Biol 432 Group 1 Project

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Project Info

Group name: Teambits

Date: 2022/3/21

GitHub Link: https://github.com/carolinetang77/BIOL432-group1

Load the packages we will need

```
library(dplyr)
library(ggplot2)
library(BiocManager)
library(genbankr)
library(rentrez)
library(muscle)
library(ape)
library(reshape2)
library(ggtree)
library(tidyverse)
library(Biostrings)
library(annotate)
```

Input the data

```
Table1 <- read.csv("./InputData/TableS1.csv")
Table9 <- read.csv("./InputData/TableS9.csv")

readLines("./InputData/TableS8.dat", n = 10) # Take a look at the .dat file
```

```
##  [1] "BCoV1.1\t0.000147672\t1939"    "BCoV1.main\t0.00016565\t1947"
##  [3] "CHIKV.1\t0.000335409\t1953"    "CHIKV.2\t8.95474e-05\t1829"
##  [5] "CHIKV.grafted\tNaN\t1829"      "CHIKV.main\t0.000117516\t1758"
##  [7] "DENV.1\t0.000678122\t1980"     "DENV.10\t0.000615014\t1962"
##  [9] "DENV.11\t0.00077911\t1974"     "DENV.12\t0.00152533\t2004"
```

```
Table8 <- read.table("./InputData/TableS8_edited.dat", header = F)
names(Table8) <- c("virus", "rate", "year")

Table11 <- read.csv("./InputData/TableS11.csv")
```

Research question 1: Are mutation rates correlated with transmission method? Do certain transmission methods have higher mutation rates?

```
# Check how many types of virus in table S9
table(Table9$virus)
```

Coding by Chenyang Wu

```
##
##   BCoV1   CHIKV    DENV   Ebola     EVA     EVB     EVC    EVCr     EVD    H3N2
##     110      54     187      32     317     121      88      75      77       3
##     HCV    HCVr     HDV    HMPV    HRSV    HRV3    MERS     MMV     MRV Norwalk
##     190     190      16      45      77      64     100      80      24     112
##    OHVA    PeVA     RVA   SARS2      SV    TBEV     WNV     YFV    ZIKV
##      86      79      33      25      71      76      66      55      55
```

```
# Check the corresponding transmission type to the abbreviation in table S1
list(Table1$Abbreviation)
```

```
## [[1]]
##  [1] "BCoV1"   "CHIKV"   "DENV"    "Ebola"   "EVA"     "EVB"     "EVC"
##  [8] "EVD"     "H3N2"    "HCV"     "HDV"     "HMPV"    "HRSV"    "HRV3"
## [15] "MERS"    "MMV"     "MRV"     "Norwalk" "OHVA"    "PeVA"    "RVA"
## [22] "SARS2"   "SV"      "TBEV"    "WNV"     "YFV"     "ZIKV"
```

```
list(Table1$Transmission)
```

