Uganda tsetse PopGen: PCA, diversity, CSE

Norah Saarman

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RStudio Configuration: - **R version:** R 4.4.0 (Geospatial packages)
- **Number of cores:** 4 (up to 32 available)
- **Account:** saarman-np
- **Partition:** saarman-shared-np (allows multiple simultaneous jobs)
- **Memory per job:** 100G (cluster limit: 1000G total; avoid exceeding half)

```
library(adegenet)
library(geosphere)
library(igraph)
library(scatterplot3d)
library(ggplot2)
library(dplyr)
library(hierfstat)
library(pegas)
library(PopGenReport)
library(poppr)
library(sf)
library(rnaturalearth)
library(rnaturalearthdata)
library(cowplot)
library(viridis)
```

Importing data as genind object from .gen

```
# 1. Genepop .gen file "./input/Gff_11loci_allsites_genepop.gen"
Gff.genind <- read.genepop("../input/Gff_11loci_allsites_genepop.gen", ncode = 3)
```

```
##
##  Converting data from a Genepop .gen file to a genind object...
##
##
## File description:  Glossina fuscipes fuscipes Uganda, all sites, 11 loci, GpCAG133 has a lot of miss
##
## ...done.
```

```
# Metadata with base R function read.csv()
Gff <- read.csv("../input/Gff_11loci_allsites_indinfo.txt", header=TRUE, sep = "\t")

# Add site code to @pop slot
Gff.genind@pop <- as.factor(Gff$SiteCode)

# Check genind object
```

```
Gff.genind
```

```
## /// GENIND OBJECT /////////
##
##  // 2,736 individuals; 11 loci; 162 alleles; size: 1.9 Mb
##
##  // Basic content
##    @tab:  2736 x 162 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 7-28)
##    @loc.fac: locus factor for the 162 columns of @tab
##    @all.names: list of allele names for each locus
##    @ploidy: ploidy of each individual  (range: 2-2)
##    @type:  codom
##    @call: read.genepop(file = "../input/Gff_11loci_allsites_genepop.gen",
##     ncode = 3)
##
##  // Optional content
##    @pop: population of each individual (group size range: 8-236)
```

```
summary(Gff.genind)
```

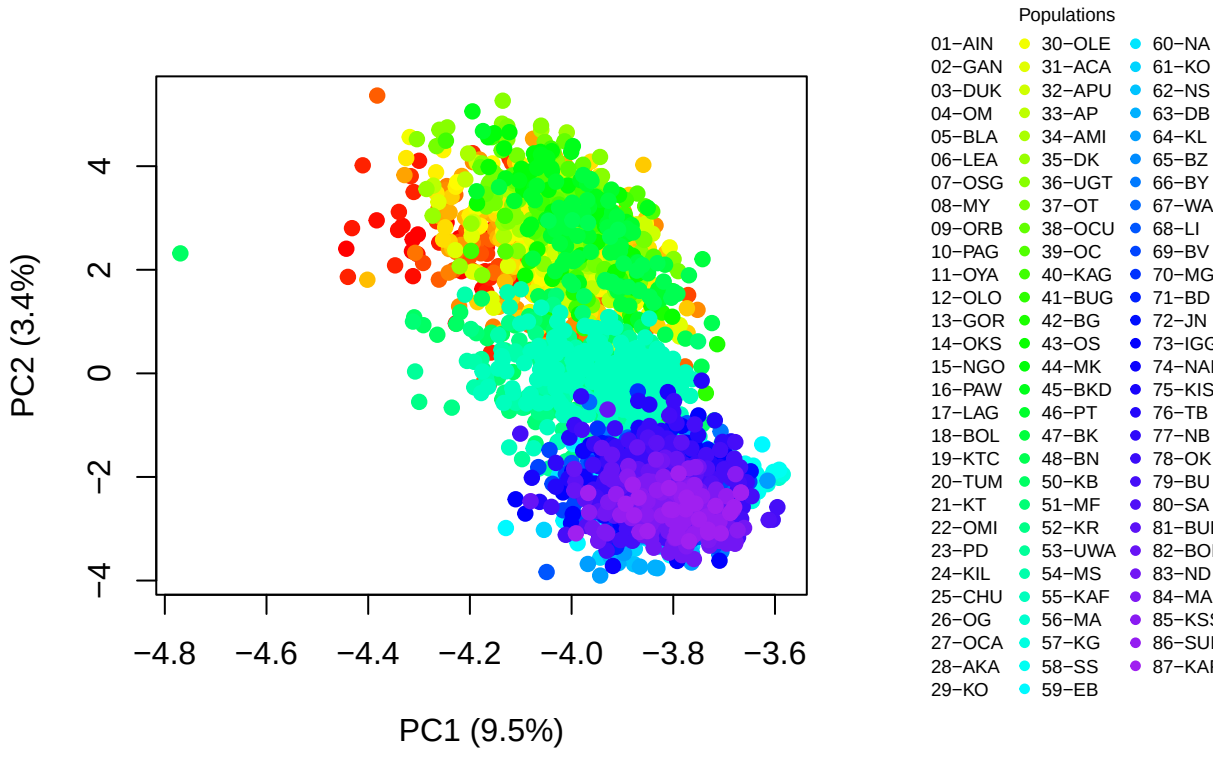
```
##
## // Number of individuals: 2736
## // Group sizes: 19 20 25 15 10 8 20 15 20 20 9 24 25 26 25 13 17 25 20 20 17 15 13 21 25 37 20 26 15
## // Number of alleles per locus: 27 7 17 8 10 15 9 13 28 10 18
## // Number of alleles per group: 65 65 65 61 52 50 60 60 59 57 52 54 69 66 57 50 50 59 56 56 55 60 59
## // Percentage of missing data: 3.25 %
## // Observed heterozygosity: 0.72 0.44 0.51 0.2 0.59 0.51 0.33 0.43 0.71 0.51 0.65
## // Expected heterozygosity: 0.85 0.63 0.69 0.23 0.71 0.73 0.5 0.56 0.87 0.7 0.84
```

Visualize genetic variation with PCA

All samples:

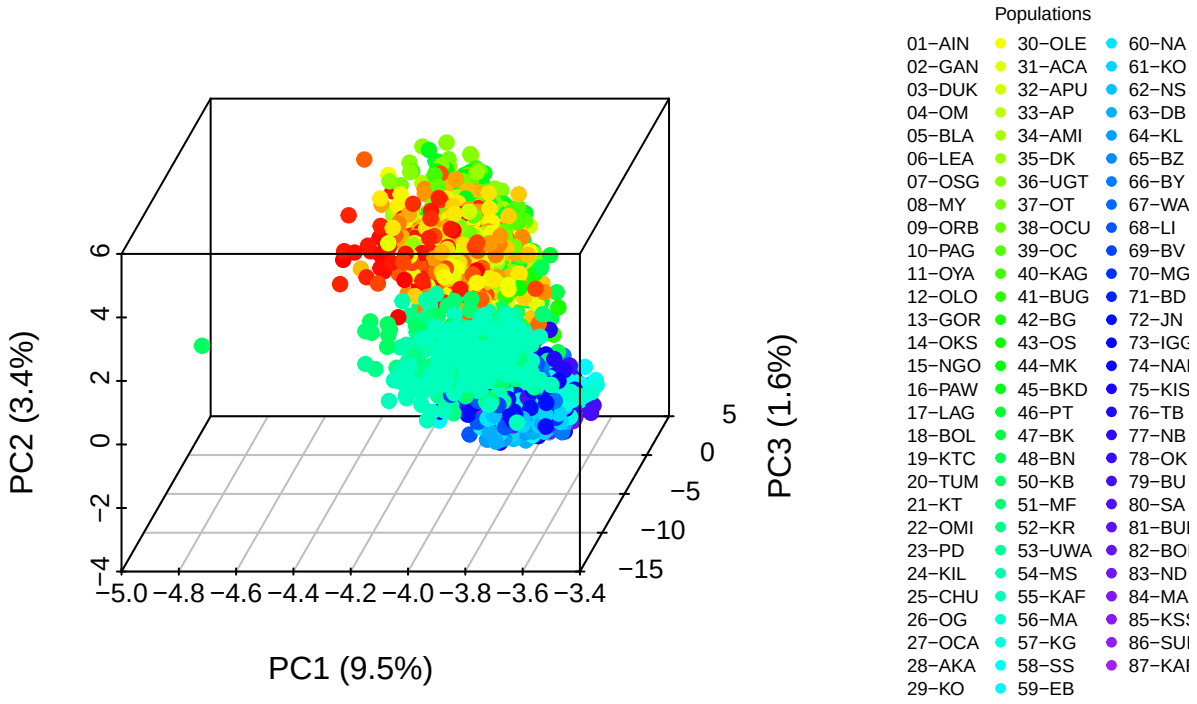
```
# replace NAs with locus means for PCA
gen_tab <- tab(Gff.genind, NA.method = "mean")

# Compute PCA on the subset
pca_result3 <- dudi.pca(gen_tab, cent = FALSE, scale = TRUE, scannf = FALSE, nf = 4)

# Extract percent variance explained for PC1 and PC2
var_explained <- round(100 * pca_result3$eig / sum(pca_result3$eig), 1)

# Generate 86 visually distinct colors
set.seed(123)  # for reproducibility
n_pops <- nlevels(pop(Gff.genind))
color_palette <- colorRampPalette(c("red", "orange", "yellow", "green", "cyan", "blue", "purple"))(n_pop

# Map population levels to colors
site_levels <- levels(pop(Gff.genind))
site_colors <- setNames(color_palette, site_levels)

# Get color for each individual
pop_colors <- site_colors[as.character(pop(Gff.genind))]
```

```r
# Layout: 2 panels (main plot + legend)
layout(matrix(c(1, 2), nrow = 1), widths = c(6, 2.5))

# PCA plot
#pdf("../figures/PCA-all-2D.pdf", width = 14, height = 10)
par(mar = c(5, 4, 4, 2))
plot(pca_result3$li[, 1:2],
     col = pop_colors,
     pch = 19,
     xlab = paste0("PC1 (", var_explained[1], "%)"),
     ylab = paste0("PC2 (", var_explained[2], "%)"))
# Legend panel
par(mar = c(1,1,1,1))  # increase space to avoid clipping
plot.new()
legend("center",
       legend = site_levels,
       col = site_colors,
       pch = 19,
       cex = 0.6,
       title = "Populations",
       ncol = 3,
       bty = "n")  # <- no border
```




```r
#dev.off()

# 3D PCA plot
#pdf("../figures/PCA-all-3D.pdf", width = 14, height = 10)
par(mar = c(5, 4, 4, 2))
s3d <- scatterplot3d(pca_result3$li[, c(1,3,2)],
```

```
                    color = pop_colors,
                    pch = 19,
                    xlab = paste0("PC1 (", var_explained[1], "%)"), # horizontal
                    ylab = paste0("PC3 (", var_explained[3], "%)"), # depth
                    zlab = paste0("PC2 (", var_explained[2], "%)"), # vertical
                    angle = 55,
                    box = TRUE)
# Legend panel
par(mar = c(1, 1, 1, 1))
plot.new()
legend("center",
       legend = site_levels,
       col = site_colors,
       pch = 19,
       cex = 0.6,
       title = "Populations",
       ncol = 3,
       bty = "n")
```




```
#dev.off()
```

