Basics of genomics & quality control: II

1st EvoGenomics Methods Workshop

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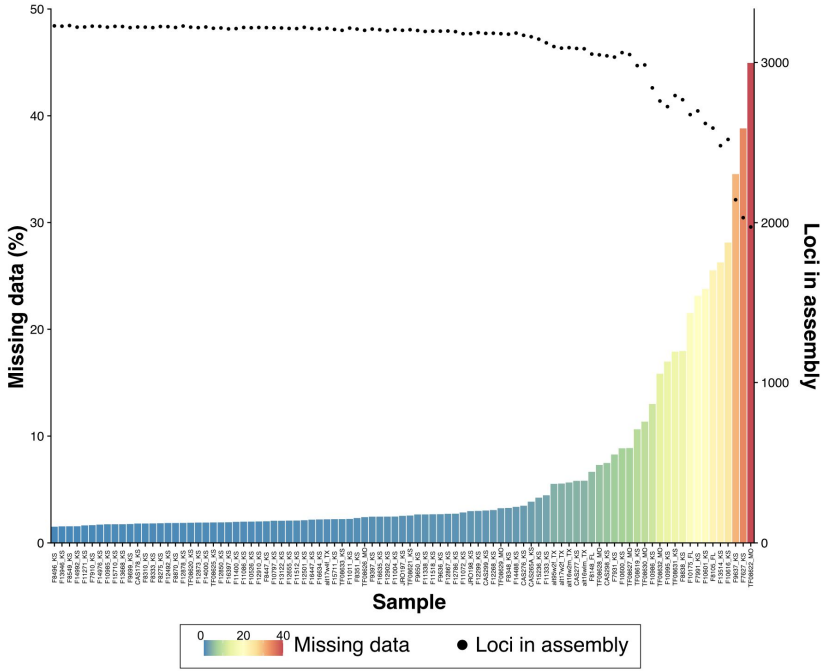
Quality control

There are several key data visualizations that can help us troubleshoot or identify potential “red flags” in our data before moving forward with analyses

Distribution of **basic summary statistics**



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Distribution of **basic summary statistics**

PCA for dimensional reduction

What types of things might correspond to different PCA groupings?

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Distribution of **basic summary statistics**

PCA for dimensional reduction

What types of things might correspond to different PCA groupings?

- Libraries
- Samples with very high amounts of missing data
- Might have real biological significance: population structure, presence of inversions, etc.

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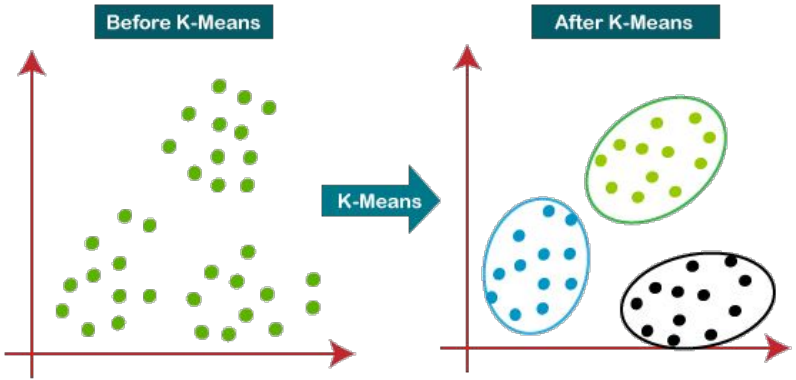
Distribution of **basic summary statistics**

PCA for dimensional reduction

Basic **clustering** or population structure analyses



https://www.javatpoint.com/k-means-clustering-algorithm-in-machine-learning

Admixture

admixture is similar to STRUCTURE, fastSTRUCTURE, etc.

