HGT (log)"
## [9] "%GC"
```

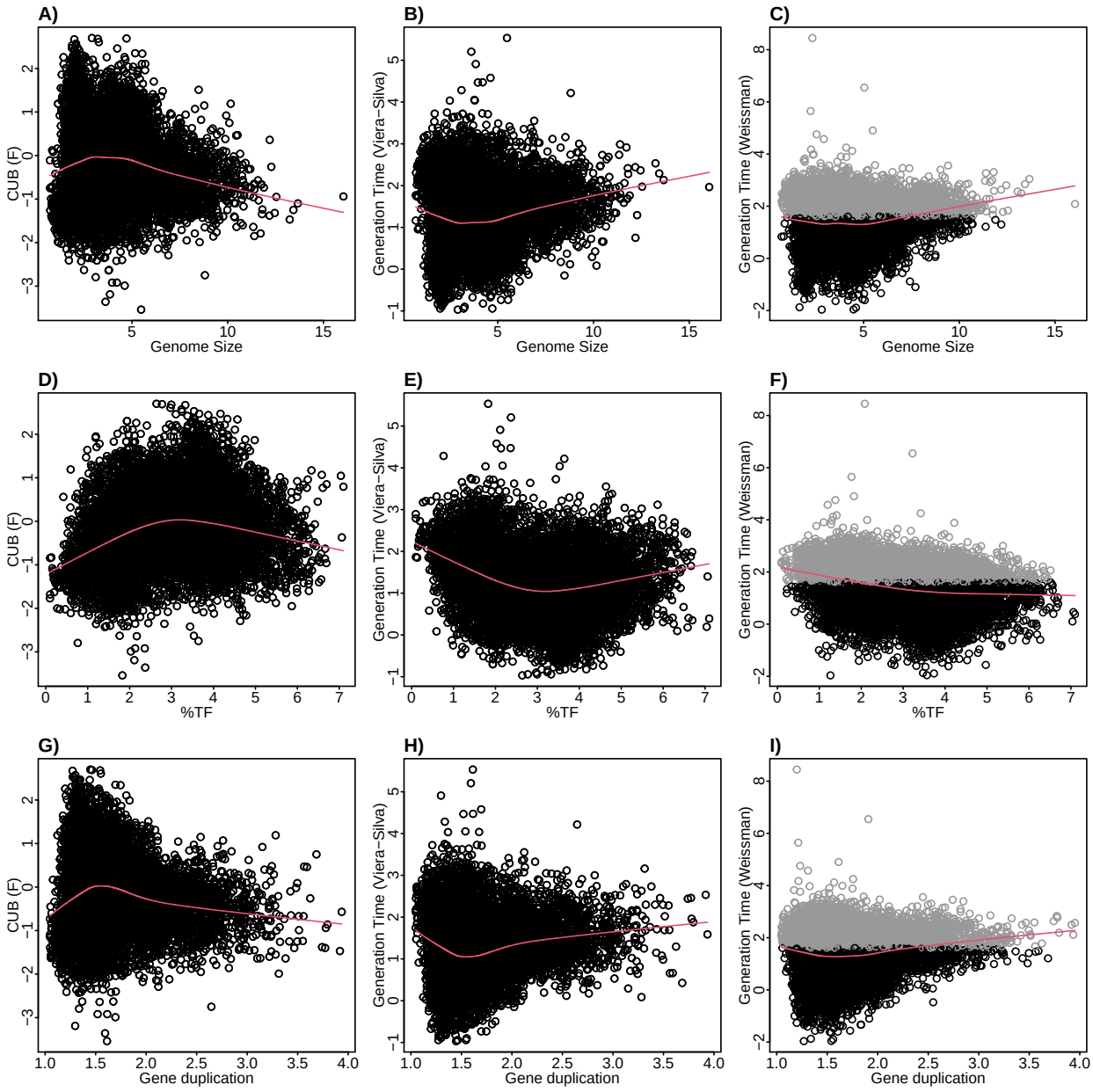
```
## pdf
##   2
```

Figure 4: Pairwise scatterplots among genomic traits.(The positions and colors of some text labels were later adjusted with an external graphic program)

8.2 Pairwise scatterplots of resistance realted tratits versus CUB parameters (Figure 5)