Considering K=2 by Evano method

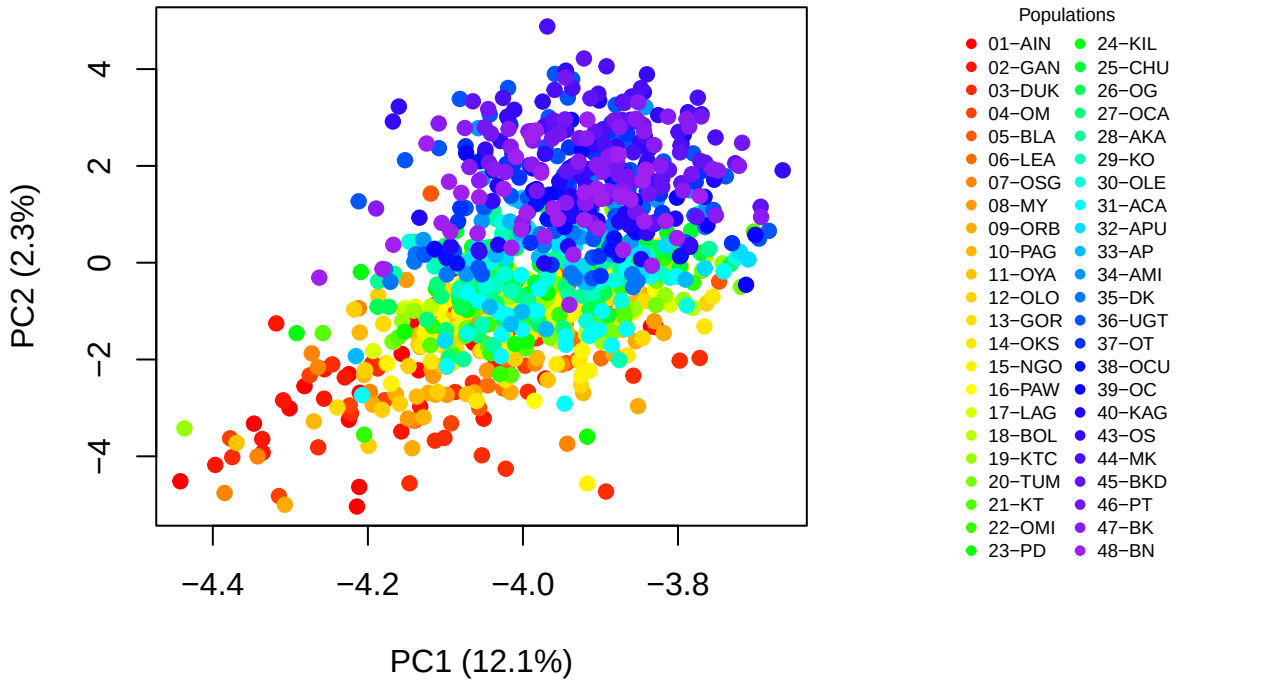
```
# Add K2 cluster combining "south" and "west" into "south"
Gff$K2 <- ifelse(Gff$SiteMajCluster %in% c("south", "west"), "south", Gff$SiteMajCluster)
```

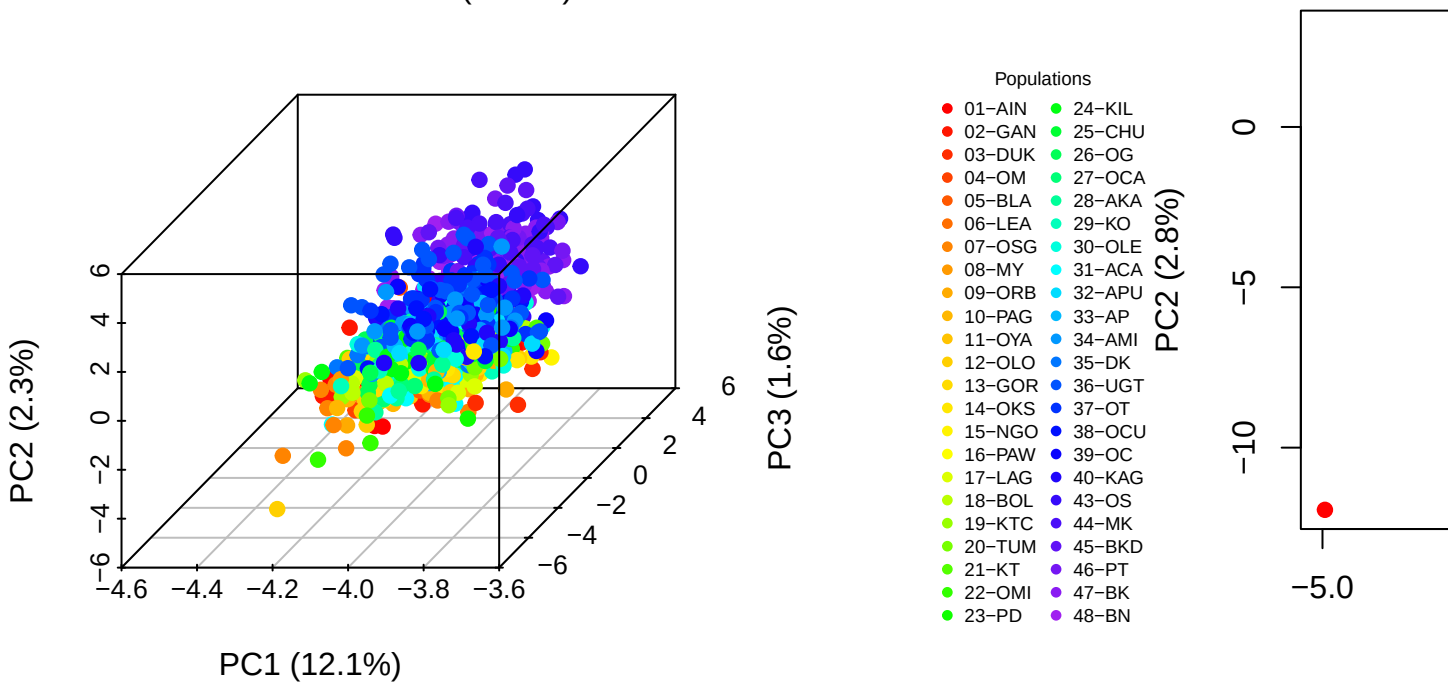
PCA for each K2 clusters separately

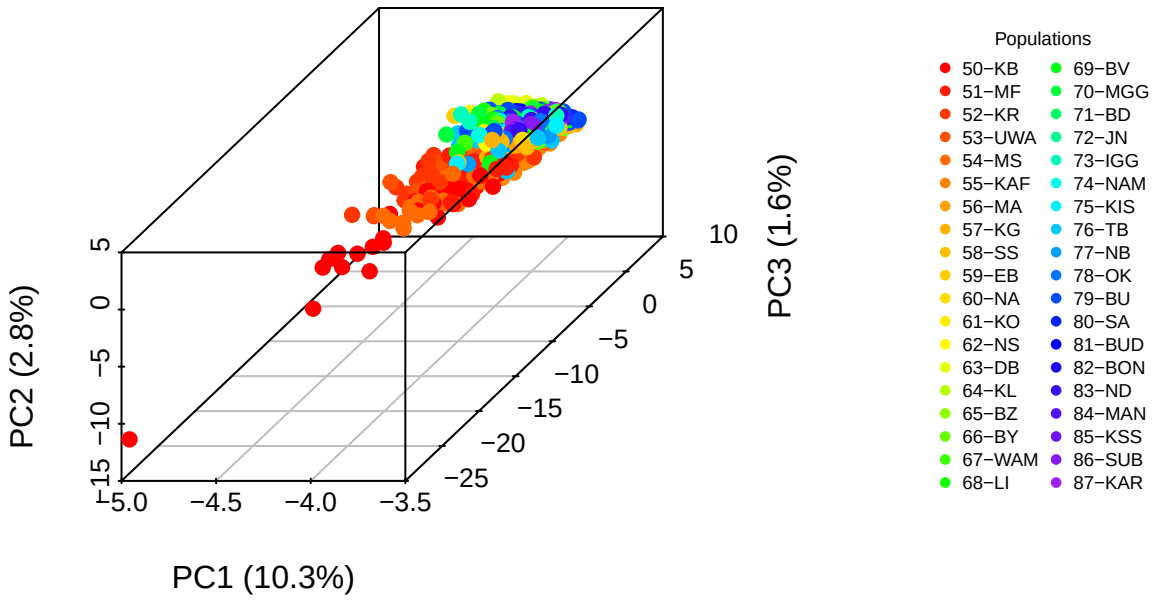
```
# PCA for each K2 cluster separately
for (region in c("north","south")) {
  genind_sub <- Gff.genind[pop(Gff.genind) %in% Gff$SiteCode[Gff$K2 == region]]
```

```r
# Replace NAs with locus means for PCA
gen_tab <- tab(genind_sub, NA.method = "mean")
pca_result <- dudi.pca(gen_tab, cent = FALSE, scale = TRUE, scannf = FALSE, nf = 4)
var_explained <- round(100 * pca_result$eig / sum(pca_result$eig), 1)
# Color palette
set.seed(123)
n_pops <- nlevels(pop(genind_sub))
palette <- colorRampPalette(c("red", "orange", "yellow", "green", "cyan", "blue", "purple"))(n_pops)
site_levels <- levels(pop(genind_sub))
site_colors <- setNames(palette, site_levels)
pop_colors <- site_colors[as.character(pop(genind_sub))]
layout(matrix(c(1, 2), nrow = 1), widths = c(6, 2.5))

# 2D PCA plot
#pdf(paste0("../figures/PCA-", region, "-2D.pdf"), width = 14, height = 10)
par(mar = c(5, 4, 4, 2))
plot(pca_result$li[, 1:2],
     col = pop_colors,
     pch = 19,
     xlab = paste0("PC1 (", var_explained[1], "%)"),
     ylab = paste0("PC2 (", var_explained[2], "%)"))
# Legend
par(mar = c(1, 1, 1, 1))
plot.new()
legend("center",
       legend = site_levels,
       col = site_colors,
       pch = 19,
       cex = 0.6,
       title = "Populations",
       ncol = 2,
       bty = "n")
#dev.off()

# 3D PCA plot
#pdf(paste0("../figures/PCA-", region, "-3D.pdf"), width = 14, height = 10)
par(mar = c(5, 1, 4, 2))
scatterplot3d(x = pca_result$li[, 1], # horizontal
              y = pca_result$li[, 3], # depth
              z = pca_result$li[, 2], # vertical
              color = pop_colors,
              pch = 19,
              xlab = paste0("PC1 (", var_explained[1], "%)"),
              ylab = paste0("PC3 (", var_explained[3], "%)"),
              zlab = paste0("PC2 (", var_explained[2], "%)"),
              angle = 55,
              box = TRUE)
par(mar = c(1, 1, 1, 1))
plot.new()
legend("center",
       legend = site_levels,
       col = site_colors,
       pch = 19,
```

```
          cex = 0.6,
          title ="Populations",
          ncol = 2,
          bty = "n")
  #dev.off()
}
```







Choosing sites for Random Forest (K=2)

Filter out any Gff$Cluster == nogroup to avoid admixed individuals, which will inflate the genetic distance within each geographic region.

