Predicting evolution using frequency-dependent selection in bacterial populations

Data analysis and simulations

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Contents

This is the code for reproducing the figures from the paper "Predicting evolution using frequency-dependent selection in bacterial populations" ## add DOI

```
require(ape)
require(car)
require(readr)
require(vegan)
require(gtools)
require(ggtree)
require(readxl)
require(gridSVG)
require(cowplot)
require(deSolve)
require(Metrics)
require(ggrepel)
require(svglite)
require(quadprog)
require(phytools)
require(devtools)
require(tidyverse)
require(wesanderson)
require(RColorBrewer)

#### Quadratic Programming function ####
## X is a matrix with rows = COGs and columns = SCs
## Y is a matrix with rows = COGs and columns = 1
QP <- function(X, Y){
  rinv <- solve(chol(t(X) %*% X)) # M to be minimized in quad. function (Choleski decomp)
```

```
  C <- cbind(rep(1,ncol(X)), diag(ncol(X))) #The contraints to minimize the quandratic function
  b <- c(1,rep(0,ncol(X)))
  d <- t(Y) %*% X  #Vector in the quadratic function to be minimized
  output <- solve.QP(Dmat = rinv, factorized = TRUE, dvec = d,
                     Amat = C, bvec = b, meq = 1)$solution
  output <- round(output, digits = 5)
  return(output)
}
```

Figure 1: Southwest US Data

```
#### Data mining ####
set.seed(9340)
df <- read_csv("data_southwestUS.csv") ## presence absence dataframe with metadata

dfE1 <- df %>% subset(Epoch1 == "E1") ## E1 prevaccine
vaccineT <- df %>% distinct(BAPS2, PCV7.actual)
vaccineT <- vaccineT %>% subset(PCV7.actual == "VT") %>%
  dplyr::rename(W = PCV7.actual) %>%
  full_join(subset(vaccineT,PCV7.actual == "NVT")) %>%
  unite("vaccine", W:PCV7.actual, na.rm = TRUE)

dfF <- df %>% select(BAPS2, Epoch1) %>% group_by(Epoch1) %>%
  count(BAPS2) %>% mutate(freq = prop.table(n)) %>% ungroup() %>%
  select(Epoch1, BAPS2, freq) %>%
  spread(Epoch1, freq, fill = 0)

zero_E1 <- dfE1 %>% distinct(BAPS2) %>%
  mutate(n = 0, freq = 0)
```

```
#### Replicates  - null expectation Pro rata ####
dfE1_NVT_all <- data.frame(NULL)
replicates <- 10000

for(i in 1:replicates){
  #sub-sampling from each epoch independently with replacemnt - best subsampling strategy
  dfE1_NVT_i <- dfE1 %>% sample_frac(1, replace = TRUE) %>%
    subset(PCV7.actual == "NVT") %>% count(BAPS2) %>%
    mutate(freq = prop.table(n)) %>% bind_rows(zero_E1) %>%
    group_by(BAPS2) %>% summarise(n = sum(n), freq = sum(freq)) %>%
    arrange(BAPS2) %>% ungroup %>% mutate(iter = i)

  dfE1_NVT_all <- bind_rows(dfE1_NVT_all, dfE1_NVT_i)
}

dfE1_NVT_all <- dfE1_NVT_all %>% group_by(BAPS2) %>%
  summarise(expected_E3 = quantile(freq, 0.5), cil = quantile(freq, 0.025),
            ciu = quantile(freq, 0.975)) %>% ungroup

dfF <- left_join(dfF,dfE1_NVT_all) %>% replace(., is.na(.), 0) %>%
  mutate(delta = E3 - E1, deltaExp = expected_E3-E1,
         CI_low = cil-E1, CI_up = ciu-E1) %>%
```

```
  mutate(SC=BAPS2) %>%
  mutate(expectation = "Null exp.", signif = ifelse(delta > CI_up, "pos",NA)) %>%
  mutate(signif = ifelse(delta < CI_low, "neg", signif)) %>%
  mutate(signif = ifelse(E1 == 0 | E3 == 0, NA, signif))

dfF <- left_join(dfF,vaccineT)

#### Plot A: Prevalence by sequence cluster ####
dat1 <- mutate(dfF, signif = ifelse(E3 > CI_up | E3 < CI_low, -0.005,NA), Epoch = "E1")
dat1 <- subset(dat1, select = c(SC, Epoch, signif))

datPlotA <- dfF %>% select(SC, E1, E3) %>%
  pivot_longer(-SC, names_to =  "Epoch", values_to = "Prevalence") %>%
  left_join(dat1) %>% left_join(select(dfF, SC, vaccine)) %>%
  mutate(SC = as.character(SC)) %>% arrange(Epoch,-Prevalence)

rank <- as.character(unique(datPlotA$SC))

datPlotA <- datPlotA %>% mutate(SC = factor(SC, levels = rank))

plot1A <- ggplot(datPlotA, aes(x=SC, y=Prevalence, alpha=Epoch, fill=vaccine)) +
  geom_bar(stat='identity', position='dodge') +
  scale_alpha_manual(values = c(1,0.4),
                     labels = c("Pre-vaccine", "Post-vaccine")) +
  scale_fill_manual(values = c("#143c77","darkred","mediumpurple4"),
                    labels = c("Non-vaccine type", "Vaccine type", "Mixed"),
                    name = "Composition") +
  xlab("Strain (SC)") + theme_classic() +
  scale_y_continuous(expand = c(0, 0), limits = c(0,0.15)) +
  theme(legend.title = element_text(face="bold", size = 10),
        legend.justification = c(1, 1), legend.box = "horizontal",
        legend.position = c(1, 1), # legend.position = c(0.725, 0.85),
        legend.spacing.y = unit(0.2, "cm"),
        legend.text=element_text(size=9),
        legend.background = element_blank(),
        legend.box.background = element_rect(fill = gray(0.96), color = NA))

#### Plot B: Change in prevalence ####
dfF <- dfF %>% mutate(SC = factor(SC, levels = rank))

plot1B <- ggplot() + ylim(c(-0.09,0.08)) +
  geom_hline(yintercept=0, lty="dashed",size=0.65) +
  scale_colour_manual(values = c("#143c77","darkred","mediumpurple4"),
                      labels = c("Non-vaccine type", "Vaccine type", "Mixed"),
                      name = "SC Composition") +
  scale_linetype_manual(values = c(1), name = NULL) +
  scale_shape_manual(values = c("-", "+")) + theme_classic() +
  geom_point(data=dfF, aes(SC, delta, col = vaccine),
             size =4, alpha  = 0.85, show.legend = F) +
  geom_pointrange(data=dfF, aes(x=SC, y=deltaExp,
                                ymin=CI_low, ymax=CI_up, lty=expectation),
                  size=.15, fatten = 6, show.legend = F) +
  geom_point(data=dfF, aes(x=SC, y=-0.09, shape = signif), size=6,
```

```
            col = "lightsteelblue4", show.legend = F) +
  labs(x="Strain (SC)") + labs(y="Change in prevalence") +
  theme(legend.title = element_text(face="bold", size = 10),
        legend.text=element_text(size=9),
        legend.position = c(0.01, 1),
        legend.justification = c(0.01, 1),
        legend.box = "horizontal",
        legend.background = element_blank(),
        legend.box.background = element_rect(fill = gray(0.95), color = NA))

#### Final figure 1 ####
figure1 <- plot_grid(plot1A, plot1B, labels = c("A","B"), ncol = 1)
ggsave("figure1.png", figure1, width = 14,height = 7)
```

Figure 2: Simulations

```
#### Funct. rootfun ####
rfun <- function(t, state, pars){
  dstate <- unlist(repEq(t, state, pars)) # rate of change vector
  return(sum(abs(dstate)) - 1e-4)
}

#### Funct. checkFeas ####
checkFeas <- function(e, g){
  g <- as.data.frame(g)
  temp <- sapply(g, function(x)(length(unique(x))))
  id <- which(temp==1)
  e[id] <- as.numeric(unique(g[,id]))
  return(e)
}

#### Funct. replicator ####
repEq <- function(t, Nf, pars){
  with(as.list(c(Nf, pars)), {
    f <- NULL
    dfdt <- rep(0, nSC)
    xifi <- rep(0, nSC)

    ## loci frequencies ##
    for(k in 1:nCOG){ f[k] <- sum(Nf*genot[,k]) }

    for(k in 1:nSC){
      for(l in 1:nCOG){
        xifi[k] <- xifi[k] + Nf[k]*(genot[k,l]*(eqbm[l] - f[l]))
      }
    }

    ## dfdt ##
    for(k in 1:nSC){ dfdt[k] <- xifi[k] - Nf[k]*sum(xifi) }

    return(list(dfdt))
```

```
  })
}


####################################

#### Pre-intervation ####
#### Parameters ####
nCOG <- 10
nSC <- 8
eqbm <- c(0.5677,0.5138,0.4050,0.4388,0.4981,
          0.5065,0.5725,0.4513,0.5811,0.4034)
vacT <- c(2,3,5)
timeSteps <- 0.5
posCom <- 2^nCOG
genot <- data.frame(permutations(n=2,r=nCOG,v=c(0,1), repeats.allowed = T))
colnames(genot) <- as.character(1:nCOG)
pres <- c(7,193,320,337,340,621,674,842)
genotF <- c(0.2342,0.1511,0.0033,0.1248, 0.1750,0.0067,0.2219,0.083)
genotP <- genot[pres,]
times <- seq(from=0, to=1000, by=timeSteps)
pars <- list(eqbm = eqbm, nCOG = nCOG, nSC = nSC, genot = genotP)

#### Simulations ####
out1 <- out1P <- as.data.frame(lsodar(func=repEq,y=genotF,times=times,parms=pars,rootfun=rfun))
out1 <- out1 %>% pivot_longer(-time, names_to = "genotype", values_to = "frequency") %>%
  mutate(genotype = paste('G', genotype, sep = "")) %>% subset(frequency > 0)

out1_pre <- out1 %>% mutate(time = time/3)
E1 <- round(as.numeric(out1P[nrow(out1P),-1]), digits = 5) ### last time in the data frame

#### Intervation ####
#### Parameters ####
E2 <- E1
E2[vacT] <- 0 ### remove genotypes G2 (001) and G6 (101) 'vaccine types'
E2 <- E2/sum(E2)
idZ <- which(E2 > 0)
timeS <- 40
genotPV <- genotP[idZ,]
eqbmPV <- checkFeas(eqbm, genotPV)
pars$eqbm <- eqbmPV
pars <- list(eqbm = eqbm, nCOG = nCOG, nSC = nSC, genot = genotP)

rfun <- function(t, state, pars){
  dstate <- unlist(repEq(t, state, pars)) # rate of change vector
  return(sum(abs(dstate)) - 1e-6)
}

### Simulations ###
out2 <- out2P <- as.data.frame(lsodar(func=repEq,y=E2,times=times,parms=pars,rootfun=rfun))
out2 <- out2 %>% mutate(time = time + timeS) %>%
  pivot_longer(-time, names_to = "genotype", values_to = "frequency") %>%
  mutate(genotype = paste('G', genotype, sep = "")) %>% subset(time <= 80)
```

```
outF <- rbind(out1_pre, out2)
E3 <- round(as.numeric(out2P[nrow(out2P),-1]), digits = 5)

lineT <- data.frame(linetype = "Non-vaccine type",
                    genotype = paste('G', 1:8, sep = ""), index = 1:8)
lineT <- lineT %>% mutate(linetype = ifelse(index %in% vacT, "Vaccine type",
                                            "Non-vaccine type"), index = NULL)

outF <- outF %>% full_join(lineT) %>%
  mutate(frequency = ifelse(frequency == 0,
                            runif(1, min = 0.00001, max = 0.00002), frequency))

E3 <- outF %>% subset(time == max(outF$time)) %>% arrange(-frequency)
alphaT <- data.frame(genotype = E3$genotype,
                     alpha = c(seq(from=1,to=0.9,length.out =5),1,1,1))
outF <- full_join(outF, alphaT)

#### Figure A ####
plot2A <- ggplot(outF, aes(time, frequency, group = genotype, alpha = alpha,
                           colour = factor(linetype), linetype = factor(linetype))) +
  annotate("rect", xmin = 31.5, xmax = 37.5, ymin = -Inf,
           ymax = 0.28, fill = "gray93", colour = NA) +
  annotate("rect", xmin = 69, xmax = 75, ymin = -Inf,
           ymax = 0.28, fill = "gray93", colour = NA) +
  annotate("rect", xmin = 40, xmax = 42, ymin = -Inf,
           ymax = 0.28, fill = "darkslategray4", colour = NA, alpha = 0.3) +
  geom_line(size = 1) + theme_classic() +
  scale_colour_manual(values = c('#143c77', "darkred")) + xlim(0,75) + #899DA4 #C93312
  #scale_linetype_manual(values = c("solid", "dashed")) +
  geom_segment(x = 38.2, y = 0.3175, xend = 38.2, yend = 0.265,
               arrow = arrow(length = unit(0.25, "cm")),
               colour = 'black', show.legend = F) +
  annotate("text", x = 38.2, y = 0.33, label="Vaccine introduction",
           fontface="bold", color="black", size = 3.7) +
  #geom_segment(aes(x = 65, y = 0.29, xend = 74, yend = 0.29), colour = 'black') +
  annotate("text", x = 34.5, y = 0.295, label="Pre-vaccine\nequilibrium",
           color="dimgrey", fontface="bold", size = 2.65) +
  annotate("text", x = 72, y = 0.295, label="Post-vaccine\nequilibrium",
           color="dimgrey", fontface="bold", size = 2.65) +
  annotate("text", x = 41, y = 0.295, label="Predicted\nFitness",
           color="darkslategray", fontface="bold", size = 2.5) +
  ylab("Strain Prevalence") + xlab("Time") +
  scale_alpha(range = c(0.15, 1), guide = 'none') +
  theme(legend.justification=c(0,0),legend.position=c(0.02,0.85),
        legend.title=element_blank(),
        axis.text = element_text(colour = "black"),
        legend.text=element_text(size=8),
        plot.title = element_text(hjust = 0, size=10, face="bold"))

###################################
COGs <- 2371 ## 2371
SCs <- 35
dataOutF <- data.frame(NULL)
```

```r
replicates <- 10
VTselect <- 3

for(int in 1:replicates){
  el0 <- runif(COGs, min = 0.05, max = 0.95)
  dat <- as.matrix(replicate(SCs, sample(c(0,1), COGs, replace = TRUE)))
  dat <- unique(dat, MARGIN = 2)
  VT <- sample(1:SCs, VTselect)
  NVT <- (1:SCs)[-VT]

  if(ncol(dat) < SCs){
    cat("run again\n")
    break
  }

  #### E1 = pre-vaccine frequencies ####
  x1 <- QP(dat, as.matrix(el0))

  ### re-calculate el ###
  el <- dat %*% as.matrix(x1)

  #### E2 - frequencies just after vaccine intro ####
  x2 <- x1[-VT]
  x2 <- round(x2/sum(x2), digits = 5)
  dat2 <- dat[,-VT]
  fl <- dat2 %*% as.matrix(x2)

  #### E3 - frequencies long-term post-vaccine ####
  x3 <- QP(dat2, el)
  el3 <- dat2 %*% as.matrix(x3)

  #### fitness function "omega" just after vaccine intro ####
  whole <- as.numeric(el - fl) ### this is similar to (el - fl) and thus fitness.
  deltaE <- x3 - x2
  omega <- as.vector(t(dat2) %*% whole) ## length SCs, similar to FFS

  phi <- sum(x2 * omega) ## average fitness
  rateOfChange <- omega - phi ## "rate of change": omega_g - phi

  ################### Relative fitness ###################
  dataOut <- data.frame(riskDif = deltaE, r = rateOfChange)
  dataOut <- subset(dataOut, riskDif != 0)
  dataOut <- mutate(dataOut, change = ifelse(riskDif < 0, "Decreased", "Increased"), rep = int)
  dataOutF <- rbind(dataOutF, dataOut)
}

dataOutF <- dataOutF %>% mutate(col = ifelse(sign(riskDif) == sign(r), "same", "diff"))

#### Figure B ####
plot2B <- ggplot(dataOutF, aes(x=change, y=r,
                               group=factor(rep), colour = col)) +
  geom_point(position = position_dodge(width = 1), size = 2.5, alpha  = 0.6) +
  geom_hline(yintercept = 0, linetype = 3) + theme_classic() +
```

```
  xlab("Observed prevalence change") +
  ylab("Standardized Fitness") +
  theme(legend.position="none", axis.text = element_text(colour = "black"),
        plot.title = element_text(hjust = 0, size=10, face="bold")) +
  scale_colour_manual(values = c("gray70", "darkslategray4"))

figure2 <- plot_grid(plot2A, plot2B, labels = c("A", "B"), rel_widths = c(1.75,1))
ggsave("figure2.png", figure2, width = 14, height = 4.5)
```