```
# Figure 5
par(mfrow = c(3, 3))
par(mar = c(2.5, 2.5, 1.5, 0.2))
par(mgp = c(0.9, 0.1, 0))
# A)
plot(gtraits.sp$Genome.size, gtraits.sp$CUB.F., ylab = "CUB (F)", xlab = "Genome Size",
    tck = -0.01)
panel.smooth(gtraits.sp$Genome.size, gtraits.sp$CUB.F.)
title("A)", adj = 0, line = 0.4)
# B)
plot(gtraits.sp$Genome.size, gtraits.sp$log.d, ylab = "Generation Time (Viera-Silva)",
    xlab = "Genome Size", tck = -0.01)
panel.smooth(gtraits.sp$Genome.size, gtraits.sp$log.d)
title("B)", adj = 0, line = 0.4)
# C)
plot(gtraits.sp$Genome.size, gtraits.sp$log.d.gRodon, ylab = "Generation Time (Weissman)",
    xlab = "Genome Size", tck = -0.01, col = ifelse(gtraits.sp$log.d.gRodon >=
        log(5), "gray60", "black"))
good <- !(is.na(gtraits.sp$log.d.gRodon) | is.na(gtraits.sp$Genome.size))
lines(lowess(gtraits.sp$log.d.gRodon[good] ~ gtraits.sp$Genome.size[good],
    f = 2/3, iter = 3), col = 2)
title("C)", adj = 0, line = 0.4)
# D)
plot(gtraits.sp$X.TF, gtraits.sp$CUB.F., ylab = "CUB (F)", xlab = "%TF",
    tck = -0.01)
panel.smooth(gtraits.sp$X.TF, gtraits.sp$CUB.F.)
# legend('topright', paste('max: ',round(exTF_F,2),'%'), bty='n')
title("D)", adj = 0, line = 0.4)
# E)
plot(gtraits.sp$X.TF, gtraits.sp$log.d, ylab = "Generation Time (Viera-Silva)",
    xlab = "%TF", tck = -0.01)
panel.smooth(gtraits.sp$X.TF, gtraits.sp$log.d)
title("E)", adj = 0, line = 0.4)
# F)
plot(gtraits.sp$X.TF, gtraits.sp$log.d.gRodon, ylab = "Generation Time (Weissman)",
    xlab = "%TF", tck = -0.01, col = ifelse(gtraits.sp$log.d.gRodon >=
        log(5), "gray60", "black"))
good <- !(is.na(gtraits.sp$log.d.gRodon) | is.na(gtraits.sp$X.TF))
lines(lowess(gtraits.sp$log.d.gRodon[good] ~ gtraits.sp$X.TF[good], f = 2/3,
    iter = 3), col = 2)
title("F)", adj = 0, line = 0.4)
```

```r
# G)
plot(gtraits.sp$Gene.duplication, gtraits.sp$CUB.F., ylab = "CUB (F)",
    xlab = "Gene duplication", tck = -0.01)
panel.smooth(gtraits.sp$Gene.duplication, gtraits.sp$CUB.F.)
title("G)", adj = 0, line = 0.4)
# H)
plot(gtraits.sp$Gene.duplication, gtraits.sp$log.d, ylab = "Generation Time (Viera-Silva)",
    xlab = "Gene duplication", tck = -0.01)
panel.smooth(gtraits.sp$Gene.duplication, gtraits.sp$log.d)
title("H)", adj = 0, line = 0.4)
# I)
plot(gtraits.sp$Gene.duplication, gtraits.sp$log.d.gRodon, ylab = "Generation Time (Weissman)",
    xlab = "Gene duplication", tck = -0.01, col = ifelse(gtraits.sp$log.d.gRodon >=
        log(5), "gray60", "black"))
good <- !(is.na(gtraits.sp$log.d.gRodon) | is.na(gtraits.sp$Gene.duplication))
lines(lowess(gtraits.sp$log.d.gRodon[good] ~ gtraits.sp$Gene.duplication[good],
    f = 2/3, iter = 3), col = 2)
title("I)", adj = 0, line = 0.4)
```




```
## pdf
##   2
```

Figure 5: Scatterplots displaying pairwise relationships between the codon usage bias (F, Vieira-Silva and Rocha, 2010) the generation time (log-transformed) estimated as detailed in Vieira-Silva et al. (Vieira-Silva and Rocha, 2010) and the generation time (log-transformed) estimated using the R package gRodon (v0.0.0.9000, Weissman et al., 2021) against genomes size, %TF and gene duplication. Genomes with low codon usage bias resulting in predicted generations times (gRodon) exceeding 5 h (highlighted in gray) represent species with generations times >5 h, while the exact value is inaccurate. The absence of genomes with elevated generation times at the extremes of the value ranges for the displayed resistance related traits is accordingly independent from these inaccuracies.

9 Partial correlations (Table 1)

```
gtraits1 <- gtraits.sp[gtraits.sp$Genome.size<=4,] #species with genome size <=4
gtraits2 <- gtraits.sp[gtraits.sp$Genome.size>=5,] #species with genome size >=5

partial.rho <-  data.frame(rho.1=c(cor.test(gtraits1$Genome.size, gtraits1$CUB.F.,
                                            method="spearman")[[4]],
                                   cor.test(gtraits1$Genome.size, gtraits1$RRN_rrnDB,
                                            method="spearman")[[4]],

                                   cor.test(gtraits1$Gene.duplication, gtraits1$CUB.F.,
                                            method="spearman")[[4]],
                                   cor.test(gtraits1$Gene.duplication, gtraits1$RRN_rrnDB,
                                            method="spearman")[[4]],

                                   cor.test(gtraits1$X.TF, gtraits1$CUB.F.,
                                            method="spearman")[[4]],
                                   cor.test(gtraits1$X.TF, gtraits1$RRN_rrnDB,
                                            method="spearman")[[4]]),

                           rho.2=c(cor.test(gtraits2$Genome.size, gtraits2$CUB.F.,
                                            method="spearman")[[4]],
                                   cor.test(gtraits2$Genome.size, gtraits2$RRN_rrnDB,
                                            method="spearman")[[4]],

                                   cor.test(gtraits2$Gene.duplication, gtraits2$CUB.F.,
                                            method="spearman")[[4]],
                                   cor.test(gtraits2$Gene.duplication, gtraits2$RRN_rrnDB,
                                            method="spearman")[[4]],