Filtering for only largest/most complete genetic sample from each 5 km radius for gps within each Gff$SiteMajCluster independently so that we get best samples from both north and south of the river/Lake Kyoga dividing north and south.

```
# Create filtered copy of Gff excluding 'nogroup'
Gff_filt <- Gff[Gff$Cluster != "nogroup", ]

# Ensure Gff$SiteCode matches pop(Gff.genind)
Gff_filt$SiteCode <- as.character(Gff_filt$SiteCode)
pop_names <- as.character(pop(Gff.genind))
if (!all(pop_names == Gff_filt$SiteCode)) {
  Gff_filt <- Gff_filt[match(pop_names, Gff_filt$SiteCode), ]
  stopifnot(all(as.character(pop(Gff.genind)) == Gff_filt$SiteCode))
}
```

```
## Warning in pop_names == Gff_filt$SiteCode: longer object length is not a
## multiple of shorter object length
```

```
# Initialize storage
selected_sites <- character()
report_list <- list()

# Loop through each K2 cluster separately (north/south)
for (region in c("north","south")) {
  Gff_sub <- Gff_filt[Gff_filt$K2 == region, ]
  # Build coordinate matrix
  xy <- cbind(lon = Gff_sub$SiteLong, lat = Gff_sub$SiteLat)
  dist_mat <- distm(xy, fun = distHaversine)
  # Define adjacency for <2 km
  threshold_m <- 2000
  adj_mat <- dist_mat < threshold_m
```

```
  # Create undirected graph of close sites
  g <- graph_from_adjacency_matrix(adj_mat, mode = "undirected", diag = FALSE)
  clust_ids <- components(g)$membership
  # For each 5km cluster, pick the SiteCode with the most individuals in Gff.genind
  for (cid in unique(clust_ids)) {
    site_group <- Gff_sub$SiteCode[clust_ids == cid]
    counts <- table(pop(Gff.genind)[pop(Gff.genind) %in% site_group])
    best_site <- names(which.max(counts))
    selected_sites <- c(selected_sites, best_site)
    report_list[[best_site]] <- site_group
  }
}

# Subset Gff and Gff.genind to selected sites
Gff_rf <- Gff_filt[Gff_filt$SiteCode %in% selected_sites, ]
Gff.genind_rf <- Gff.genind[pop(Gff.genind) %in% selected_sites]

# Report selected SiteCodes by region
print(split(Gff_rf$SiteCode, Gff_rf$K2))
```

```
## $north
##   [1] "01-AIN" "01-AIN" "01-AIN" "01-AIN" "01-AIN" "01-AIN" "01-AIN" "01-AIN"
##   [9] "01-AIN" "01-AIN" "01-AIN" "01-AIN" "01-AIN" "01-AIN" "01-AIN" "01-AIN"
##  [17] "01-AIN" "01-AIN" "01-AIN" "02-GAN" "02-GAN" "02-GAN" "02-GAN" "02-GAN"
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##  [817] "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"
##  [825] "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"
##  [833] "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"  "68-LI"
##  [841] "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"
##  [849] "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"
##  [857] "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"
##  [865] "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"
##  [873] "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"
##  [881] "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"
##  [889] "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"  "69-BV"
##  [897] "69-BV"  "69-BV"  "69-BV"  "69-BV"  "70-MGG" "70-MGG" "70-MGG" "70-MGG"
##  [905] "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG"
##  [913] "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG"
##  [921] "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG"
##  [929] "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG"
##  [937] "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG"
##  [945] "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG"
##  [953] "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "70-MGG" "71-BD"
##  [961] "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"
##  [969] "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"
##  [977] "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"
##  [985] "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"  "71-BD"
##  [993] "71-BD"  "71-BD"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"
## [1001] "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"
## [1009] "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"
## [1017] "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"
## [1025] "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"  "72-JN"
```

```
## [1033] "72-JN"  "72-JN"  "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG"
## [1041] "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG"
## [1049] "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG"
## [1057] "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG"
## [1065] "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG"
## [1073] "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG"
## [1081] "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG"
## [1089] "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG" "73-IGG"
## [1097] "73-IGG" "73-IGG" "73-IGG" "74-NAM" "74-NAM" "74-NAM" "74-NAM" "74-NAM"
## [1105] "74-NAM" "74-NAM" "74-NAM" "74-NAM" "74-NAM" "74-NAM" "74-NAM" "74-NAM"
## [1113] "74-NAM" "74-NAM" "75-KIS" "75-KIS" "75-KIS" "75-KIS" "75-KIS" "75-KIS"
## [1121] "75-KIS" "75-KIS" "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"
## [1129] "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"
## [1137] "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"
## [1145] "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"  "76-TB"  "78-OK"  "78-OK"
## [1153] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1161] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1169] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1177] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1185] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1193] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1201] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1209] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1217] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1225] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1233] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1241] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1249] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1257] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"
## [1265] "78-OK"  "78-OK"  "78-OK"  "78-OK"  "78-OK"  "79-BU"  "79-BU"  "79-BU"
## [1273] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1281] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1289] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1297] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1305] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1313] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1321] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1329] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1337] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1345] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1353] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1361] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1369] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1377] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"  "79-BU"
## [1385] "79-BU"  "79-BU"  "79-BU"  "79-BU"  "81-BUD" "81-BUD" "81-BUD" "81-BUD"
## [1393] "81-BUD" "81-BUD" "81-BUD" "81-BUD" "81-BUD" "81-BUD" "81-BUD" "81-BUD"
## [1401] "81-BUD" "81-BUD" "81-BUD" "81-BUD" "81-BUD" "81-BUD" "81-BUD" "81-BUD"
## [1409] "81-BUD" "81-BUD" "81-BUD" "81-BUD" "82-BON" "82-BON" "82-BON" "82-BON"
## [1417] "82-BON" "82-BON" "82-BON" "82-BON" "82-BON" "82-BON" "82-BON" "82-BON"
## [1425] "82-BON" "82-BON" "82-BON" "82-BON" "82-BON" "82-BON" "82-BON" "82-BON"
## [1433] "82-BON" "82-BON" "82-BON" "82-BON" "83-ND"  "83-ND"  "83-ND"  "83-ND"
## [1441] "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"
## [1449] "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"
## [1457] "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"
```

```
## [1465] "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"  "83-ND"
## [1473] "83-ND"  "83-ND"  "83-ND"  "83-ND"  "84-MAN" "84-MAN" "84-MAN" "84-MAN"
## [1481] "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN"
## [1489] "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN"
## [1497] "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN"
## [1505] "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "84-MAN" "85-KSS"
## [1513] "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS"
## [1521] "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS"
## [1529] "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS"
## [1537] "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS" "85-KSS"
## [1545] "85-KSS" "85-KSS" "86-SUB" "86-SUB" "86-SUB" "86-SUB" "86-SUB" "86-SUB"
## [1553] "86-SUB" "86-SUB" "86-SUB" "86-SUB" "86-SUB" "86-SUB" "86-SUB" "86-SUB"
## [1561] "86-SUB" "86-SUB" "86-SUB" "86-SUB" "86-SUB" "86-SUB" "86-SUB" "86-SUB"
## [1569] "86-SUB" "86-SUB" "87-KAR" "87-KAR" "87-KAR" "87-KAR" "87-KAR" "87-KAR"
## [1577] "87-KAR" "87-KAR" "87-KAR" "87-KAR" "87-KAR" "87-KAR" "87-KAR" "87-KAR"
## [1585] "87-KAR" "87-KAR" "87-KAR" "87-KAR" "87-KAR" "87-KAR" "87-KAR" "87-KAR"
## [1593] "87-KAR" "87-KAR"
```

```r
# Report groupings and selected representative from each 5km cluster
report_df <- data.frame(
  selected = names(report_list),
  group = sapply(report_list, function(x) paste(sort(unique(x)), collapse = ", ")),
  row.names = NULL
)
print(report_df)
```

```
##    selected                group
## 1    01-AIN               01-AIN
## 2    02-GAN               02-GAN
## 3    03-DUK         03-DUK, 04-OM
## 4    05-BLA               05-BLA
## 5    06-LEA               06-LEA
## 6    07-OSG               07-OSG
## 7     08-MY                08-MY
## 8    09-ORB               09-ORB
## 9    10-PAG               10-PAG
## 10   11-OYA               11-OYA
## 11   12-OLO               12-OLO
## 12   14-OKS        13-GOR, 14-OKS
## 13   15-NGO               15-NGO
## 14   16-PAW               16-PAW
## 15   17-LAG               17-LAG
## 16   18-BOL               18-BOL
## 17   19-KTC               19-KTC
## 18   20-TUM               20-TUM
## 19    21-KT                21-KT
## 20   22-OMI               22-OMI
## 21    23-PD                23-PD
## 22   24-KIL               24-KIL
## 23   25-CHU               25-CHU
## 24    26-OG                26-OG
## 25   27-OCA               27-OCA
## 26   28-AKA               28-AKA
## 27   30-OLE         29-KO, 30-OLE
## 28   31-ACA               31-ACA
```

```
## 29   32-APU                32-APU
## 30    33-AP                 33-AP
## 31   36-UGT 34-AMI, 35-DK, 36-UGT
## 32    37-OT                 37-OT
## 33   38-OCU         38-OCU, 39-OC
## 34   40-KAG                40-KAG
## 35    43-OS                 43-OS
## 36    44-MK                 44-MK
## 37   45-BKD                45-BKD
## 38    46-PT                 46-PT
## 39    47-BK                 47-BK
## 40    48-BN                 48-BN
## 41    50-KB                 50-KB
## 42    51-MF                 51-MF
## 43    52-KR         52-KR, 53-UWA
## 44    54-MS                 54-MS
## 45   55-KAF                55-KAF
## 46    56-MA                 56-MA
## 47    57-KG                 57-KG
## 48    58-SS                 58-SS
## 49    59-EB                 59-EB
## 50    60-NA                 60-NA
## 51    61-KO                 61-KO
## 52    62-NS                 62-NS
## 53    63-DB                 63-DB
## 54    64-KL                 64-KL
## 55    65-BZ                 65-BZ
## 56    66-BY                 66-BY
## 57   67-WAM                67-WAM
## 58    68-LI                 68-LI
## 59    69-BV                 69-BV
## 60   70-MGG                70-MGG
## 61    71-BD                 71-BD
## 62    72-JN                 72-JN
## 63   73-IGG                73-IGG
## 64   74-NAM                74-NAM
## 65   75-KIS                75-KIS
## 66    76-TB          76-TB, 77-NB
## 67    78-OK                 78-OK
## 68    79-BU          79-BU, 80-SA
## 69   81-BUD                81-BUD
## 70   82-BON                82-BON
## 71    83-ND                 83-ND
## 72   84-MAN                84-MAN
## 73   85-KSS                85-KSS
## 74   86-SUB                86-SUB
## 75   87-KAR                87-KAR
```

