wilding_urbano_outline_results

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1. Samples

- **AG1000g phase 2: 1142 genomes and 16 populations**

GNcol (4), GQgam (9), GHgam (12), FRgam (24), GNgam (40), KE (48), GHcol (55), GM (65), GAgam (69), Icol (71), BFcol (75), AOcol (78), GW (91), BFgam (92), UGgam (112), CMgam (297)

- **Wilding genomes: 96 genomes and 3 populations**

32 LVBdom (Libreville, Gabon domestic)
32 LPdom (La lope, Gabon domestic)
32 LPfor (La lope, Gabon forest)

- **Urbano genomes: 88 genomes and 3 populations**

10 BZV (Brazzaville, Congo)
36 DLA (Douala, Cameroon)
42 LBV (Libreville, Gabon)

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2. Dataset creation: reads mapping, SNP calling and filtering

2.1 Reads mapping

2.1.1 Bash script to perform: FASTQC, cutadapt, bwa mem, gatk realigner, bam report

- FASTQC report:

-> Wilding fastqc report: *not available because we've got mapped reads.* -> Urbano fastqc report: fastqc report git hub link.

- cutadapt:

```
cutadapt -a AGATCGGAAGAGCACACGTCTGAA -A AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT  -q 3
```

- bwa mem:

```
header=$(zcat $sampleId.R1.fastq.gz | head -n 1)
id=$(echo $header | head -n 1 | cut -f 1-4 -d":" | sed 's/@//' | sed 's/:/_/g')
sm=$(echo $header | head -n 1 | grep -Eo "[ATGCN]+$")
echo "Read Group @RG\tID:$id\tSM:$id"_"$sm\tLB:$id"_"$sm\tPL:ILLUMINA"

bwa mem -t 1 Anopheles_gambiae.AgamP4.dna.chr.fna $sampleId.R1.fastq.gz $sampleId.R2.fastq.gz -R $(echo
m\tPL:ILLUMINA") | samtools view -F 4 -b - | samtools sort - -o $sampleId.map.sort.bam
```

- gatk realigner:

```
java -jar ~/bioInf/bin/GenomeAnalysisTK-3.8-0-ge9d806836/GenomeAnalysisTK.jar -T RealignerTargetCreator
 -I $sampleId.map.sort.bam -o $sampleId.realignertargetcreator.intervals

java -Xmx8G -Djava.io.tmpdir=/tmp -jar ~/bioInf/bin/GenomeAnalysisTK-3.8-0-ge9d806836/GenomeAnalysisTK.j
ae.AgamP4.dna.chr.fna -targetIntervals $sampleId.realignertargetcreator.intervals -I $sampleId.map.sort
```

- bam file report (qualimap):

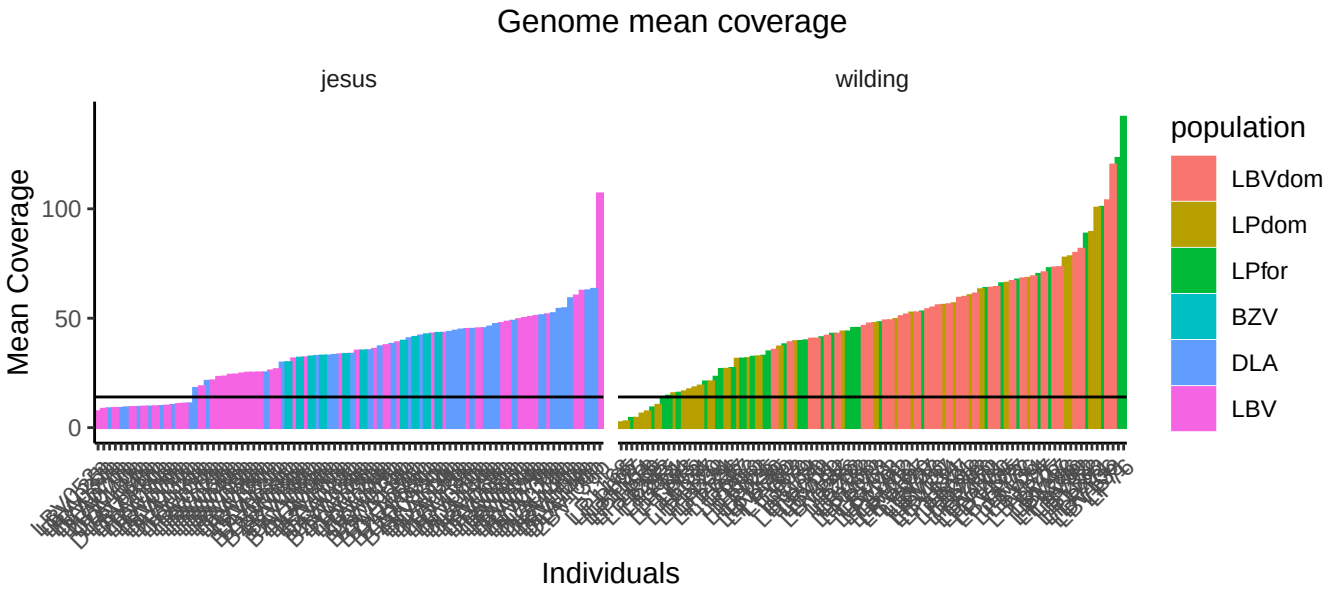
```
qualimap bamqc -bam /scratch/daron_anopheles/bam/$inputFile.indelrealigner.bam -c --java-mem-size=8G -ou
es/fastqBamInfo/bamInfo/jesus_qualimap/$inputFile.outqualimap -nt 2 -outformat HTML
```

-> wilding bam report: qualimap report git hub link.

-> urbano bam report: qualimap report git hub link.

2.1.2 Bam files analysis (genome depth, sex determination)

- Genome mean coverage:

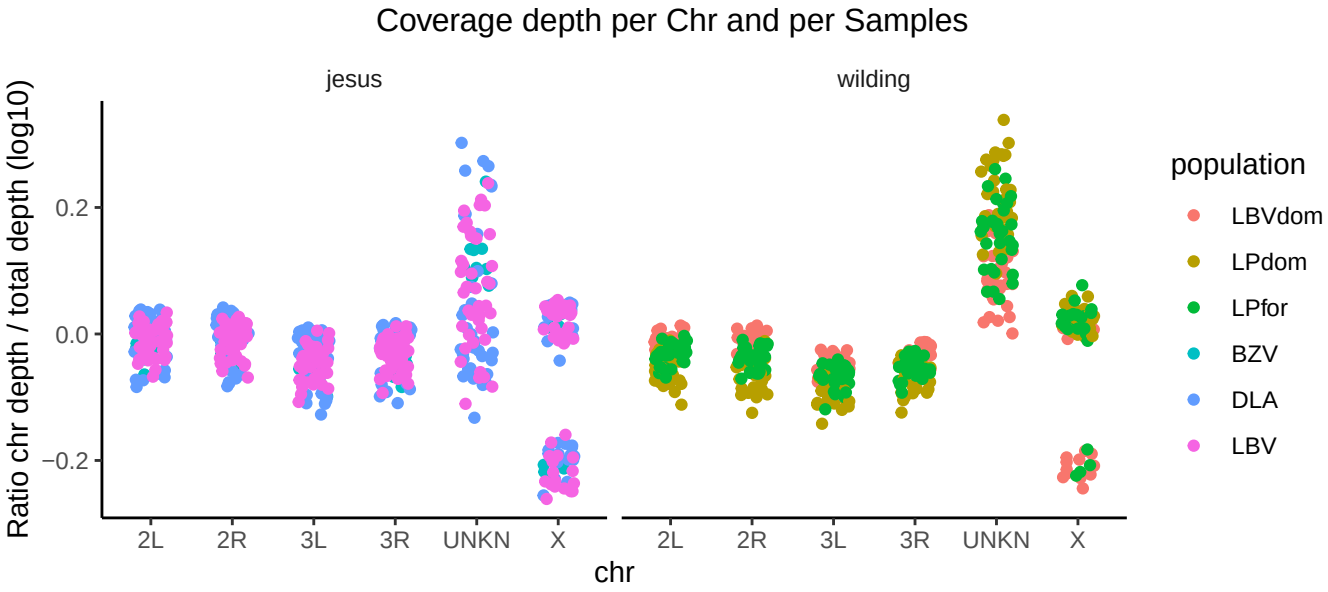


–> Filtering individuals: Remove individuals with mean coverage lower than 14x

9 individuals from Wilding: LP69, LP243, LP697, LP1118, LP1125, LP1164, LP1165, LP1168, LP1285

