evidence2

2025-05-02

Load all of the libraries that we'll be using for this project

```
##
##    /// adegenet 2.1.11 is loaded ////////////
##
##    > overview: '?adegenet'
##    > tutorials/doc/questions: 'adegenetWeb()'
##    > bug reports/feature requests: adegenetIssues()
```

```
## Loading required package: BiocGenerics
```

```
##
## Attaching package: 'BiocGenerics'
```

```
## The following object is masked from 'package:ade4':
##
##     score
```

```
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
```

```
## The following objects are masked from 'package:base':
##
##     anyDuplicated, aperm, append, as.data.frame, basename, cbind,
##     colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
##     get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
##     match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
##     Position, rank, rbind, Reduce, rownames, sapply, saveRDS, setdiff,
##     table, tapply, union, unique, unsplit, which.max, which.min
```

```
## Loading required package: S4Vectors
```

```
## Loading required package: stats4
```

```
##
## Attaching package: 'S4Vectors'
```

```
## The following object is masked from 'package:utils':
##
##     findMatches
```

```
## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname
```

```
## Loading required package: IRanges
```

```
## Loading required package: XVector
```

```
## Loading required package: GenomeInfoDb
```

```
##
## Attaching package: 'Biostrings'
## The following object is masked from 'package:ape':
##
##     complement
## The following object is masked from 'package:base':
##
##     strsplit
##
## Attaching package: 'dplyr'
## The following objects are masked from 'package:Biostrings':
##
##     collapse, intersect, setdiff, setequal, union
## The following object is masked from 'package:GenomeInfoDb':
##
##     intersect
## The following object is masked from 'package:XVector':
##
##     slice
## The following objects are masked from 'package:IRanges':
##
##     collapse, desc, intersect, setdiff, slice, union
## The following objects are masked from 'package:S4Vectors':
##
##     first, intersect, rename, setdiff, setequal, union
## The following objects are masked from 'package:BiocGenerics':
##
##     combine, intersect, setdiff, union
## The following object is masked from 'package:ape':
##
##     where
## The following objects are masked from 'package:stats':
##
##     filter, lag
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
##
## Attaching package: 'tidyr'
## The following object is masked from 'package:reshape2':
##
##     smiths
## The following object is masked from 'package:S4Vectors':
##
##     expand
```

```
##
## Attaching package: 'phangorn'

## The following object is masked from 'package:adegenet':
##
##     AICc

## ggtree v3.14.0 Learn more at https://yulab-smu.top/contribution-tree-data/
##
## Please cite:
##
## Shuangbin Xu, Lin Li, Xiao Luo, Meijun Chen, Wenli Tang, Li Zhan, Zehan
## Dai, Tommy T. Lam, Yi Guan, Guangchuang Yu. Ggtree: A serialized data
## object for visualization of a phylogenetic tree and annotation data.
## iMeta 2022, 1(4):e56. doi:10.1002/imt2.56

##
## Attaching package: 'ggtree'

## The following object is masked from 'package:tidyr':
##
##     expand

## The following object is masked from 'package:Biostrings':
##
##     collapse

## The following object is masked from 'package:IRanges':
##
##     collapse

## The following object is masked from 'package:S4Vectors':
##
##     expand

## The following object is masked from 'package:ape':
##
##     rotate

## treeio v1.30.0 Learn more at https://yulab-smu.top/contribution-tree-data/
##
## Please cite:
##
## LG Wang, TTY Lam, S Xu, Z Dai, L Zhou, T Feng, P Guo, CW Dunn, BR
## Jones, T Bradley, H Zhu, Y Guan, Y Jiang, G Yu. treeio: an R package
## for phylogenetic tree input and output with richly annotated and
## associated data. Molecular Biology and Evolution. 2020, 37(2):599-603.
## doi: 10.1093/molbev/msz240

##
## Attaching package: 'treeio'

## The following object is masked from 'package:Biostrings':
##
##     mask
```

Performing sequence loading

```
# List of countries where COVID killed the most people
countries <- c("usa", "china", "india", "france", "germany", "brazil", "south_korea", "japan", "italy",
```

```r
# Map the names to DNA String Sets
sequences <- sapply(countries, function(x) readDNAStringSet(paste("./sequences/", x, ".fasta", sep="")))

# Convert to DNABin types
dna_bins <- do.call(c, sapply(sequences, as.DNAbin))

# Labeling and Display
country_titles <- sapply(countries, function(string) str_to_title(str_replace(string, "_", " ")))

# Create a  Dataframe from the Sequences
sequence_frame <- data.frame(
  country_key = countries,
  country_title  = country_titles,
  sequence_length = sapply(sequences, width),
  a_frequency = sapply(sequences, function(seq) letterFrequency(seq, 'A')),
  c_frequency = sapply(sequences, function(seq) letterFrequency(seq, 'C')),
  g_frequency = sapply(sequences, function(seq) letterFrequency(seq, 'G')),
  t_frequency = sapply(sequences, function(seq) letterFrequency(seq, 'T'))
)

# Print Genome Sequence Lengths
print("The sequence lengths for each genome are as follows")
```

```
## [1] "The sequence lengths for each genome are as follows"
```

```r
sequence_frame[3]
```

```
##             sequence_length
## usa                   29750
## china                 29903
## india                 29834
## france                29759
## germany               29741
## brazil                29835
## south_korea           29738
## japan                 29784
## italy                 29741
## uk                    29750
```

```r
# Print Genome Sequence Nitrogenous Base Frequencies
print("The frequencies for each base in each genome are as follows:")
```

```
## [1] "The frequencies for each base in each genome are as follows:"
```