Remove smaller sample size(s) of any 2-3 sites within 2 km of each other: 04-OM, 29-KO, 13-GOR, 34-AMI, 35-DK, 39-OC, 53-UWA, 77-NB, 80-SA

Brings total site count down to 75.

```
Gff.genind_rf # group size 8-236
```

```
## /// GENIND OBJECT /////////
```

```
##
##  // 2,532 individuals; 11 loci; 162 alleles; size: 1.8 Mb
##
##  // Basic content
##    @tab:  2532 x 162 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 7-28)
##    @loc.fac: locus factor for the 162 columns of @tab
##    @all.names: list of allele names for each locus
##    @ploidy: ploidy of each individual  (range: 2-2)
##    @type:  codom
##    @call: .local(x = x, i = i, j = j, drop = drop)
##
##  // Optional content
##    @pop: population of each individual (group size range: 8-236)
```

```
table(pop(Gff.genind_rf))
```

```
##
## 01-AIN 02-GAN 03-DUK 05-BLA 06-LEA 07-OSG  08-MY 09-ORB 10-PAG 11-OYA 12-OLO
##     19     20     25     10      8     20     15     20     20      9     24
## 14-OKS 15-NGO 16-PAW 17-LAG 18-BOL 19-KTC 20-TUM  21-KT 22-OMI  23-PD 24-KIL
##     26     25     13     17     25     20     20     17     15     13     21
## 25-CHU  26-OG 27-OCA 28-AKA 30-OLE 31-ACA 32-APU  33-AP 36-UGT  37-OT 38-OCU
##     25     37     20     26     24     25     29     15     64     40     25
## 40-KAG  43-OS  44-MK 45-BKD  46-PT  47-BK  48-BN  50-KB  51-MF  52-KR  54-MS
##     20     32     24     24     26     40     40     40     38     55     30
## 55-KAF  56-MA  57-KG  58-SS  59-EB  60-NA  61-KO  62-NS  63-DB  64-KL  65-BZ
##    236     33     32     40     35     33     40     16     32     40     18
##  66-BY 67-WAM  68-LI  69-BV 70-MGG  71-BD  72-JN 73-IGG 74-NAM 75-KIS  76-TB
##     62     14     46     60     59     35     40     65     15      8     28
##  78-OK  79-BU 81-BUD 82-BON  83-ND 84-MAN 85-KSS 86-SUB 87-KAR
##    119    119     24     24     40     35     35     24     24
```

Too imbalanced... - Downsample 55-KAF, 78-OK, 79-BU to N individuals (e.g., 50) - Remove sites with fewer than n=15

```
# Set thresholds
min_n <- 15
max_n <- 50

# Compute sample sizes per population
pop_sizes <- table(pop(Gff.genind_rf))

# Keep populations with n >= min_n
pops_to_keep <- names(pop_sizes[pop_sizes >= min_n])

# Indices to retain
inds_keep <- unlist(lapply(pops_to_keep, function(p) {
  inds <- which(pop(Gff.genind_rf) == p)
  if (length(inds) > max_n) {
    sample(inds, max_n)  # downsample
  } else {
    inds  # keep as-is
  }
}))
```

```
#Subset both genind and metadata
Gff.genind_rf <- Gff.genind_rf[inds_keep]
Gff_rf <- Gff_rf[inds_keep, ]

# Re-check population sizes
Gff.genind_rf
```

```
## /// GENIND OBJECT /////////
##
##  // 2,068 individuals; 11 loci; 162 alleles; size: 1.5 Mb
##
##  // Basic content
##    @tab:  2068 x 162 matrix of allele counts
##    @loc.n.all: number of alleles per locus (range: 7-28)
##    @loc.fac: locus factor for the 162 columns of @tab
##    @all.names: list of allele names for each locus
##    @ploidy: ploidy of each individual  (range: 2-2)
##    @type:  codom
##    @call: .local(x = x, i = i, j = j, drop = drop)
##
##  // Optional content
##    @pop: population of each individual (group size range: 15-50)
```

```
summary(Gff.genind_rf)
```

```
##
## // Number of individuals: 2068
## // Group sizes: 19 20 25 20 15 20 20 24 26 25 17 25 20 20 17 15 21 25 37 20 26 24 25 29 15 50 40 25 1
## // Number of alleles per locus: 26 7 16 8 10 12 9 13 28 9 18
## // Number of alleles per group: 65 65 65 60 60 59 57 54 66 57 50 59 56 56 55 60 61 62 70 59 61 58 66
## // Percentage of missing data: 2.51 %
## // Observed heterozygosity: 0.71 0.47 0.5 0.19 0.59 0.52 0.32 0.39 0.71 0.51 0.63
## // Expected heterozygosity: 0.85 0.63 0.69 0.23 0.71 0.73 0.51 0.55 0.87 0.7 0.84
```

```
table(pop(Gff.genind_rf))
```

```
##
## 01-AIN 02-GAN 03-DUK 07-OSG  08-MY 09-ORB 10-PAG 12-OLO 14-OKS 15-NGO 17-LAG
##     19     20     25     20     15     20     20     24     26     25     17
## 18-BOL 19-KTC 20-TUM  21-KT 22-OMI 24-KIL 25-CHU  26-OG 27-OCA 28-AKA 30-OLE
##     25     20     20     17     15     21     25     37     20     26     24
## 31-ACA 32-APU  33-AP 36-UGT  37-OT 38-OCU 40-KAG  43-OS  44-MK 45-BKD  46-PT
##     25     29     15     50     40     25     20     32     24     24     26
##  47-BK  48-BN  50-KB  51-MF  52-KR  54-MS 55-KAF  56-MA  57-KG  58-SS  59-EB
##     40     40     40     38     50     30     50     33     32     40     35
##  60-NA  61-KO  62-NS  63-DB  64-KL  65-BZ  66-BY  68-LI  69-BV 70-MGG  71-BD
##     33     40     16     32     40     18     50     46     50     50     35
##  72-JN 73-IGG 74-NAM  76-TB  78-OK  79-BU 81-BUD 82-BON  83-ND 84-MAN 85-KSS
##     40     50     15     28     50     50     24     24     40     35     35
## 86-SUB 87-KAR
##     24     24
```

```
length(unique(Gff_rf$SiteCode))
```

```
## [1] 68
```

Now 68 pops with group size ranges from 15-50.

Genetic diversity

Estimate allelic richness (standardized/rarefied) with pegas

```
genind_hf <- genind2hierfstat(Gff.genind_rf)

# This function rarefies allelic richness to the smallest sample size
allelic_richness <- allelic.richness(genind_hf)

# Extract mean rarefied allelic richness per population
mean_ar <- colMeans(allelic_richness$Ar, na.rm = TRUE)
mean_ar
```

```
##   01-AIN   02-GAN   03-DUK   07-OSG    08-MY   09-ORB   10-PAG   12-OLO
## 4.583157 4.260748 4.324919 4.298275 4.085127 4.124468 3.989337 3.799450
##   14-OKS   15-NGO   17-LAG   18-BOL   19-KTC   20-TUM    21-KT   22-OMI
## 4.158996 4.035677 3.671817 3.970963 4.001584 3.920779 3.954186 4.306697
##   24-KIL   25-CHU    26-OG   27-OCA   28-AKA   30-OLE   31-ACA   32-APU
## 4.053850 3.882766 4.005494 4.195913 3.955369 3.790632 4.011672 3.736532
##    33-AP   36-UGT    37-OT   38-OCU   40-KAG    43-OS    44-MK   45-BKD
## 3.819058 3.615759 3.491427 3.415275 3.498529 3.352508 2.731920 2.899221
##    46-PT    47-BK    48-BN    50-KB    51-MF    52-KR    54-MS   55-KAF
## 2.999925 3.098557 3.452087 3.821452 4.047632 4.130584 3.607622 3.158181
##    56-MA    57-KG    58-SS    59-EB    60-NA    61-KO    62-NS    63-DB
## 2.297347 2.260882 2.463630 2.910498 2.463815 2.444742 2.715141 2.465209
##    64-KL    65-BZ    66-BY    68-LI    69-BV   70-MGG    71-BD    72-JN
## 2.742293 2.972306 3.202754 3.409572 3.228719 3.301905 3.203263 2.776018
##   73-IGG   74-NAM    76-TB    78-OK    79-BU   81-BUD   82-BON    83-ND
## 3.238009 3.443531 3.045357 3.088576 3.127337 3.124326 3.088781 2.251466
##   84-MAN   85-KSS   86-SUB   87-KAR
## 2.628454 2.433326 2.108145 2.310008
```