Model-based clustering method, agnostic to sampling

User-specified number of clusters (*K*), method will cluster samples accordingly

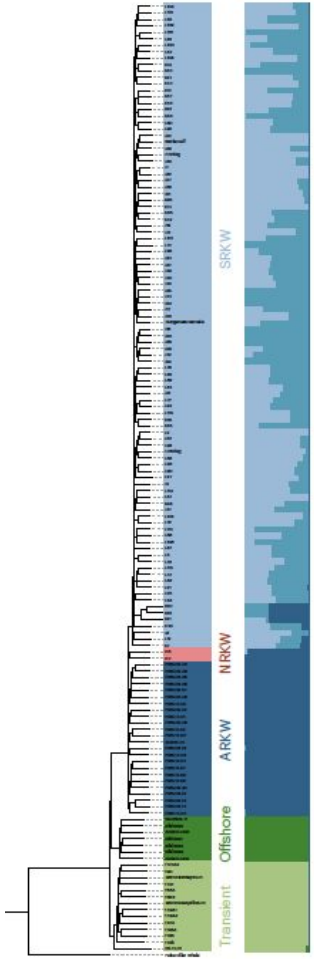


Figure from Kardos et al. (2023) https://doi.org/10.1038/s41559-023-01995-0

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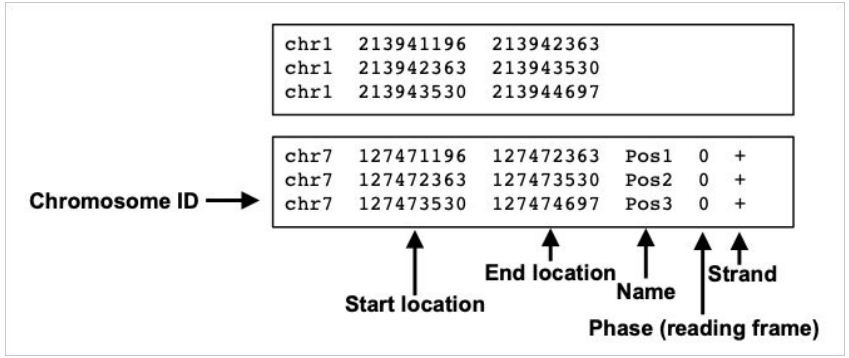
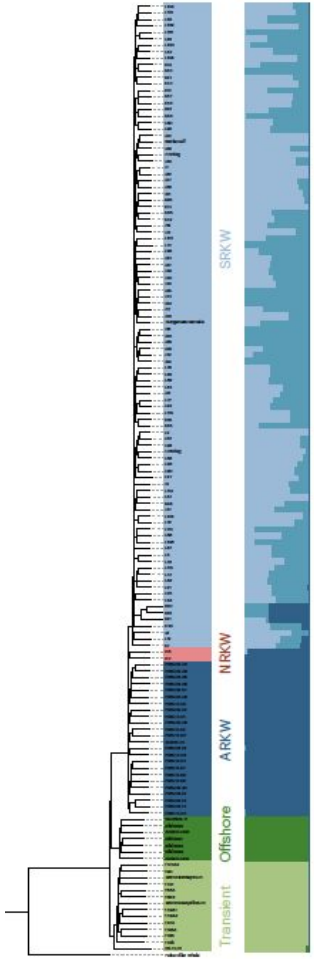
Model-based clustering method, agnostic to sampling

User-specified number of clusters (*K*), method will cluster samples accordingly

```
admixture file.bed 5
```





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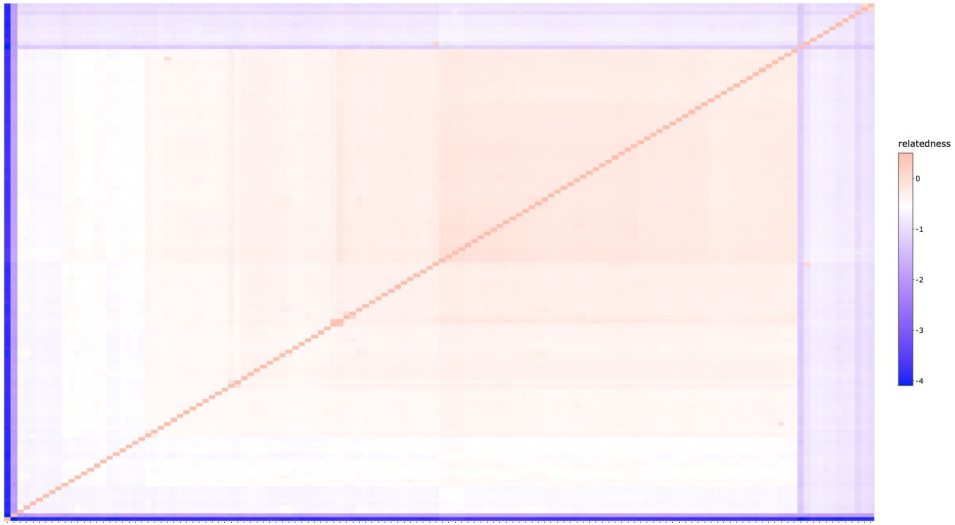
Other good QC visualizations that can be used