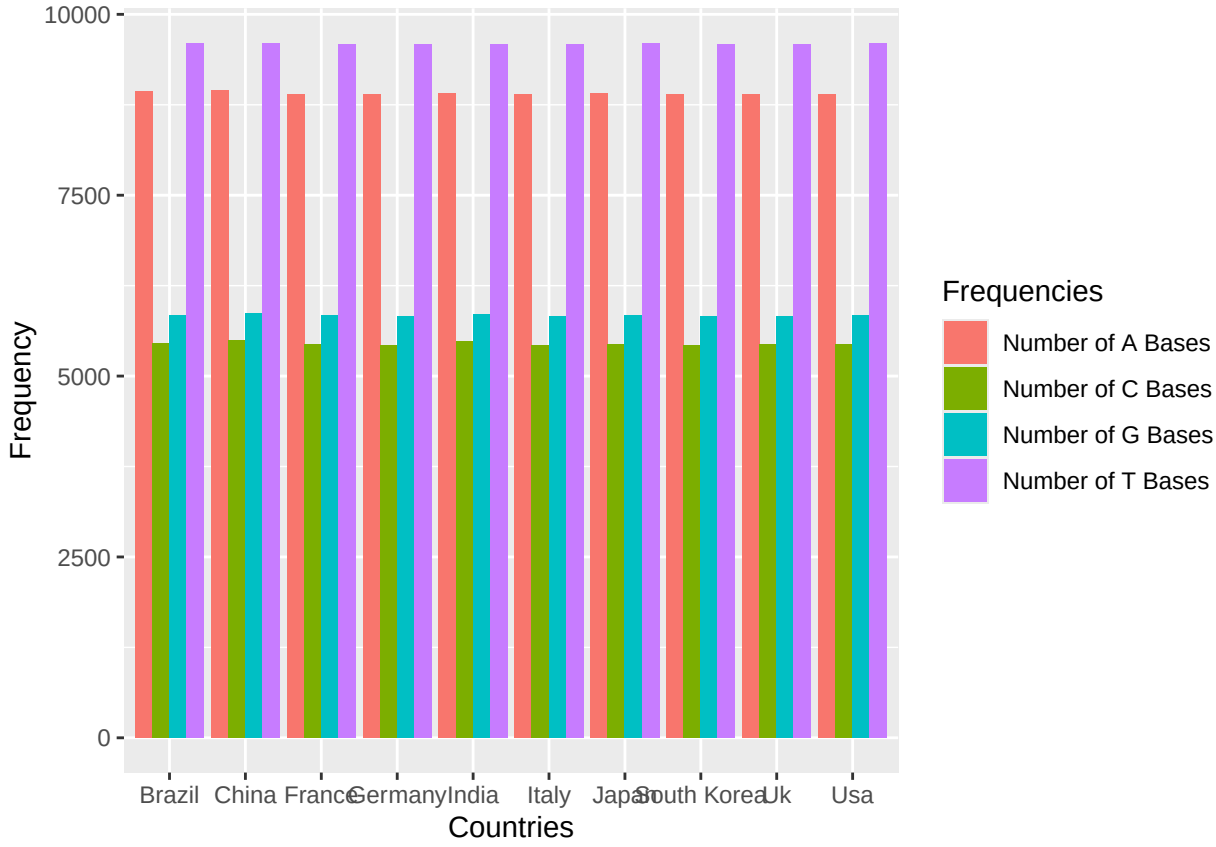
```r
sequence_frame[c(4, 5, 6, 7)]
```

```
##             a_frequency c_frequency g_frequency t_frequency
## usa                8890        5433        5834        9593
## china              8954        5492        5863        9594
## india              8909        5482        5851        9592
## france             8895        5443        5834        9587
## germany            8896        5429        5828        9588
## brazil             8943        5457        5841        9594
## south_korea        8891        5429        5829        9589
## japan              8905        5441        5834        9604
```

```
## italy               8893        5425        5830        9592
## uk                  8893        5433        5830        9592
```