Figure 3: Prediction (Southwest US data)

```
#### Data ####
dfFVT <- df %>% select(BAPS2,PCV7.actual, Epoch1) %>%
  group_by(Epoch1) %>%  count(BAPS2,PCV7.actual) %>%
  mutate(freq = round(prop.table(n), digits = 3)) %>% ungroup() %>%
  select(Epoch1, BAPS2, PCV7.actual, freq) %>%
  spread(Epoch1, freq, fill = 0) %>% arrange(BAPS2,PCV7.actual)

#### Present at E1 ####
SCE1 <- dfFVT %>% subset(E1 > 0) %>% ## & BAPS2 !="27"
  select(BAPS2, PCV7.actual) %>%
  mutate(Epoch1 = "E1")

#### NVTs Present at E1 ####
SCE2 <- SCE1 %>% subset(PCV7.actual == "NVT"  & BAPS2 !="27")


SC_freq_df <- df %>% select(BAPS2, PCV7.actual, Epoch1,
                            HMPREF0837_12128:HMPREF0837_10616) %>%
  arrange(BAPS2) %>% group_by(BAPS2,PCV7.actual,Epoch1) %>%
  mutate(SC_n = n()) %>% ungroup() %>%
  group_by(BAPS2,PCV7.actual,Epoch1,SC_n) %>%
  summarise_at(vars(HMPREF0837_12128:HMPREF0837_10616),mean) %>%
  ungroup()

### Get the matrix and the SC for the pre-vaccine epch "E1"
df_preV <- SCE1 %>% left_join(SC_freq_df)
SC_freq_preV <- as.matrix(df_preV %>% mutate(SC_freq=SC_n/sum(SC_n)) %>% select(SC_freq))
SC_COG_preV <- as.matrix(t(df_preV %>% select(HMPREF0837_12128:HMPREF0837_10616)))

#### Get e_l ####
el <- SC_COG_preV %*% SC_freq_preV

#### Figure A ####
dfImputed <- dfFVT %>% subset(PCV7.actual == "NVT" & E1 == 0) %>%
  select(BAPS2,PCV7.actual) %>% mutate(Epoch1 = "E1", n=1) %>%
  select(Epoch1,BAPS2,PCV7.actual,n)

dfFVTImputed <- df %>% select(BAPS2,PCV7.actual, Epoch1) %>%
  group_by(Epoch1) %>%  count(BAPS2,PCV7.actual) %>%
  ungroup() %>% bind_rows(dfImputed) %>% group_by(Epoch1) %>%
```

```
  mutate(freq = round(prop.table(n), digits = 3)) %>% ungroup() %>%
  select(Epoch1, BAPS2, PCV7.actual, freq) %>%
  spread(Epoch1, freq, fill = 0) %>% arrange(BAPS2,PCV7.actual)

dfFNVTImputed <- dfFVTImputed %>%
  subset(PCV7.actual == "NVT" & BAPS2 !="27") %>%
  mutate(deltaE = E3 - E1) %>% arrange(BAPS2)

#### E2 - frequencies just after vaccine intro ####
x_imputed <- dfFNVTImputed$E1
x_imputed <- as.matrix(round(x_imputed/sum(x_imputed), digits = 5))

dat2_imputed <- dfImputed %>% mutate(Epoch1 = "E2") %>%
  select(BAPS2,PCV7.actual,Epoch1) %>% bind_rows(SCE2) %>%
  arrange(BAPS2) %>% left_join(SC_freq_df) %>%
  select(HMPREF0837_12128:HMPREF0837_10616)
dat2_imputed <- as.matrix(dat2_imputed)

fl_imp <- t(dat2_imputed) %*% x_imputed

#### fitness function "omega" just after vaccine intro ####
whole <- as.numeric(el - fl_imp) ### this is similar to (el - fl) and thus fitness.
deltaE <- dfFNVTImputed$deltaE
omega <- as.vector(dat2_imputed %*% whole) ## length SCs, similar to FFS

phi <- sum(x_imputed * omega) ## average fitness
rateOfChange <- omega - phi ## "rate of change": omega_g - phi

dat3AB <- dfFNVTImputed %>% select(BAPS2, deltaE) %>%
  mutate(r = rateOfChange) %>% subset(deltaE != 0) %>%
  mutate(change = ifelse(deltaE < 0, "Decreased", "Increased")) %>%
  mutate(col = ifelse(sign(deltaE) == sign(r), "same", "diff")) %>%
  left_join(vaccineT)

highlight <- dat3AB %>% filter(col == "diff")

lm3 <- lm(r~deltaE,dat3AB)
summary(lm3)
```

Call: lm(formula = r ~ deltaE, data = dat3AB)

Residuals: Min 1Q Median 3Q Max -12.7964 -1.8504 -0.1459 2.3969 10.6031

Coefficients: Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.4151 1.0220 0.406 0.688
deltaE 182.1310 38.6050 4.718 5.55e-05 *** — Signif. codes: 0 ‘’ ***0.001*** ’’ *0.01* ’’ 0.05 ‘.’ 0.1 ’ ’ 1

Residual standard error: 4.995 on 29 degrees of freedom Multiple R-squared: 0.4342, Adjusted R-squared: 0.4147 F-statistic: 22.26 on 1 and 29 DF, p-value: 5.555e-05

```
#### Figure A ####
plot3A <- ggplot(dat3AB, aes(x=deltaE, y=r, colour = vaccine)) +
  theme_classic() + xlab("Observed Prevalence Change") +
  ylab("Standardized Fitness") + xlim(-0.03, 0.065) + ylim(-12,20) +
  geom_hline(yintercept=0, lty="dotted", alpha=.6) +
  geom_vline(xintercept=0, lty="dotted", alpha=.6) +
```

```
  annotate("rect", xmin=-0.03,xmax=0,ymin=-12,ymax=0,
           fill="darkslategray4", alpha= 0.15) +
  annotate("rect", xmin=0,xmax=0.065,ymin=0,ymax=20,
           fill="darkslategray4", alpha= 0.15) +
  geom_smooth(aes(group = 1), color="grey70", method=lm,
              formula = y~x,  show.legend=FALSE, se=FALSE) +
  geom_point(size = 3) + theme(legend.position = "none",
                               axis.text = element_text(colour = "black"),) +
  scale_colour_manual(values = c("#143c77","mediumorchid4")) +
  geom_text_repel(aes(label = paste("SC", BAPS2, sep="-")), data = highlight, size = 3.5)


#### Figure B ####
df_postV <- SCE2 %>% left_join(SC_freq_df)
SC_COG_postV <- as.matrix(t(df_postV %>% select(HMPREF0837_12128:HMPREF0837_10616)))

SC_freq_postV_obs <- SCE2 %>% mutate(Epoch1 = "E3") %>%
  left_join(SC_freq_df) %>%
  mutate(SC_freq=SC_n/sum(SC_n, na.rm = T)) %>%
  select(BAPS2, PCV7.actual, SC_freq) %>%
  mutate(SC_freq = replace_na(SC_freq, 0))

## Predict postV frequencies
SC_freq_postV_pred <- QP(SC_COG_postV, el) #Matrix: rows = COGs, columns = (SCs - VT)
SC_freq_postV_obs <- SC_freq_postV_obs %>%
  mutate(SC_pred = SC_freq_postV_pred) %>%
  left_join(vaccineT)

outlier3B <- SC_freq_postV_obs %>% mutate(diff = abs(SC_freq - SC_pred))
outlier3B <- outlier3B %>% filter(diff %in% boxplot(outlier3B$diff, plot = FALSE)$out)

W_model3B <- lm(data=SC_freq_postV_obs,SC_freq~SC_pred);
summary(W_model3B)
```

Call: lm(formula = SC_freq ~ SC_pred, data = SC_freq_postV_obs)

Residuals: Min 1Q Median 3Q Max -0.059467 -0.014478 -0.002457 0.015118 0.053442

Coefficients: Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.012366 0.008616 1.435 0.16364
SC_pred 0.666107 0.198686 3.353 0.00255 ** — Signif. codes: 0 ‘’ ***0.001*** ’’ *0.01* ’’ 0.05 ‘.’ 0.1 ’ ’ 1

Residual standard error: 0.02329 on 25 degrees of freedom Multiple R-squared: 0.3101, Adjusted R-squared: 0.2826 F-statistic: 11.24 on 1 and 25 DF, p-value: 0.002551

```
confint(W_model3B)
```

```
                2.5 %     97.5 %
```

(Intercept) -0.005380061 0.03011185 SC_pred 0.256905970 1.07530899

```
linearHypothesis(W_model3B, c("(Intercept) = 0", "SC_pred = 1"), test = "Chisq")
```

Linear hypothesis test

Hypothesis: (Intercept) = 0 SC_pred = 1

Model 1: restricted model Model 2: SC_freq ~ SC_pred

Res.Df RSS Df Sum of Sq Chisq Pr(>Chisq) 1 27 0.015093
2 25 0.013561 2 0.0015319 2.8241 0.2436

```
linearHypothesis(W_model3B, c("(Intercept) = 0", "SC_pred = 1"))
```

Linear hypothesis test

Hypothesis: (Intercept) = 0 SC_pred = 1

Model 1: restricted model Model 2: SC_freq ~ SC_pred

Res.Df RSS Df Sum of Sq F Pr(>F) 1 27 0.015093
2 25 0.013561 2 0.0015319 1.412 0.2624

```
plot3B <- ggplot(SC_freq_postV_obs,
                 aes(x = SC_pred, y = SC_freq, colour = vaccine)) +
  geom_segment(aes(x=0,xend=0.12,y=0,yend=0.12),
               color="black",alpha=.7,lwd=0.5,lty=3) +
  theme(legend.position = "none") + theme_classic() +
  geom_smooth(method='lm', color="#899DA4" ,
              formula=y~x, alpha=0.3, lwd=.6,
              fullrange=T, linetype="blank", show.legend=F) +
  annotate(geom = "text", x=0.11, y =0.114,
           label = "1:1 line", angle = 45, size = 3) +
  geom_point(size=3) +  ##
  scale_x_continuous("Predicted Prevalence (NFDS)")+
  scale_y_continuous("Observed Prevalence") +
  coord_fixed(ratio = 1, xlim=c(0,0.12), ylim=c(0,0.12)) +
  scale_colour_manual(values = c("#143c77","mediumorchid4")) +
  theme(legend.position = "none",
        axis.text = element_text(colour = "black"),) +
  geom_text_repel(aes(label = paste("SC", BAPS2, sep = "-")), data = outlier3B, size = 3.5)


#### Figure C ####

SC_freq_E1 <- df_preV %>%
  mutate(SC_freq_E1=SC_n/sum(SC_n)) %>%
  select(BAPS2,PCV7.actual,SC_freq_E1)

SC_freq_postV_diff <- SC_freq_postV_obs %>%
  left_join(SC_freq_E1) %>%
  mutate(diff_pred = SC_freq_E1 - SC_pred,
         diff_obs = SC_freq_E1 - SC_freq) %>%
  left_join(vaccineT)

SC_freq_postV_diff <- SC_freq_postV_diff %>%
  mutate(diff = abs(diff_pred - diff_obs)) %>%
  arrange(diff)

stats <- summary(lm(SC_freq_postV_diff$diff_pred~SC_freq_postV_diff$diff_obs))
ars <- round(stats$adj.r.squared, digits = 3)
sseE <- round(sse(SC_freq_postV_diff$diff_obs, SC_freq_postV_diff$diff_pred), digits = 3)
maeE <- round(mae(SC_freq_postV_diff$diff_obs, SC_freq_postV_diff$diff_pred),digits = 3)##Mean Absolute
rmseE <- round(rmse(SC_freq_postV_diff$diff_obs, SC_freq_postV_diff$diff_pred),digits = 3) ##Root Mean

accNFDS <- data.frame(Model = "Accesory genome (NFDS)", nloci = length(el),
```

```
                      adj.r.squared = ars, SSE = sseE, RMSE = rmseE)


W_model3C <- lm(data=SC_freq_postV_diff,diff_obs~diff_pred);
summary(W_model3C)
```