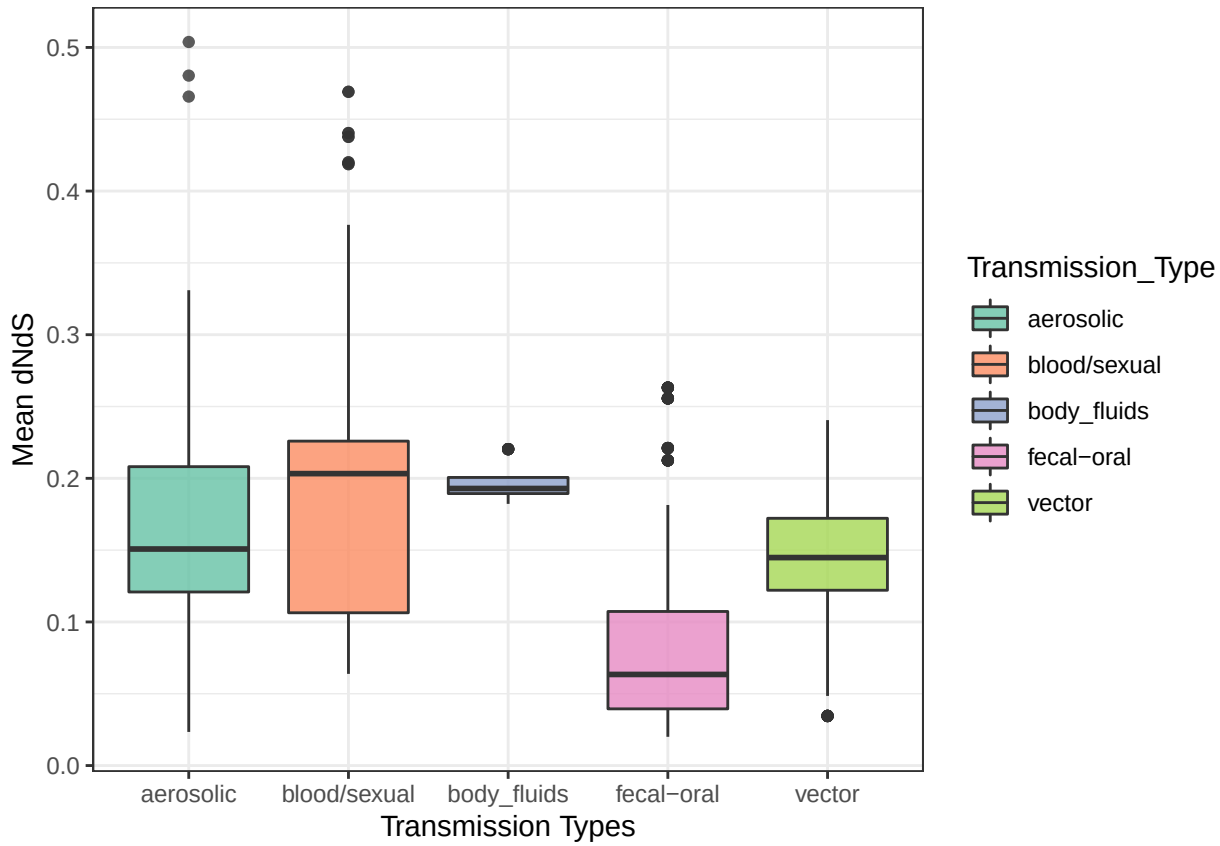
```
## [[1]]
##  [1] "aerosolic"     "vector"        "vector"        "body fluids"
##  [5] "fecal-oral"    "fecal-oral"    "fecal-oral"    "fecal-oral"
##  [9] "aerosolic"     "blood/ sexual" "blood/ sexual" "aerosolic"
## [13] "aerosolic"     "aerosolic"     "aerosolic"     "aerosolic"
## [17] "aerosolic"     "fecal-oral"    "fecal-oral"    "aerosolic"
## [21] "aerosolic"     "aerosolic"     "fecal-oral"    "vector"
## [25] "vector"        "vector"        "vector"
```