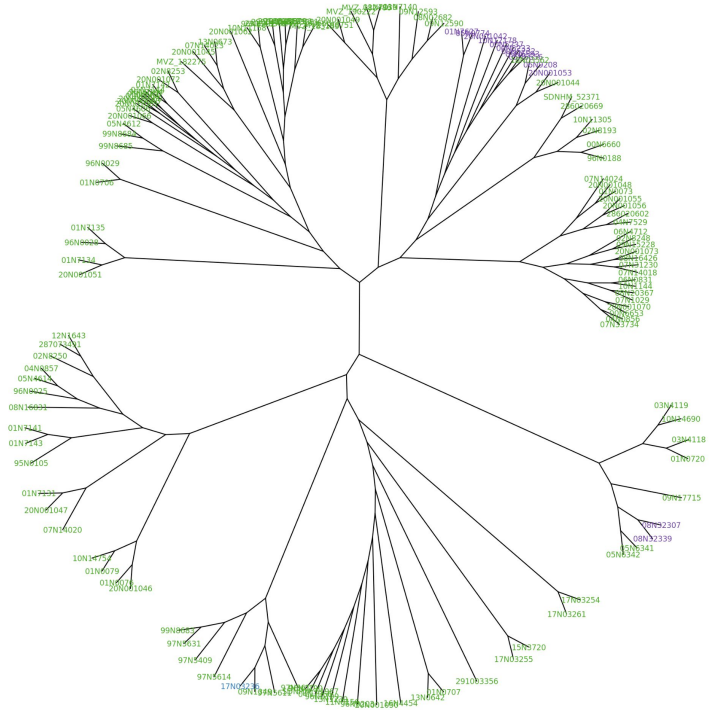
Relatedness plot




```
plink2 --vcf file.vcf --out outfile_name --make-king-square
```

https://www.cog-genomics.org/plink/2.0/distance

Neighbor-joining tree



EXERCISE 2: data visualization for quality control

Download the worksheet here:
https://github.com/eachambers/EvoGeno-Methods-Workshop/blob/main/Workshop1/Exercises/EvoGenomics_Ws1_Ex2.txt

Download the R script here:
https://github.com/eachambers/EvoGeno-Methods-Workshop/blob/main/Workshop1/Exercises/Workshop1_Exercise2.R

```
# ================================================================================
#                                    EXERCISE 2
# ================================================================================

# ================================ 1. RUNNING ADMIXTURE ================================

# Admixture takes in a bed file, which you should have created using plink. We're going to
# run it on both our LD-pruned and un-pruned datasets just to see how the results compare.
# Sometimes, chromosome names aren't supported by admixture, and this is the case with the
# Lampropeltis data. The problem is with the bim file, which is the file that accompanies
# a bed file. The first column of the bim file needs to be the chromosome code, which must
# be 'X'/'Y'/'XY'/'MT'; '0' indicates unknown. If you take a look at the bim files that were
# made by Plink in exercise 1, you'll notice that the first column has RAD tag numbers. The
# easiest way to get around this is to replace all of these RAD tag numbers with 0s. To do
# so, run the following on **BOTH** your pruned and unpruned bim files:

$ awk '{$1=0;print $0}' file.bim > file.bim.tmp # adds zeros to first column
$ mv file.bim.tmp file.bim # replaces original bim file

#       (1a) Run admixture with K=3 for both the pruned and unpruned bed files.
#               *** YOUR ANSWER HERE ***
```

```
# === 2. DATA VISUALIZATION OF SUMMARY STATISTICS ===

# Be sure to set your working directory (wherever you saved your output files!)
# and load relevant libraries. The following questions can be answered (and
# visualized using only the tidyverse package).

# 2a ----------------------------------------------------------------------

#   Using the missing data stats you generated in step 1.3a, plot the data however you
#   would like. What is the average proportion of missing data across the entire
#   dataset?
# TODO: **YOUR ANSWER HERE**


# 2b ----------------------------------------------------------------------

#   Using the depth statistics you generated in step 1.3b, plot the data in whichever
#   way you would like. What is the average read depth across the entire dataset?
# TODO: **YOUR ANSWER HERE**
```

Further processing for downstream population genetics analyses

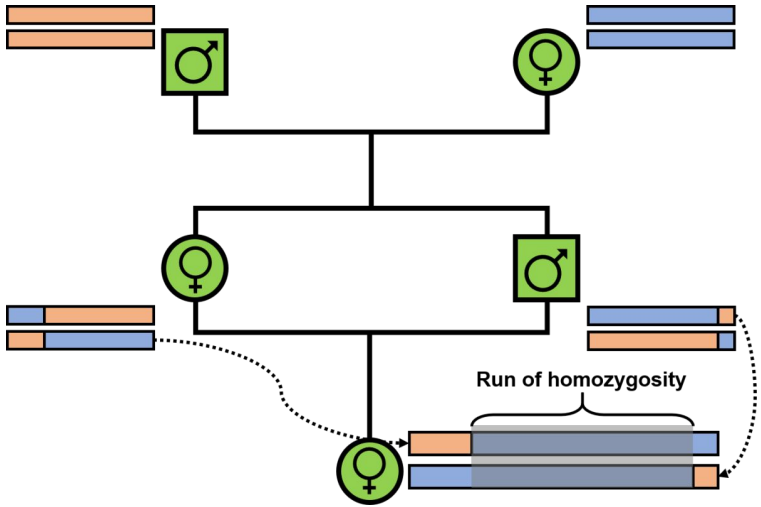
- Genetic distance matrix (plink)

```
plink --vcf file.vcf --out prefix_name --distance square
```