17 individuals from Urbano: DLA037p, DLA076p, DLA077p, DLA102p, DLA105p, DLA130p, DLA132p, DLA155Bp, LBV001p, LBV007p, LBV009p, LBV052p, LBV125p, LBV127p, LBV137p, LBV140p, LBV142p

- Determining sex of each samples:



Result: **wilding**: 16/96 males ; **urbano**: 36/88 males

2.2 SNPs calling and filtering

2.2.1 SNPs calling script

- gatk unifiedGenotyper:

```
java -jar ~/bin/GenomeAnalysisTK-3.8-0-ge9d806836/GenomeAnalysisTK.jar -T UnifiedGenotyper -R Anopheles_
ist -L $interval --genotyping_mode DISCOVERY --downsampling_type BY_SAMPLE -dcov 250 --output_mode EMIT_
17 --genotype_likelihoods_model BOTH --heterozygosity 0.01 --indel_heterozygosity 0.001 -stand_call_con
rose.$out.unifiedGenotyper.vcf
```

2.2.2 SNP filtering:

- Jupyter-notebook script to generate html report on the newly created VCF file: Jupyter notebook VCF stat report code.

```
launch_ipynb.py -i vcfStats_slurm.ipynb -o wilding.chr3R.vcfStats_slurm.html
```

-> Wilding and Urbano samples SNPs stat report: Wilding and Urbano SNP stat report.

Table 1: Final number of SNPs per chr after filtration

chr	NbSNP
2L	2723196
2R	3485073
3L	2540839
3R	3524803
X	639905

- 4 inds removed because imiss > 10%: LP1124 LP1145 LP47 LP63
  /! for X chr 10 samples are removed: BZV093bu DLA136u DLA137u LBV066u LBV072u LBV131u LP1124w LP1145w LP47w LP63w
- Bash script to perform SNP and ind filtering (cause scikit is only outputing stats)

```
#!/bin/bash

# input file list
IN_VCF=$1         # input VCF file
AG_VCF_ACCESS=$2  # AG1000G VCF for genome accessibility, downloaded at ftp://ngs.sanger.ac.uk/producti
ssibility.X.vcf.gz
REF=$3
IND=$4

IN_PREFIX=`echo $IN_VCF | sed 's,\(.*\).vcf.gz,\1,'`

# Step 1: Select variants using GATK
echo "--> Step 1: Select variant based on GATK metrics `date`"
echo "Filter Expression QD < 5.00 || FS > 60.000 || ReadPosRankSum < -8.000"

java -jar ~/bioInf/bin/GenomeAnalysisTK-3.8-0-ge9d806836/GenomeAnalysisTK.jar -T SelectVariants -R $REF
 -selectType SNP

tabix $IN_PREFIX.snponly.vcf.gz
```

```
java -jar ~/bioInf/bin/GenomeAnalysisTK-3.8-0-ge9d806836/GenomeAnalysisTK.jar -T VariantFiltration -R $
PREFIX.annot.vcf.gz --filterExpression "QD < 5.00 || FS > 60.000 || ReadPosRankSum < -8.000 " --filterNa

zcat $IN_PREFIX.annot.vcf.gz | egrep -v "LOW_QUAL" | bgzip > $IN_PREFIX.passQC.vcf.gz

# Step 2: Select variants from inputed VCF based on genome accessibility
echo "--> Step 2: Choose variant based on genome accessibility `date`"
echo "minGQ 20 min DP 10"

zcat $AG_VCF_ACCESS | awk '{if($7=="PASS"){print $1"\t"$2}}' > $IN_PREFIX.pos

vcftools --gzvcf $IN_PREFIX.passQC.vcf.gz --positions $IN_PREFIX.pos --minGQ 20 --non-ref-ac-any 1 --re
> $IN_PREFIX.snpPassQC.vcf.gz

rm $IN_PREFIX.annot.vcf.gz $IN_PREFIX.snponly.vcf.gz $IN_PREFIX.passQC.vcf.gz

vcftools --gzvcf $IN_PREFIX.snpPassQC.vcf.gz --missing-site --stdout | awk '{if($6<0.05){print $0}}' | c

vcftools --gzvcf $IN_PREFIX.snpPassQC.vcf.gz --positions $IN_PREFIX.lmiss --remove $IND --non-ref-ac-any
| bgzip > $IN_PREFIX.passQC.vcf.gz

rm $IN_PREFIX.snpPassQC.vcf.gz
```

- Summary of filtering step:

1. Remove individual with mean coverage lower than 14x
2. Discard SNPs present in none accessible area (defined in ag1000g), QD $<$ 5.00, FS $>$ 60.000 and ReadPosRankSum $<$ -8.000
3. Replace by NA genotypes with low call confidence (GQ$<$20)
4. Remove SNPs with $>$5% lmiss
5. Remove Inds with $>$10% imiss

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3. Structure of genetic variation

Global genetic structure

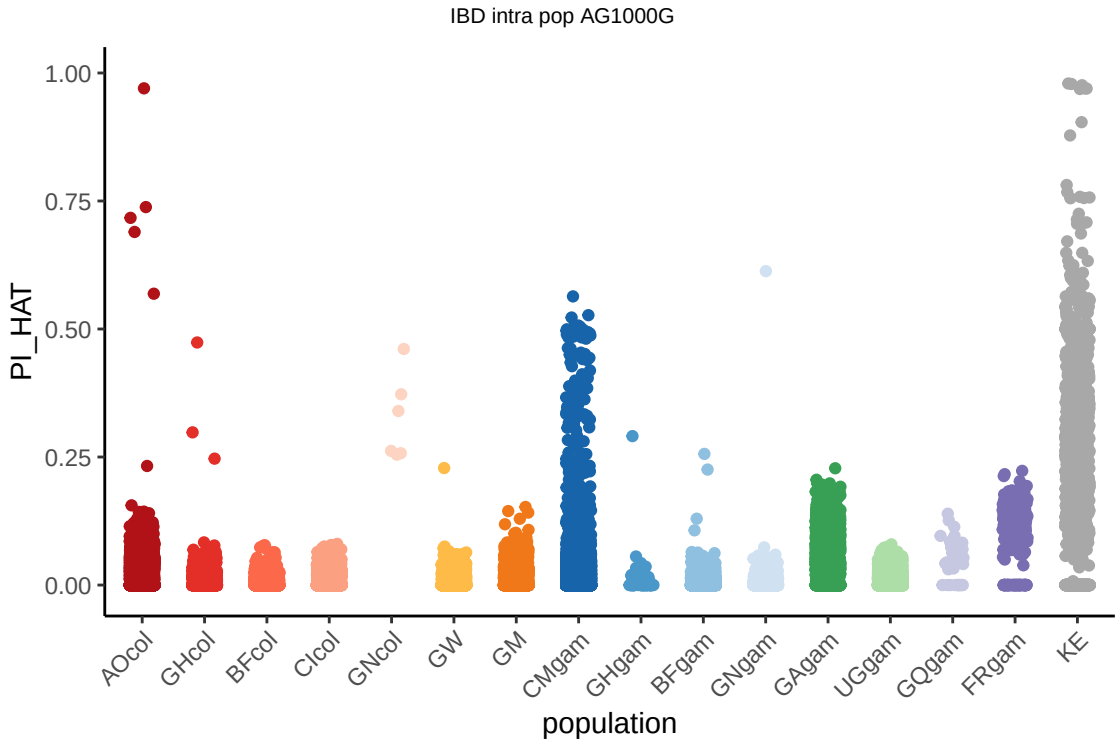
IBD anlaysis to identify closely related samples