                                   cor.test(gtraits2$X.TF, gtraits2$CUB.F.,
                                            method="spearman")[[4]],
                                   cor.test(gtraits2$X.TF, gtraits2$RRN_rrnDB,
                                            method="spearman")[[4]]))

rownames(partial.rho) <- c('Genome size vs CUB', 'Genome size vs RRN',
                           'Gene duplication vs CUB', 'Gene duplication vs RRN',
                           '%TF vs CUB', '%TF vs RRN')

partial.p <-  data.frame(p.1=c(cor.test(gtraits1$Genome.size, gtraits1$CUB.F.,
                                        method="spearman")[[3]],
                               cor.test(gtraits1$Genome.size, gtraits1$RRN_rrnDB,
                                        method="spearman")[[3]],

                               cor.test(gtraits1$Gene.duplication, gtraits1$CUB.F.,
                                        method="spearman")[[3]],
                               cor.test(gtraits1$Gene.duplication, gtraits1$RRN_rrnDB,
                                        method="spearman")[[3]],

                               cor.test(gtraits1$X.TF, gtraits1$CUB.F.,
                                        method="spearman")[[3]],
                               cor.test(gtraits1$X.TF, gtraits1$RRN_rrnDB,
                                        method="spearman")[[3]]),
```

```
                        p.2=c(cor.test(gtraits2$Genome.size, gtraits2$CUB.F.,
                                       method="spearman")[[3]],
                                     cor.test(gtraits2$Genome.size, gtraits2$RRN_rrnDB,
                                              method="spearman")[[3]],

                                     cor.test(gtraits2$Gene.duplication, gtraits2$CUB.F.,
                                              method="spearman")[[3]],
                                     cor.test(gtraits2$Gene.duplication, gtraits2$RRN_rrnDB,
                                              method="spearman")[[3]],

                                     cor.test(gtraits2$X.TF, gtraits2$CUB.F.,
                                              method="spearman")[[3]],
                                     cor.test(gtraits2$X.TF, gtraits2$RRN_rrnDB,
                                              method="spearman")[[3]]))

rownames(partial.p) <- c('Genome size vs CUB', 'Genome size vs RRN',
                         'Gene duplication vs CUB', 'Gene duplication vs RRN',
                         '%TF vs CUB', '%TF vs RRN')
partial.p <- partial.p *12 #correct for multiple pairwise comparisons
cbind (partial.rho, partial.p)
```

```
##                              rho.1       rho.2           p.1          p.2
## Genome size vs CUB      0.05498845 -0.27456072  5.366894e-03 1.460472e-44
## Genome size vs RRN      0.08957200 -0.02276168  2.658807e-05 3.655556e+00
## Gene duplication vs CUB 0.12251446 -0.22906158  5.191756e-14 7.633830e-31
## Gene duplication vs RRN 0.07371961 -0.15904954  1.673868e-03 8.422135e-12
## %TF vs CUB              0.44281760 -0.12183041 4.261743e-194 7.043748e-09
## %TF vs RRN              0.38248210  0.22648473  1.670563e-92 9.207386e-24
```

Table 1 displays correlation coefficients and p-values between selected resistance and resilience related traits for species with small (<=4 Mbp) and large (>=5 Mbp) genomes

10 Habitat specific PCAs (Figure 6)

To visualize trait covariation patterns in dependence of the habitat type we sorted genomes based on information given in the column 'Habitat' of Table 1 into three habitat types: all genomes containing the strings *'soil'* or *'rhizosphere'* in the habitat description where classified as originating from *soil habitats*; all genomes containing the strings *'aquatic'* or *'marine'* or *'water'* where classified as originating from *aquatic habitats*; all genomes containing the strings *'oral'* or *'stomach'* or *'gut'* or *'intestinal'* or *'feces'* were classified as originating from the intestinal tract. Genomes that could not be assigned to either of these three habitats were not further classified. Trait values were summarized at the species level and the habitat type. Genomes affiliating with the same species that were not consistently assigned to the same habitat type were accordingly not aggregated.

```
vars.habitat <-  gtraits %>% select(IMG.Genome.ID,Habitat,
  GTDB_division,GTDB_phylum,GTDB_class,GTDB_order,GTDB_family,GTDB_genus, GTDB_species, FASTANI_species
  Genome.size,X.GC,RRN_IMG,RRN_rrnDB,per.HGT.corr,CUB.F.,Generation.time..Vieira.Silva., Generation.tim
  Gene.duplication,Gene.richness,X.TF,Prophages.corr) %>% #
  mutate(Habitat.color = ifelse (grepl('aquatic|marine|water', gtraits$Habitat, ignore.case=T),'steelblu
  mutate(Habitat.color = ifelse (grepl('soil|rhizosphere', gtraits$Habitat, ignore.case=T),'tomato4',Hal
  mutate(Habitat.color = ifelse (grepl('intestinal|feces|stomach|gut|oral', gtraits$Habitat, ignore.cas
  group_by(GTDB_division,GTDB_phylum, GTDB_class, GTDB_order, GTDB_family, GTDB_genus, GTDB_species, FA
  summarise_at(vars(Genome.size:Prophages.corr), mean, na.rm = TRUE) %>%
  ungroup() %>%
```

```
  mutate(log.HGT_perc= log(per.HGT.corr+0.01)) %>%
  select(Genome.size, X.TF, Gene.duplication, Gene.richness,
         CUB.F., RRN_rrnDB ,Prophages.corr, log.HGT_perc ,X.GC,  Habitat.color) %>%
  arrange((Habitat.color))