Combine the relevant columns into 1 dataframe

```
# Label the transmission type to the dataset
dNdSData <- Table9 %>%
  mutate(Transmission_Type = recode(virus,
                                    "BCoV1" = "aerosolic",
                                    "CHIKV" = "vector",
                                    "DENV" = "vector",
                                    "Ebola" = "body_fluids",
                                    "EVA" = "fecal-oral",
                                    "EVB" = "fecal-oral",
                                    "EVC" = "fecal-oral",
                                    "EVCr" = "fecal-oral",
                                    "EVD" = "fecal-oral",
                                    "H3N2" = "aerosolic",
                                    "HCV" = "blood/sexual",
                                    "HCVr" = "blood/sexual",
```

```
                                    "HDV" = "blood/sexual",
                                    "HMPV" = "aerosolic",
                                    "HRSV" = "aerosolic",
                                    "HRV3" = "aerosolic",
                                    "MERS" = "aerosolic",
                                    "MMV" = "aerosolic",
                                    "MRV" = "aerosolic",
                                    "Norwalk" = "fecal-oral",
                                    "OHVA" = "fecal-oral",
                                    "PeVA" = "aerosolic",
                                    "RVA" = "aerosolic",
                                    "SARS2" = "aerosolic",
                                    "SV" = "fecal-oral",
                                    "TBEV" = "vector",
                                    "WNV" = "vector",
                                    "YFV" = "vector",
                                    "ZIKV" = "vector"))

# Draw a box plot for mean dNdS data with different transmission types
ggplot(dNdSData, aes(x = Transmission_Type, y = meandNdS,
                     na.rm = TRUE, fill = Transmission_Type)) +
  geom_boxplot(alpha = 0.8) +
  theme_bw() +
  scale_fill_brewer(palette = "Set2") +
  labs(x = "Transmission Types", y = "Mean dNdS")
```



```r
MutaRate <- Table8 %>%
  mutate(Transmission_Type = recode(virus,
                                    "BCoV1" = "aerosolic",
                                    "CHIKV" = "vector",
                                    "DENV" = "vector",
                                    "Ebola" = "body_fluids",
                                    "EVA" = "fecal-oral",
                                    "EVB" = "fecal-oral",
                                    "EVC" = "fecal-oral",
                                    "EVCr" = "fecal-oral",
                                    "EVD" = "fecal-oral",
                                    "H3N2" = "aerosolic",
                                    "HCV" = "blood/sexual",
                                    "HCVr" = "blood/sexual",
                                    "HDV" = "blood/sexual",
                                    "HMPV" = "aerosolic",
                                    "HRSV" = "aerosolic",
                                    "HRV3" = "aerosolic",
                                    "MERS" = "aerosolic",
                                    "MMV" = "aerosolic",
                                    "MRV" = "aerosolic",
                                    "Norwalk" = "fecal-oral",
                                    "OHVA" = "fecal-oral",
                                    "PeVA" = "aerosolic",
                                    "RVA" = "aerosolic",
                                    "SARS2" = "aerosolic",
                                    "SV" = "fecal-oral",
                                    "TBEV" = "vector",
                                    "WNV" = "vector",
                                    "YFV" = "vector",
                                    "ZIKV" = "vector"))

# Draw a box plot for the mutation rate under different transmission types
ggplot(MutaRate, aes(x = Transmission_Type,
                     y = rate, na.rm = TRUE,
                     fill = Transmission_Type)) +
  geom_boxplot(alpha = 0.8) +
  theme_bw() +
  scale_fill_brewer(palette = "Set2") +
  labs(x = "Transmission Types", y = "Mutation Rate")
```

Fig.1 Boxplot of mean dNdS values among different transmission types.

```
## Warning: Removed 21 rows containing non-finite values (stat_boxplot).
```