Call: lm(formula = diff_obs ~ diff_pred, data = SC_freq_postV_diff)

Residuals: Min 1Q Median 3Q Max -0.057236 -0.011661 0.002054 0.011921 0.067643

Coefficients: Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.003684 0.006783 -0.543 0.5919
diff_pred 0.795065 0.273307 2.909 0.0075 ** — Signif. codes: 0 ‘’ ***0.001*** ’’ *0.01* ’’ 0.05 ‘.’ 0.1 ’ ’ 1

Residual standard error: 0.0243 on 25 degrees of freedom Multiple R-squared: 0.2529, Adjusted R-squared: 0.223 F-statistic: 8.463 on 1 and 25 DF, p-value: 0.007504

```
confint(W_model3C)
```

```
               2.5 %     97.5 %
```

(Intercept) -0.01765401 0.01028661 diff_pred 0.23217925 1.35795061

```
linearHypothesis(W_model3C, c("(Intercept) = 0", "diff_pred = 1"), test = "Chisq")
```

Linear hypothesis test

Hypothesis: (Intercept) = 0 diff_pred = 1

Model 1: restricted model Model 2: diff_obs ~ diff_pred

Res.Df RSS Df Sum of Sq Chisq Pr(>Chisq) 1 27 0.015093
2 25 0.014761 2 0.00033197 0.5623 0.7549

```
outlier3C <- SC_freq_postV_diff %>%
  filter(diff %in% boxplot(SC_freq_postV_diff$diff, plot = FALSE)$out)

#### Figure C ####
plot3C <- ggplot(SC_freq_postV_diff, aes(x = diff_pred,
                                         y = diff_obs, colour = vaccine)) +
  geom_segment(aes(x=-0.1,xend=0.05,y=-0.1,yend=0.05),
               color="black",alpha=.7,lwd=0.5,lty=3) +
  geom_smooth(method='lm', color="gray80" ,formula=y~x,
              alpha=0.3, lwd=.6, fullrange=T,
              linetype="blank", show.legend=F) +
  annotate(geom = "text", x=0.0375, y =0.0425,
           label = "1:1 line", angle = 45, size = 3) +
  geom_point(size=3) + theme_classic() +
  scale_colour_manual(values = c("#143c77","mediumorchid4"),
                      labels = c("Non-vaccine type", "Mixed"),
                      name = "SC Composition") +
  theme(legend.position = "none",
        axis.text = element_text(colour = "black"),) +
  xlab("Predicted Prevalence Change (NFDS)")+
  ylab("Observed Prevalence Change") +
  coord_fixed(ratio = 1, xlim=c(-0.1,0.05), ylim=c(-0.1,0.05)) +
  annotate("text",x=-0.097,y=0.04, size=2.5,hjust = 0,
           label=paste("SSE = ", sseE, "\nRMSE = ",
                       rmseE, "\nAdj. R2 = ", ars)) +
```

```
  geom_text_repel(aes(label = paste("SC", BAPS2, sep = "-")), data = outlier3C, size = 3.5)

#### Figure D ####
SC_freq_postV_diff <- SC_freq_postV_diff %>%
  mutate(prorata = SC_freq_E1/sum(SC_freq_E1)) %>%
  mutate(diff_predPro = SC_freq_E1 - prorata) %>%
  mutate(diffP = abs(diff_predPro - diff_obs))

stats <- summary(lm(SC_freq_postV_diff$diff_predPro~SC_freq_postV_diff$diff_obs))
ars <- round(stats$adj.r.squared, digits = 3)
sseE <- round(sse(SC_freq_postV_diff$diff_obs, SC_freq_postV_diff$diff_predPro), digits = 3)
maeE <- round(mae(SC_freq_postV_diff$diff_obs, SC_freq_postV_diff$diff_predPro),digits = 3)##Mean Absol
rmseE <- round(rmse(SC_freq_postV_diff$diff_obs, SC_freq_postV_diff$diff_predPro),digits = 3) ##Root Me

accProrata <- data.frame(Model = "Accesory genome (Pro rata)", nloci = length(el),
                         adj.r.squared = ars, SSE = sseE, RMSE = rmseE)

W_model3D <- lm(data=SC_freq_postV_diff,diff_obs~diff_predPro);
summary(W_model3D)
```

Call: lm(formula = diff_obs ~ diff_predPro, data = SC_freq_postV_diff)

Residuals: Min 1Q Median 3Q Max -0.053397 -0.014551 -0.001313 0.021763 0.038569

Coefficients: Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.027719 0.009368 -2.959 0.00666 ** diff_predPro -0.541862 0.431660 -1.255 0.22098
— Signif. codes: 0 ‘’ ***0.001*** ’’ *0.01* ’’ 0.05 ‘.’ 0.1 ’ ’ 1

Residual standard error: 0.02727 on 25 degrees of freedom Multiple R-squared: 0.05929, Adjusted R-squared: 0.02167 F-statistic: 1.576 on 1 and 25 DF, p-value: 0.221

```
confint(W_model3D)
```

```
                2.5 %       97.5 %
```

(Intercept) -0.04701286 -0.008425814 diff_predPro -1.43088194 0.347158089

```
linearHypothesis(W_model3D, c("(Intercept) = 0", "diff_predPro = 1"), test = "Chisq")
```

Linear hypothesis test

Hypothesis: (Intercept) = 0 diff_predPro = 1

Model 1: restricted model Model 2: diff_obs ~ diff_predPro

Res.Df RSS Df Sum of Sq Chisq Pr(>Chisq)
1 27 0.028071
2 25 0.018586 2 0.0094854 12.759 0.001696 ** — Signif. codes: 0 ‘’ ***0.001*** ’’ *0.01* ’’ 0.05 ‘.’ 0.1 ’ ’ 1

```
plot3D <- ggplot(SC_freq_postV_diff, aes(x = diff_predPro,
                                         y = diff_obs, colour = vaccine)) +
  geom_segment(aes(x=-0.1,xend=0.05,y=-0.1,yend=0.05),
               color="black",alpha=.7,lwd=0.5,lty=3) +
  geom_smooth(method='lm', color="gray80" ,formula=y~x,
              alpha=0.3, lwd=.6, fullrange=T,
              linetype="blank", show.legend=F) +
  annotate(geom = "text", x=0.0375, y =0.0425,
           label = "1:1 line", angle = 45, size = 3) +
  geom_point(size=3) + theme_classic() +
```

```
  scale_colour_manual(values = c("#143c77","mediumorchid4"),
                      labels = c("Non-vaccine type", "Mixed"),
                      name = "SC Composition") +
  theme(legend.position = "none",
        axis.text = element_text(colour = "black"),) +
  xlab("Predicted Prevalence Change (Pro rata)")+
  coord_fixed(ratio = 1, ylim=c(-0.1,0.05), xlim=c(-0.1,0.05)) +
  ylab("Observed Prevalence Change") +
  annotate("text",x=-0.097,y=0.04, size=2.5,hjust = 0,
           label=paste("SSE = ", sseE, "\nRMSE = ",
                       rmseE, "\nAdj. R2 = ", ars))


#### Combine figure 3 ####
ptitle1 <- ggplot() + theme_void() +
  annotate("rect", fill = "darkslategray4", alpha = 0.3,
           xmin = 0, xmax = 1, ymin = 0, ymax = 1)
ptitle1 <- ggdraw(ptitle1) + draw_label("Predicted Fitness",
                                        fontface='bold', colour = "darkslategrey") ## ,
pA <- plot_grid(plot3A, labels = c("A"))
pA <- plot_grid(ptitle1, pA, ncol = 1, scale = c(1,0.9), rel_heights=c(0.075, 1))


ptitle2 <- ggplot() + theme_void() +
  annotate("rect", fill = "gray93", xmin = 0, xmax = 1, ymin = 0, ymax = 1)
ptitle2 <- ggdraw(ptitle2) + draw_label("Post-vaccine Equilibrium Frequencies",
                                        fontface='bold', colour = "gray30") ## ,
pBCD <- plot_grid(plot3B, plot3C, plot3D, ncol = 3, labels = c("B","C","D"))
pBCD <- plot_grid(ptitle2, pBCD, ncol = 1, scale = c(1,0.9), rel_heights=c(0.075, 1))
plot3 <- plot_grid(pA, pBCD, rel_widths = c(1,3), rel_heights = c(1,1))

ggsave("figure3.png", plot3, width = 16, height = 4)
```