Most diversity stats use poppr

```
# Compute per-population stats
div_stats <- poppr(Gff.genind_rf)

# View a summary table
div_stats
```

```
##       Pop    N  MLG eMLG       SE    H      G lambda   E.5  Hexp       Ia
## 1  01-AIN   19   19 15.0 1.33e-07 2.94   19.0  0.947 1.000 0.721  0.04162
## 2  02-GAN   20   20 15.0 1.50e-07 3.00   20.0  0.950 1.000 0.695 -0.02912
## 3  03-DUK   25   25 15.0 4.18e-07 3.22   25.0  0.960 1.000 0.687  0.01829
## 4  07-OSG   20   20 15.0 1.50e-07 3.00   20.0  0.950 1.000 0.685 -0.28441
## 5   08-MY   15   15 15.0 0.00e+00 2.71   15.0  0.933 1.000 0.638  0.00679
## 6  09-ORB   20   20 15.0 1.50e-07 3.00   20.0  0.950 1.000 0.646 -0.06216
## 7  10-PAG   20   20 15.0 1.50e-07 3.00   20.0  0.950 1.000 0.649  0.00277
## 8  12-OLO   24   24 15.0 4.63e-07 3.18   24.0  0.958 1.000 0.608  0.01274
## 9  14-OKS   26   26 15.0 2.46e-07 3.26   26.0  0.962 1.000 0.622 -0.08768
## 10 15-NGO   25   25 15.0 4.18e-07 3.22   25.0  0.960 1.000 0.645 -0.06576
## 11 17-LAG   17   17 15.0 0.00e+00 2.83   17.0  0.941 1.000 0.570  0.65876
## 12 18-BOL   25   25 15.0 4.18e-07 3.22   25.0  0.960 1.000 0.614  0.06185
## 13 19-KTC   20   20 15.0 1.50e-07 3.00   20.0  0.950 1.000 0.634  0.17083
```

```
## 14 20-TUM   20   20 15.0 1.50e-07 3.00   20.0  0.950 1.000 0.603  0.12220
## 15  21-KT   17   17 15.0 0.00e+00 2.83   17.0  0.941 1.000 0.633  0.07529
## 16 22-OMI   15   15 15.0 0.00e+00 2.71   15.0  0.933 1.000 0.632  0.31473
## 17 24-KIL   21   21 15.0 0.00e+00 3.04   21.0  0.952 1.000 0.642  0.09284
## 18 25-CHU   25   25 15.0 4.18e-07 3.22   25.0  0.960 1.000 0.599  0.09597
## 19  26-OG   37   37 15.0 9.13e-07 3.61   37.0  0.973 1.000 0.621 -0.11078
## 20 27-OCA   20   20 15.0 1.50e-07 3.00   20.0  0.950 1.000 0.666 -0.08867
## 21 28-AKA   26   26 15.0 2.46e-07 3.26   26.0  0.962 1.000 0.618  0.06542
## 22 30-OLE   24   24 15.0 4.63e-07 3.18   24.0  0.958 1.000 0.597  0.11002
## 23 31-ACA   25   25 15.0 4.18e-07 3.22   25.0  0.960 1.000 0.606  0.20404
## 24 32-APU   29   29 15.0 9.38e-07 3.37   29.0  0.966 1.000 0.601 -0.06391
## 25  33-AP   15   15 15.0 0.00e+00 2.71   15.0  0.933 1.000 0.606  0.34404
## 26 36-UGT   50   50 15.0 0.00e+00 3.91   50.0  0.980 1.000 0.585  0.08853
## 27  37-OT   40   40 15.0 1.80e-06 3.69   40.0  0.975 1.000 0.571  0.06462
## 28 38-OCU   25   25 15.0 4.18e-07 3.22   25.0  0.960 1.000 0.568 -0.05678
## 29 40-KAG   20   20 15.0 1.50e-07 3.00   20.0  0.950 1.000 0.570  0.29471
## 30  43-OS   32   32 15.0 0.00e+00 3.47   32.0  0.969 1.000 0.540  0.01320
## 31  44-MK   24   24 15.0 4.63e-07 3.18   24.0  0.958 1.000 0.431 -0.10228
## 32 45-BKD   24   24 15.0 4.63e-07 3.18   24.0  0.958 1.000 0.451 -0.06728
## 33  46-PT   26   26 15.0 2.46e-07 3.26   26.0  0.962 1.000 0.511 -0.01831
## 34  47-BK   40   40 15.0 1.80e-06 3.69   40.0  0.975 1.000 0.504  0.11078
## 35  48-BN   40   40 15.0 1.80e-06 3.69   40.0  0.975 1.000 0.578  0.28780
## 36  50-KB   40   40 15.0 1.80e-06 3.69   40.0  0.975 1.000 0.583 -0.10537
## 37  51-MF   38   38 15.0 0.00e+00 3.64   38.0  0.974 1.000 0.636 -0.01558
## 38  52-KR   50   50 15.0 0.00e+00 3.91   50.0  0.980 1.000 0.655 -0.00793
## 39  54-MS   30   30 15.0 6.09e-07 3.40   30.0  0.967 1.000 0.581  0.14182
## 40 55-KAF   50   50 15.0 0.00e+00 3.91   50.0  0.980 1.000 0.497  0.03315
## 41  56-MA   33   33 15.0 0.00e+00 3.50   33.0  0.970 1.000 0.304  0.24065
## 42  57-KG   32   32 15.0 0.00e+00 3.47   32.0  0.969 1.000 0.315 -0.00445
## 43  58-SS   40   40 15.0 1.80e-06 3.69   40.0  0.975 1.000 0.341  0.04044
## 44  59-EB   35   35 15.0 9.66e-07 3.56   35.0  0.971 1.000 0.487  0.42852
## 45  60-NA   33   33 15.0 0.00e+00 3.50   33.0  0.970 1.000 0.467  0.13936
## 46  61-KO   40   40 15.0 1.80e-06 3.69   40.0  0.975 1.000 0.383 -0.07328
## 47  62-NS   16   16 15.0 0.00e+00 2.77   16.0  0.938 1.000 0.407  0.16855
## 48  63-DB   32   32 15.0 0.00e+00 3.47   32.0  0.969 1.000 0.424 -0.04743
## 49  64-KL   40   40 15.0 1.80e-06 3.69   40.0  0.975 1.000 0.479  0.05266
## 50  65-BZ   18   18 15.0 1.05e-07 2.89   18.0  0.944 1.000 0.486  0.06400
## 51  66-BY   50   50 15.0 0.00e+00 3.91   50.0  0.980 1.000 0.509  0.09059
## 52  68-LI   46   46 15.0 0.00e+00 3.83   46.0  0.978 1.000 0.524 -0.00226
## 53  69-BV   50   50 15.0 0.00e+00 3.91   50.0  0.980 1.000 0.534 -0.02767
## 54 70-MGG   50   50 15.0 0.00e+00 3.91   50.0  0.980 1.000 0.519 -0.05398
## 55  71-BD   35   35 15.0 9.66e-07 3.56   35.0  0.971 1.000 0.511 -0.11040
## 56  72-JN   40   40 15.0 1.80e-06 3.69   40.0  0.975 1.000 0.444  0.06943
## 57 73-IGG   50   50 15.0 0.00e+00 3.91   50.0  0.980 1.000 0.528  0.04277
## 58 74-NAM   15   15 15.0 0.00e+00 2.71   15.0  0.933 1.000 0.545 -0.17373
## 59  76-TB   28   28 15.0 0.00e+00 3.33   28.0  0.964 1.000 0.512 -0.00191
## 60  78-OK   50   50 15.0 0.00e+00 3.91   50.0  0.980 1.000 0.514 -0.09053
## 61  79-BU   50   50 15.0 0.00e+00 3.91   50.0  0.980 1.000 0.502  0.11748
## 62 81-BUD   24   24 15.0 4.63e-07 3.18   24.0  0.958 1.000 0.511  0.06376
## 63 82-BON   24   24 15.0 4.63e-07 3.18   24.0  0.958 1.000 0.537  0.34967
## 64  83-ND   40   40 15.0 1.80e-06 3.69   40.0  0.975 1.000 0.354  0.20419
## 65 84-MAN   35   35 15.0 9.66e-07 3.56   35.0  0.971 1.000 0.396  0.07113
## 66 85-KSS   35   35 15.0 9.66e-07 3.56   35.0  0.971 1.000 0.362 -0.05269
## 67 86-SUB   24   24 15.0 4.63e-07 3.18   24.0  0.958 1.000 0.289  0.04648
```

```
## 68 87-KAR   24   23 14.6 4.85e-01 3.12   22.2  0.955 0.977 0.375  0.03373
## 69  Total 2068 2067 15.0 7.01e-03 7.63 2066.0  1.000 1.000 0.664  0.75189
##        rbarD          File
## 1   0.004200 Gff.genind_rf
## 2  -0.002956 Gff.genind_rf
## 3   0.001847 Gff.genind_rf
## 4  -0.028581 Gff.genind_rf
## 5   0.000700 Gff.genind_rf
## 6  -0.006376 Gff.genind_rf
## 7   0.000282 Gff.genind_rf
## 8   0.001282 Gff.genind_rf
## 9  -0.008886 Gff.genind_rf
## 10 -0.006648 Gff.genind_rf
## 11  0.067972 Gff.genind_rf
## 12  0.006248 Gff.genind_rf
## 13  0.017287 Gff.genind_rf
## 14  0.012386 Gff.genind_rf
## 15  0.007744 Gff.genind_rf
## 16  0.032072 Gff.genind_rf
## 17  0.009369 Gff.genind_rf
## 18  0.009782 Gff.genind_rf
## 19 -0.011138 Gff.genind_rf
## 20 -0.009091 Gff.genind_rf
## 21  0.006631 Gff.genind_rf
## 22  0.011256 Gff.genind_rf
## 23  0.020782 Gff.genind_rf
## 24 -0.006480 Gff.genind_rf
## 25  0.035299 Gff.genind_rf
## 26  0.009062 Gff.genind_rf
## 27  0.006541 Gff.genind_rf
## 28 -0.005804 Gff.genind_rf
## 29  0.029830 Gff.genind_rf
## 30  0.001346 Gff.genind_rf
## 31 -0.010768 Gff.genind_rf
## 32 -0.007719 Gff.genind_rf
## 33 -0.001955 Gff.genind_rf
## 34  0.011300 Gff.genind_rf
## 35  0.032032 Gff.genind_rf
## 36 -0.010561 Gff.genind_rf
## 37 -0.001562 Gff.genind_rf
## 38 -0.000800 Gff.genind_rf
## 39  0.014381 Gff.genind_rf
## 40  0.003590 Gff.genind_rf
## 41  0.028274 Gff.genind_rf
## 42 -0.000513 Gff.genind_rf
## 43  0.004714 Gff.genind_rf
## 44  0.048785 Gff.genind_rf
## 45  0.015967 Gff.genind_rf
## 46 -0.008711 Gff.genind_rf
## 47  0.024362 Gff.genind_rf
## 48 -0.005514 Gff.genind_rf
## 49  0.005902 Gff.genind_rf
## 50  0.007225 Gff.genind_rf
## 51  0.009545 Gff.genind_rf
```

```
## 52 -0.000231 Gff.genind_rf
## 53 -0.002889 Gff.genind_rf
## 54 -0.006035 Gff.genind_rf
## 55 -0.012401 Gff.genind_rf
## 56  0.007418 Gff.genind_rf
## 57  0.004767 Gff.genind_rf
## 58 -0.019502 Gff.genind_rf
## 59 -0.000215 Gff.genind_rf
## 60 -0.010430 Gff.genind_rf
## 61  0.012353 Gff.genind_rf
## 62  0.008090 Gff.genind_rf
## 63  0.043995 Gff.genind_rf
## 64  0.025826 Gff.genind_rf
## 65  0.009257 Gff.genind_rf
## 66 -0.006982 Gff.genind_rf
## 67  0.007154 Gff.genind_rf
## 68  0.004515 Gff.genind_rf
## 69  0.075992 Gff.genind_rf
```