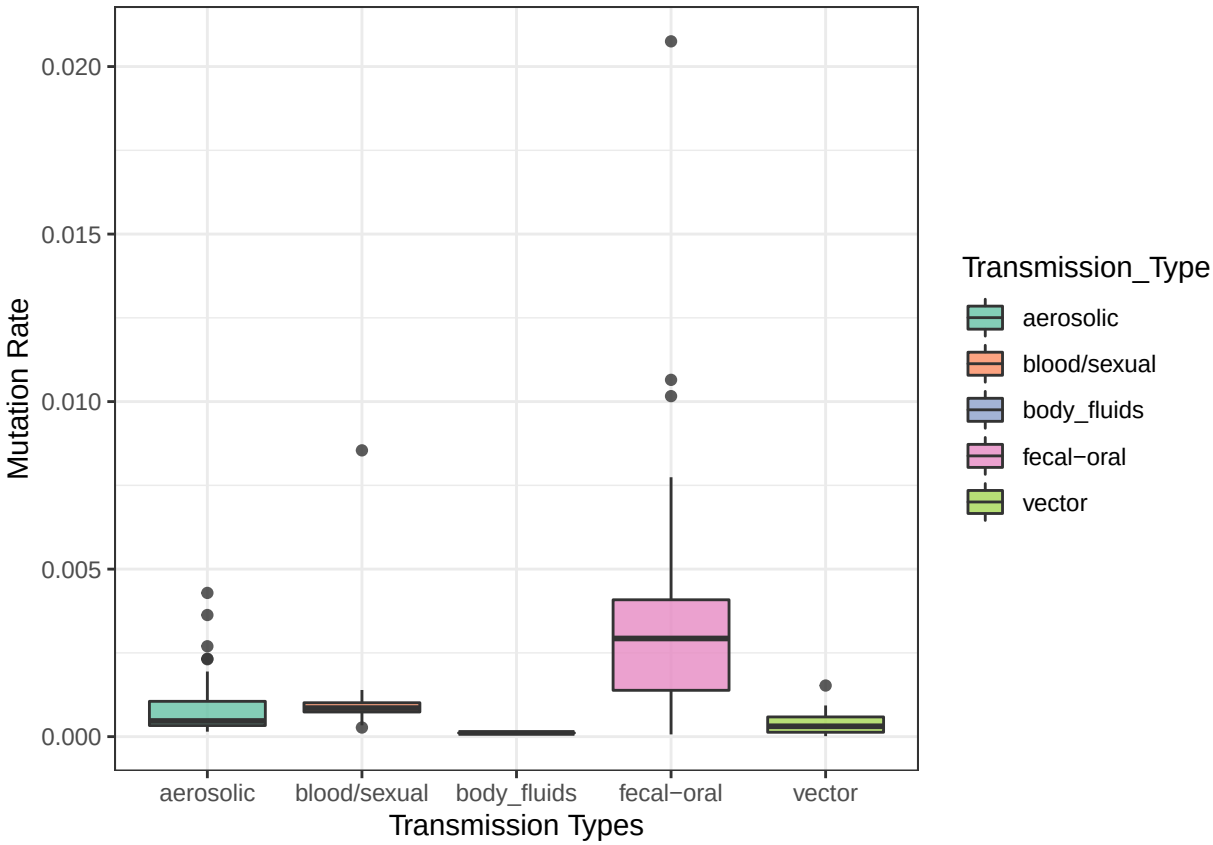


Fig.2 Boxplot of mutation rate among different Transmission Types

Adjust the sequence ID for SARS2

Covid sequences have different IDs from other viruses, not based on genbank accession number so we have to fix that

```
# Load the virus table and the COVID sequence acknowledgement table
Table3 <- read.delim("InputData/TableS3_GISAID_acknowledgements.dat")

# Merge the COVID rows in the virus table with the acknowledgement table
DataMerged <- merge(Table11[Table11$virus == "SARS2",], Table3, by.x = "ID", by.y = "internID")

# Filter for only rows with GenBank accession IDs
Datafilter <- DataMerged %>%
  filter(genbank_accession != "?")

# Replace the alldb ID with the accession ID
Datafilter$ID <- Datafilter$genbank_accession

# Select only the columns from the original virus table and rename them
Datafilter <- Datafilter %>%
  dplyr::select(1:12)
names(Datafilter) <- names(Table11)
```

```r
# Replace the original covid rows with the ones we just made
finalIDs <- bind_rows(Table11[Table11$virus != "SARS2",], Datafilter)
```

```r
# Check the number of IDs in table S11 for each virus
table(finalIDs$virus)
```

This chunk was done with the help of Caroline Tang

```
##
##   BCoV1   CHIKV    DENV   Ebola     EVA     EVB     EVC     EVD    H3N2     HCV
##     321    1127    7263    2512    2866     959    1019     974   29706    4494
##     HDV    HMPV    HRSV    HRV3    MERS     MMV     MRV Norwalk    OHVA    PeVA
##     740     224    2751     477     629     329     608    2384     677     323
##     RVA   SARS2      SV    TBEV     WNV     YFV    ZIKV
##     337    1542     289     266    2731     435    1106
```

```r
# Since there are so many IDs, We decided to pick 5 IDs from each virus for question 1

# Random pick 5 IDs for each virus
set.seed(1) # Set seed to make sure the output are constant
IDs <- finalIDs %>%
  group_by(virus) %>%
  sample_n(5)
```

```r
# Double check if the code correctly pick 5 IDs from each virus
table(IDs$virus)
```

```
##
##   BCoV1   CHIKV    DENV   Ebola     EVA     EVB     EVC     EVD    H3N2     HCV
##       5       5       5       5       5       5       5       5       5       5
##     HDV    HMPV    HRSV    HRV3    MERS     MMV     MRV Norwalk    OHVA    PeVA
##       5       5       5       5       5       5       5       5       5       5
##     RVA   SARS2      SV    TBEV     WNV     YFV    ZIKV
##       5       5       5       5       5       5       5
```

```r
# Create the id list
ncbi_ids <- IDs$ID
```

```r
# Search the sequence info from NCBI
Q1Vir <- entrez_fetch(db = "nuccore", id = ncbi_ids, rettype = "fasta")
Q1Seq <- strsplit(Q1Vir, split = "\n\n", fixed = T)
Q1Seq <- unlist(Q1Seq)

# Use regular expression to edit the search result
header <- gsub("(^>.*genome|*cds|*sequence|*SEQUENCES|*RNA)\\n[ATCG].*", "\\1", Q1Seq)

seq <- gsub("^>.*genome\\n([ATCG].*)", "\\1", Q1Seq)
seq <- gsub("^>.*cds\\n([ATCG].*)", "\\1", seq)
seq <- gsub("^>.*sequence\\n([ATCG].*)", "\\1", seq)
seq <- gsub("^>.*SEQUENCES\\n([ATCG].*)", "\\1", seq)
seq <- gsub("^>.*RNA\\n([ATCG].*)", "\\1", seq)

Q1SeqTable <- data.frame(Name = header, Sequence = seq)
Q1SeqTable$Sequence <- gsub("\n", "", Q1SeqTable$Sequence)

# There are several lines have different ending words, so we will output the data set and adjust it man
```

```
write.csv(Q1SeqTable, "./InputData/Q1Seq.csv", row.names = F)
```

```
# Input the edited data
Q1Sequence <- read.csv("./InputData/Q1Seq_edited.csv")
```