Fig. Suppl 1: Tree

```
## The tree was modified after being produced (style purposes)

readMatrix<-function(heatmapData){
  if (is.matrix(heatmapData)) {
    x = data.frame(heatmapData)
  }
  else if (is.data.frame(heatmapData)) {
    x = heatmapData
  }
  else {
    x<-read.csv(heatmapData,row.names=1)
  }
  x
}
getLayout<-function(infoFile,infoCols,heatmapData,barData,doBlocks,treeWidth=10,infoWidth=10,dataWidth=

  # m = layout matrix
  # w = layout widths vector
```

```
# h = layout height vector

# tree
w = c(edgeWidth,treeWidth)
m<-cbind(c(0,0,0),c(0,1,0)) # first two columns, edge + tree
x = 1

# info
if (!is.null(infoFile)) { # info is provided

  printCols = TRUE
  if (!is.null(infoCols)) {
    if (is.na(infoCols)) {
      printCols = FALSE
    }}

  if (printCols) {
    x = x + 1
    m<-cbind(m,c(0,x,0))
    w = c(w,infoWidth)
  }
}

# heatmap
if (!is.null(heatmapData)) {
  x = x + 1
  m<-cbind(m,c(x+1,x,0)) # add heatmap & labels
  x = x + 2
  m[1,2] = x # add heatmap scale above tree
  w = c(w,dataWidth)
}

# barplot
if (!is.null(barData)) {
  x = x + 1
  m<-cbind(m,c(0,x,x+1)) # barplot and scale bar
  x = x + 1
  w = c(w,barDataWidth)
}

if (doBlocks) {
  x = x + 1
  m<-cbind(m,c(0,x,0)) # recomb blocks
  w = c(w,blockPlotWidth)
}

# empty edge column
m<-cbind(m,c(0,0,0))
w = c(w,edgeWidth)

if (!is.null(heatmapData) | !is.null(barData)) { h = c(labelHeight,mainHeight,labelHeight) }
else { h = c(edgeWidth,mainHeight,edgeWidth) }
```

```
  return(list(m=as.matrix(m),w=w,h=h))
}
plotTree<-function(tree,ladderise=NULL,heatmapData=NULL,barData=NULL,infoFile=NULL,blockFile=NULL,snpFi

  require(ape)

  # PREPARE TREE, CHOOSE LADDERISATION OR NOT, AND GET TIP ORDER
  if (is.character(tree)){
    t<-read.tree(tree)
  }
  else t<-tree
  if (is.null(ladderise))
  {
    tl<-t
  }
  else if (ladderise=="descending")
  {
    tl<-ladderize(t, T)
  }
  else if (ladderise=="ascending")
  {
    tl<-ladderize(t, F)
  }
  else if (!is.null(ladderise))
  {
    print("Ladderise option should be exactly 'ascending' or 'descending'.  Any other command will raise
  }
  tips<-tl$edge[,2]
  tip.order<-tips[tips<=length(tl$tip.label)]
  tip.label.order<-tl$tip.label[tip.order] # for ordering data. note that for tiplabel(), the order is


  # PREPARE HEATMAP DATA
  if (!is.null(heatmapData)) {

    # read heatmap data and convert to data frame
    x<-readMatrix(heatmapData)

    # order rows of heatmap matrix to match tree
    y.ordered<-x[tip.label.order,]

    # reorder columns?
    if (!is.null(cluster)) {
      if (!(cluster==FALSE)) {

        if (cluster=="square" & ncol(y.ordered)==nrow(y.ordered)) {
          # order columns to match row order
          original_order<-1:nrow(x)
          names(original_order)<-rownames(x)
          reordered<-original_order[tip.label.order]
          y.ordered<-y.ordered[,rev(as.numeric(reordered))]
        }
```

```
      else {
        # cluster columns
        if (cluster==TRUE) {cluster="ward"} # set default clustering algorithm
        h<-hclust(dist(t(na.omit(y.ordered))),cluster)
        y.ordered<-y.ordered[,h$order]
      }

    }} # finished reordering columns

} # finished setting up heatmap data


# PREPARE BAR PLOT
if (!is.null(barData)) {
  b<-readMatrix(barData)
  barData<-b[,1]
  names(barData)<-rownames(b)
}

# PREPARE INFO TO PRINT
if (!is.null(infoFile)) {
  info<-readMatrix(infoFile)
  info.ordered<-info[rev(tip.label.order),]
}
else {info.ordered=NULL}


# PREPARE DISCRETE TRAIT FOR COLOURING NODES AND INFERRING ANCESTRAL STATES
ancestral=NULL
nodeColourSuccess=NULL
if (!is.null(colourNodesBy) & !is.null(infoFile)) {

  if (colourNodesBy %in% colnames(info.ordered)) {
    nodeColourSuccess = TRUE
    loc1<-info.ordered[,which(colnames(info.ordered)==colourNodesBy)]

    # assign values
    tipLabelSet <- character(length(loc1))
    names(tipLabelSet) <- rownames(info.ordered)
    groups<-table(loc1,exclude="")
    n<-length(groups)
    groupNames<-names(groups)

    # set colours
    if (is.null(tipColours)){ colours<-rainbow(n) }
    else{ colours<-tipColours }

    # assign colours based on values
    for (i in 1:n) {
      g<-groupNames[i]
      tipLabelSet[loc1==g]<-colours[i]
    }
    tipLabelSet <- tipLabelSet[tl$tip]
```

```
    # ancestral reconstruction
    if (ancestral.reconstruction) { ancestral<-ace(loc1,tl,type="discrete") }

  }}
# finished with trait labels and ancestral reconstruction


# OPEN EXTERNAL DEVICE FOR DRAWING
# open PDF for drawing
if (!is.null(outputPDF)) {
  pdf(width=w,height=h,file=outputPDF)
}
# open PNG for drawing
if (!is.null(outputPNG)) {
  png(width=w,height=h,file=outputPNG)
}


# SET UP LAYOUT FOR PLOTTING
doBlocks <- (!is.null(blockFile) | !is.null(snpFile))
l <- getLayout(infoFile,infoCols,heatmapData,barData,doBlocks,treeWidth=treeWidth,infoWidth=infoWidth
layout(l$m, widths=l$w, heights=l$h)


# PLOT TREE
par(mar=rep(0,4))
tlp<-plot.phylo(tl,no.margin=T,show.tip.label=tip.labels,label.offset=offset,edge.width=lwd,edge.colo

# colour by trait
if (!is.null(nodeColourSuccess)) {
  tiplabels(col= tipLabelSet,pch=16,cex=tip.colour.cex)
  if (ancestral.reconstruction) { nodelabels(pie=ancestral$lik.anc, cex=pie.cex, piecol=colours) }
  if (legend) { legend(legend.pos,legend=groupNames,fill=colours) }
}

if (axis) { axisPhylo(axisPos) }

# PLOT INFO
if (!is.null(infoFile)) { # info is provided

  printCols = TRUE
  if (!is.null(infoCols)) {
    if (is.na(infoCols)) {
      printCols = FALSE
    }}

  if (printCols) {

    par(mar=rep(0,4))

    if (!is.null(infoCols)) {infoColNumbers = which(colnames(info.ordered) %in% infoCols)}
    else { infoColNumbers = 1:ncol(info.ordered)}
```

```
    plot(NA,axes=F,pch="",xlim=c(0,length(infoColNumbers)+1.5),ylim=c(0.5,length(tl$tip)+0.5),xaxs="i"

    # plot all info columns
    for (i in 1:length(infoColNumbers)) {
      j<-infoColNumbers[i]
      text(x=rep(i+1,nrow(info.ordered)+1),y=c((nrow(info.ordered)):1),info.ordered[,j],cex=infoCex)
    }

  }
}


# PLOT HEATMAP
if (!is.null(heatmapData)) {

  if (is.null(heatmapBreaks)) { heatmapBreaks = seq(min(y.ordered,na.rm=T),max(y.ordered,na.rm=T),leng

  # plot heatmap
  par(mar=rep(0,4), xpd=TRUE)
  image((1:ncol(y.ordered))-0.5,(1:nrow(y.ordered))-0.5, as.matrix(t(y.ordered)),col=heatmap.colours,l

  # draw vertical lines over heatmap
  if (!is.null(vlines.heatmap)) {
    for (v in vlines.heatmap) {abline(v=v, col=vlines.heatmap.col)}
  }

  # overlay blocks on heatmap
  if (!is.null(heatmap.blocks)) {
    for (coords in heatmap.blocks) {rect(xleft=coords[1], 0, coords[2], ncol(y.ordered), col=vlines.he
  }


  # data labels for heatmap
  par(mar=rep(0,4))
  plot(NA, axes=F, xaxs="i", yaxs="i", ylim=c(0,2), xlim=c(0.5,ncol(y.ordered)+0.5))
  text(1:ncol(y.ordered)-0.5,rep(0,ncol(x)),colnames(y.ordered), srt=90, cex=colLabelCex, pos=4)

  # scale for heatmap
  par(mar=c(2,0,0,2))
  #image(as.matrix(seq(min(y.ordered,na.rm=T),max(y.ordered,na.rm=T),length.out=length(heatmap.colours
  image(as.matrix(seq(min(y.ordered,na.rm=T),max(y.ordered,na.rm=T),length.out=length(heatmap.colours)
  axis(1,at=heatmapBreaks[-length(heatmapBreaks)]/max(y.ordered,na.rm=T),labels=round(heatmapBreaks[-1
}

# BARPLOT
if (!is.null(barData)) {
  par(mar=rep(0,4))
  barplot(barData[tip.label.order], horiz=T, axes=F, xaxs="i", yaxs="i", xlab="", ylab="", ylim=c(0.25

  # scale for barData plot
  par(mar=c(2,0,0,0))
  plot(NA, yaxt="n", xaxs="i", yaxs="i", xlab="", ylab="", ylim=c(0,2), xlim=c((-1)*max(barData,na.rm=
}
```

```
  # SNPS AND RECOMBINATION BLOCKS
  if (doBlocks) {
    par(mar=rep(0,4))
    plot(NA,axes=F,pch="",xlim=c(genome_offset,genome_offset+genome_size+1.5),ylim=c(0.5,length(tl$tip)+

    # plot snps
    if (!is.null(snpFile)) {
      snps<-read.csv(snpFile,header=F,row.names=1) # in case colnames start with numbers or contain das
      snps_strainCols <- snps[1,] # column names = strain names
      snps<-snps[-1,] # drop strain names

      for (strain in tip.label.order){
        # print SNPs compared to ancestral alleles in column 1
        s<-rownames(snps)[(as.character(snps[,1]) != as.character(snps[,which(snps_strainCols==strain)])]
        y <- which(tip.label.order==strain)
        if (length(s)>0) {
          for (x in s) {
            points(x,y,pch="|",col=snp_colour,cex=0.25)
          }
        }
      }
    }

    # plot blocks
    if (!is.null(blockFile)){
      blocks<-read.delim(blockFile,header=F)
      for (i in 1:nrow(blocks)) {
        if (as.character(blocks[i,1]) %in% tip.label.order) {
          y <- which(tip.label.order==as.character(blocks[i,1]))
          x1 <- blocks[i,2]
          x2 <- blocks[i,3]
          lines(c(x1,x2),c(y,y),lwd=blwd,lend=2,col=block_colour)
        }
      }
    }

  } # finished with SNPs and recomb blocks

  # CLOSE EXTERNAL DRAWING DEVICE
  if (!is.null(outputPDF) | !is.null(outputPNG)) {
    dev.off()
  }

  # RETURN ordered info and ancestral reconstruction object
  if (!is.null(heatmapData)){mat=as.matrix(t(y.ordered))}
  else {mat=NULL}
  return(list(info=info.ordered,anc=ancestral,mat=mat,strain_order=tip.label.order))
}


tree<-read.tree("RAxML_bestTree.All.Core.tre") #Core genome tree
#tree<-read.tree("RAxML_bestTree.All.Binary.tre") #Accessory genome tree
#tree <- ladderize(midpoint.root(tree), right = FALSE) #Ladderized the tree and midpoint root
```

```
heatmap_colors <- c("#ECCBAE", "#A42820", "#5F5647", "#9A8822", "#74A089", "#D8B70A",
                    "#046C9A", "#3F5151", "#4E2A1E", "#F2300F", "#FF0000", "#02401B",
                    "#D69C4E", "#FAD510", "#CB2314", "#273046", "#00A08A", "#A2A475",
                    "#ABDDDE", "#550307", "#354823", "#F5CDB4", "#F2AD00", "#972D15",
                    "#000000", "#E1BD6D", "#EABE94", "#F8AFA8", "#F98400", "#F1BB7B",
                    "#81A88D", "#0B775E", "#35274A", "#FDDDA0", "#f0f0f0", "#377eb8",
                    "#e41a1c","#899DA4", "#D67236")

plotTree(tree,
         heatmapData="NWMA_metadata_numerated_revised.csv",
         heatmap.colours=heatmap_colors,
         legend=T,
         tip.labels = FALSE,
         #tipColours=, tip.colour.cex=.8, legend.pos="bottomright",
         lwd=.85, treeWidth=10, dataWidth=3
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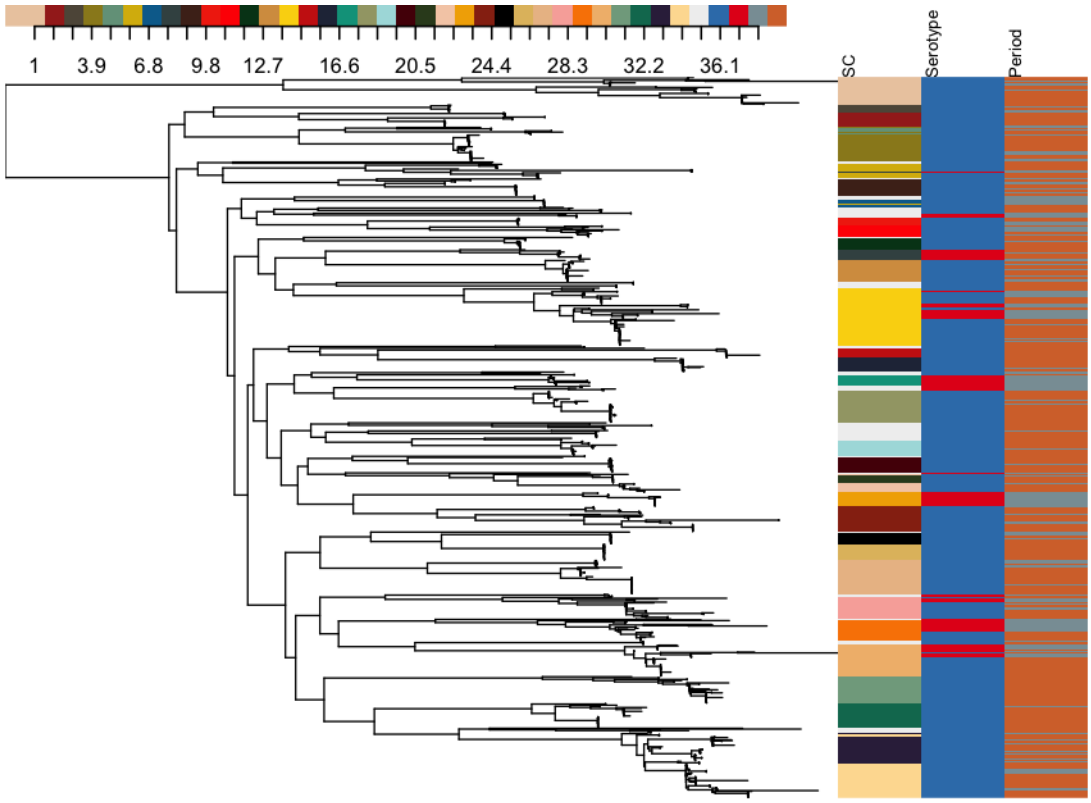

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[778] “ERR907077_G1_19A_1998” “ERR907096_G1_9I_1998” “ERR907250_G1_9I_1999” [781]
“ERR907090_G1_9I_1998” “ERR907071_G1_9I_1998” “ERR907454_G1_9I_1998” [784] “ERR907615_G2_31_2006”
“ERR907494_G3_31_2007” “ERR907114_G1_31_1999” [787] “ERR907117_G1_31_1999” “ERR906980_G5_31_2011”
“ERR907032_G5_31_2011” [790] “ERR907604_G2_31_2006” “ERR907854_G3_31_2007” “ERR907426_G1_31_1999”
[793] “ERR907028_G5_31_2011” “ERR907302_G5_31_2012” “ERR907299_G5_31_2011” [796]

"ERR907185_G5_31_2012" "ERR907612_G3_31_2007" "ERR907109_G1_31_1999" [799] "ERR907204_G1_31_1999"
"ERR907087_G1_31_1998" "ERR907767_G3_31_2007" [802] "ERR907739_G2_31_2007" "ERR907684_G4_31_2007"
"ERR907700_G3_31_2007" [805] "ERR907049_G1_31_1998" "ERR907812_G2_7C_2007" "ERR907843_G3_7C_2007"
[808] "ERR907832_G2_7C_2007" "ERR906954_G5_7C_2010" "ERR907041_G5_7C_2011" [811]
"ERR907040_G5_7C_2011" "ERR907182_G5_7C_2012" "ERR907355_G5_7C_2011" [814] "ERR907205_G1_25F_1999"
"ERR907391_G1_5_1999" "ERR907199_G5_5_2011"
[817] "ERR907201_G5_5_2011" "ERR907575_G1_19F_1999" "ERR907356_G5_7C_2011" [820]
"ERR907674_G4_7C_2007" "ERR907162_G5_7C_2011" "ERR907160_G5_7C_2011" [823] "ERR907317_G5_7C_2010"
"ERR907118_G5_7C_2011" "ERR907588_G3_8_2006"
[826] "ERR907286_G1_8_1999" "ERR907151_G5_11A_2010" "ERR907392_G1_11D_1999" [829]
"ERR907570_G1_11D_1999" "ERR907228_G1_11D_1998" "ERR907866_G2_11D_2007" [832]
"ERR907350_G5_11D_2011" "ERR906963_G5_11D_2010" "ERR906962_G5_11D_2010" [835]
"ERR907613_G3_11D_2007" "ERR907259_G1_11D_1999" "ERR907256_G1_11D_1999" [838]
"ERR907253_G1_11D_1999" "ERR907229_G1_11D_1998" "ERR907273_G1_11D_1999" [841]
"ERR907337_G5_11D_2010" "ERR907136_G5_11D_2010" "ERR907135_G5_11D_2010" [844]
"ERR907652_G4_11D_2006" "ERR907539_G3_11D_2007" "ERR907679_G4_11D_2007" [847]
"ERR907608_G4_11D_2006" "ERR907694_G2_11D_2007" "ERR907668_G3_11D_2007" [850]
"ERR907708_G3_11D_2007" "ERR907578_G2_11D_2006" "ERR907581_G4_11D_2006" [853]
"ERR907042_G5_11D_2012" "ERR907534_G3_11D_2006" "ERR907188_G5_11D_2010" [856]
"ERR907770_G4_11D_2007" "ERR907732_G3_11D_2007" "ERR907726_G2_11D_2007" [859]
"ERR907706_G2_11D_2007" "ERR907720_G3_11D_2007" "ERR907133_G5_11D_2010" [862]
"ERR907571_G1_11D_1999" "ERR907523_G2_37_2007" "ERR907653_G2_37_2007" [865] "ERR907673_G4_33A_2007"
"ERR907174_G5_37_2011" "ERR907799_G3_37_2007" [868] "ERR907750_G3_37_2007" "ERR907274_G1_37_1999"
"ERR907828_G4_37_2007" [871] "ERR907576_G1_37_2000" "ERR907208_G1_33F_1999" "ERR907591_G4_3_2006"
[874] "ERR907261_G1_3_1999" "ERR907331_G5_3_2010" "ERR907805_G3_3_2007"
[877] "ERR907007_G5_3_2010" "ERR907022_G5_3_2011" "ERR907512_G2_3_2006"
[880] "ERR907315_G5_3_2010" "ERR907830_G4_3_2007" "ERR907689_G4_3_2006"
[883] "ERR907688_G4_3_2006" "ERR907466_G2_3_2007" "ERR907297_G5_3_2011"
[886] "ERR907351_G5_3_2011" "ERR907134_G5_3_2010" "ERR907786_G2_3_2007"
[889] "ERR907492_G4_3_2006" "ERR907857_G3_3_2007" "ERR907482_G4_3_2007"
[892] "ERR907074_G1_10A_1998" "ERR907073_G1_10A_1998" "ERR907230_G1_10A_1998"
[895] "ERR906979_G5_10A_2011" "ERR907646_G2_10A_2006" "ERR907881_G3_10A_2007"
[898] "ERR907241_G1_10A_1998" "ERR907565_G1_10A_1999" "ERR907491_G3_10A_2007"
[901] "ERR907067_G1_10A_1998" "ERR907000_G5_NT_2010" "ERR907329_G5_NT_2010"
[904] "ERR906981_G5_NT_2011" "ERR906949_G5_NT_2010" "ERR907346_G5_NT_2011"
[907] "ERR907301_G5_NT_2011" "ERR907611_G4_NT_2007" "ERR907597_G3_NT_2007"
[910] "ERR907023_G5_NT_2011" "ERR907428_G1_NT_2000" "ERR907580_G4_NT_2007"
[913] "ERR907143_G5_NT_2010" "ERR907502_G2_NT_2007" "ERR907692_G4_NT_2007"
[916] "ERR907691_G4_NT_2007" "ERR906983_G5_NT_2011" "ERR907596_G2_NT_2007"
[919] "ERR907614_G3_NT_2007" "ERR907427_G1_NT_1999" "ERR907221_G1_NT_1998"
[922] "ERR907176_G5_NT_2011" "ERR907177_G5_NT_2011" "ERR906982_G5_NT_2011"
[925] "ERR907347_G5_NT_2011" "ERR907412_G1_NT_2000" "ERR907411_G1_NT_2000"
[928] "ERR907283_G1_NT_1999" "ERR907052_G1_NT_1998" "ERR907303_G5_NT_2012"
[931] "ERR907319_G5_NT_2010" "ERR907401_G1_NT_1999" "ERR907091_G1_NT_1998"
[934] "ERR906970_G5_NT_2011" "ERR907753_G3_NT_2007" "ERR907754_G2_NT_2007" [937]
"ERR907148_G5_NT_2010"

Fig. Suppl 2: Mass US Data