Context: Downstream analysis required the absence of high related samples (i.e. more than we would expect by chance in a random sample). Goal: Identify putative closely related pair of sample using plink –genome

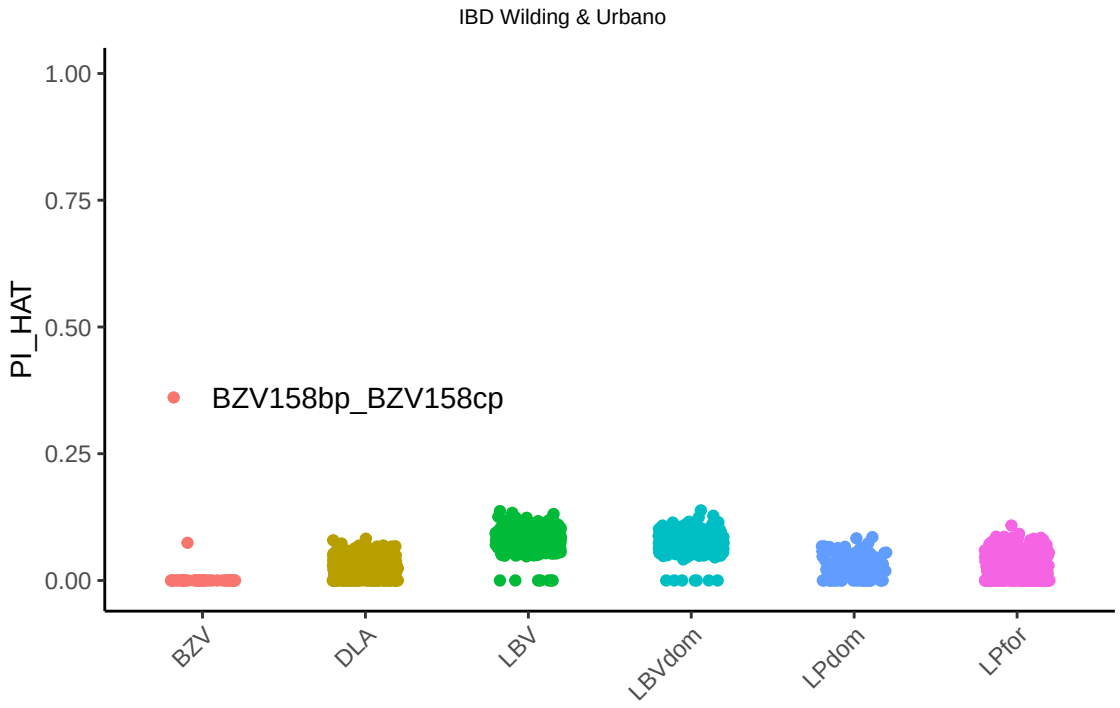
Bellow is the script for the IBD analysis:

```
# 1. Prune VCF using customized script. The program output the list of the coordinate of unlinked SNPs
prune_SNPs.py --snp wilding_urbano.3L.unifiedGenotyper.cov14x.passQC.vcf.gz --pop all --meta wilding_url

prune_SNPs.py --snp wilding_urbano.3R.unifiedGenotyper.cov14x.passQC.vcf.gz --pop all --meta wilding_url

# 2. Fetch pruned SNPs using VCFtools
vcftools --gzvcf wilding_urbano.3L.unifiedGenotyper.cov14x.passQC.vcf.gz --positions wilding_urbano.3L.p
 --recode --stdout | bgzip > wilding_urbano.3L.pruned.vcf.gz

vcftools --gzvcf wilding_urbano.3R.unifiedGenotyper.cov14x.passQC.vcf.gz --positions wilding_urbano.3R.p
 --recode --stdout | bgzip > wilding_urbano.3R.pruned.vcf.gz

vcf-concat wilding_urbano.3L.unifiedGenotyper.cov14x.passQC.vcf.gz wilding_urbano.3R.unifiedGenotyper.co

# 3. IBD with plink --genome
plink --vcf wilding_urbano.3.pruned.vcf.gz --allow-extra-chr --genome --out wilding_urbano.chr3.ibd
```

1. AG1000g



2. Wilding & Urbano



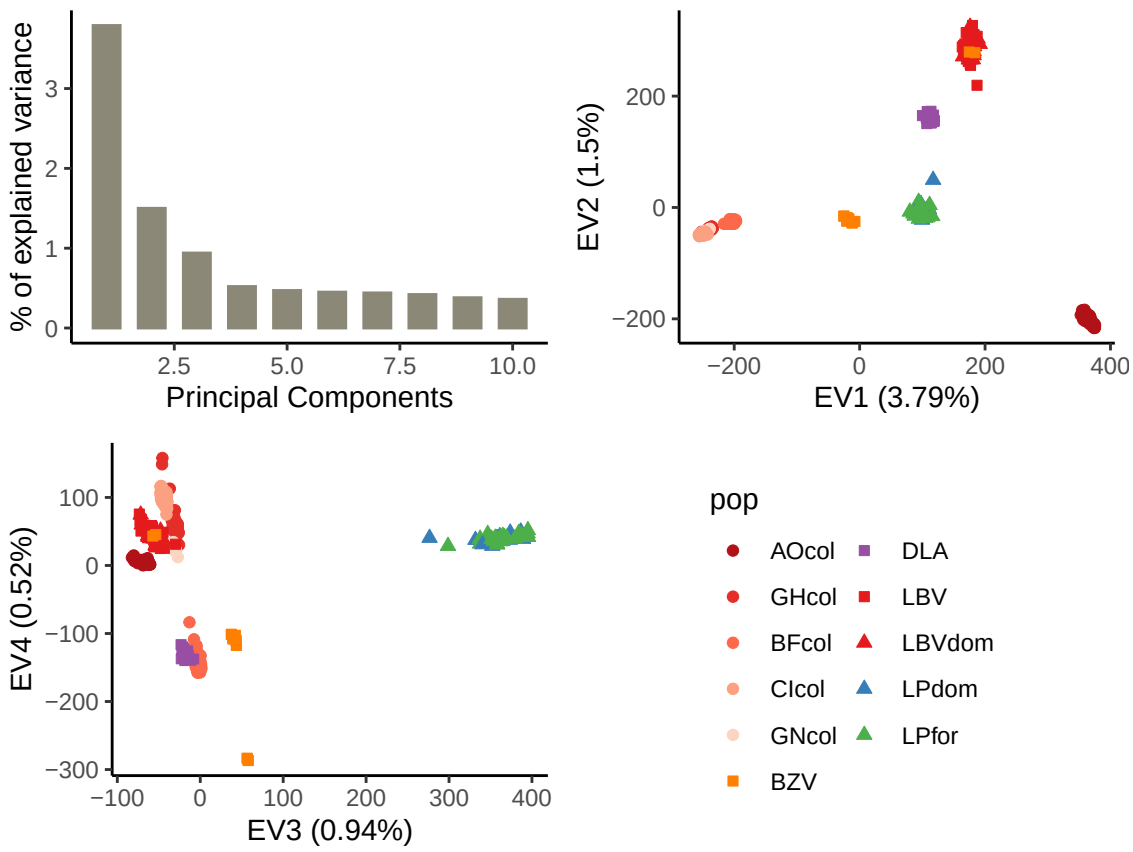
- Conclusion: Most sample pairs have low IBD value, meaning they are not related.