Hierfstat, separating by pop and rarefying to min samples size with 100 replications with own code:

```
# Set rarefaction parameters
# Minimum number of individuals per population to include
min_n <- 15

# Number of random resampling repetitions per population
n_reps <- 100

# Split the genind object by population
# seppop() returns a list of genind objects, one for each population
pop_list <- seppop(Gff.genind_rf)

# For each pop, repeat random sampling n_reps times
# For each replicate:
# - Randomly sample min_n individuals (with no replacement)
# - Convert to hierfstat format
# - Run basic.stats()
# - Extract and average FIS, Ho (observed heterozygosity), and He (expected heterozygosity)
results <- lapply(names(pop_list), function(pname) {
  g <- pop_list[[pname]]
  # Skip populations with fewer than min_n individuals
  if (nInd(g) < min_n) return(NULL)
  # Repeat rarefied sampling n_reps times
  replicate(n_reps, {
    # Randomly sample min_n individuals
    inds <- sample(1:nInd(g), min_n)
    g_sub <- g[inds, ]
    # Convert to hierfstat format and compute basic stats
    hf <- genind2hierfstat(g_sub)
    bs <- basic.stats(hf)
    # Return vector of average values across loci
    c(
      FIS = mean(bs$Fis, na.rm = TRUE),
      Ho  = mean(bs$Ho, na.rm = TRUE),
      He  = mean(bs$Hs, na.rm = TRUE)
```

```
    )
  }) |>
    t() |>                  # transpose so each row = one replicate
    as.data.frame()         # convert to data frame for easy averaging
})

# Average results across replicates for each population
# Keep only populations where stats were successfully computed
names(results) <- names(pop_list)[sapply(results, is.data.frame)]

# Compute mean FIS, Ho, He across replicates
summary_stats <- do.call(rbind, lapply(results, function(df) {
  colMeans(df, na.rm = TRUE)
}))

# Format as a final summary table
summary_df <- data.frame(
  Population = rownames(summary_stats),
  round(summary_stats, 3),  # round to 3 decimal places
  row.names = NULL
)
```

Compile results from the three code chunks into one data frame

```
# Convert allelic richness (mean_ar) to a data frame
ar_df <- data.frame(
  Population = names(mean_ar),
  Allelic_Richness = as.numeric(mean_ar),
  row.names = NULL
)

# Convert poppr results (div_stats) to a data frame
#    (assumes div_stats has rownames corresponding to populations)
div_stats_df <- data.frame(
  Population = div_stats$Pop,
  div_stats[ , setdiff(names(div_stats), "Pop") ],
  row.names = NULL
)

# Merge all three data frames by "Population"
#    Use Reduce() for a sequential full join
summary_df <- Reduce(function(x, y) {
  merge(x, y, by = "Population", all = TRUE)
}, list(ar_df, div_stats_df, summary_df))

# Save the combined summary to a CSV file
#write.csv(summary_df, "../input/summary_df.csv", row.names = FALSE)

# Print the final table
print(summary_df)
```

```
##    Population Allelic_Richness    N  MLG     eMLG           SE        H
## 1      01-AIN         4.583157   19   19 15.00000 1.332800e-07 2.944439
## 2      02-GAN         4.260748   20   20 15.00000 1.504944e-07 2.995732
```

```
## 3      03-DUK        4.324919   25   25 15.00000 4.182955e-07 3.218876
## 4      07-OSG        4.298275   20   20 15.00000 1.504944e-07 2.995732
## 5       08-MY        4.085127   15   15 15.00000 0.000000e+00 2.708050
## 6      09-ORB        4.124468   20   20 15.00000 1.504944e-07 2.995732
## 7      10-PAG        3.989337   20   20 15.00000 1.504944e-07 2.995732
## 8      12-OLO        3.799450   24   24 15.00000 4.626565e-07 3.178054
## 9      14-OKS        4.158996   26   26 15.00000 2.457562e-07 3.258097
## 10     15-NGO        4.035677   25   25 15.00000 4.182955e-07 3.218876
## 11     17-LAG        3.671817   17   17 15.00000 0.000000e+00 2.833213
## 12     18-BOL        3.970963   25   25 15.00000 4.182955e-07 3.218876
## 13     19-KTC        4.001584   20   20 15.00000 1.504944e-07 2.995732
## 14     20-TUM        3.920779   20   20 15.00000 1.504944e-07 2.995732
## 15      21-KT        3.954186   17   17 15.00000 0.000000e+00 2.833213
## 16     22-OMI        4.306697   15   15 15.00000 0.000000e+00 2.708050
## 17     24-KIL        4.053850   21   21 15.00000 0.000000e+00 3.044522
## 18     25-CHU        3.882766   25   25 15.00000 4.182955e-07 3.218876
## 19      26-OG        4.005494   37   37 15.00000 9.127493e-07 3.610918
## 20     27-OCA        4.195913   20   20 15.00000 1.504944e-07 2.995732
## 21     28-AKA        3.955369   26   26 15.00000 2.457562e-07 3.258097
## 22     30-OLE        3.790632   24   24 15.00000 4.626565e-07 3.178054
## 23     31-ACA        4.011672   25   25 15.00000 4.182955e-07 3.218876
## 24     32-APU        3.736532   29   29 15.00000 9.377082e-07 3.367296
## 25      33-AP        3.819058   15   15 15.00000 0.000000e+00 2.708050
## 26     36-UGT        3.615759   50   50 15.00000 0.000000e+00 3.912023
## 27      37-OT        3.491427   40   40 15.00000 1.803964e-06 3.688879
## 28     38-OCU        3.415275   25   25 15.00000 4.182955e-07 3.218876
## 29     40-KAG        3.498529   20   20 15.00000 1.504944e-07 2.995732
## 30      43-OS        3.352508   32   32 15.00000 0.000000e+00 3.465736
## 31      44-MK        2.731920   24   24 15.00000 4.626565e-07 3.178054
## 32     45-BKD        2.899221   24   24 15.00000 4.626565e-07 3.178054
## 33      46-PT        2.999925   26   26 15.00000 2.457562e-07 3.258097
## 34      47-BK        3.098557   40   40 15.00000 1.803964e-06 3.688879
## 35      48-BN        3.452087   40   40 15.00000 1.803964e-06 3.688879
## 36      50-KB        3.821452   40   40 15.00000 1.803964e-06 3.688879
## 37      51-MF        4.047632   38   38 15.00000 0.000000e+00 3.637586
## 38      52-KR        4.130584   50   50 15.00000 0.000000e+00 3.912023
## 39      54-MS        3.607622   30   30 15.00000 6.085806e-07 3.401197
## 40     55-KAF        3.158181   50   50 15.00000 0.000000e+00 3.912023
## 41      56-MA        2.297347   33   33 15.00000 0.000000e+00 3.496508
## 42      57-KG        2.260882   32   32 15.00000 0.000000e+00 3.465736
## 43      58-SS        2.463630   40   40 15.00000 1.803964e-06 3.688879
## 44      59-EB        2.910498   35   35 15.00000 9.657056e-07 3.555348
## 45      60-NA        2.463815   33   33 15.00000 0.000000e+00 3.496508
## 46      61-KO        2.444742   40   40 15.00000 1.803964e-06 3.688879
## 47      62-NS        2.715141   16   16 15.00000 0.000000e+00 2.772589
## 48      63-DB        2.465209   32   32 15.00000 0.000000e+00 3.465736
## 49      64-KL        2.742293   40   40 15.00000 1.803964e-06 3.688879
## 50      65-BZ        2.972306   18   18 15.00000 1.053671e-07 2.890372
## 51      66-BY        3.202754   50   50 15.00000 0.000000e+00 3.912023
## 52      68-LI        3.409572   46   46 15.00000 0.000000e+00 3.828641
## 53      69-BV        3.228719   50   50 15.00000 0.000000e+00 3.912023
## 54     70-MGG        3.301905   50   50 15.00000 0.000000e+00 3.912023
## 55      71-BD        3.203263   35   35 15.00000 9.657056e-07 3.555348
## 56      72-JN        2.776018   40   40 15.00000 1.803964e-06 3.688879
```

```
## 57     73-IGG         3.238009   50   50 15.00000 0.000000e+00 3.912023
## 58     74-NAM         3.443531   15   15 15.00000 0.000000e+00 2.708050
## 59      76-TB         3.045357   28   28 15.00000 0.000000e+00 3.332205
## 60      78-OK         3.088576   50   50 15.00000 0.000000e+00 3.912023
## 61      79-BU         3.127337   50   50 15.00000 0.000000e+00 3.912023
## 62     81-BUD         3.124326   24   24 15.00000 4.626565e-07 3.178054
## 63     82-BON         3.088781   24   24 15.00000 4.626565e-07 3.178054
## 64      83-ND         2.251466   40   40 15.00000 1.803964e-06 3.688879
## 65     84-MAN         2.628454   35   35 15.00000 9.657056e-07 3.555348
## 66     85-KSS         2.433326   35   35 15.00000 9.657056e-07 3.555348
## 67     86-SUB         2.108145   24   24 15.00000 4.626565e-07 3.178054
## 68     87-KAR         2.310008   24   23 14.61957 4.854937e-01 3.120292
## 69      Total               NA 2068 2067 14.99995 7.011983e-03 7.633667
##             G    lambda       E.5      Hexp           Ia          rbarD
## 1    19.00000 0.9473684 1.0000000 0.7208808  0.041616591  0.0042002802
## 2    20.00000 0.9500000 1.0000000 0.6948870 -0.029117292 -0.0029556143
## 3    25.00000 0.9600000 1.0000000 0.6866765  0.018290805  0.0018470585
## 4    20.00000 0.9500000 1.0000000 0.6853279 -0.284413207 -0.0285812567
## 5    15.00000 0.9333333 1.0000000 0.6384535  0.006791379  0.0006995413
## 6    20.00000 0.9500000 1.0000000 0.6458834 -0.062158096 -0.0063755282
## 7    20.00000 0.9500000 1.0000000 0.6489510  0.002766823  0.0002824040
## 8    24.00000 0.9583333 1.0000000 0.6077979  0.012742568  0.0012822709
## 9    26.00000 0.9615385 1.0000000 0.6217202 -0.087681707 -0.0088864910
## 10   25.00000 0.9600000 1.0000000 0.6452916 -0.065758420 -0.0066475919
## 11   17.00000 0.9411765 1.0000000 0.5699336  0.658758970  0.0679723412
## 12   25.00000 0.9600000 1.0000000 0.6142299  0.061851960  0.0062484109
## 13   20.00000 0.9500000 1.0000000 0.6344010  0.170825520  0.0172873245
## 14   20.00000 0.9500000 1.0000000 0.6033110  0.122202089  0.0123856772
## 15   17.00000 0.9411765 1.0000000 0.6328068  0.075291161  0.0077435310
## 16   15.00000 0.9333333 1.0000000 0.6319086  0.314726418  0.0320724946
## 17   21.00000 0.9523810 1.0000000 0.6418541  0.092842924  0.0093691731
## 18   25.00000 0.9600000 1.0000000 0.5991458  0.095967779  0.0097815036
## 19   37.00000 0.9729730 1.0000000 0.6206347 -0.110778501 -0.0111377650
## 20   20.00000 0.9500000 1.0000000 0.6660260 -0.088671148 -0.0090910872
## 21   26.00000 0.9615385 1.0000000 0.6180584  0.065423613  0.0066307659
## 22   24.00000 0.9583333 1.0000000 0.5974624  0.110023484  0.0112558835
## 23   25.00000 0.9600000 1.0000000 0.6062798  0.204039654  0.0207819184
## 24   29.00000 0.9655172 1.0000000 0.6007143 -0.063913591 -0.0064798638
## 25   15.00000 0.9333333 1.0000000 0.6058752  0.344038061  0.0352989160
## 26   50.00000 0.9800000 1.0000000 0.5845777  0.088533996  0.0090617988
## 27   40.00000 0.9750000 1.0000000 0.5707955  0.064618140  0.0065406477
## 28   25.00000 0.9600000 1.0000000 0.5679406 -0.056782035 -0.0058040628
## 29   20.00000 0.9500000 1.0000000 0.5702670  0.294709961  0.0298300587
## 30   32.00000 0.9687500 1.0000000 0.5400839  0.013204821  0.0013463203
## 31   24.00000 0.9583333 1.0000000 0.4307979 -0.102279586 -0.0107682308
## 32   24.00000 0.9583333 1.0000000 0.4514068 -0.067275704 -0.0077192455
## 33   26.00000 0.9615385 1.0000000 0.5114544 -0.018314825 -0.0019553909
## 34   40.00000 0.9750000 1.0000000 0.5043689  0.110779966  0.0113004959
## 35   40.00000 0.9750000 1.0000000 0.5778481  0.287803253  0.0320317006
## 36   40.00000 0.9750000 1.0000000 0.5830840 -0.105366399 -0.0105605272
## 37   38.00000 0.9736842 1.0000000 0.6357320 -0.015579471 -0.0015622365
## 38   50.00000 0.9800000 1.0000000 0.6545189 -0.007927994 -0.0008004312
## 39   30.00000 0.9666667 1.0000000 0.5809571  0.141824546  0.0143805742
## 40   50.00000 0.9800000 1.0000000 0.4967772  0.033145778  0.0035901824
```

```
## 41   33.00000 0.9696970 1.0000000 0.3043507  0.240653955  0.0282741339
## 42   32.00000 0.9687500 1.0000000 0.3154762 -0.004454101 -0.0005129762
## 43   40.00000 0.9750000 1.0000000 0.3409243  0.040438533  0.0047139646
## 44   35.00000 0.9714286 1.0000000 0.4873703  0.428520444  0.0487853405
## 45   33.00000 0.9696970 1.0000000 0.4669210  0.139357083  0.0159667239
## 46   40.00000 0.9750000 1.0000000 0.3827947 -0.073278338 -0.0087109335
## 47   16.00000 0.9375000 1.0000000 0.4065526  0.168554828  0.0243624995
## 48   32.00000 0.9687500 1.0000000 0.4241162 -0.047432175 -0.0055144322
## 49   40.00000 0.9750000 1.0000000 0.4789701  0.052661988  0.0059018944
## 50   18.00000 0.9444444 1.0000000 0.4859334  0.063996759  0.0072245000
## 51   50.00000 0.9800000 1.0000000 0.5089285  0.090590732  0.0095454847
## 52   46.00000 0.9782609 1.0000000 0.5244826 -0.002258499 -0.0002305685
## 53   50.00000 0.9800000 1.0000000 0.5339183 -0.027672346 -0.0028892877
## 54   50.00000 0.9800000 1.0000000 0.5193046 -0.053979384 -0.0060350933
## 55   35.00000 0.9714286 1.0000000 0.5112005 -0.110396274 -0.0124005298
## 56   40.00000 0.9750000 1.0000000 0.4444438  0.069428837  0.0074180714
## 57   50.00000 0.9800000 1.0000000 0.5277465  0.042767647  0.0047670215
## 58   15.00000 0.9333333 1.0000000 0.5446010 -0.173733618 -0.0195023913
## 59   28.00000 0.9642857 1.0000000 0.5119924 -0.001908985 -0.0002147069
## 60   50.00000 0.9800000 1.0000000 0.5141629 -0.090534755 -0.0104298411
## 61   50.00000 0.9800000 1.0000000 0.5019481  0.117483054  0.0123526285
## 62   24.00000 0.9583333 1.0000000 0.5110280  0.063755017  0.0080899078
## 63   24.00000 0.9583333 1.0000000 0.5366809  0.349669262  0.0439954174
## 64   40.00000 0.9750000 1.0000000 0.3537687  0.204194236  0.0258259005
## 65   35.00000 0.9714286 1.0000000 0.3963515  0.071133644  0.0092574112
## 66   35.00000 0.9714286 1.0000000 0.3622693 -0.052692176 -0.0069824976
## 67   24.00000 0.9583333 1.0000000 0.2894485  0.046481546  0.0071543028
## 68   22.15385 0.9548611 0.9769483 0.3752051  0.033730644  0.0045145283
## 69 2066.00193 0.9995160 0.9997036 0.6643510  0.751892157  0.0759915177
##             File    FIS    Ho    He
## 1  Gff.genind_rf  0.041 0.691 0.721
## 2  Gff.genind_rf  0.189 0.570 0.698
## 3  Gff.genind_rf  0.155 0.575 0.688
## 4  Gff.genind_rf  0.137 0.585 0.689
## 5  Gff.genind_rf  0.082 0.576 0.641
## 6  Gff.genind_rf  0.195 0.532 0.649
## 7  Gff.genind_rf  0.160 0.550 0.651
## 8  Gff.genind_rf  0.036 0.587 0.608
## 9  Gff.genind_rf  0.074 0.562 0.620
## 10 Gff.genind_rf  0.044 0.620 0.645
## 11 Gff.genind_rf  0.138 0.488 0.569
## 12 Gff.genind_rf  0.046 0.582 0.614
## 13 Gff.genind_rf  0.072 0.575 0.636
## 14 Gff.genind_rf  0.043 0.564 0.606
## 15 Gff.genind_rf  0.126 0.557 0.637
## 16 Gff.genind_rf  0.155 0.560 0.635
## 17 Gff.genind_rf  0.048 0.604 0.641
## 18 Gff.genind_rf  0.003 0.593 0.601
## 19 Gff.genind_rf  0.016 0.623 0.622
## 20 Gff.genind_rf  0.014 0.641 0.667
## 21 Gff.genind_rf -0.011 0.621 0.617
## 22 Gff.genind_rf  0.027 0.577 0.597
## 23 Gff.genind_rf  0.044 0.569 0.607
## 24 Gff.genind_rf  0.068 0.556 0.603
```

```
## 25 Gff.genind_rf  0.085 0.556 0.608
## 26 Gff.genind_rf  0.082 0.536 0.584
## 27 Gff.genind_rf  0.050 0.538 0.570
## 28 Gff.genind_rf -0.003 0.555 0.569
## 29 Gff.genind_rf  0.204 0.479 0.574
## 30 Gff.genind_rf  0.066 0.514 0.547
## 31 Gff.genind_rf -0.064 0.459 0.432
## 32 Gff.genind_rf  0.068 0.417 0.453
## 33 Gff.genind_rf  0.068 0.484 0.514
## 34 Gff.genind_rf  0.087 0.458 0.503
## 35 Gff.genind_rf  0.084 0.524 0.578
## 36 Gff.genind_rf -0.011 0.591 0.581
## 37 Gff.genind_rf  0.009 0.629 0.635
## 38 Gff.genind_rf  0.096 0.593 0.657
## 39 Gff.genind_rf  0.056 0.553 0.578
## 40 Gff.genind_rf  0.081 0.464 0.495
## 41 Gff.genind_rf -0.100 0.340 0.304
## 42 Gff.genind_rf  0.009 0.309 0.314
## 43 Gff.genind_rf  0.031 0.322 0.341
## 44 Gff.genind_rf  0.024 0.469 0.488
## 45 Gff.genind_rf -0.105 0.508 0.466
## 46 Gff.genind_rf  0.023 0.375 0.385
## 47 Gff.genind_rf  0.102 0.386 0.407
## 48 Gff.genind_rf -0.027 0.440 0.424
## 49 Gff.genind_rf  0.023 0.482 0.478
## 50 Gff.genind_rf  0.074 0.457 0.487
## 51 Gff.genind_rf  0.032 0.489 0.510
## 52 Gff.genind_rf  0.002 0.529 0.525
## 53 Gff.genind_rf  0.072 0.498 0.534
## 54 Gff.genind_rf  0.014 0.511 0.520
## 55 Gff.genind_rf  0.014 0.506 0.510
## 56 Gff.genind_rf  0.024 0.433 0.443
## 57 Gff.genind_rf  0.081 0.490 0.530
## 58 Gff.genind_rf -0.026 0.559 0.544
## 59 Gff.genind_rf  0.026 0.495 0.512
## 60 Gff.genind_rf  0.012 0.507 0.512
## 61 Gff.genind_rf  0.073 0.463 0.498
## 62 Gff.genind_rf  0.066 0.485 0.511
## 63 Gff.genind_rf -0.072 0.574 0.537
## 64 Gff.genind_rf -0.012 0.361 0.354
## 65 Gff.genind_rf -0.008 0.402 0.394
## 66 Gff.genind_rf  0.053 0.339 0.360
## 67 Gff.genind_rf  0.030 0.287 0.294
## 68 Gff.genind_rf  0.296 0.260 0.377
## 69 Gff.genind_rf     NA    NA    NA
```

