wilding_paper

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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.
ae.AgamP4.dna.chr.fna -targetIntervals $sampleId.realignertargetcreator.intervals -I $sampleId.map.sort
```

- bam file report (qualimap):

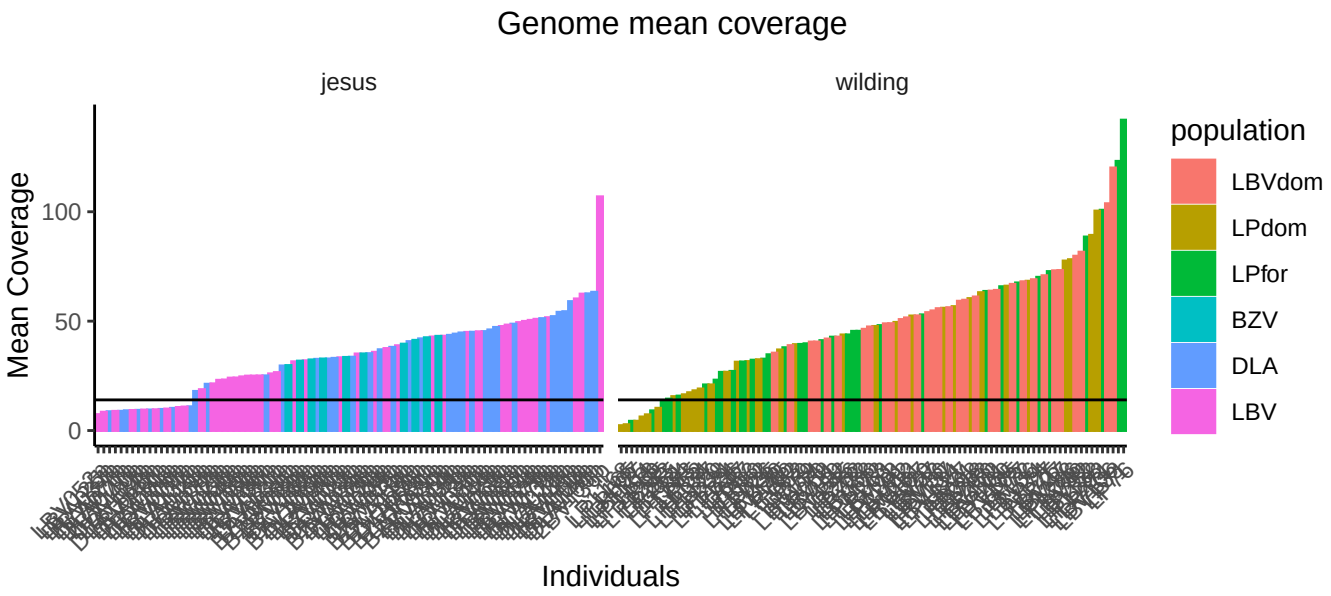
```
qualimap bamqc -bam /scratch/daron_anopheles/bam/$inputFile.indelrealigner.bam -c --java-mem-size=8G -ou
es/fastqBamInfo/bamInfo/jesus_qualimap/$inputFile.outqualimap -nt 2 -outformat HTML
```

-> 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:

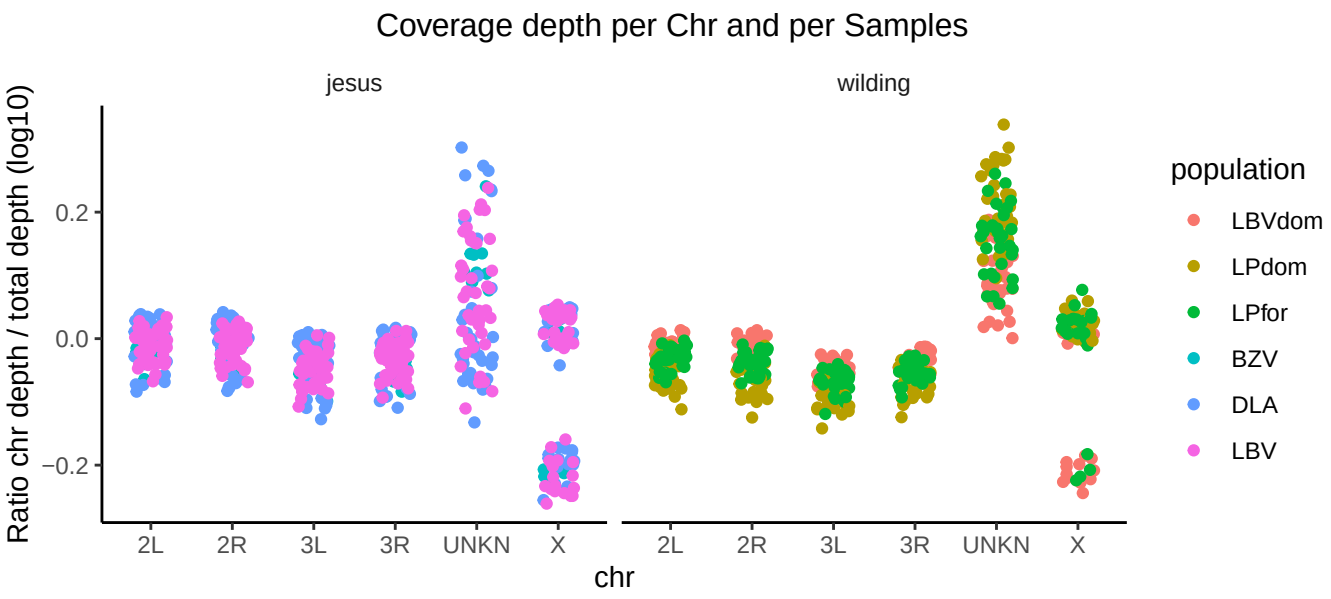


–> Filtering individuals: Remove individuals with mean coverage lower than 14x

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:



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 samples only SNPs stat report: Wilding SNP stat report.

Final number of SNPs per chr:
2L 1,228,916
2R 1,605,477
3L 1,159,765
3R 1,610,164
X 295,618

9 inds removed because imiss > 10%: LP1120 LP1134 LP1283 LP255 LP51 LP53 LP65 LP934 LP937

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

Final number of SNPs per chr