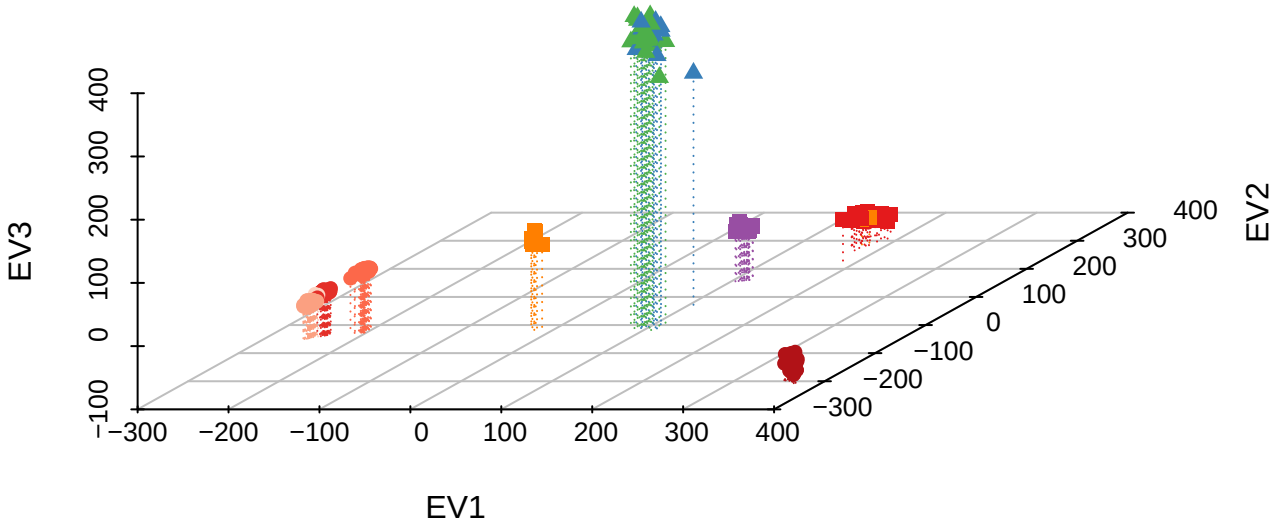
PCA

```
# PCA analysis was made using the jupyter-notebook script pca.ipynb
```

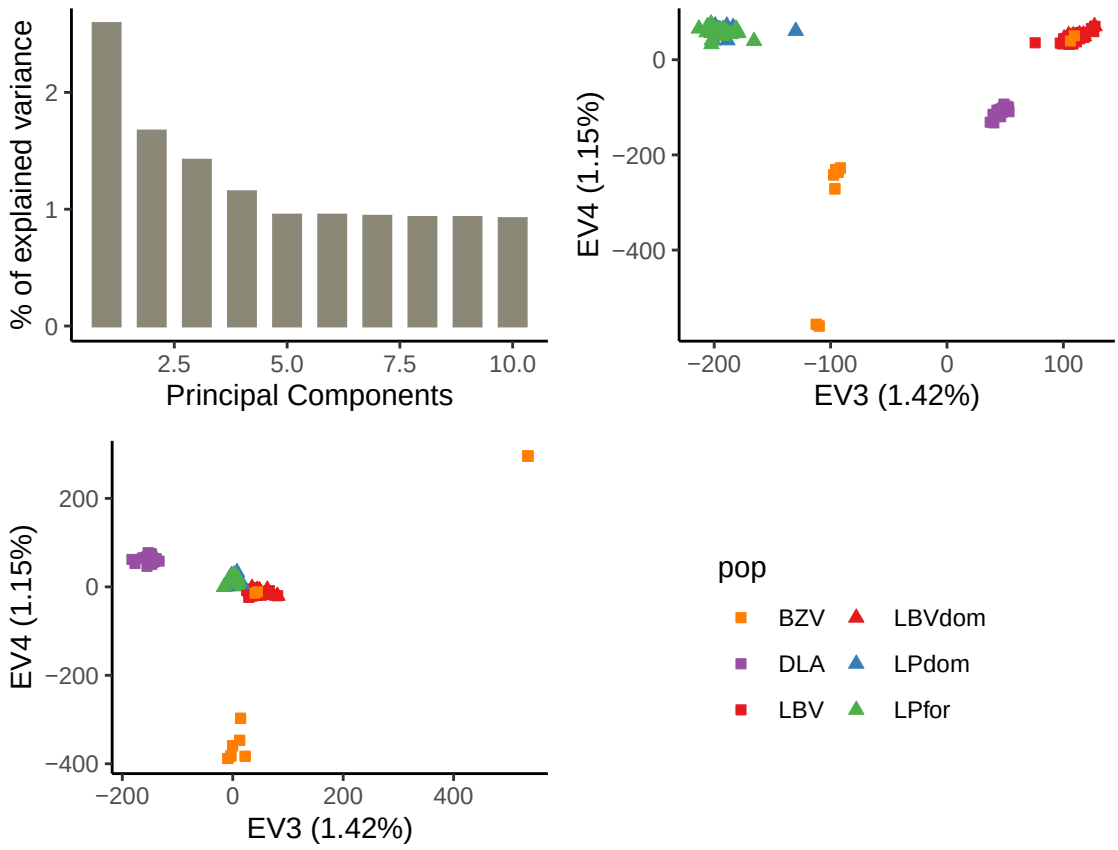
Question: How does the genetics variation of wilding/urbano samples is structured alone, or within the AG1000G samples?

1. Wilding and Urbano samples within ag1000g colluzzi


% of explained variance
Principal Components
EV2 (1.5%)
EV1 (3.79%)
EV4 (0.52%)
EV3 (0.94%)
pop
AOcol
GHcol
BFcol
CIcol
GNcol
BZV
DLA
LBV
LBVdom
LPdom
LPfor



2. Wilding and Urbano



- Conclusion:

1. Wilding genome are located between the AOcol and GHcol population, as expected.
2. Based on the wilding pca, we can distinguish clearly two pop: (i) LBVdom and (ii) LPdom-LPfor. LPdom and LPfor even they have been samples at 15km away from each other they represent one single population

Admixture

Question: Same question than above adding the admixture component.