Multiple Alignments

```
Q1DF <- data.frame(ID = IDs$ID,
                   Virus = IDs$virus,
                   Seq = Q1Sequence$Sequence,
                   stringsAsFactors = FALSE)

Q1DF <- Q1DF %>%
  mutate(Transmission_Type = recode(Virus,
                                    "BCoV1" = "aerosolic",
                                    "CHIKV" = "vector",
                                    "DENV" = "vector",
                                    "Ebola" = "body_fluids",
                                    "EVA" = "fecal-oral",
                                    "EVB" = "fecal-oral",
                                    "EVC" = "fecal-oral",
                                    "EVCr" = "fecal-oral",
                                    "EVD" = "fecal-oral",
                                    "H3N2" = "aerosolic",
                                    "HCV" = "blood/sexual",
                                    "HCVr" = "blood/sexual",
                                    "HDV" = "blood/sexual",
                                    "HMPV" = "aerosolic",
                                    "HRSV" = "aerosolic",
                                    "HRV3" = "aerosolic",
                                    "MERS" = "aerosolic",
                                    "MMV" = "aerosolic",
                                    "MRV" = "aerosolic",
                                    "Norwalk" = "fecal-oral",
                                    "OHVA" = "fecal-oral",
                                    "PeVA" = "aerosolic",
                                    "RVA" = "aerosolic",
                                    "SARS2" = "aerosolic",
                                    "SV" = "fecal-oral",
                                    "TBEV" = "vector",
                                    "WNV" = "vector",
                                    "YFV" = "vector",
                                    "ZIKV" = "vector"))

# Convert DNASbin to DNAStringSet
VirString <- Q1DF$Seq %>%
  as.character %>%
  lapply(., paste0, collapse = "") %>%
  unlist %>%
  DNAStringSet

# Give each sequence a unique names
names(VirString) <- paste(1:nrow(Q1DF), Q1DF$ID, sep = "_")
```

```
# Use MUSCLE to align the sequences
# This line will take about 1 hour for R to run it.
VirAlign <- muscle::muscle(stringset = VirString, diags = T, gapopen = -10)

# Convert our DNA Multiple Alignment object to a DNA Bin object
VirAlignBin <- as.DNAbin(VirAlign)
```

```
SeqLen <- as.numeric(lapply(VirString, length))
# Show the distribution of sequence length
qplot(SeqLen) + theme_bw()
```