# Change column names for Figure 6
colnames(vars.habitat) <- c('Genome size', '%TF', 'Gene duplication', 'Gene richness',
                    'CUB (F)','RRN', 'Prophages', '%HGT (log)','%GC','Habitat.color')
vars.habitat
```

```
## # A tibble: 9,453 x 10
##    `Genome size` `%TF` `Gene duplication` `Gene richness` `CUB (F)`   RRN
##            <dbl> <dbl>              <dbl>           <dbl>     <dbl> <dbl>
##  1          2.09  1.60               1.40            745      -0.9    NaN
##  2          1.46  1.60               1.27            790      -1.32   NaN
##  3          3.26  1.34               1.82            925      -1.2    NaN
##  4          1.86  1.93               1.36           1084      -1.19     1
##  5          1.51  1.47               1.27            932.     -1.27   NaN
##  6          4.67  2.32               2.04           1256       0.27   NaN
##  7          5.19  2.57               2.06           1239       0.17   NaN
##  8          3.67  2.30               1.76           1199       0.54     1
##  9          3.98  2.76               1.87           1225       0.83     1
## 10          3.45  2.43               1.64           1163      -0.16     1
## # ... with 9,443 more rows, and 4 more variables: Prophages <dbl>,
## #   `%HGT (log)` <dbl>, `%GC` <dbl>, Habitat.color <chr>
```

```
vars.aqua <- vars.habitat[vars.habitat$Habitat.color=='steelblue3',]
vars.soil <- vars.habitat[vars.habitat$Habitat.color=='tomato4',]
vars.digest <- vars.habitat[vars.habitat$Habitat.color=='lightpink3',]

# Mean genome size in each habitat type
mean.gs <-na.omit(vars.habitat[,c(1:10)]) %>%
  tibble() %>%
  select('Genome size', Habitat.color) %>%
  mutate(Habitat = ifelse (Habitat.color=='steelblue3','aquatic','undefined')) %>%
  mutate(Habitat = ifelse (Habitat.color=='tomato4','soil',Habitat))  %>%
  mutate(Habitat = ifelse (Habitat.color=='lightpink3','digestive tract',Habitat))  %>%
  select(-Habitat.color) %>%
  group_by(Habitat) %>%
  summarise_each(funs(n(),mean,sd))
mean.gs
```

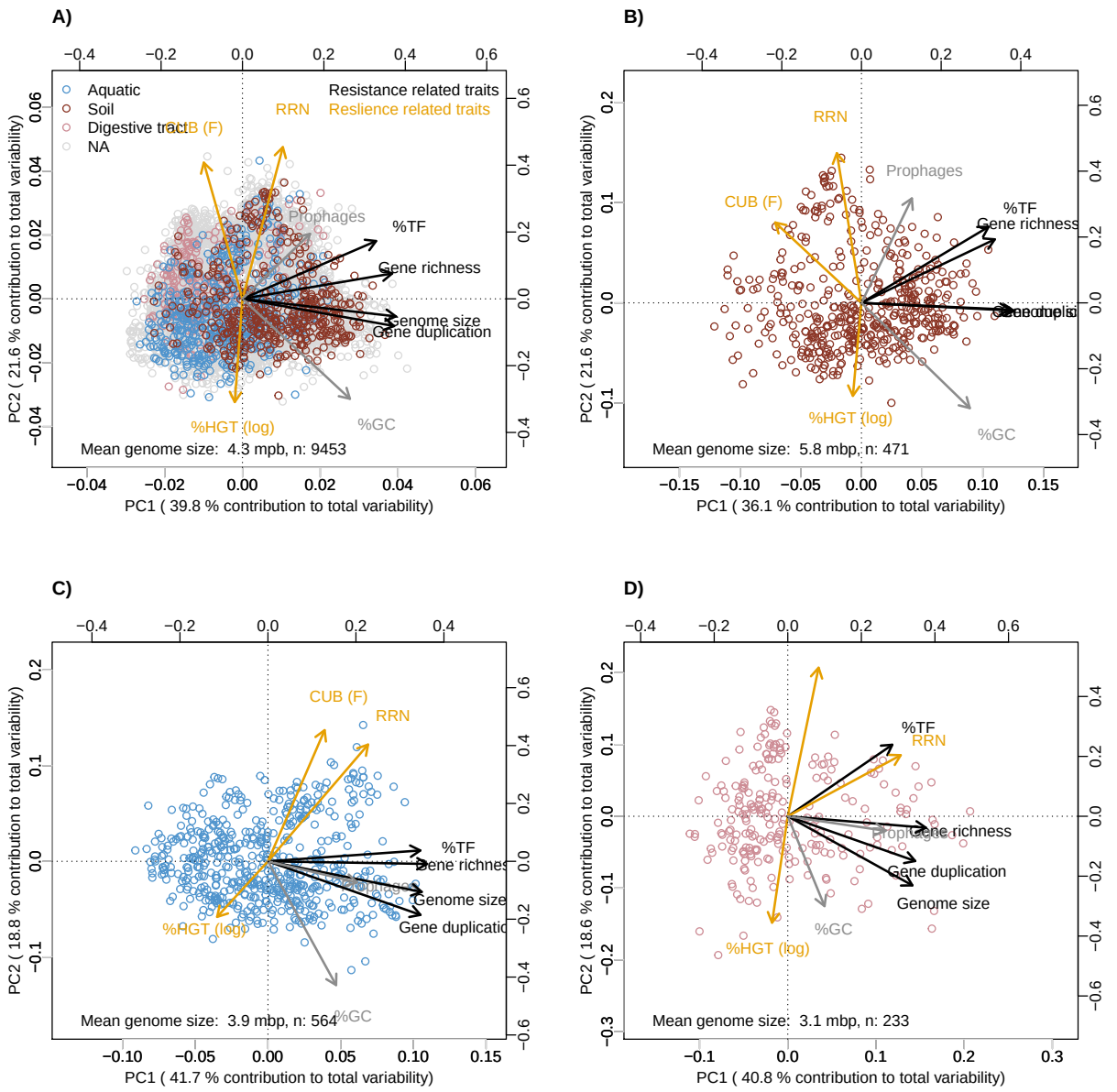
```
## # A tibble: 4 x 4
##   Habitat             n  mean    sd
##   <chr>           <int> <dbl> <dbl>
## 1 aquatic           564  3.93  1.43
## 2 digestive tract   233  3.07  1.24
## 3 soil              471  5.82  1.97
## 4 undefined        4915  4.47  1.90
```