- lauch admixture on the cluster, 1 job per k, 10 time a random set of 100kSNPs and 10 seed per samples set (N=100 per k):

```
path_to_tmp="/scratch/daron_$SLURM_JOB_ID"
IN_PREFIX="ag1000g.phase2.anopheles-rose.merged.biallelic.3.pruned"
K=${1}

# download vcf file
scp nas3:/data3/projects/plasmodium/anopheles/vcf_store/$IN_PREFIX.vcf.gz $path_to_tmp

# run admixture 10 resamples of 100kSNPs with 10 different seed; N=100
N=0
for i in {1..10}; do
    zcat $IN_PREFIX.vcf.gz | egrep -v "#" | cut -f 1,2 | shuf -n 100000 | sort -k 1n,1n -k 2n,2n > pos
    vcftools --gzvcf $IN_PREFIX.vcf.gz --positions pos --stdout --recode | sed 's,^3L,1,' | sed 's,^3R,
```

```
    tabix samp.vcf.gz
    plink --vcf samp.vcf.gz --make-bed  --allow-extra-chr --out samp

    for j in {1..10}; do
        N=$(( $N + 1 ))
        admixture --cv --seed=$RANDOM -j9 samp.bed $K | tee $IN_PREFIX.n$N.log${K}.out
        mv samp.$K.P $IN_PREFIX.n$N.$K.P
        mv samp.$K.Q $IN_PREFIX.n$N.$K.Q
        scp $IN_PREFIX.n$N.$K.P $path_to_dir/
        scp $IN_PREFIX.n$N.$K.Q $path_to_dir/
        scp $IN_PREFIX.n$N.log${K}.out $path_to_dir/
    done
done
```

- reorder ind based on a customized order:

```
R --vanilla --args $PWD/'admixture output file' $PWD/'ind ordered as in the admix' $PWD/'ind new order'
```

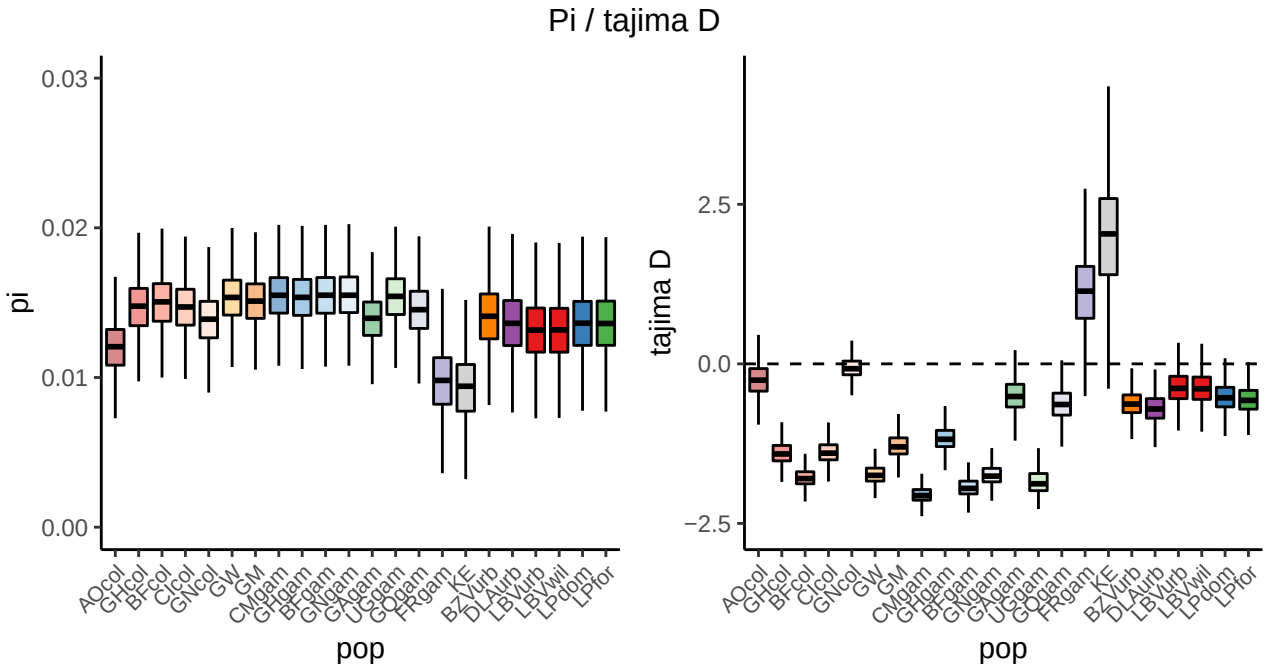
- run clumpack

```
CLUMPAK.pl -id 10 -dir ./ -file out_ordered_ag1000gCol_wilding.zip -inputtype Admixture -indtopop ag1000

option help:
-id Job id (defaut random number generated)
-inputtype Input file format [Admixture|Structure]
-indtopop population label (only pop name in the same order than input file)
```

Stat descriptives

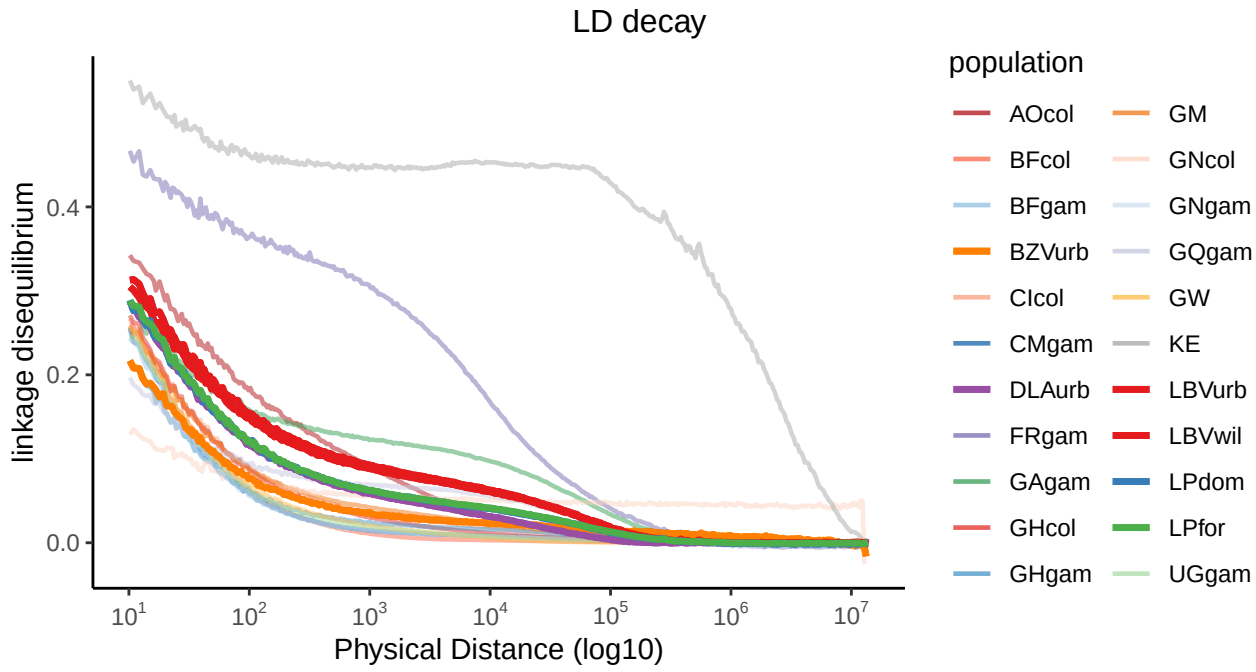
- Pi and tajima D

```
popStatsDescWindows.py --snp anopheles-rose.unifiedGenotyper.cov14x.passQC.zarr --keep LPdom.ind --bed a
```