Cavalli-Sforza and Edwards' Distance (CSE)

Pairwise within cluster "north"

```
# Subset genind and metadata
meta_sub <- Gff_rf[Gff_rf$K2 == "north", ]
gen_sub <- Gff.genind_rf[pop(Gff.genind_rf) %in% meta_sub$SiteCode]
```

```r
# Convert to genpop (aggregates by population)
genpop_sub <- genind2genpop(gen_sub)
```

```
##
##  Converting data from a genind to a genpop object...
##
## ...done.
```

```r
# Compute Cavalli-Sforza and Edwards' distance
cse_dist <- dist.genpop(genpop_sub, method = 2)  # method = 2 (CSE distance

# Display as a matrix
cse_north <- as.matrix(cse_dist)
```

Pairwise within cluster "south"

```r
# Subset genind and metadata
meta_sub <- Gff_rf[Gff_rf$K2 == "south", ]
gen_sub <- Gff.genind_rf[pop(Gff.genind_rf) %in% meta_sub$SiteCode]

# Convert to genpop (aggregates by population)
genpop_sub <- genind2genpop(gen_sub)
```

```
##
##  Converting data from a genind to a genpop object...
##
## ...done.
```

```r
# Compute Cavalli-Sforza and Edwards' distance
cse_dist <- dist.genpop(genpop_sub, method = 2)  # method = 2 (CSE distance)

# Display as a matrix
cse_south <- as.matrix(cse_dist)
```