```
# Principal component analyses
pca<-prcomp(na.omit(vars.habitat[,-10]),center=TRUE, scale=TRUE)
pca.aqua<-prcomp(na.omit(vars.aqua[,-10]),center=TRUE, scale=TRUE)
pca.soil<-prcomp(na.omit(vars.soil[,-10]),center=TRUE, scale=TRUE)
pca.digest<-prcomp(na.omit(vars.digest[,-10]),center=TRUE, scale=TRUE)
```

```
# Proportion variance explained in components 1 and 2
prop.1<-summary(pca)$importance[2]*100
prop.2<-summary(pca)$importance[5]*100
prop.aqua.1<-summary(pca.aqua)$importance[2]*100
prop.aqua.2<-summary(pca.aqua)$importance[5]*100
prop.soil.1<-summary(pca.soil)$importance[2]*100
prop.soil.2<-summary(pca.soil)$importance[5]*100
prop.digest.1<-summary(pca.digest)$importance[2]*100
prop.digest.2<-summary(pca.digest)$importance[5]*100

# Extract the eigenvectors and the loading factors and normalize PC axes to unit length
pca.scores<-pca$x[,1:2]
pca.loadings<-pca$rotation[,1:2]
pca.scores[,1]<-pca.scores[,1]*(1/sqrt(sum(pca.scores[,1]^2)))
pca.scores[,2]<-pca.scores[,2]*(1/sqrt(sum(pca.scores[,2]^2)))

pca.aqua.scores<-pca.aqua$x[,1:2]
pca.aqua.loadings<-pca.aqua$rotation[,1:2]
pca.aqua.scores[,1]<-pca.aqua.scores[,1]*(1/sqrt(sum(pca.aqua.scores[,1]^2)))
pca.aqua.scores[,2]<-pca.aqua.scores[,2]*(1/sqrt(sum(pca.aqua.scores[,2]^2)))

pca.soil.scores<-pca.soil$x[,1:2]
pca.soil.scores[,2] <- -pca.soil.scores[,2] #multiply with -1 to mirror PCA biplot
pca.soil.loadings<-pca.soil$rotation[,1:2]
pca.soil.loadings[,2] <- -pca.soil.loadings[,2] #multiply with -1 to mirror PCA biplot
pca.soil.scores[,1]<-pca.soil.scores[,1]*(1/sqrt(sum(pca.soil.scores[,1]^2)))
pca.soil.scores[,2]<-pca.soil.scores[,2]*(1/sqrt(sum(pca.soil.scores[,2]^2)))

pca.digest.scores<-pca.digest$x[,1:2]
pca.digest.scores[,2] <- -pca.digest.scores[,2] #multiply with -1 to mirror PCA biplot
pca.digest.loadings<-pca.digest$rotation[,1:2]
pca.digest.loadings[,2] <- -pca.digest.loadings[,2] #multiply with -1 to mirror PCA biplot
pca.digest.scores[,1]<-pca.digest.scores[,1]*(1/sqrt(sum(pca.digest.scores[,1]^2)))
pca.digest.scores[,2]<-pca.digest.scores[,2]*(1/sqrt(sum(pca.digest.scores[,2]^2)))

# Set scaling parameters for biplots
sc<-1.5
xlim <- unsigned.range(pca.scores[,1])*sc
ylim <- unsigned.range(pca.scores[,2])*sc
ratio <-max(abs(unsigned.range(pca.loadings[,1])[1]
                -unsigned.range(pca.loadings[,1])[2])/abs(xlim[1]-xlim[2]),
            abs(unsigned.range(pca.loadings[,2])[1]
                -unsigned.range(pca.loadings[,2])[2])/abs(ylim[1]-ylim[2]))*sc/1.3

xlim.aqua <- unsigned.range(pca.aqua.scores[,1])*sc
ylim.aqua <- unsigned.range(pca.aqua.scores[,2])*sc
ratio.aqua <-max(abs(unsigned.range(pca.aqua.loadings[,1])[1]
                     -unsigned.range(pca.aqua.loadings[,1])[2])/abs(xlim.aqua[1]
                                                                    -xlim.aqua[2]),
            abs(unsigned.range(pca.aqua.loadings[,2])[1]
                -unsigned.range(pca.aqua.loadings[,2])[2])/abs(ylim.aqua[1]
                                                               -ylim.aqua[2]))*sc/1.3
```