```
dfMA <- read_csv("data_MassUS.csv")

vaccineMA <- dfMA %>% distinct(SC, PCV7.actual)
vaccineMA <- vaccineMA %>% subset(PCV7.actual == "VT") %>%
```

```
  dplyr::rename(W = PCV7.actual) %>%
  full_join(subset(vaccineMA,PCV7.actual == "NVT")) %>%
  unite("vaccine", W:PCV7.actual, na.rm = TRUE)

dfE1MA <- dfMA %>% subset(Epoch1 == "E1") ## E1 prevaccine

dfFMA <- dfMA %>% select(SC, Epoch1) %>% group_by(Epoch1) %>%
  count(SC) %>% mutate(freq = prop.table(n)) %>% ungroup() %>%
  select(Epoch1, SC, freq) %>%
  spread(Epoch1, freq, fill = 0)


zero_E1MA <- dfE1MA %>% distinct(SC) %>%
  mutate(n = 0, freq = 0)

#### Replicates  - null expectation Pro rata ####
dfE1_NVT_allMA <- data.frame(NULL)
replicates <- 10000

for(i in 1:replicates){
  #sub-sampling from each epoch independently with replacemnt - best subsampling strategy
  dfE1_NVTMA_i <- dfE1MA %>% sample_frac(1, replace = TRUE) %>%
    subset(PCV7.actual == "NVT") %>% count(SC) %>%
    mutate(freq = prop.table(n)) %>% bind_rows(zero_E1MA) %>%
    group_by(SC) %>% summarise(n = sum(n), freq = sum(freq)) %>%
    arrange(SC) %>% ungroup %>% mutate(iter = i)

  dfE1_NVT_allMA <- bind_rows(dfE1_NVT_allMA, dfE1_NVTMA_i)
}

dfE1_NVT_allMA <- dfE1_NVT_allMA %>% group_by(SC) %>%
  summarise(expected_E3 = quantile(freq, 0.5), cil = quantile(freq, 0.025),
            ciu = quantile(freq, 0.975)) %>% ungroup

dfFMA <- left_join(dfFMA,dfE1_NVT_allMA) %>%
  replace(., is.na(.), 0) %>%
  mutate(delta = E3 - E1, deltaExp = expected_E3-E1,
         CI_low = cil-E1, CI_up = ciu-E1) %>%
  mutate(expectation = "Null exp.",
         signif = ifelse(delta > CI_up, "pos",NA)) %>%
  mutate(signif = ifelse(delta < CI_low, "neg", signif)) %>%
  mutate(signif = ifelse(E1 == 0 | E3 == 0, NA, signif)) %>%
  as_tibble(dfFMA) %>% mutate(SC = as.double(SC))

dfFMA <- left_join(as_tibble(dfFMA),vaccineMA)

#### Plot A: Prevalence by sequence cluster ####
dat1MA <- mutate(dfFMA, signif = ifelse(E3 > CI_up | E3 < CI_low, -0.005,NA), Epoch = "E1")
dat1MA <- subset(dat1MA, select = c(SC, Epoch, signif))

datPlotAMA <- dfFMA %>% select(SC, E1, E3) %>%
  pivot_longer(-SC, names_to =  "Epoch", values_to = "Prevalence") %>%
  left_join(dat1MA) %>% left_join(select(dfFMA, SC, vaccine)) %>%
```

```
  mutate(SC = as.character(SC)) %>% arrange(Epoch,-Prevalence)

rank <- as.character(unique(datPlotAMA$SC))

datPlotAMA <- datPlotAMA %>% mutate(SC = factor(SC, levels = rank))
dfFMA <- dfFMA %>% mutate(SC = factor(SC, levels = rank))

figureSA <- ggplot(datPlotAMA, aes(x=SC, y=Prevalence, alpha=Epoch, fill=vaccine)) +
  geom_bar(stat='identity', position='dodge') +
  scale_alpha_manual(values = c(1,0.4),
                     labels = c("Pre-vaccine", "Post-vaccine")) +
  scale_fill_manual(values = c("NVT"="#143c77","VT"="darkred","VT_NVT"="mediumpurple4"),
                    labels = c("NVT"="Non-vaccine type", "VT"="Vaccine type", "VT_NVT"="Mixed"),
                    name = "Composition") +
  xlab("Strain (SC)") + theme_classic() +
  scale_y_continuous(expand = c(0, 0), limits = c(0,0.3)) +
  theme(legend.title = element_text(face="bold", size = 10),
        legend.justification = c(1, 1), legend.box = "horizontal",
        legend.position = c(1, 1), # legend.position = c(0.725, 0.85),
        legend.spacing.y = unit(0.2, "cm"),
        legend.text=element_text(size=9),
        legend.background = element_blank(),
        legend.box.background = element_rect(fill = gray(0.96), color = NA))


#### Plot B: Change in prevalence ####
figureSB <- ggplot() + ylim(c(-0.2,0.35)) +
  geom_hline(yintercept=0, lty="dashed",size=0.65) +
  scale_colour_manual(values = c("NVT"="#143c77","VT"="darkred","VT_NVT"="mediumpurple4"),
                      labels = c("NVT"="Non-vaccine type", "VT"="Vaccine type", "VT_NVT"="Mixed"),
                      name = "SC Composition") +
  scale_linetype_manual(values = c(1), name = NULL) +
  scale_shape_manual(values = c("-", "+")) + theme_classic() +
  geom_point(data=dfFMA, aes(SC, delta, col = vaccine),
             size =4, alpha  = 0.85, show.legend = F) +
  geom_pointrange(data=dfFMA, aes(x=SC, y=deltaExp,
                                  ymin=CI_low, ymax=CI_up, lty=expectation),
                  size=.15, fatten = 6, show.legend = F) +
  geom_point(data=dfFMA, aes(x=SC, y=-0.2, shape = signif), size=6,
             col = "lightsteelblue4", show.legend = F) +
  labs(x="Strain (SC)") + labs(y="Change in prevalence") +
  theme(legend.title = element_text(face="bold", size = 10),
        legend.text=element_text(size=9),
        legend.position = c(0.01, 1),
        legend.justification = c(0.01, 1),
        legend.box = "horizontal",
        legend.background = element_blank(),
        legend.box.background = element_rect(fill = gray(0.95), color = NA))

######################

dfFVTMA <- dfMA %>% select(SC,PCV7.actual, Epoch1) %>%
  group_by(Epoch1) %>%  count(SC,PCV7.actual) %>%
```

```
  mutate(freq = round(prop.table(n), digits = 3)) %>%
  ungroup() %>% select(Epoch1, SC, PCV7.actual, freq) %>%
  spread(Epoch1, freq, fill = 0) %>% arrange(SC,PCV7.actual)

#### Present at E1 (17 SCs)####
SCE1MA <- dfFVTMA %>% subset(E1 > 0) %>% ##
  select(SC, PCV7.actual) %>%
  mutate(Epoch1 = "E1")

#### NVT present at E1 (9 SCs)####
SCE2MA <- SCE1MA %>% subset(PCV7.actual == "NVT")


SC_freq_dfMA <- dfMA %>% select(SC,PCV7.actual,Epoch1,pbp1a:CLS343169) %>%
  arrange(SC) %>% group_by(SC,PCV7.actual,Epoch1) %>%
  mutate(SC_n = n()) %>% ungroup() %>%
  group_by(SC,PCV7.actual,Epoch1,SC_n) %>%
  summarise_at(vars(pbp1a:CLS343169),mean) %>%
  ungroup()

### Get the matrix and the SC for the pre-vaccine epch "E1"
df_preVMA <- SCE1MA %>% left_join(SC_freq_dfMA)
SC_freq_preVMA <- as.matrix(df_preVMA %>%
                              mutate(SC_freq=SC_n/sum(SC_n)) %>% select(SC_freq))
SC_COG_preVMA <- as.matrix(t(df_preVMA %>% select(pbp1a:CLS343169)))

## Get e_l for the Mass data (1056 COGs)
el_MA <- SC_COG_preVMA %*% SC_freq_preVMA

#### Imputed data ####
dfImputedMA <- dfFVTMA %>%
  subset(PCV7.actual == "NVT" & E1 == 0 & E2 > 0) %>%
  select(SC,PCV7.actual) %>% mutate(Epoch1 = "E1", n=1) %>%
  select(Epoch1,SC,PCV7.actual,n)

dfFVTImputedMA <- dfMA %>% select(SC,PCV7.actual, Epoch1) %>%
  group_by(Epoch1) %>%  count(SC,PCV7.actual) %>%
  ungroup() %>% bind_rows(dfImputedMA) %>% group_by(Epoch1) %>%
  mutate(freq = round(prop.table(n), digits = 3)) %>% ungroup() %>%
  select(Epoch1, SC, PCV7.actual, freq) %>%
  spread(Epoch1, freq, fill = 0) %>% arrange(SC,PCV7.actual)

### SC13 is not present at E2
dfFNVTImputedMA <- dfFVTImputedMA %>%
  subset(PCV7.actual == "NVT" & SC != "13") %>%
  mutate(deltaE = E3 - E1) %>% arrange(SC)

#### E2 - frequencies just after vaccine intro ####
x_imputedMA <- dfFNVTImputedMA$E1
x_imputedMA <- as.matrix(round(x_imputedMA/sum(x_imputedMA), digits = 5))

dat2_imputedMA <- dfImputedMA %>% mutate(Epoch1 = "E2") %>%
  select(SC,PCV7.actual,Epoch1) %>% bind_rows(SCE2MA) %>%
```

```
  arrange(SC) %>% left_join(SC_freq_dfMA) %>%
  select(pbp1a:CLS343169)
dat2_imputedMA <- as.matrix(dat2_imputedMA)

fl_impMA <- t(dat2_imputedMA) %*% x_imputedMA

#### fitness function "omega" just after vaccine intro ####
wholeMA <- as.numeric(el_MA - fl_impMA)
omegaMA <- as.vector(dat2_imputedMA %*% wholeMA) ## length SCs, similar to FFS

phiMA <- sum(x_imputedMA * omegaMA) ## average fitness
rateOfChangeMA <- omegaMA - phiMA ## "rate of change": omega_g - phi

dat3ABMA <- dfFNVTImputedMA %>% select(SC, deltaE) %>%
  mutate(r = rateOfChangeMA) %>% subset(deltaE != 0) %>%
  mutate(change = ifelse(deltaE < 0, "Decreased", "Increased")) %>%
  mutate(col = ifelse(sign(deltaE) == sign(r), "same", "diff")) %>%
  left_join(vaccineMA)


lmS2 <- lm(r~deltaE,dat3ABMA)
summary(lmS2)
```

Call: lm(formula = r ~ deltaE, data = dat3ABMA)

Residuals: Min 1Q Median 3Q Max -14.1551 -1.9226 0.3457 3.0135 8.5055

Coefficients: Estimate Std. Error t value Pr(>|t|) (Intercept) 4.413 2.481 1.779 0.101 deltaE 20.799 58.154 0.358 0.727

Residual standard error: 6.156 on 12 degrees of freedom Multiple R-squared: 0.01055, Adjusted R-squared: -0.07191 F-statistic: 0.1279 on 1 and 12 DF, p-value: 0.7268

```
highlightS <- dat3ABMA %>% filter(col == "diff")