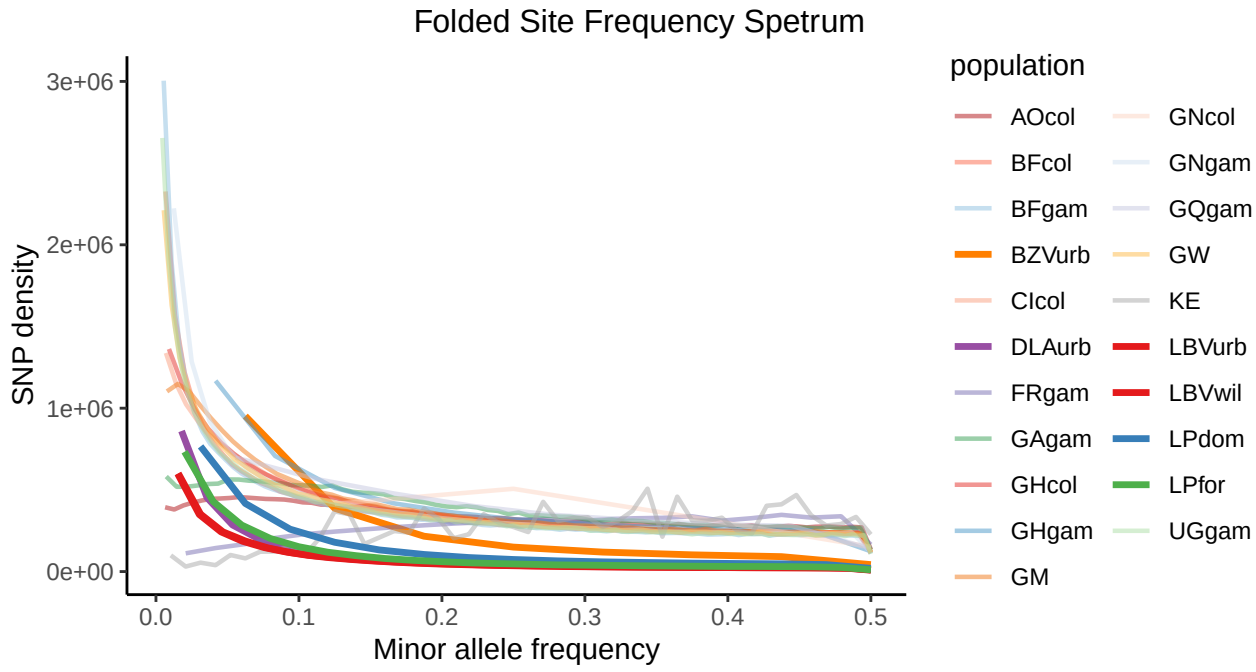
- LDdecay Here is the link to the jupyter-notebook script: LdDecay.

```
ldDecay.ipynb
```



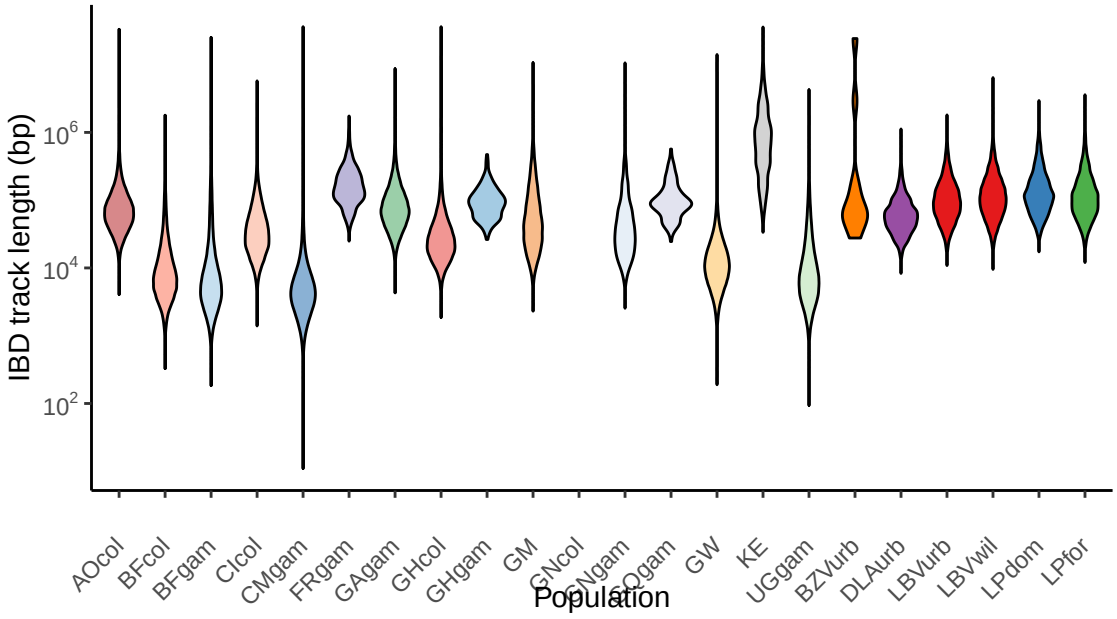
- Folde Site Frequency spetrum

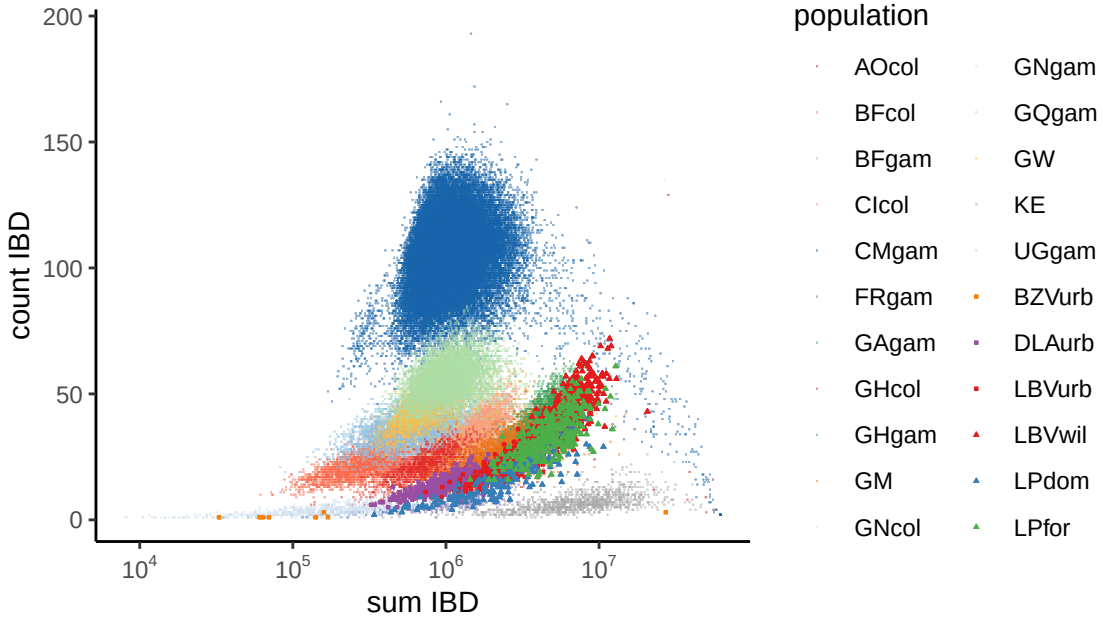
```
sfs.py --zarr ag1000g.phase2.anopheles-rose.merged.biallelic.zarr --keep LPdom.ind --bed ag1000g.chr3pe
```