```
xlim.soil <- unsigned.range(pca.soil.scores[,1])*sc
ylim.soil <- unsigned.range(pca.soil.scores[,2])*sc
ratio.soil <-max(abs(unsigned.range(pca.soil.loadings[,1])[1]
                     -unsigned.range(pca.soil.loadings[,1])[2])/abs(xlim.soil[1]
                                                                    -xlim.soil[2]),
            abs(unsigned.range(pca.soil.loadings[,2])[1]
                -unsigned.range(pca.soil.loadings[,2])[2])/abs(ylim.soil[1]
                                                               -ylim.soil[2]))*sc/1.3

xlim.digest <- unsigned.range(pca.digest.scores[,1])*sc
ylim.digest <- unsigned.range(pca.digest.scores[,2])*sc
ratio.digest <-max(abs(unsigned.range(pca.digest.loadings[,1])[1]
                       -unsigned.range(pca.digest.loadings[,1])[2])/abs(xlim.digest[1]
                                                                        -xlim.digest[2]),
            abs(unsigned.range(pca.digest.loadings[,2])[1]
                -unsigned.range(pca.digest.loadings[,2])[2])/abs(ylim.digest[1]
                                                                 -ylim.digest[2]))*sc/1.3

# Figure 6
par(mfrow=c(2,2))
par(mgp=c(1.5,0.4,0))

col=c(rep("black",4), rep("#E69F00",2), c("gray55","#E69F00","gray55"))
col.genomes <- na.omit(vars.habitat) %>% pull(Habitat.color)
plot(pca.scores,xlim=xlim,ylim=ylim,col=col.genomes,cex=1,
     ylab=paste("PC2 (",round(prop.2,1),"% contribution to total variability)"),
     xlab=paste("PC1 (",round(prop.1,1),"% contribution to total variability)"))
axis(1, col = "gray85")
axis(2, col = "gray85")
title("A)", line=2.5, adj=0)
legend('topleft',c("Aquatic","Soil","Digestive tract","NA"),pch = c(1,1,1,1),
       col=c('steelblue3','tomato4','lightpink3',"gray85"),bty="n")
legend('topright',c("Resistance related traits","Reslience related traits"),
       text.col=c('black','#E69F00'),bty="n")
legend('bottomleft', paste(c('Mean genome size: '),
                           round(mean(vars.habitat[,1, drop=T]),1),c('mpb, n:'),
                           nrow(vars.habitat)),bty="n")
par(new=TRUE)
plot(pca.loadings,axes=FALSE,type="n",xlim=xlim*ratio,ylim=ylim*ratio,
     xlab = "", ylab = "")
axis(3, col = "black")
axis(4, col = "black")
text(pca.loadings, labels = rownames(pca.loadings), cex = 1, col = col)
arrows(0, 0, pca.loadings[, 1] * 0.8, pca.loadings[, 2] * 0.8, col = col,
       length = 0.1,lwd =2)
abline(v=0,h=0,lty="dotted")

plot(pca.soil.scores,xlim=xlim.soil,ylim=ylim.soil,col='tomato4',cex=1,
     ylab=paste("PC2 (",round(prop.soil.2,1),"% contribution to total variability)"),
     xlab=paste("PC1 (",round(prop.soil.1,1),"% contribution to total variability)"))
axis(1, col = "gray85")
axis(2, col = "gray85")
title("B)", line=2.5, adj=0)
```

```
legend('bottomleft', paste(c('Mean genome size: '),round(mean.gs[3,3,drop=T],1),
                           c('mbp, n:'),mean.gs[3,2,drop=T]),bty="n")
par(new=TRUE)
plot(pca.soil.loadings,axes=FALSE,type="n",xlim=xlim.soil*ratio.soil,
     ylim=ylim.soil*ratio.soil,xlab = "", ylab = "")
axis(3, col = "black")
axis(4, col = "black")
text(pca.soil.loadings, labels = rownames(pca.soil.loadings), cex = 1, col = col)
arrows(0, 0, pca.soil.loadings[, 1] * 0.8, pca.soil.loadings[, 2] * 0.8, col = col,
       length = 0.1,lwd =2)
abline(v=0,h=0,lty="dotted")

plot(pca.aqua.scores,xlim=xlim.aqua,ylim=ylim.aqua,col='steelblue3',cex=1,
     ylab=paste("PC2 (",round(prop.aqua.2,1),"% contribution to total variability)"),
     xlab=paste("PC1 (",round(prop.aqua.1,1),"% contribution to total variability)"))
axis(1, col = "gray85")
axis(2, col = "gray85")
title("C)", line=2.5, adj=0)
legend('bottomleft', paste(c('Mean genome size: '),round(mean.gs[1,3,drop=T],1),
                           c('mbp, n:'),mean.gs[1,2,drop=T]),bty="n")
par(new=TRUE)
plot(pca.aqua.loadings,axes=FALSE,type="n",xlim=xlim.aqua*ratio.aqua,
     ylim=ylim.aqua*ratio.aqua,
     xlab = "", ylab = "")
axis(3, col = "black")
axis(4, col = "black")
text(pca.aqua.loadings, labels = rownames(pca.aqua.loadings), cex = 1, col = col)
arrows(0, 0, pca.aqua.loadings[, 1] * 0.8, pca.aqua.loadings[, 2] * 0.8, col = col,
       length = 0.1,lwd =2)
abline(v=0,h=0,lty="dotted")