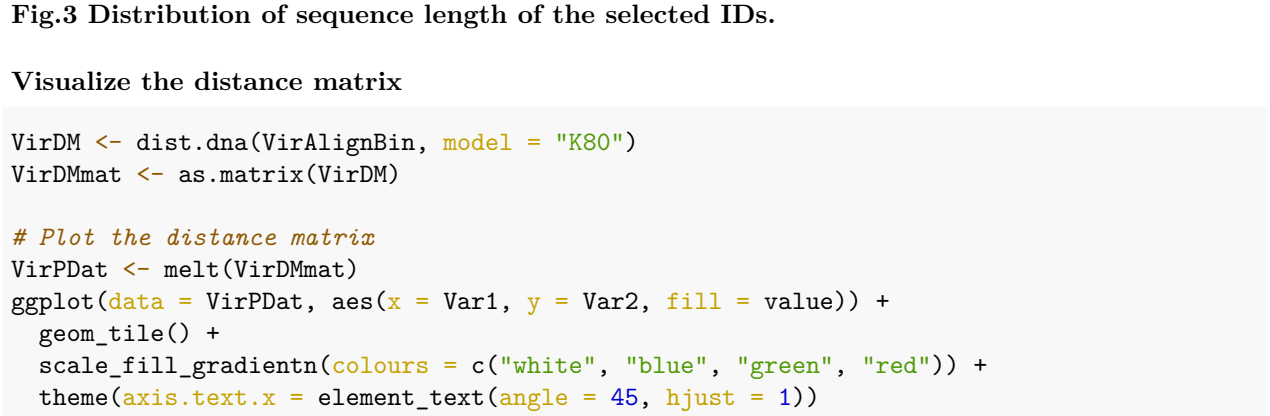


Fig.3 Distribution of sequence length of the selected IDs.

Visualize the distance matrix

```
VirDM <- dist.dna(VirAlignBin, model = "K80")
VirDMmat <- as.matrix(VirDM)

# Plot the distance matrix
VirPDat <- melt(VirDMmat)
ggplot(data = VirPDat, aes(x = Var1, y = Var2, fill = value)) +
  geom_tile() +
  scale_fill_gradientn(colours = c("white", "blue", "green", "red")) +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```

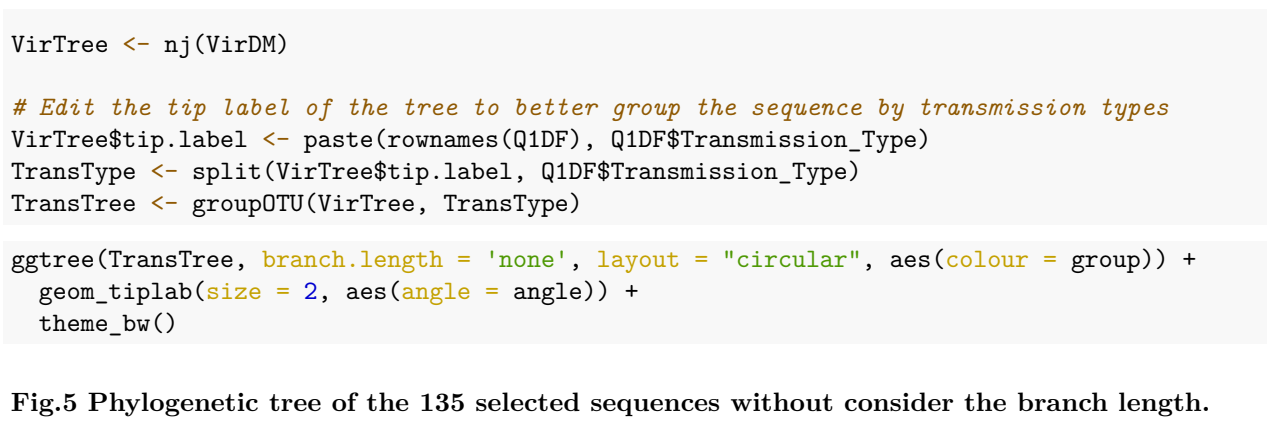


Fig.4 Figure of the distance matrix.

Create the phlogeny tree

```
VirTree <- nj(VirDM)

# Edit the tip label of the tree to better group the sequence by transmission types
VirTree$tip.label <- paste(rownames(Q1DF), Q1DF$Transmission_Type)
TransType <- split(VirTree$tip.label, Q1DF$Transmission_Type)
TransTree <- groupOTU(VirTree, TransType)
```