Convert to data frame and graph

```
# Plot Frequencies
ggplot(
  data = sequence_frame %>% gather(Frequencies, Frequency, -country_title, -country_key, -sequence_leng
  aes(x = country_title, y = Frequency, fill = Frequencies)
) +
  geom_bar(stat = 'identity', position = 'dodge') +
  xlab("Countries") +
  scale_fill_discrete(
    labels = c(
      "a_frequency" = "Number of A Bases",
      "c_frequency" = "Number of C Bases",
      "g_frequency" = "Number of G Bases",
      "t_frequency" = "Number of T Bases"
  ))
```



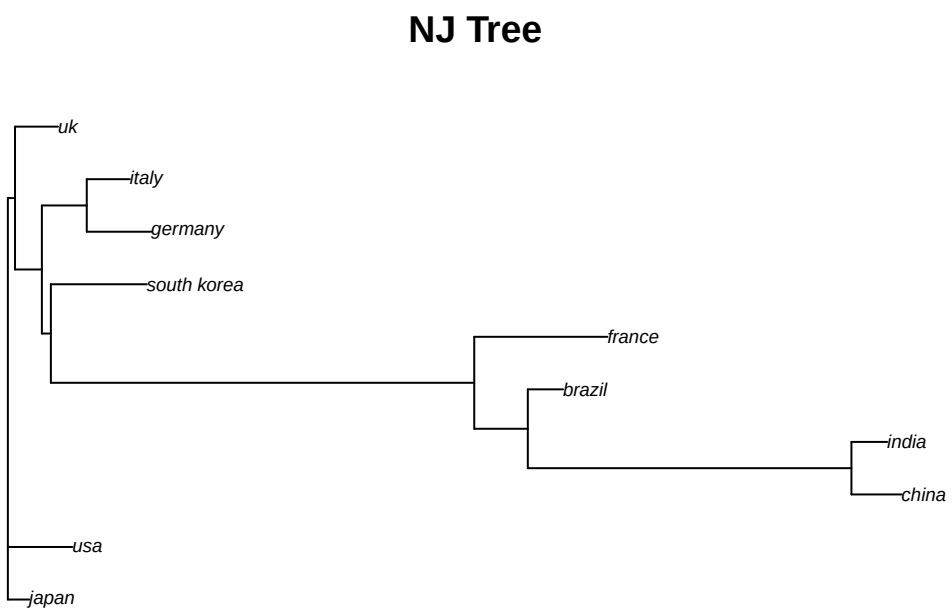

```
# Read FASTAs Again but in a Different Format (idealy, this shouldn't be the case but we couldn't find
countries <- c("usa", "china", "india", "france", "germany", "brazil", "south_korea", "japan", "italy",

# Place Sequences into a DNAStringSet
all_sequences <- DNAStringSet()
for (country in countries) {
  seq <- readDNAStringSet(paste0("./sequences/", country, ".fasta"))
  names(seq) <- country  # Explicitly name each sequence
```

```
  all_sequences <- c(all_sequences, seq)
}

# Load Mortality Rates
# 3. Prepare mortality data with EXACT MATCH to tip labels
mortality_data <- data.frame(
  label = countries,  # Must match tree$tip.label
  Cases = c(103436829, 99380363, 45043415, 39016278, 38437756, 37511921,
            34571873, 33803572, 26826486, 24992089),
  Deaths = c(1201488, 122358, 533641, 168091, 174979, 702116,
             35934, 74694, 197542, 232112)

) %>%
  mutate(
    Mortality = round(Deaths/Cases * 100, 2),
    Country = stringr::str_to_title(gsub("_", " ", label))
  )

mortality_data$Rates = mortality_data$Deaths / mortality_data$Cases

# Align DNA Sequences
aligned <- AlignSeqs(all_sequences)
```

```
## Determining distance matrix based on shared 11-mers:
## ================================================================================
##
## Time difference of 0.01 secs
##
## Clustering into groups by similarity:
## ================================================================================
##
## Time difference of 0 secs
##
## Aligning Sequences:
## ================================================================================
##
## Time difference of 0.18 secs
##
## Iteration 1 of 2:
##
## Determining distance matrix based on alignment:
## ================================================================================
##
## Time difference of 0 secs
##
## Reclustering into groups by similarity:
## ================================================================================
##
## Time difference of 0 secs
##
## Realigning Sequences:
## ================================================================================
##
## Time difference of 0 secs
```