- IBD

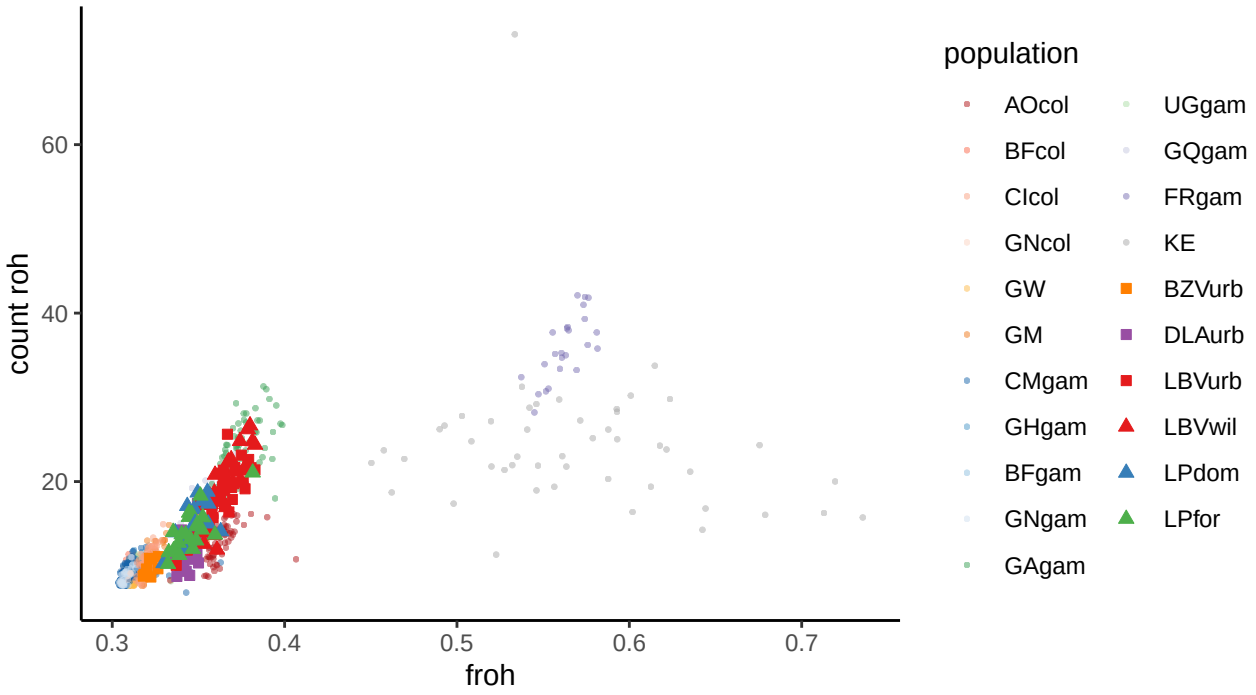
```
egrep -w $pop wilding.samples.meta.txt | cut -f 1 > k

vcftools --gzvcf anopheles-rose.3L.unifiedGenotyper.cov14x.passQC.vcf.gz --chr 3L --from-bp 15000000 --

vcftools --gzvcf anopheles-rose.3R.unifiedGenotyper.cov14x.passQC.vcf.gz --chr 3R --from-bp 1000000 --t

java -Xmx8g -jar ~/bioInf/bin/ibdseq.r1206.jar gt=$pop.3L.vcf.gz out=$pop.ibdseq.3L
java -Xmx8g -jar ~/bioInf/bin/ibdseq.r1206.jar gt=$pop.3R.vcf.gz out=$pop.ibdseq.3R
```





- roh

```
get_roh.py --snp ag1000g.phase2.anopheles-rose.merged.biallelic.zarr --keep LPdom.ind --bed ag1000g.chr
```

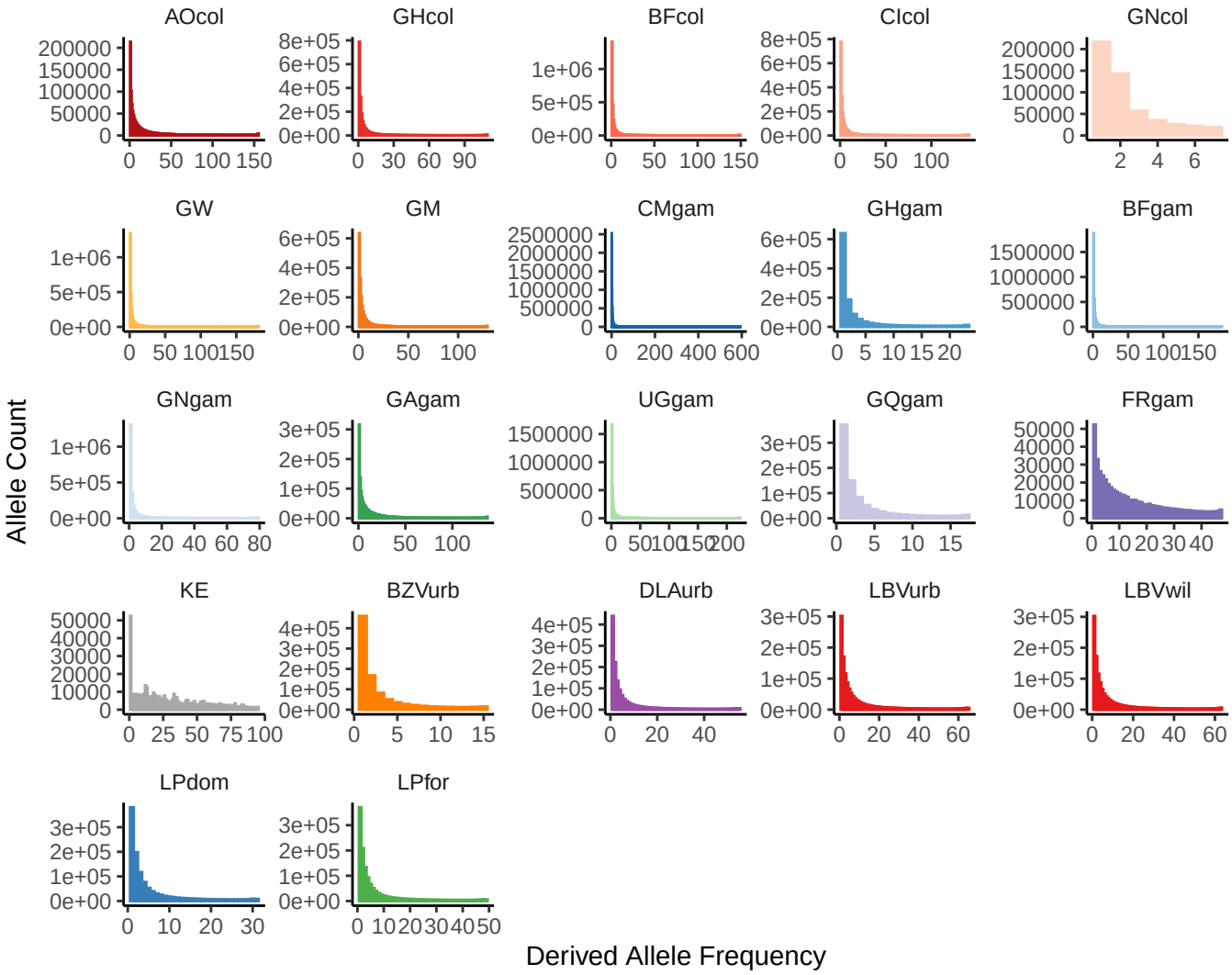


Deomgraphic history:

Stairway plot

SNP polarization: In order to generate a unfolded site frequency spetrum we need to polarize the SNPs using an outgroup. Here we used A. merus as outgroup with N=10 individuals.