#### Figure C ####
plotSC <- ggplot(dat3ABMA, aes(x=deltaE, y=r, colour = vaccine)) +
  theme_classic() +
  xlab("Observed Prevalence Change") +
  ylab("Standardized Fitness") + #xlim(-0.03, 0.065) + ylim(-12,20) +
  geom_hline(yintercept=0, lty="dotted", alpha=.6) +
  geom_vline(xintercept=0, lty="dotted", alpha=.6) +
  annotate("rect", xmin=-0.025,xmax=0,ymin=-10,ymax=0,
           fill="darkslategray4", alpha= 0.15) +
  annotate("rect", xmin=0,xmax=0.09,ymin=0,ymax=14,
           fill="darkslategray4", alpha= 0.15) +
  geom_smooth(aes(group = 1), color="grey70", method=lm,
              formula = y~x,  show.legend=FALSE, se=FALSE) +
  geom_point(size = 3) +
  theme(legend.position = "none",
        axis.text = element_text(colour = "black"),) +
  scale_colour_manual(values = c("#143c77","mediumorchid4")) +
  geom_text_repel(aes(label = paste("SC", SC, sep="-")),
                  data = highlightS, size = 3.5)

##################################
```

```
df_postVMA <- SCE2MA %>% left_join(SC_freq_dfMA)
SC_COG_postVMA <- as.matrix(t(df_postVMA %>% select(pbp1a:CLS343169)))

#### Predict postV frequencies ####
SC_freq_postV_predMA <- QP(SC_COG_postVMA, el_MA) #Matrix: rows = COGs, columns = (SCs - VT)

SC_freq_postV_obsMA <- SCE2MA %>% mutate(Epoch1 = "E3") %>%
  left_join(SC_freq_dfMA) %>%
  mutate(SC_freq=SC_n/sum(SC_n, na.rm = T)) %>%
  select(SC, PCV7.actual, SC_freq) %>%
  mutate(SC_freq = replace_na(SC_freq, 0))

SC_freq_postV_obsMA <- SC_freq_postV_obsMA %>%
  mutate(SC_pred = SC_freq_postV_predMA) %>%
  left_join(vaccineMA)

W_modelSB <- lm(data=SC_freq_postV_obsMA,SC_freq~SC_pred);
summary(W_modelSB)
```

Call: lm(formula = SC_freq ~ SC_pred, data = SC_freq_postV_obsMA)

Residuals: Min 1Q Median 3Q Max -0.073407 -0.018085 -0.008067 0.027381 0.068476

Coefficients: Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.02328 0.02959 0.787 0.4572
SC_pred 0.79044 0.22626 3.494 0.0101 * — Signif. codes: 0 ‘’ ***0.001*** ’’ *0.01* ’’ 0.05 ‘.’ 0.1 ’ ’ 1

Residual standard error: 0.04684 on 7 degrees of freedom Multiple R-squared: 0.6355, Adjusted R-squared: 0.5834 F-statistic: 12.2 on 1 and 7 DF, p-value: 0.01008

```
confint(W_modelSB)
```

```
               2.5 %     97.5 %
```

(Intercept) -0.04669562 0.09326407 SC_pred 0.25542617 1.32545776

```
linearHypothesis(W_modelSB, c("(Intercept) = 0", "SC_pred = 1"), test = "Chisq")
```

Linear hypothesis test

Hypothesis: (Intercept) = 0 SC_pred = 1

Model 1: restricted model Model 2: SC_freq ~ SC_pred

Res.Df RSS Df Sum of Sq Chisq Pr(>Chisq) 1 9 0.017243
2 7 0.015361 2 0.0018824 0.8578 0.6512

```
## no outliers
outlierSB <- SC_freq_postV_obsMA %>% mutate(diff = abs(SC_freq - SC_pred))
outlierSB <- outlierSB %>%
  filter(diff %in% boxplot(outlierSB$diff, plot = FALSE)$out)

#### Figure D ####
plotSD <- ggplot(SC_freq_postV_obsMA,
                 aes(x = SC_pred, y = SC_freq, colour = vaccine)) +
  theme_classic() +
  geom_segment(aes(x=0,xend=0.3,y=0,yend=0.3),
               color="black",alpha=.7,lwd=0.5,lty=3) +
  geom_smooth(method='lm',color="#899DA4",
```

```
              formula=y~x, alpha=0.3, lwd=.6,
              fullrange=T, linetype="blank", show.legend=F) +
  annotate(geom = "text", x=0.28, y =0.29,
           label = "1:1 line", angle = 45, size = 3) +
  geom_point(size=3) +

  scale_x_continuous("Predicted Prevalence (NFDS)")+
  scale_y_continuous("Observed Prevalence") +
  coord_fixed(ratio = 1, ylim=c(0,0.3), xlim=c(0,0.3)) +
  scale_colour_manual(values = c("#143c77","mediumorchid4")) +
  theme(legend.position = "none",
        axis.text = element_text(colour = "black"),)

###################################

SC_freq_E1MA <- df_preVMA %>%
  mutate(SC_freq_E1=SC_n/sum(SC_n)) %>%
  select(SC,PCV7.actual,SC_freq_E1)

SC_freq_postV_diffMA <- SC_freq_postV_obsMA %>%
  left_join(SC_freq_E1MA) %>%
  mutate(diff_pred = SC_freq_E1 - SC_pred,
         diff_obs = SC_freq_E1 - SC_freq) %>%
  left_join(vaccineMA)

SC_freq_postV_diffMA <- SC_freq_postV_diffMA %>%
  mutate(diff = abs(diff_pred - diff_obs)) %>%
  arrange(diff)

stats <- summary(lm(SC_freq_postV_diffMA$diff_pred~SC_freq_postV_diffMA$diff_obs))
ars <- round(stats$adj.r.squared, digits = 3)
sseE <- round(sse(SC_freq_postV_diffMA$diff_obs, SC_freq_postV_diffMA$diff_pred), digits = 3)
maeE <- round(mae(SC_freq_postV_diffMA$diff_obs, SC_freq_postV_diffMA$diff_pred),digits = 3)##Mean Abso
rmseE <- round(rmse(SC_freq_postV_diffMA$diff_obs, SC_freq_postV_diffMA$diff_pred),digits = 3) ##Root M

W_modelSC <- lm(data=SC_freq_postV_diffMA,diff_obs~diff_pred);
summary(W_modelSC)
```

Call: lm(formula = diff_obs ~ diff_pred, data = SC_freq_postV_diffMA)

Residuals: Min 1Q Median 3Q Max -0.065605 -0.017260 -0.003470 0.006301 0.071991

Coefficients: Estimate Std. Error t value Pr(>|t|) (Intercept) -0.02685 0.02422 -1.108 0.304 diff_pred 0.54087 0.33000 1.639 0.145

Residual standard error: 0.04393 on 7 degrees of freedom Multiple R-squared: 0.2773, Adjusted R-squared: 0.1741 F-statistic: 2.686 on 1 and 7 DF, p-value: 0.1452

```
confint(W_modelSC)
```

```
               2.5 %     97.5 %
```

(Intercept) -0.08413255 0.03043303 diff_pred -0.23946801 1.32120619

```
linearHypothesis(W_modelSC, c("(Intercept) = 0", "diff_pred = 1"), test = "Chisq")
```

Linear hypothesis test

Hypothesis: (Intercept) = 0 diff_pred = 1

Model 1: restricted model Model 2: diff_obs ~ diff_pred

Res.Df RSS Df Sum of Sq Chisq Pr(>Chisq) 1 9 0.017243
2 7 0.013508 2 0.0037353 1.9357 0.3799

```
#### No outlier ####
outlier3C <- SC_freq_postV_diffMA %>%
  filter(diff %in% boxplot(SC_freq_postV_diffMA$diff, plot = FALSE)$out)

#### Figure E ####
plotSE <- ggplot(SC_freq_postV_diffMA,
                 aes(x = diff_pred, y = diff_obs, colour = vaccine)) +
  geom_segment(aes(x=-0.2,xend=0.025,y=-0.2,yend=0.025),
               color="black",alpha=.7,lwd=0.5,lty=3) +
  geom_smooth(method='lm', color="gray80" ,formula=y~x,
              alpha=0.3, lwd=.6, fullrange=T,
              linetype="blank", show.legend=F) +
  annotate(geom = "text", x=0.01, y =0.0175,
           label = "1:1 line", angle = 45, size = 3) +
  geom_point(size=3) + theme_classic() +
  scale_colour_manual(values = c("#143c77","mediumorchid4"),
                      labels = c("Non-vaccine type", "Mixed"),
                      name = "SC Composition") +
  theme(legend.position = "none",
        axis.text = element_text(colour = "black"),) +
  xlab("Predicted Prevalence Change (NFDS)")+
  ylab("Observed Prevalence Change") +
  coord_fixed(ratio = 1, xlim=c(-0.2,0.025), ylim=c(-0.2,0.025)) +
  annotate("text",x=-0.19,y=0.01, size=2.5,hjust = 0,
           label=paste("SSE = ", sseE, "\nRMSE = ",
                       rmseE, "\nAdj. R2 = ", ars))

#### Figure F ####
SC_freq_postV_diffMA <- SC_freq_postV_diffMA %>%
  mutate(prorata = SC_freq_E1/sum(SC_freq_E1)) %>%
  mutate(diff_predPro = SC_freq_E1 - prorata) %>%
  mutate(diffP = abs(diff_predPro - diff_obs))

stats <- summary(lm(SC_freq_postV_diffMA$diff_predPro~SC_freq_postV_diffMA$diff_obs))
ars <- round(stats$adj.r.squared, digits = 3)
sseE <- round(sse(SC_freq_postV_diffMA$diff_obs, SC_freq_postV_diffMA$diff_predPro), digits = 3)
maeE <- round(mae(SC_freq_postV_diffMA$diff_obs, SC_freq_postV_diffMA$diff_predPro),digits = 3)##Mean A
rmseE <- round(rmse(SC_freq_postV_diffMA$diff_obs, SC_freq_postV_diffMA$diff_predPro),digits = 3) ##Roo

W_modelSD <- lm(data=SC_freq_postV_diffMA,diff_obs~diff_predPro);
summary(W_modelSD)
```

Call: lm(formula = diff_obs ~ diff_predPro, data = SC_freq_postV_diffMA)

Residuals: Min 1Q Median 3Q Max -0.05935 -0.04736 0.00284 0.01786 0.07442

Coefficients: Estimate Std. Error t value Pr(>|t|) (Intercept) -0.04741 0.02675 -1.772 0.12 diff_predPro 0.18930 0.35483 0.533 0.61

Residual standard error: 0.05065 on 7 degrees of freedom Multiple R-squared: 0.03907, Adjusted R-squared:

-0.0982 F-statistic: 0.2846 on 1 and 7 DF, p-value: 0.6102

```
confint(W_modelSD)
```

```
               2.5 %     97.5 %
```

(Intercept) -0.1106683 0.01584975 diff_predPro -0.6497446 1.02834770

```
linearHypothesis(W_modelSD, c("(Intercept) = 0", "diff_predPro = 1"), test = "Chisq")
```

Linear hypothesis test

Hypothesis: (Intercept) = 0 diff_predPro = 1

Model 1: restricted model Model 2: diff_obs ~ diff_predPro

Res.Df RSS Df Sum of Sq Chisq Pr(>Chisq)
1 9 0.031356
2 7 0.017961 2 0.013394 5.22 0.07353 . — Signif. codes: 0 ‘’ ***0.001*** ’’ *0.01* ’’ 0.05 ‘.’ 0.1 ’ ’ 1

```
plotSF <- ggplot(SC_freq_postV_diffMA, aes(x = diff_predPro,
                                           y = diff_obs, colour = vaccine)) +
  geom_segment(aes(x=-0.2,xend=0.025,y=-0.2,yend=0.025),
               color="black",alpha=.7,lwd=0.5,lty=3) +
  geom_smooth(method='lm', color="gray80" ,formula=y~x,
              alpha=0.3, lwd=.6, fullrange=T,
              linetype="blank", show.legend=F) +
  annotate(geom = "text", x=0.01, y =0.0175,
           label = "1:1 line", angle = 45, size = 3) +
  geom_point(size=3) + theme_classic() +
  scale_colour_manual(values = c("#143c77","mediumorchid4"),
                      labels = c("Non-vaccine type", "Mixed"),
                      name = "SC Composition") +
  theme(legend.position = "none",
        axis.text = element_text(colour = "black"),) +
  xlab("Predicted Prevalence Change (Pro rata)")+
  ylab("Observed Prevalence Change") +
  coord_fixed(ratio = 1, xlim=c(-0.2,0.025), ylim=c(-0.2,0.025)) +
  annotate("text",x=-0.19,y=0.01, size=2.5,hjust = 0,
           label=paste("SSE = ", sseE, "\nRMSE = ",
                       rmseE, "\nAdj. R2 = ", ars))



figureSAB <- plot_grid(figureSA, figureSB, labels = c("A","B"), nrow = 1)
figureSCF <- plot_grid(plotSC, plotSD, plotSE, plotSF,
                       nrow = 1, labels=c("C","D","E","F"))

plotSPARC <- plot_grid(figureSAB,figureSCF, ncol=1)
ggsave("figureS_MASS.png", plotSPARC, width = 14, height = 7)
```

Fig. Suppl 3: Similarity of accessory genes among strains

```
#Accessory genome phylogeny
AG.tree <- read.tree("RAxML_bestTree.All.Binary.tre") #All

#Creating distance matrix from Tee
```

```
PatristicDistMatrix <- cophenetic(AG.tree) # patristic distances
PatristicDist <- as.dist(PatristicDistMatrix, diag = TRUE, upper = TRUE)

#Setting up group/SC assignments for between SC patristic distance
seq.labels <- as.data.frame(rownames(PatristicDistMatrix)) #Obtaining ordered taxa
colnames(seq.labels) <- "taxa"
clades <- as.data.frame(cbind(df$FinalName,df$BAPS2)) #All data set
colnames(clades) <- c("taxa","clade")
seq.labels$id  <- 1:nrow(clades) #adding row number to maintain order for sorting after merge
labels.clades <- merge(seq.labels, clades, by="taxa") #merging
labels.clades <- labels.clades[order(labels.clades$id), ] #ordering