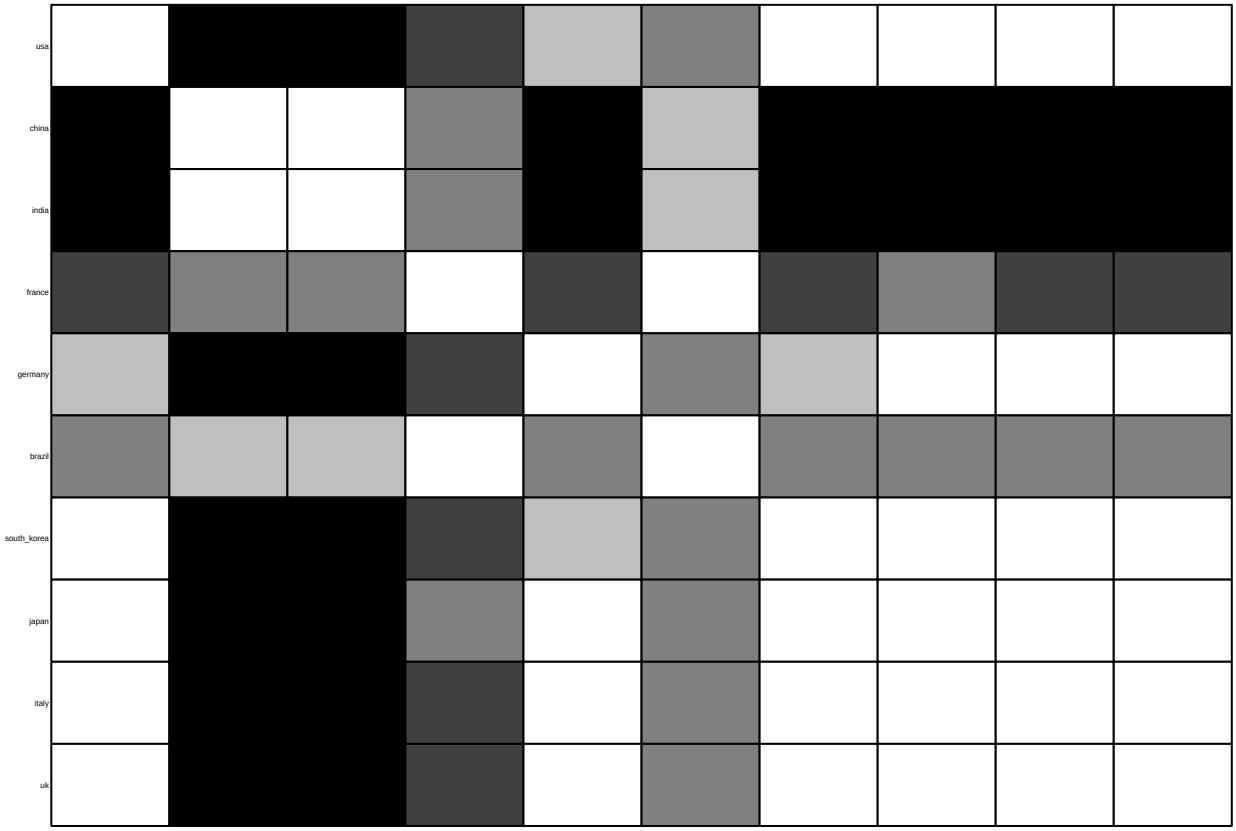
```
##
## Alignment converged - skipping remaining iteration.
```

```
dna <- as.matrix(as.DNAbin(aligned))

# Stop if Our Sequences are not Aligned
stopifnot(length(unique(ncol(dna))) == 1)

# DNA Distribution
dna_distro <- dist.dna(dna, model = "TN93")

# Create NJ Tree From DNA Distro
phylotree <- nj(dna_distro)



# Calculate Bootstrapping Values
boots <- boot.phylo(
  phylotree,
  dna,
  function(e) root(nj(dist.dna(e, model = "TN93")), 1),
  B = 100,
  quiet = TRUE
)

# Plot our Phylo Tree with Bootstrap Values
myPal <- colorRampPalette(c("red","yellow","green","blue"))
mortalityPalette <- colorRampPalette(c("red", "blue"))
plot(phylotree, cex = 0.6, main = "NJ Tree")
```




```
# Convert our DNA Distribution and Plot it in a Table
adj_matrix <- as.matrix(dna_distro)
table.paint(
  adj_matrix,
  cleg = 0,
```

```
  clabel.row = 0.25,
  clabel.col = 0.25
)
```




```
p <- ggtree(phylotree) %<+% mortality_data +
  geom_tiplab(aes(label = paste0(Country, "\n", Mortality, "%")),
              size = 3, hjust = -0.1) +
  geom_tippoint(aes(color = Mortality), size = 5) +
  scale_color_gradient(low = "blue", high = "red", na.value = "gray") +
  xlim(0, max(dna_distro) * 1.3) +
  labs(title = "Phylogenetic Tree with Mortality Rates",
      color = "Mortality (%)") +
  theme_tree2()

print(p)
```

Phylogenetic Tree with Mortality Rates