plot(pca.digest.scores,xlim=xlim.digest,ylim=ylim.digest,col='lightpink3',cex=1,
     ylab=paste("PC2 (",round(prop.digest.2,1),"% contribution to total variability)"),
     xlab=paste("PC1 (",round(prop.digest.1,1),"% contribution to total variability)"))
axis(1, col = "gray85")
axis(2, col = "gray85")
title("D)", line=2.5, adj=0)
legend('bottomleft', paste(c('Mean genome size: '), round(mean.gs[2,3,drop=T],1),
                           c('mbp, n:'),mean.gs[2,2,drop=T]),bty="n")
par(new=TRUE)
plot(pca.digest.loadings,axes=FALSE,type="n",xlim=xlim.digest*ratio.digest,
     ylim=ylim.digest*ratio.digest,
     xlab = "", ylab = "")
axis(3, col = "black")
axis(4, col = "black")
text(pca.digest.loadings, labels = rownames(pca.digest.loadings), cex = 1, col = col)
arrows(0, 0, pca.digest.loadings[, 1] * 0.8, pca.digest.loadings[, 2] * 0.8, col = col,length = 0.1,
       lwd =2)
abline(v=0,h=0,lty="dotted")
```




```
## pdf
##   2
```

```
## pdf
##   2
```

Figure 6: Principal component analyses illustrating covariations among genomic traits from JGI/IMG prokaryotic genomes dependence on the habitat type.(The positions of some text labels were later adjusted with an external graphic program)

11 Mantel correlograms (Figure 7)

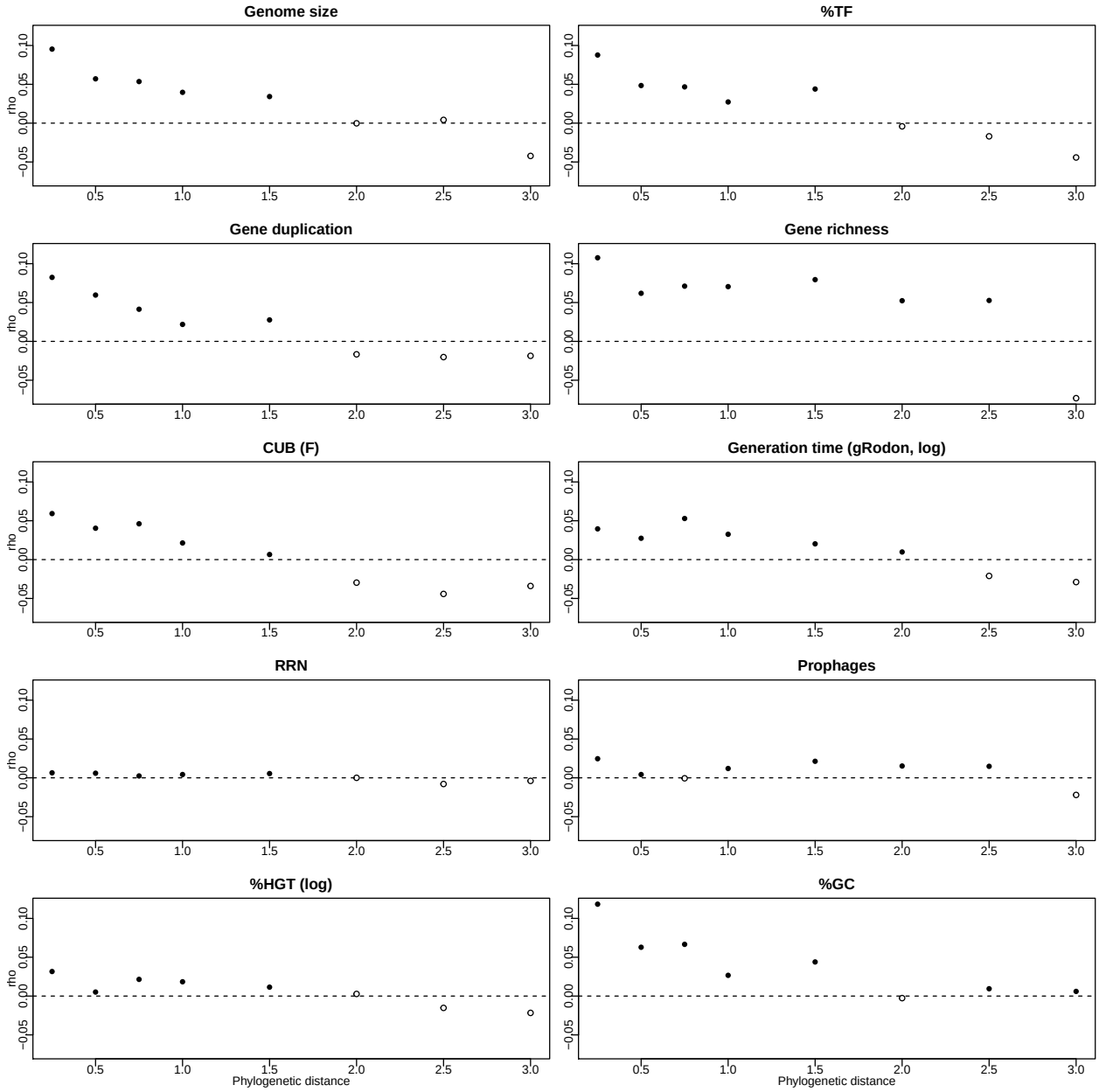
Input files for Figure 7 were created as detailed in scripts_phylogenetic.signal.md