#Creating Final Matrix
md <- meandist(PatristicDist, labels.clades$clade) #calculating mean distance between clades
md.matrix <- as.dist(md,diag = FALSE, upper = TRUE)
md.matrix <- as.matrix(md.matrix)
diag(md.matrix) <- NA

#Saving Accessory geneome distances in long format - needed for Sup Figure 4
PatristicDist.long <- as.data.frame(as.table(md.matrix))
PatristicDist.long <- PatristicDist.long[! (PatristicDist.long$Var1 == PatristicDist.long$Var2),]
PatristicDist.long <- PatristicDist.long[!is.na(PatristicDist.long$Freq),]
AccGenomeDistances <- PatristicDist.long #Sup figure 4

#Heatmap of between strain accessory genome distances
my_palette <- colorRampPalette(c("#4d004b", "#8c96c6", "#e0ecf4"))(n = 100)

pdf('figureS_Heatmap.pdf', width = 10, height = 10) #Change as needed
gplots::heatmap.2(md.matrix,
                  col = my_palette,
                  scale = "none",
                  na.rm = TRUE,
                  dendrogram = "row",
                  trace="none",
                  cexCol = .7, cexRow=.7,
                  labRow = rownames(md),
                  srtCol = 70, #Changes angle of X-axis
                  key=TRUE,
                  key.title=NA,
                  key.xlab = "Patristic \nDistance"
)
dev.off()
```

```
## quartz_off_screen
##                 2
```

Fig. Suppl 4: Core versus Accessory Genome distances

```
##Scatterplot comparing core genome patristic
## distance and accessory genome distance

#### Figure A ####
#Core genome distances
```

```
CG.tree<-read.tree("RAxML_bestTree.All.Core.tre") #Core genome tree

PatristicDistMatrix <- cophenetic(CG.tree) #patristic distances
PatristicDist <- as.dist(PatristicDistMatrix,diag = TRUE, upper = TRUE)
#Setting up group/SC assignments for between SC patristic distance
seq.labels <- as.data.frame(rownames(PatristicDistMatrix)) #Obtaining ordered taxa
colnames(seq.labels) <- "taxa"
clades <- as.data.frame(cbind(df$FinalName,df$BAPS2)) #All data set
colnames(clades) <- c("taxa","clade")
seq.labels$id  <- 1:nrow(clades) #adding row number to maintain order for sorting after merge
labels.clades <- merge(seq.labels, clades, by="taxa") #merging
labels.clades <- labels.clades[order(labels.clades$id), ] #ordering

md <- meandist(PatristicDist, labels.clades$clade) #calculating mean distance between clades
md.matrix <- as.dist(md,diag = FALSE, upper = TRUE)
md.matrix <- as.matrix(md.matrix)
diag(md.matrix) <- NA

#Accessory geneome distances
PatristicDist.long <- as.data.frame(as.table(md.matrix))
PatristicDist.long <- PatristicDist.long[! (PatristicDist.long$Var1 == PatristicDist.long$Var2),]
PatristicDist.long <- PatristicDist.long[!is.na(PatristicDist.long$Freq),]
CoreGenomeDistances <- PatristicDist.long

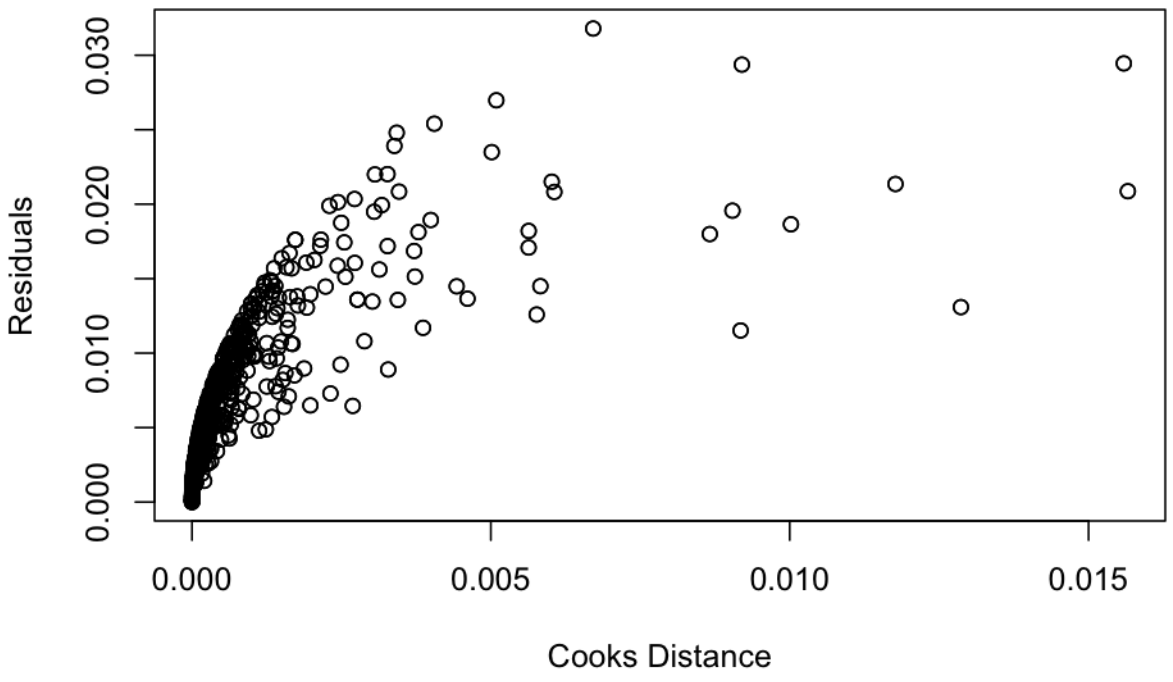
MergedDistances <- as.data.frame(cbind(CoreGenomeDistances,AccGenomeDistances))
colnames(MergedDistances) <- c("Var1a", "Var2b", "PCore", "Var1c", "Var2d","PAcc")

MergedDistances$MSE  <- (MergedDistances$PCore-MergedDistances$PAcc)^2
MergedDistancesMedian <- MergedDistances[(MergedDistances$PCore > 0.06 & MergedDistances$PCore < 0.15),]
MergedDistancesMedian <- within(MergedDistancesMedian, A.quartile <- as.integer(cut(MergedDistancesMedia
MergedDistancesMedian <- within(MergedDistancesMedian, P.quartile <- as.integer(cut(MergedDistancesMedia

model <- lm(MergedDistancesMedian$PCore~MergedDistancesMedian$PAcc)
car::outlierTest(model)
```

```
## No Studentized residuals with Bonferroni p < 0.05
## Largest |rstudent|:
##      rstudent unadjusted p-value Bonferroni p
## 207 -3.554855         0.00039412      0.43826
```

```
MergedDistancesMedian$residuals <- abs(resid(model)) #Risiduals
MergedDistancesMedian$cooks <- cooks.distance(model)
plot(MergedDistancesMedian$cooks, MergedDistancesMedian$residuals,
     xlab="Cooks Distance", ylab="Residuals")
```




```
#Make name varianble
MergedDistancesMedian$comp <- paste(MergedDistancesMedian$Var1a, MergedDistancesMedian$Var2b, sep = "-")

R2 <- round(cor(MergedDistancesMedian$PCore,MergedDistancesMedian$PAcc,method = "pearson"),2)

plotS4A <- ggplot(MergedDistancesMedian, aes(PCore,PAcc)) +
  geom_point(color="black",size=1) +
  geom_density_2d(color="#08519c", alpha=.7, show.legend =FALSE) +
  geom_smooth(color="#636363", method=lm, alpha=.3, linetype="dashed",
              size=.6, formula = y~x,  show.legend=FALSE, se=TRUE) +
  labs(x="Core Genome Divergence (Patristic Distance)") +
  labs(y="Accessory Genome Divergence \n(Patristic Distance)") +
  annotate("text", x = .075, y = 2.3,
           label = paste("R^2 == ", R2), parse=TRUE)

#### Figure B ####

RelativeFitness.E1 <- dat3AB %>% filter(BAPS2!="01")

RF.E1.distmat <- as.matrix(dist(RelativeFitness.E1$r))
RF.E1.distmat[upper.tri(RF.E1.distmat)] <- NA; diag(RF.E1.distmat) <- NA
rownames(RF.E1.distmat) <- RelativeFitness.E1$BAPS2; colnames(RF.E1.distmat) <- RelativeFitness.E1$BAPS
RF.E1.distlong <- na.omit(as.data.frame.table(RF.E1.distmat))
colnames(RF.E1.distlong) <- c("Var1a","Var2b","RelFit")

CorDist_RelFit <- merge(RF.E1.distlong, MergedDistances, by=c("Var1a", "Var2b"))
```

```
CorDist_RelFit <- CorDist_RelFit[,c("Var1a", "Var2b","RelFit","PCore","PAcc")]

####Supplemental figures - Core and accessory divergence including fitness
plotS4B <- ggplot(CorDist_RelFit, aes(PCore,RelFit)) +
  geom_point(color="black",size=1) +
  geom_density_2d(color="#08519c", alpha=.7, show.legend =FALSE) +
  labs(x="Core Genome Divergence (Patristic Distance)") +
  labs(y="Absolute fitness difference")

plotS4C <- ggplot(CorDist_RelFit, aes(PAcc,RelFit)) +
  geom_point(color="black",size=1) +
  geom_density_2d(color="#08519c", alpha=.7, show.legend =FALSE) +
  labs(x="Accessory Genome Divergence (Patristic Distance)") +
  labs(y="Absolute fitness difference")

#theme_set(theme_cowplot(font_size=13))
figureS4 <- plot_grid(ncol=3, nrow=1, plotS4A, plotS4B,
                      plotS4C, labels = "AUTO", align = 'h')
ggsave("figureS_distance.png", figureS4, width = 15, height = 5)
```

Fig. Suppl 5: COGs by SCs

```
SC_freq_E1 <- SC_freq_postV_obs %>% select(BAPS2, PCV7.actual) %>%
  left_join(df) %>% subset(Epoch1 == "E1") %>%
  select(BAPS2, HMPREF0837_12128:HMPREF0837_10616) %>%
  arrange(BAPS2) %>% group_by(BAPS2) %>%
  summarise_at(vars(HMPREF0837_12128:HMPREF0837_10616),mean) %>%
  ungroup() %>%
  pivot_longer(cols=HMPREF0837_12128:HMPREF0837_10616, names_to = "COG", values_to = "f") %>%
  mutate(Epoch = "Pre-vaccine")

SC_freq_E3 <- SC_freq_postV_obs %>% select(BAPS2, PCV7.actual) %>%
  left_join(df) %>% subset(Epoch1 == "E3") %>%
  select(BAPS2, HMPREF0837_12128:HMPREF0837_10616) %>%
  arrange(BAPS2) %>% group_by(BAPS2) %>%
  summarise_at(vars(HMPREF0837_12128:HMPREF0837_10616),mean) %>%
  ungroup() %>%
  pivot_longer(cols=HMPREF0837_12128:HMPREF0837_10616, names_to = "COG", values_to = "f") %>%
  mutate(Epoch = "Post-vaccine")

SC_freq_E13 <- bind_rows(SC_freq_E1, SC_freq_E3)

plotCOGs_SC <- ggplot(SC_freq_E13, aes(f, fill = Epoch)) +
  geom_histogram(aes(y = ..count..), position = "dodge", bins = 10) +
  facet_wrap(~BAPS2, nrow = 3) +
  xlab("COG frequency") + theme_minimal() +
  scale_fill_manual("", values = c("#D3723D", "#8A9DA4")) +
  annotate("segment", x=-Inf, xend=Inf, y=-Inf, yend=-Inf)+
  annotate("segment", x=-Inf, xend=-Inf, y=-Inf, yend=Inf) +
  theme(legend.position = "bottom")

ggsave("figureS_COGsSCs.png", plotCOGs_SC, width = 15, height = 5)
```

Table Statistics

```
#### Accessory genome SA ####
## Sensitivity analysis using a subsample of 119 isolates
## collected in 2010 prior to the initiation of PCV13

#### Data ####
dfSenst <- df %>% separate(FinalName, c("ID1", "ID2", "ID3", "Year"))
dfSenstE12 <- dfSenst %>% filter(Epoch1 != "E3")
dfSenstE3 <- dfSenst %>% filter(Epoch1 == "E3" & Year == "2010")
dfS <- bind_rows(dfSenstE12,dfSenstE3)

dfFVTS <- dfS %>% select(BAPS2,PCV7.actual, Epoch1) %>%
  group_by(Epoch1) %>%  count(BAPS2,PCV7.actual) %>%
  mutate(freq = round(prop.table(n), digits = 3)) %>% ungroup() %>%
  select(Epoch1, BAPS2, PCV7.actual, freq) %>%
  spread(Epoch1, freq, fill = 0) %>% arrange(BAPS2,PCV7.actual)

#### Present at E1 ####
SCE1S <- dfFVTS %>% subset(E1 > 0) %>% ## & BAPS2 !="27"
  select(BAPS2, PCV7.actual) %>%
  mutate(Epoch1 = "E1")

#### NVTs Present at E1 ####
SCE2S <- SCE1S %>% subset(PCV7.actual == "NVT"  & BAPS2 !="27")

SC_freq_dfS <- dfS %>% select(BAPS2, PCV7.actual, Epoch1,
                              HMPREF0837_12128:HMPREF0837_10616) %>%
  arrange(BAPS2) %>% group_by(BAPS2,PCV7.actual,Epoch1) %>%
  mutate(SC_n = n()) %>% ungroup() %>%
  group_by(BAPS2,PCV7.actual,Epoch1,SC_n) %>%
  summarise_at(vars(HMPREF0837_12128:HMPREF0837_10616),mean) %>%
  ungroup()

### Get the matrix and the SC for the pre-vaccine epch "E1"
df_preVS <- SCE1S %>% left_join(SC_freq_dfS)
SC_freq_preVS <- as.matrix(df_preVS %>% mutate(SC_freq=SC_n/sum(SC_n)) %>% select(SC_freq))
SC_COG_preVS <- as.matrix(t(df_preVS %>% select(HMPREF0837_12128:HMPREF0837_10616)))