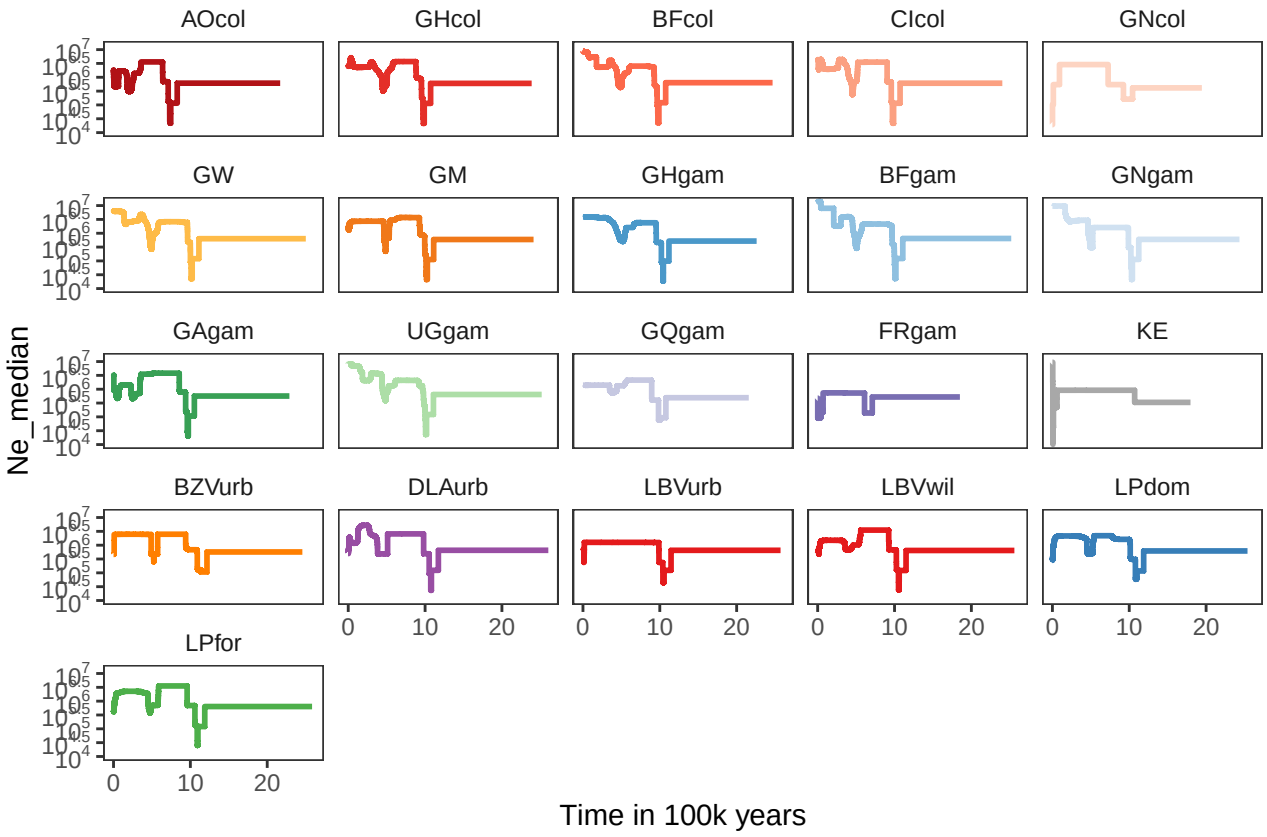
```
### merge wilding VCF with merus VCF
join -1 1 -2 1 anopheles-rose.$chr.unifiedGenotyper.cov14x.passQC.pos chri_meru.merged.pos | sed 's,_,\t

bcftools merge -i - -m snps anopheles-rose.$chr.unifiedGenotyper.cov14x.passQC.vcf.gz chri_meru.merged.v

### polarize REF allele using outgroup
# samll header printing issue in the polarizeVCF.py scrit so header need to be adding separatly
# use major allel as ancestral allel, discar 0.5:0.5 SNPs, site need a MAF of 0.1 to be considered
polarizeVCF.py --vcf anopheles-rose.$chr.unifiedGenotyper.cov14x.passQC.merge.vcf.gz --keep chri_meru.i


sfs.py --zarr ag1000g.phase2.anopheles-rose.merged.biallelic.zarr --keep LPdom.ind --bed ag1000g.chr3pe
```



b. Stairway plot

```
pop=${1}
inputSFS=${2}
sfs=$(cat /data3/projects/plasmodium/anopheles/popstructure/statDesc/usfs/$inputSFS | cut -f 3 | sed '1d
nbseq=$(cat /data3/projects/plasmodium/anopheles/popstructure/statDesc/usfs/$inputSFS | cut -f 3 | sed
nbSite=30484401
nrand_1=$(( ($nbseq-2)/4 ))
nrand_2=$(( ($nbseq-2)/2 ))
nrand_3=$(( (($nbseq-2)*3)/4 ))
nrand_4=$(($nbseq-2))

### create blueprint file
echo "popid: $pop # id of the population (no white space)" > run.blueprint
echo "nseq: $nbseq # number of sequences" >> run.blueprint
echo "L: $nbSite # total number of observed nucleic sites, including polymorphic and monomorphic"  >> ru
echo "whether_folded: false # whethr the SFS is folded (true or false)" >> run.blueprint
```

```
echo "SFS: $sfs # snp frequency spectrum: number of singleton, number of doubleton, etc. (separated by u
echo "#smallest_size_of_SFS_bin_used_for_estimation: 1 # default is 1; to ignore singletons, uncomment t
> run.blueprint
echo "#largest_size_of_SFS_bin_used_for_estimation: 29 # default is n-1; to ignore singletons, uncommen
eq-2" >> run.blueprint
echo "nrand: $nrand_1   $nrand_2    $nrand_3    $nrand_4 # number of random break points for each try (s
n.blueprint
echo "project_dir: $pop # project directory" >> run.blueprint
echo "stairway_plot_dir: /home/daron/bioInf/bin/stairway_plot_v2.1.1/stairway_plot_es # directory to the
echo "ninput: 200 # number of input files to be created for each estimation" >> run.blueprint
echo "#random_seed: $RANDOM" >> run.blueprint
echo "#output setting" >> run.blueprint
echo "mu: 2.8e-9 # assumed mutation rate per site per generation" >> run.blueprint
echo "year_per_generation: 11 # assumed generation time (in years)" >> run.blueprint
echo "#plot setting" >> run.blueprint
echo "plot_title: $pop.plot # title of the plot" >> run.blueprint
echo "xrange: 0.1,10000 # Time (1k year) range; format: xmin,xmax; "0,0" for default" >> run.blueprint
echo "yrange: 0,0 # Ne (1k individual) range; format: xmin,xmax; "0,0" for default" >> run.blueprint
echo "xspacing: 2 # X axis spacing" >> run.blueprint
echo "yspacing: 2 # Y axis spacing" >> run.blueprint
echo "fontsize: 12 # Font size" >> run.blueprint

### Run stairwayplot2
java -cp /home/daron/bioInf/bin/stairway_plot_v2.1.1/stairway_plot_es Stairbuilder run.blueprint
bash run.blueprint.sh
```


AOcol
GHcol
BFcol
CIcol
GNcol
GW
GM
GHgam
BFgam
GNgam
GAgam
UGgam
GQgam
FRgam
KE
BZVurb
DLAurb
LBVurb
LBVwil
LPdom
LPfor
Ne_median
Time in 100k years