```
ggtree(TransTree, branch.length = 'none', layout = "circular", aes(colour = group)) +
  geom_tiplab(size = 2, aes(angle = angle)) +
  theme_bw()
```

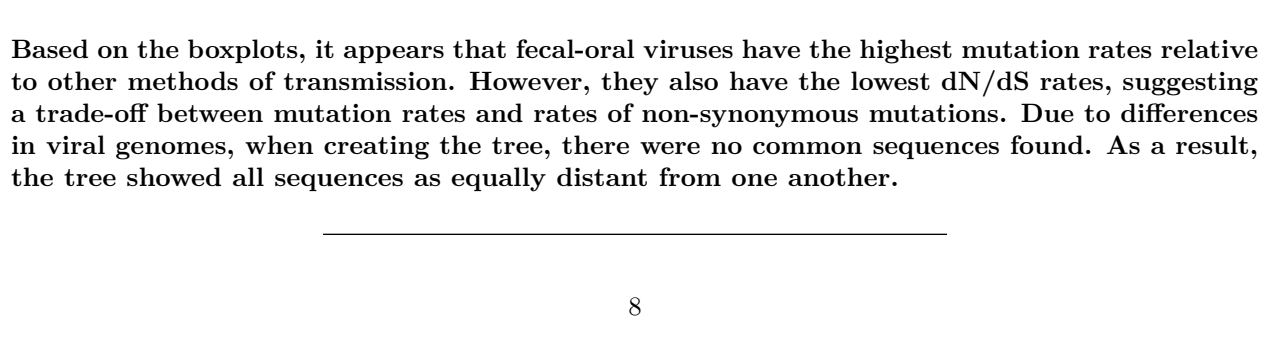


Fig.5 Phylogenetic tree of the 135 selected sequences without consider the branch length.

Output the phylogeny tree

```
write.tree(TransTree, "./Output/Transmission_Type_Tree.tre")
```

Based on the boxplots, it appears that fecal-oral viruses have the highest mutation rates relative to other methods of transmission. However, they also have the lowest dN/dS rates, suggesting a trade-off between mutation rates and rates of non-synonymous mutations. Due to differences in viral genomes, when creating the tree, there were no common sequences found. As a result, the tree showed all sequences as equally distant from one another.

Research question 2: Is the mutation rate correlated with guanine-cytosine content?

Coding By Caroline Tang

Load libraries

```
library(tidyverse)
library(rentrez)
library(genbankr)
library(Biostrings)
library(annotate)
library(ape)
```

Load in/format data on accession sequences

```
# Load the virus table and the COVID sequence acknowledgement table
virus <- read.csv("InputData/TableS11.csv")
covid <- read.delim("InputData/TableS3_GISAID_acknowledgements.dat")

# Merge the COVID rows in the virus table with the acknowledgement table
covidMerged <- merge(virus[virus$virus == "SARS2", ], covid, by.x = "ID", by.y = "internID")

# Filter for only rows with GenBank accession IDs
covidfilter <- covidMerged %>%
  filter(genbank_accession != "?")

# Replace the alldb ID with the accession ID
covidfilter$ID <- covidfilter$genbank_accession

# Select only the columns from the original virus table and rename them
covidfilter <- covidfilter %>%
  dplyr::select(1:12)
names(covidfilter) <- names(virus)

# Replace the original covid rows with the ones we just made
finalvirus <- bind_rows(virus[virus$virus != "SARS2", ], covidfilter)
```

The COVID-19 sequences have different IDs from other viruses, not based on GenBank accession number so we have to replace them.

Subset sequences (10 per virus)

```
set.seed(1)
virusSubset <- finalIDs %>%
  group_by(virus) %>%
  slice_sample(n = 10)
```

Get sequences from GenBank

```
virusID <- GBAccession(virusSubset$ID)
virusGBK <- read.GenBank(virusID, as.character = TRUE)
```

Calculate GC content of each sequence and merge with virus types

```
gc <- vector(length = length(virusGBK))
for (i in 1:length(virusGBK)) {
  gc[i] <- length(grep("[gc]", unlist(virusGBK[[i]]))) / length(virusGBK[[i]])
}
gcContent <- data.frame(ID = names(virusGBK), gc = gc)
virusSubset <- merge(virusSubset, gcContent, by = "ID")
```

Scatterplot of GC content vs. mutation rate and transmission method

```
# Calculate mean mutation rate per virus and merge with GC content
mutationMean <- Table8 %>%
  group_by(virus) %>%
  summarise(meanRate = mean(rate, na.rm = T))
virusSubset <- merge(virusSubset, mutationMean, by = "virus")