```r
# Change column names for Figure labels
mpm.2$trait <- c("Genome size")
tibble(mpm.2)  #inspect input file
```

```
## # A tibble: 8 x 6
##   clases              rM pvalues pval.Bonferroni    pd trait
##   <chr>            <dbl>   <dbl>           <dbl> <dbl> <chr>
## 1 0  -  0.25    0.0953   0.00498         0.00498  0.25 Genome size
## 2 0.25  -  0.5  0.0570   0.00498         0.00995  0.5  Genome size
## 3 0.5  -  0.75  0.0535   0.00498         0.0149   0.75 Genome size
## 4 0.75  -  1    0.0397   0.00498         0.0199   1    Genome size
## 5 1  -  1.5     0.0342   0.00498         0.0249   1.5  Genome size
## 6 1.5  -  2    -0.000217 0.473           2.84     2    Genome size
## 7 2  -  2.5     0.00419  0.0149          0.104    2.5  Genome size
## 8 2.5  -  3    -0.0422   0.00498         0.0398   3    Genome size
```

```r
mpm.3$trait <- c("%GC")
mpm.4$trait <- c("RRN")
mpm.5$trait <- c("%HGT (log)")
mpm.6$trait <- c("CUB (F)")
mpm.7$trait <- c("Generation time (gRodon, log)")
mpm.8$trait <- c("Gene duplication")
mpm.9$trait <- c("Gene richness")
mpm.10$trait <- c("%TF")
mpm.11$trait <- c("Prophages")

# Set common ylim range for all plots
ylim = range(rbind(mpm.2, mpm.3, mpm.4, mpm.5, mpm.6, mpm.7, mpm.8, mpm.9,
    mpm.10, mpm.11)[, 2])

# Figure 7
par(mfrow = c(5, 2))
par(lwd = 1)
par(mar = c(2, 1.8, 2, 0.2))
par(mgp = c(1, 0.2, 0))

mpm <- mpm.2
plot(mpm$pd, mpm$rM, ylim = ylim, pch = ifelse(mpm$pval.Bonferroni < 0.1 &
    mpm$rM > 0, 16, 1), main = mpm$trait[1], ylab = "rho", xlab = "")
abline(h = 0, lty = 2)

mpm <- mpm.10
plot(mpm$pd, mpm$rM, ylim = ylim, pch = ifelse(mpm$pval.Bonferroni < 0.1 &
    mpm$rM > 0, 16, 1), main = mpm$trait[1], ylab = "", xlab = "")
abline(h = 0, lty = 2)

mpm <- mpm.8
plot(mpm$pd, mpm$rM, ylim = ylim, pch = ifelse(mpm$pval.Bonferroni < 0.1 &
    mpm$rM > 0, 16, 1), main = mpm$trait[1], ylab = "rho", xlab = "")
abline(h = 0, lty = 2)

mpm <- mpm.9
plot(mpm$pd, mpm$rM, ylim = ylim, pch = ifelse(mpm$pval.Bonferroni < 0.1 &
    mpm$rM > 0, 16, 1), main = mpm$trait[1], ylab = "", xlab = "")
```

```
abline(h = 0, lty = 2)

mpm <- mpm.6
plot(mpm$pd, mpm$rM, ylim = ylim, pch = ifelse(mpm$pval.Bonferroni < 0.1 &
    mpm$rM > 0, 16, 1), main = mpm$trait[1], ylab = "rho", xlab = "")
abline(h = 0, lty = 2)

mpm <- mpm.7
plot(mpm$pd, mpm$rM, ylim = ylim, pch = ifelse(mpm$pval.Bonferroni < 0.1 &
    mpm$rM > 0, 16, 1), main = mpm$trait[1], ylab = "", xlab = "")
abline(h = 0, lty = 2)

mpm <- mpm.4
plot(mpm$pd, mpm$rM, ylim = ylim, pch = ifelse(mpm$pval.Bonferroni < 0.1 &
    mpm$rM > 0, 16, 1), main = mpm$trait[1], ylab = "rho", xlab = "")
abline(h = 0, lty = 2)

mpm <- mpm.11
plot(mpm$pd, mpm$rM, ylim = ylim, pch = ifelse(mpm$pval.Bonferroni < 0.1 &
    mpm$rM > 0, 16, 1), main = mpm$trait[1], ylab = "", xlab = "")
abline(h = 0, lty = 2)

mpm <- mpm.5
plot(mpm$pd, mpm$rM, ylim = ylim, pch = ifelse(mpm$pval.Bonferroni < 0.1 &
    mpm$rM > 0, 16, 1), main = mpm$trait[1], ylab = "rho", xlab = "Phylogenetic distance")
abline(h = 0, lty = 2)

mpm <- mpm.3
plot(mpm$pd, mpm$rM, ylim = ylim, pch = ifelse(mpm$pval.Bonferroni < 0.1 &
    mpm$rM > 0, 16, 1), main = mpm$trait[1], ylab = "", xlab = "Phylogenetic distance")
abline(h = 0, lty = 2)
```




```
## pdf
##   2
```

Figure 7: Results from Mantel correlograms. Filled data points indicate positive significant correlations (Bonferroni adjusted p-values Padj < 0.1) of pairwise distances of traits values against pairwise phylogenetic distances among the reference genomes. The Mantel correlograms were computed for 10,000 randomly selected genomes and with 200 permutations.