Combine CSE output into a single dataframe for downstream analysis:

```r
# Create a metadata data frame for coordinates and cluster
meta_coords <- unique(Gff_rf[, c("SiteCode", "SiteLat", "SiteLong", "K2")])
names(meta_coords) <- c("SiteCode", "lat", "long", "cluster")

# Process the "north" CSE matrix
north_df <- as.data.frame(as.table(cse_north), stringsAsFactors = FALSE)
names(north_df) <- c("Var1", "Var2", "CSEdistance")

# Keep only one triangle (Var1 < Var2 alphabetically)
north_df <- subset(north_df, Var1 < Var2)

# Merge to get lat1, long1, and Pop1_cluster
north_df <- merge(
  north_df,
  meta_coords,
  by.x = "Var1",
  by.y = "SiteCode",
  all.x = TRUE,
  suffixes = c("", ".ignore")
)
names(north_df)[names(north_df) == "lat"]       <- "lat1"
```

```r
names(north_df)[names(north_df) == "long"]      <- "long1"
names(north_df)[names(north_df) == "cluster"]   <- "Pop1_cluster"

# Merge to get lat2, long2, and Pop2_cluster
north_df <- merge(
  north_df,
  meta_coords,
  by.x = "Var2",
  by.y = "SiteCode",
  all.x = TRUE,
  suffixes = c("", ".ignore")
)
names(north_df)[names(north_df) == "lat"]       <- "lat2"
names(north_df)[names(north_df) == "long"]      <- "long2"
names(north_df)[names(north_df) == "cluster"]   <- "Pop2_cluster"
# Select and order columns
north_df <- north_df[, c(
  "Var1", "Var2", "CSEdistance",
  "lat1", "long1", "lat2", "long2",
  "Pop1_cluster", "Pop2_cluster"
)]

# Process the "south" CSE matrix (analogous steps)
south_df <- as.data.frame(as.table(cse_south), stringsAsFactors = FALSE)
names(south_df) <- c("Var1", "Var2", "CSEdistance")
south_df <- subset(south_df, Var1 < Var2)
south_df <- merge(
  south_df,
  meta_coords,
  by.x = "Var1",
  by.y = "SiteCode",
  all.x = TRUE,
  suffixes = c("", ".ignore")
)
names(south_df)[names(south_df) == "lat"]       <- "lat1"
names(south_df)[names(south_df) == "long"]      <- "long1"
names(south_df)[names(south_df) == "cluster"]   <- "Pop1_cluster"
south_df <- merge(
  south_df,
  meta_coords,
  by.x = "Var2",
  by.y = "SiteCode",
  all.x = TRUE,
  suffixes = c("", ".ignore")
)
names(south_df)[names(south_df) == "lat"]       <- "lat2"
names(south_df)[names(south_df) == "long"]      <- "long2"
names(south_df)[names(south_df) == "cluster"]   <- "Pop2_cluster"
south_df <- south_df[, c(
  "Var1", "Var2", "CSEdistance",
  "lat1", "long1", "lat2", "long2",
  "Pop1_cluster", "Pop2_cluster"
)]
```

```r
# Combine "north" and "south" results
cse_combined <- rbind(north_df, south_df)

# Save to CSV
#write.csv(cse_combined, "../input/Gff_11loci_68sites_cse.csv", row.names = FALSE)

# View the first few rows
head(cse_combined)
```

```
##     Var1   Var2 CSEdistance   lat1   long1   lat2   long2 Pop1_cluster
## 1 01-AIN 02-GAN   0.2424627 3.3044 31.1191 3.2521 31.1231        north
## 2 01-AIN 03-DUK   0.3169412 3.3044 31.1191 3.2671 31.1352        north
## 3 02-GAN 03-DUK   0.3076377 3.2521 31.1231 3.2671 31.1352        north
## 4 03-DUK 07-OSG   0.4064882 3.2671 31.1352 3.2110 31.7253        north
## 5 02-GAN 07-OSG   0.3732382 3.2521 31.1231 3.2110 31.7253        north
## 6 01-AIN 07-OSG   0.3726589 3.3044 31.1191 3.2110 31.7253        north
##   Pop2_cluster
## 1        north
## 2        north
## 3        north
## 4        north
## 5        north
## 6        north
```

Create density plots of CSEdistance for each cluster

```r
# Capitalize or rename cluster labels ("Northern"/"Southern")
cse_combined <- cse_combined %>%
  mutate(
    Pop1_cluster = case_when(
      Pop1_cluster == "north" ~ "Northern",
      Pop1_cluster == "south" ~ "Southern",
      TRUE ~ Pop1_cluster
    ),
    Pop2_cluster = case_when(
      Pop2_cluster == "north" ~ "Northern",
      Pop2_cluster == "south" ~ "Southern",
      TRUE ~ Pop2_cluster
    )
  )
# Create two density plots (one for "Northern", one for "Southern")
cluster_names <- unique(
  cse_combined$Pop1_cluster[cse_combined$Pop1_cluster == cse_combined$Pop2_cluster]
)
density_plots <- lapply(cluster_names, function(cl) {
  df <- filter(cse_combined, Pop1_cluster == cl & Pop2_cluster == cl)
  ggplot(df, aes(x = CSEdistance)) +
    geom_density(fill = "grey80", color = "black") +
    labs(
      title = paste0(cl, " Genetic Cluster"),
      x = "CSE Distance",
      y = "Density"
    ) +
    theme_minimal() +
    theme(
```

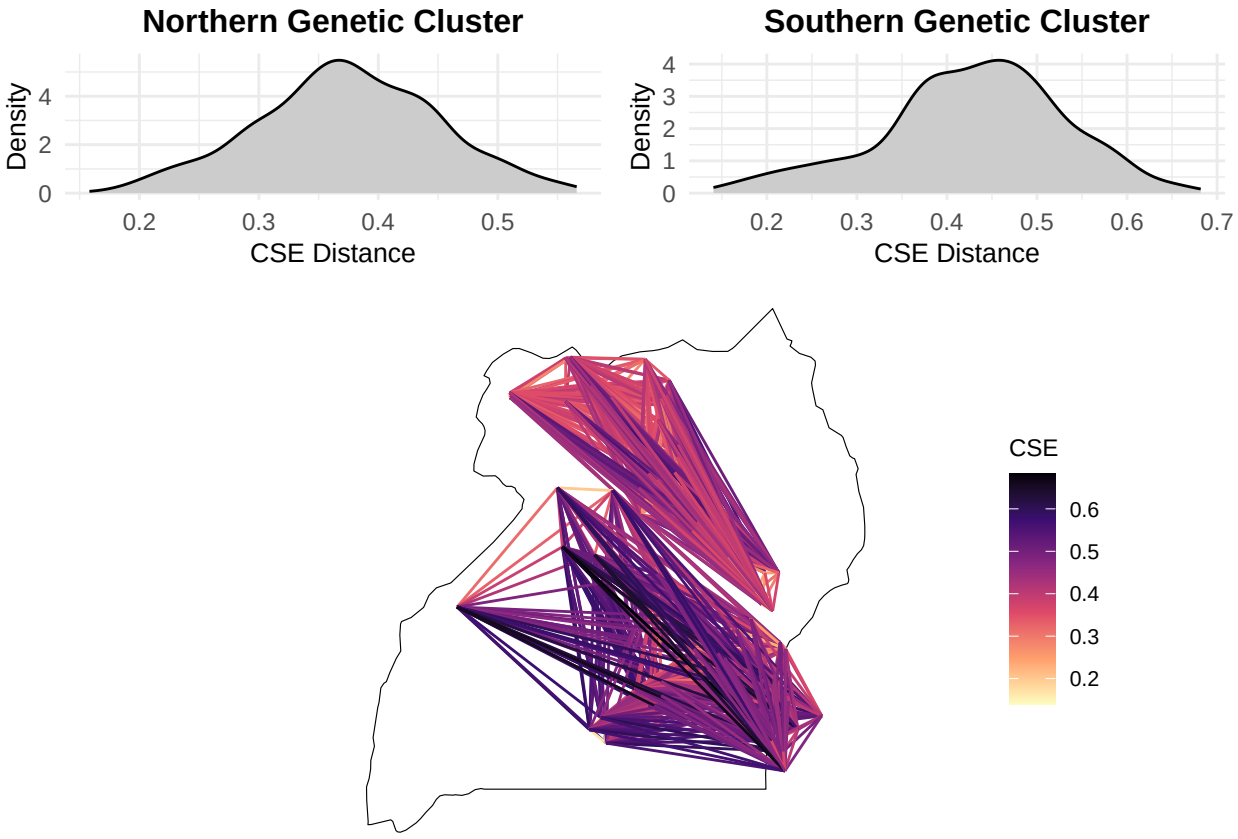
```
      plot.title = element_text(size = 12, face = "bold", hjust = 0.5),
      axis.title = element_text(size = 10),
      axis.text = element_text(size = 9)
    )
})
# Turn each row into an sf LINESTRING, colored by CSE
lines_list <- lapply(seq_len(nrow(cse_combined)), function(i) {
  coords <- matrix(
    c(
      cse_combined$long1[i], cse_combined$lat1[i],
      cse_combined$long2[i], cse_combined$lat2[i]
    ),
    ncol = 2,
    byrow = TRUE
  )
  st_linestring(coords)
})
lines_sf <- st_sf(
  geometry = st_sfc(lines_list, crs = 4326),
  CSE = cse_combined$CSEdistance
)
# 5. Load Uganda outline as an sf object
uganda <- ne_countries(scale = "medium", country = "Uganda", returnclass = "sf")

# 6. Build the map with reversed "magma" (yellow→purple) and
#    move legend to the right, vertically centered
map_plot <- ggplot() +
  geom_sf(data = uganda, fill = "white", color = "black") +
  geom_sf(
    data = lines_sf,
    aes(color = CSE),
    size = 0.6
  ) +
  scale_color_viridis_c(
    name = "CSE",
    option = "magma",   # use the "magma" palette
    direction = -1      # reverse so low = yellow, high = purple
  ) +
  theme_void() +
  theme(
    # place legend at (x=0.95, y=0.50) relative to entire panel
    legend.position = c(1.3, 0.50),
    legend.background = element_rect(fill = alpha("white", 0.6), color = NA),
    legend.title = element_text(size = 9),
    legend.text = element_text(size = 8)
  )
```

```
## Warning: A numeric ‘legend.position‘ argument in ‘theme()‘ was deprecated in ggplot2
## 3.5.0.
## i Please use the ‘legend.position.inside‘ argument of ‘theme()‘ instead.
## This warning is displayed once every 8 hours.
## Call ‘lifecycle::last_lifecycle_warnings()‘ to see where this warning was
## generated.
```

```r
# 7. Combine the two density plots (top) and the map (bottom)
top_row <- plot_grid(
  plotlist = density_plots,
  nrow = 1,
  rel_widths = rep(1, length(density_plots))
)
final_figure <- plot_grid(
  top_row,
  map_plot,
  ncol = 1,
  rel_heights = c(1, 2)
)

# Display CSE density plots
#pdf("../figures/CSE_density.pdf", width = 14, height = 10)
final_figure
```




```r
#dev.off()
```