# Merge transmission data
virusSubset <- merge(virusSubset, Table1, by.x = "virus", by.y = "Abbreviation")

# Create scatter plot
ggplot(data = virusSubset, aes(x = gc, y = meanRate)) +
  geom_point(aes(colour = Transmission), alpha = 0.8) +
  theme_classic() +
  geom_smooth(method = "lm") +
  scale_fill_brewer(palette = "Set2") +
  labs(x = "GC content", y = "Mean mutation rate")
```

```
## `geom_smooth()` using formula 'y ~ x'
```

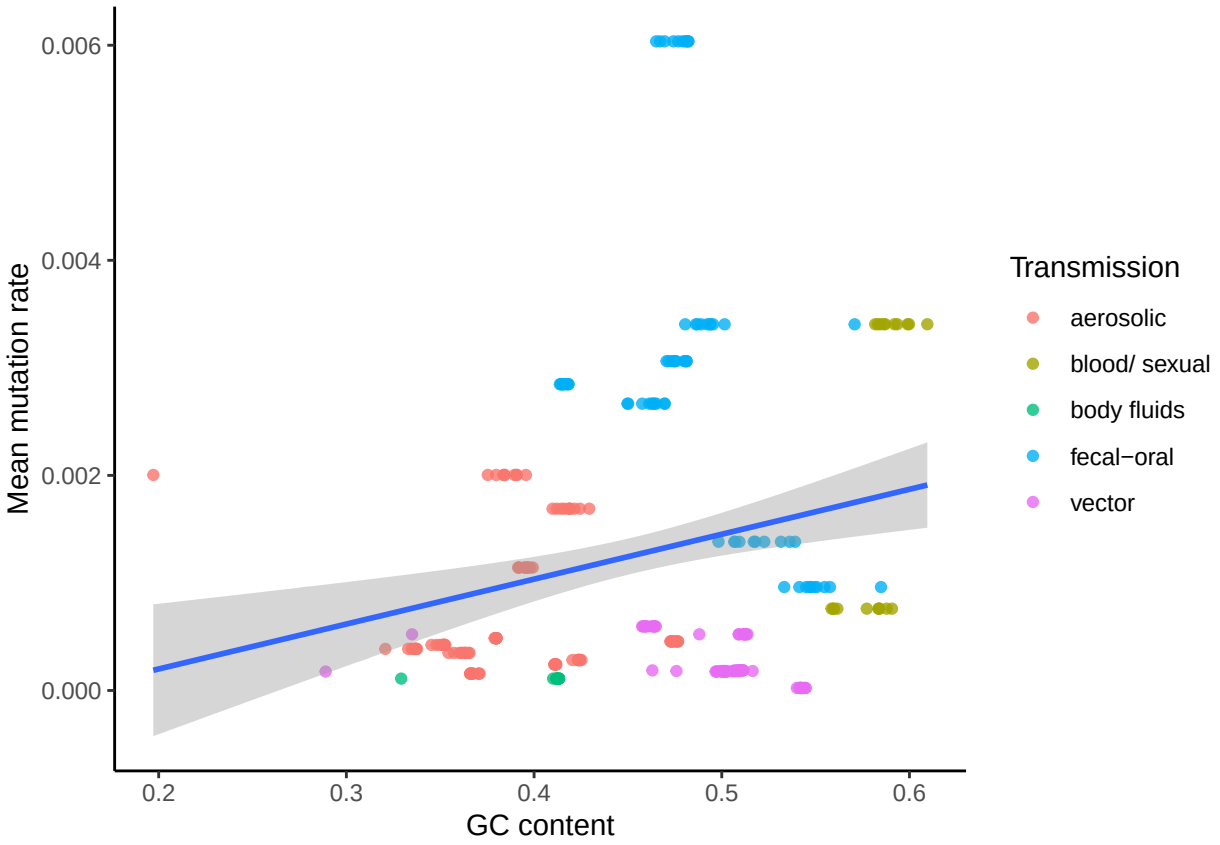


Fig.6 Scatter plot of the 130 selected sequences' GC content and mutation rates, colour coded by transmission type.

Based on the scatter plot, there is a slightly positive correlation between GC content and mutation rate, which supports the hypothesis that mutation rate increases with GC content. However, this trend varied among transmission methods, and the overall trend be skewed by the fecal-oral viruses with high mutation rates.