#### Get e_l ####
el_S <- SC_COG_preVS %*% SC_freq_preVS

df_postVS <- SCE2S %>% left_join(SC_freq_dfS)
SC_COG_postVS <- as.matrix(t(df_postVS %>% select(HMPREF0837_12128:HMPREF0837_10616)))

SC_freq_postV_obsS <- SCE2S %>% mutate(Epoch1 = "E3") %>%
  left_join(SC_freq_dfS) %>%
  mutate(SC_freq=SC_n/sum(SC_n, na.rm = T)) %>%
  select(BAPS2, PCV7.actual, SC_freq) %>%
  mutate(SC_freq = replace_na(SC_freq, 0))

## Predict postV frequencies
SC_freq_postV_predS <- QP(SC_COG_postVS, el_S) #Matrix: rows = COGs, columns = (SCs - VT)
SC_freq_postV_obsS <- SC_freq_postV_obsS %>%
```

```
  mutate(SC_pred = SC_freq_postV_predS)

SC_freq_E1S <- df_preVS %>%
  mutate(SC_freq_E1=SC_n/sum(SC_n)) %>%
  select(BAPS2,PCV7.actual,SC_freq_E1)

SC_freq_postV_diffS <- SC_freq_postV_obsS %>%
  left_join(SC_freq_E1S) %>%
  mutate(diff_pred = SC_freq_E1 - SC_pred,
         diff_obs = SC_freq_E1 - SC_freq)

SC_freq_postV_diffS <- SC_freq_postV_diffS %>%
  mutate(diff = abs(diff_pred - diff_obs)) %>%
  arrange(diff)

stats <- summary(lm(SC_freq_postV_diffS$diff_pred~SC_freq_postV_diffS$diff_obs))
ars <- round(stats$adj.r.squared, digits = 3)
sseE <- round(sse(SC_freq_postV_diffS$diff_obs, SC_freq_postV_diffS$diff_pred), digits = 3)
rmseE <- round(rmse(SC_freq_postV_diffS$diff_obs, SC_freq_postV_diffS$diff_pred),digits = 3)

accNFDSSA <- data.frame(Model = "Accesory genome (NFDS) SA",
                        nloci = length(el_S), adj.r.squared = ars,
                        SSE = sseE, RMSE = rmseE)

SC_freq_postV_diffS <- SC_freq_postV_diffS %>%
  mutate(prorata = SC_freq_E1/sum(SC_freq_E1)) %>%
  mutate(diff_predPro = SC_freq_E1 - prorata) %>%
  mutate(diffP = abs(diff_predPro - diff_obs))

stats <- summary(lm(SC_freq_postV_diffS$diff_predPro~SC_freq_postV_diffS$diff_obs))
ars <- round(stats$adj.r.squared, digits = 3)
sseE <- round(sse(SC_freq_postV_diffS$diff_obs, SC_freq_postV_diffS$diff_predPro), digits = 3)
rmseE <- round(rmse(SC_freq_postV_diffS$diff_obs, SC_freq_postV_diffS$diff_predPro),digits = 3)

accProrataSA <- data.frame(Model = "Accesory genome (Prorata) SA",
                           nloci = length(el_S), adj.r.squared = ars,
                           SSE = sseE, RMSE = rmseE)

#####################

#### Core genome ####
dfcore <- read.csv("CoreSNPpresenceAbsence.txt", header = F)
dfcore <- dfcore %>% as_tibble() %>% rename(FinalName = V1)
dfcore <- df %>% select(FinalName, BAPS2, PCV7.actual, Epoch1) %>%
  left_join(dfcore)

dfFVTcore <- dfcore %>% select(BAPS2,PCV7.actual, Epoch1) %>%
  group_by(Epoch1) %>%  count(BAPS2,PCV7.actual) %>%
  mutate(freq = round(prop.table(n), digits = 3)) %>% ungroup() %>%
  select(Epoch1, BAPS2, PCV7.actual, freq) %>%
  spread(Epoch1, freq, fill = 0) %>% arrange(BAPS2,PCV7.actual)

#### Present at E1 ####
```

```
SCE1core <- dfFVTcore %>% subset(E1 > 0) %>% ## & BAPS2 !="27"
  select(BAPS2, PCV7.actual) %>%
  mutate(Epoch1 = "E1")

#### NVTs Present at E1 ####
SCE2core <- SCE1core %>% subset(PCV7.actual == "NVT"  & BAPS2 !="27")


SC_freq_dfcore <- dfcore %>% select(BAPS2, PCV7.actual, Epoch1,
                                    V2:V62654) %>%
  arrange(BAPS2) %>% group_by(BAPS2,PCV7.actual,Epoch1) %>%
  mutate(SC_n = n()) %>% ungroup() %>%
  group_by(BAPS2,PCV7.actual,Epoch1,SC_n) %>%
  summarise_at(vars(V2:V62654),mean) %>%
  ungroup()

### Get the matrix and the SC for the pre-vaccine epch "E1"
df_preVcore <- SCE1core %>% left_join(SC_freq_dfcore)
SC_freq_preVcore <- as.matrix(df_preVcore %>% mutate(SC_freq=SC_n/sum(SC_n)) %>% select(SC_freq))
SC_COG_preVcore <- as.matrix(t(df_preVcore %>% select(V2:V62654)))

#### Get e_l for the core (62653 loci) ####
el_core <- SC_COG_preVcore %*% SC_freq_preVcore

#### Observed versus prediced prevalence ####
df_postVcore <- SCE2core %>% left_join(SC_freq_dfcore)
SC_COG_postVcore <- as.matrix(t(df_postVcore %>% select(V2:V62654)))

SC_freq_postV_obsCore <- SCE2core %>% mutate(Epoch1 = "E3") %>%
  left_join(SC_freq_dfcore) %>%
  mutate(SC_freq=SC_n/sum(SC_n, na.rm = T)) %>%
  select(BAPS2, PCV7.actual, SC_freq) %>%
  mutate(SC_freq = replace_na(SC_freq, 0))

## Predict postV frequencies
SC_freq_postV_predCore <- QP(SC_COG_postVcore, el_core)
SC_freq_postV_obsCore <- SC_freq_postV_obsCore %>%
  mutate(SC_pred = SC_freq_postV_predCore)

SC_freq_E1core <- df_preVcore %>%
  mutate(SC_freq_E1=SC_n/sum(SC_n)) %>%
  select(BAPS2,PCV7.actual,SC_freq_E1)

SC_freq_postV_diffcore <- SC_freq_postV_obsCore %>%
  left_join(SC_freq_E1core) %>%
  mutate(diff_pred = SC_freq_E1 - SC_pred,
         diff_obs = SC_freq_E1 - SC_freq)

SC_freq_postV_diffcore <- SC_freq_postV_diffcore %>%
  mutate(diff = abs(diff_pred - diff_obs)) %>%
  arrange(diff)

#### Pro rata ####
```

```
SC_freq_postV_diffcore <- SC_freq_postV_diffcore %>%
  mutate(prorata = SC_freq_E1/sum(SC_freq_E1)) %>%
  mutate(diff_predPro = SC_freq_E1 - prorata) %>%
  mutate(diffP = abs(diff_predPro - diff_obs))

#### Stats core NFDS ####
stats <- summary(lm(SC_freq_postV_diffcore$diff_pred~SC_freq_postV_diffcore$diff_obs))
ars <- round(stats$adj.r.squared, digits = 3)
sseE <- round(sse(SC_freq_postV_diffcore$diff_obs, SC_freq_postV_diffcore$diff_pred), digits = 3)
rmseE <- round(rmse(SC_freq_postV_diffcore$diff_obs, SC_freq_postV_diffcore$diff_pred),digits = 3)

coreNFDS <- data.frame(Model = "Core genome (NFDS)", nloci = length(el_core),
                       adj.r.squared = ars, SSE = sseE, RMSE = rmseE)

#### Stats core Pro rata ####
stats <- summary(lm(SC_freq_postV_diffcore$diff_predPro~SC_freq_postV_diffcore$diff_obs))
ars <- round(stats$adj.r.squared, digits = 3)
sseE <- round(sse(SC_freq_postV_diffcore$diff_obs, SC_freq_postV_diffcore$diff_predPro), digits = 3)
rmseE <- round(rmse(SC_freq_postV_diffcore$diff_obs, SC_freq_postV_diffcore$diff_predPro),digits = 3)

coreProrata <- data.frame(Model = "Core genome (Pro rata)", nloci = length(el_core),
                          adj.r.squared = ars, SSE = sseE, RMSE = rmseE)

##############################

#### Metabolic loci ####
dfmeta <- read.csv("Core_Metabolic_SNPpresenceAbsence.txt", header = F)
dfmeta <- dfmeta %>% as_tibble() %>% rename(FinalName = V1)
dfmeta <- df %>% select(FinalName, BAPS2, PCV7.actual, Epoch1) %>%
  left_join(dfmeta)

dfFVTmeta <- dfmeta %>% select(BAPS2,PCV7.actual, Epoch1) %>%
  group_by(Epoch1) %>%  count(BAPS2,PCV7.actual) %>%
  mutate(freq = round(prop.table(n), digits = 3)) %>% ungroup() %>%
  select(Epoch1, BAPS2, PCV7.actual, freq) %>%
  spread(Epoch1, freq, fill = 0) %>% arrange(BAPS2,PCV7.actual)

#### Present at E1 ####
SCE1meta <- dfFVTmeta %>% subset(E1 > 0) %>% ## & BAPS2 !="27"
  select(BAPS2, PCV7.actual) %>%
  mutate(Epoch1 = "E1")

#### NVTs Present at E1 ####
SCE2meta <- SCE1meta %>% subset(PCV7.actual == "NVT"  & BAPS2 !="27")


SC_freq_dfmeta <- dfmeta %>% select(BAPS2, PCV7.actual, Epoch1,
                                    V2:V22434) %>%
  arrange(BAPS2) %>% group_by(BAPS2,PCV7.actual,Epoch1) %>%
  mutate(SC_n = n()) %>% ungroup() %>%
  group_by(BAPS2,PCV7.actual,Epoch1,SC_n) %>%
  summarise_at(vars(V2:V22434),mean) %>%
  ungroup()
```

```
### Get the matrix and the SC for the pre-vaccine epch "E1"
df_preVmeta <- SCE1meta %>% left_join(SC_freq_dfmeta)
SC_freq_preVmeta <- as.matrix(df_preVmeta %>% mutate(SC_freq=SC_n/sum(SC_n)) %>% select(SC_freq))
SC_COG_preVmeta <- as.matrix(t(df_preVmeta %>% select(V2:V22434)))

#### Get e_l for the meta (62653 loci) ####
el_meta <- SC_COG_preVmeta %*% SC_freq_preVmeta

#### Observed versus prediced prevalence ####
df_postVmeta <- SCE2meta %>% left_join(SC_freq_dfmeta)
SC_COG_postVmeta <- as.matrix(t(df_postVmeta %>% select(V2:V22434)))

SC_freq_postV_obsMeta <- SCE2meta %>% mutate(Epoch1 = "E3") %>%
  left_join(SC_freq_dfmeta) %>%
  mutate(SC_freq=SC_n/sum(SC_n, na.rm = T)) %>%
  select(BAPS2, PCV7.actual, SC_freq) %>%
  mutate(SC_freq = replace_na(SC_freq, 0))

## Predict postV frequencies
SC_freq_postV_predMeta <- QP(SC_COG_postVmeta, el_meta)
SC_freq_postV_obsMeta <- SC_freq_postV_obsMeta %>%
  mutate(SC_pred = SC_freq_postV_predMeta)

SC_freq_E1meta <- df_preVmeta %>%
  mutate(SC_freq_E1=SC_n/sum(SC_n)) %>%
  select(BAPS2,PCV7.actual,SC_freq_E1)

SC_freq_postV_diffmeta <- SC_freq_postV_obsMeta %>%
  left_join(SC_freq_E1meta) %>%
  mutate(diff_pred = SC_freq_E1 - SC_pred,
         diff_obs = SC_freq_E1 - SC_freq)

SC_freq_postV_diffmeta <- SC_freq_postV_diffmeta %>%
  mutate(diff = abs(diff_pred - diff_obs)) %>%
  arrange(diff)

#### Pro rata ####
SC_freq_postV_diffmeta <- SC_freq_postV_diffmeta %>%
  mutate(prorata = SC_freq_E1/sum(SC_freq_E1)) %>%
  mutate(diff_predPro = SC_freq_E1 - prorata) %>%
  mutate(diffP = abs(diff_predPro - diff_obs))

#### Stats metabolic loci NFDS ####
stats <- summary(lm(SC_freq_postV_diffmeta$diff_pred~SC_freq_postV_diffmeta$diff_obs))
ars <- round(stats$adj.r.squared, digits = 3)
sseE <- round(sse(SC_freq_postV_diffmeta$diff_obs, SC_freq_postV_diffmeta$diff_pred), digits = 3)
rmseE <- round(rmse(SC_freq_postV_diffmeta$diff_obs, SC_freq_postV_diffmeta$diff_pred),digits = 3)

metaNFDS <- data.frame(Model = "Metabolic loci (NFDS)", nloci = length(el_meta),
                       adj.r.squared = ars, SSE = sseE, RMSE = rmseE)

#### Stats metabolic loci Pro rata ####
stats <- summary(lm(SC_freq_postV_diffmeta$diff_predPro~SC_freq_postV_diffmeta$diff_obs))
```

```r
ars <- round(stats$adj.r.squared, digits = 3)
sseE <- round(sse(SC_freq_postV_diffmeta$diff_obs, SC_freq_postV_diffmeta$diff_predPro), digits = 3)
rmseE <- round(rmse(SC_freq_postV_diffmeta$diff_obs, SC_freq_postV_diffmeta$diff_predPro),digits = 3)

metaProrata <- data.frame(Model = "Metabolic loci (Pro rata)", nloci = length(el_meta),
                          adj.r.squared = ars, SSE = sseE, RMSE = rmseE)

##############################

#### create table ####
dfOut <- bind_rows(accNFDS, accProrata, accNFDSSA,
                   accProrataSA, coreNFDS, coreProrata,
                   metaNFDS, metaProrata)
write.csv(dfOut, "statsTable.csv", row.names = F)
```