Further processing for downstream population genetics analyses

- Genetic distance matrix (plink)
- Runs of homozygosity (bcftools or plink)

```
bcftools roh --threads 10 -G30 --AF-dflt 0.4 -O z -o output.roh file.vcf
```



https://theg-cat.com/2022/06/02/runaway-inbreeding-how-runs-of-homozygosity-impact-conservation-management/

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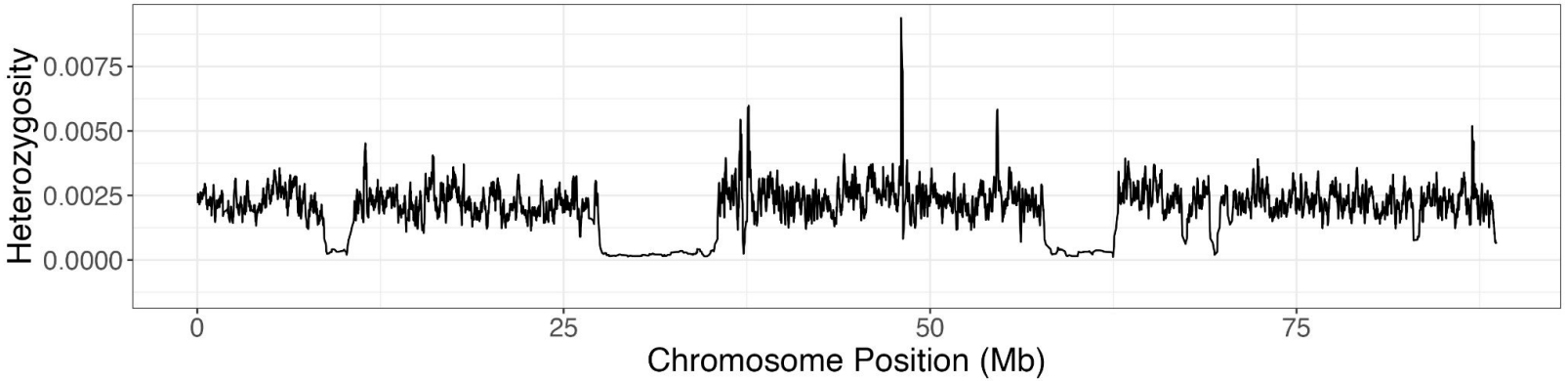

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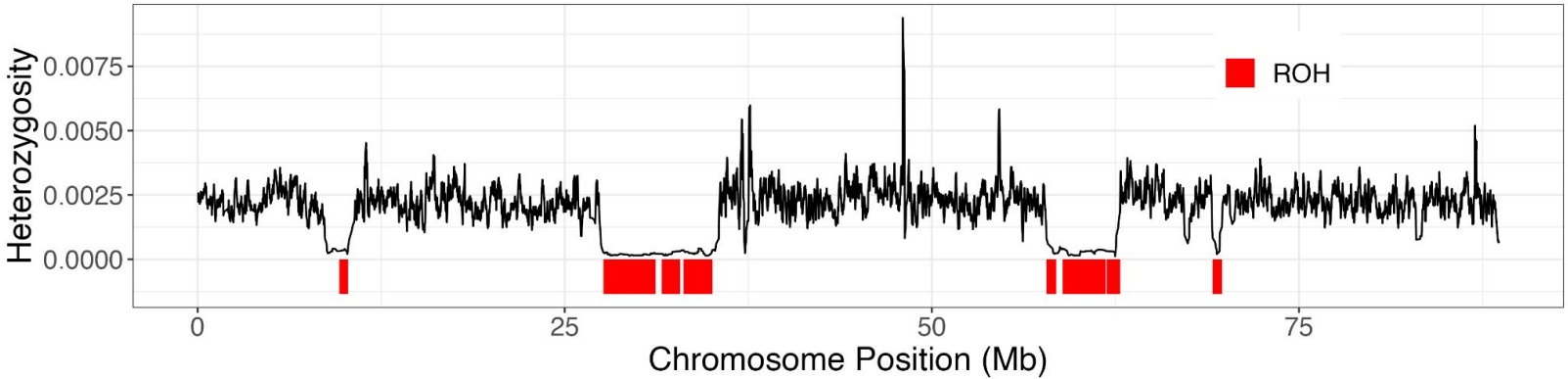

Further processing for downstream population genetics analyses

- Genetic distance matrix (plink)
- Runs of homozygosity (bcftools or plink)
- Imputation of missing genotypes