2L 2,723,196
2R 3,485,073

3L 2,540,839
3R 3,524,803
X 639,905

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}}' |

vcftools --gzvcf $IN_PREFIX.snpPassQC.vcf.gz --positions $IN_PREFIX.lmiss --remove $IND --non-ref-ac-an
| 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

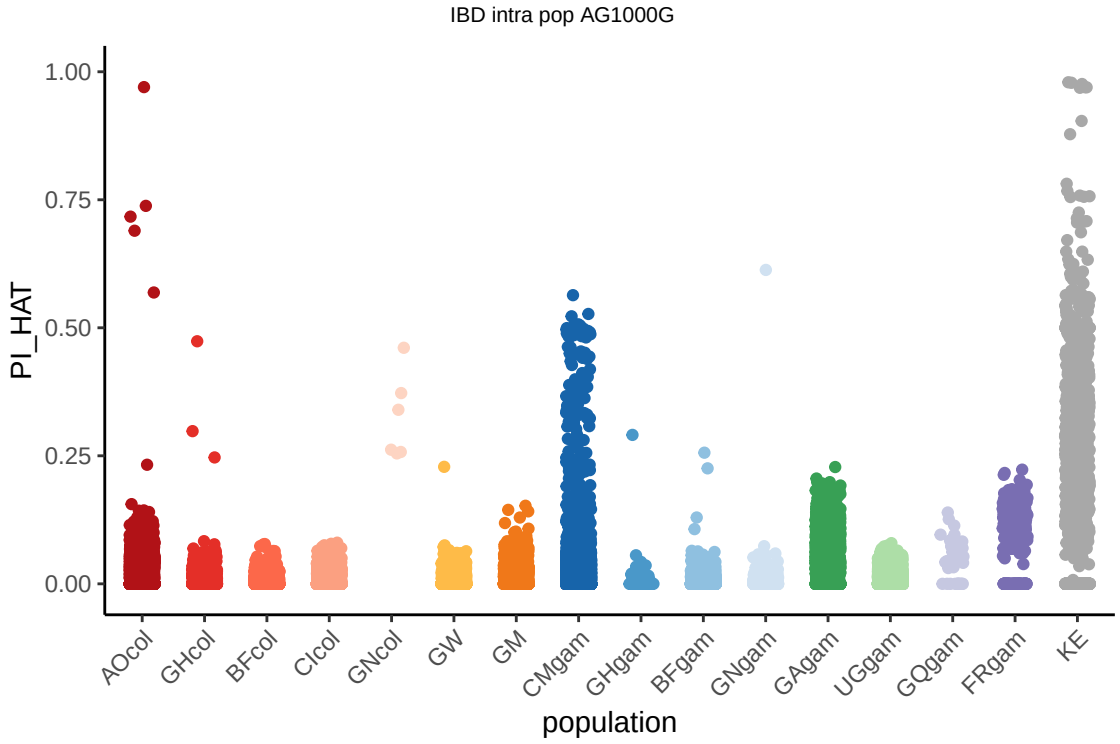
Global genetic structure

IBD anlaysis to identify closely related samples

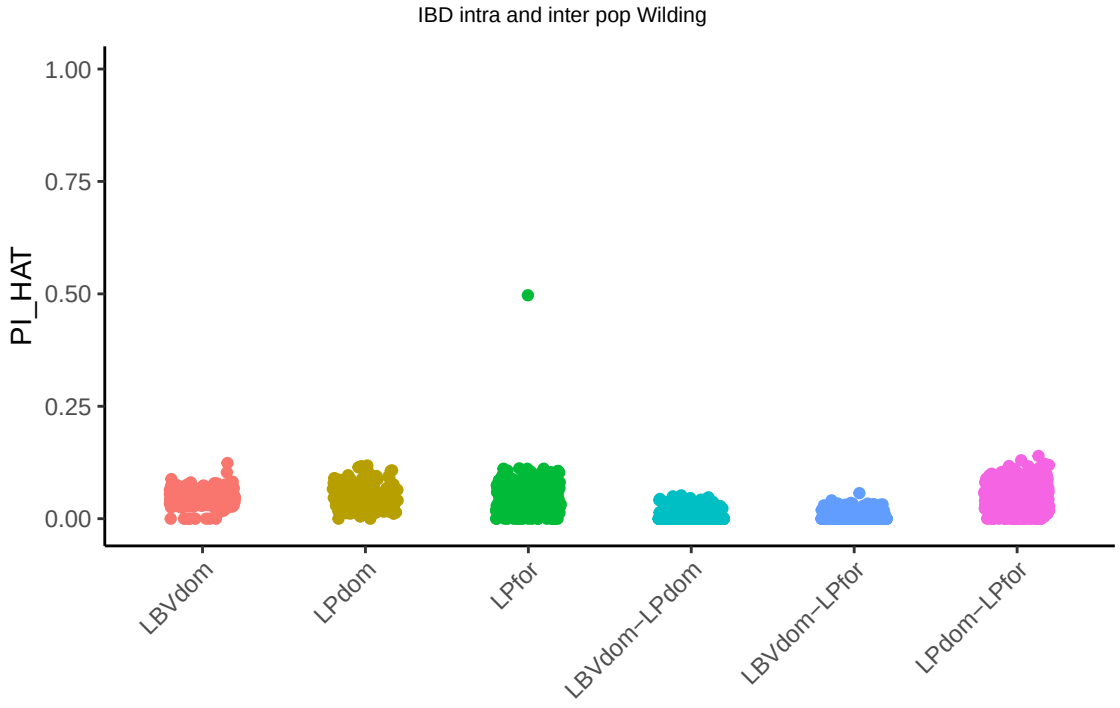
Bellow is the script for the IBD analysis:

```
# 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
```

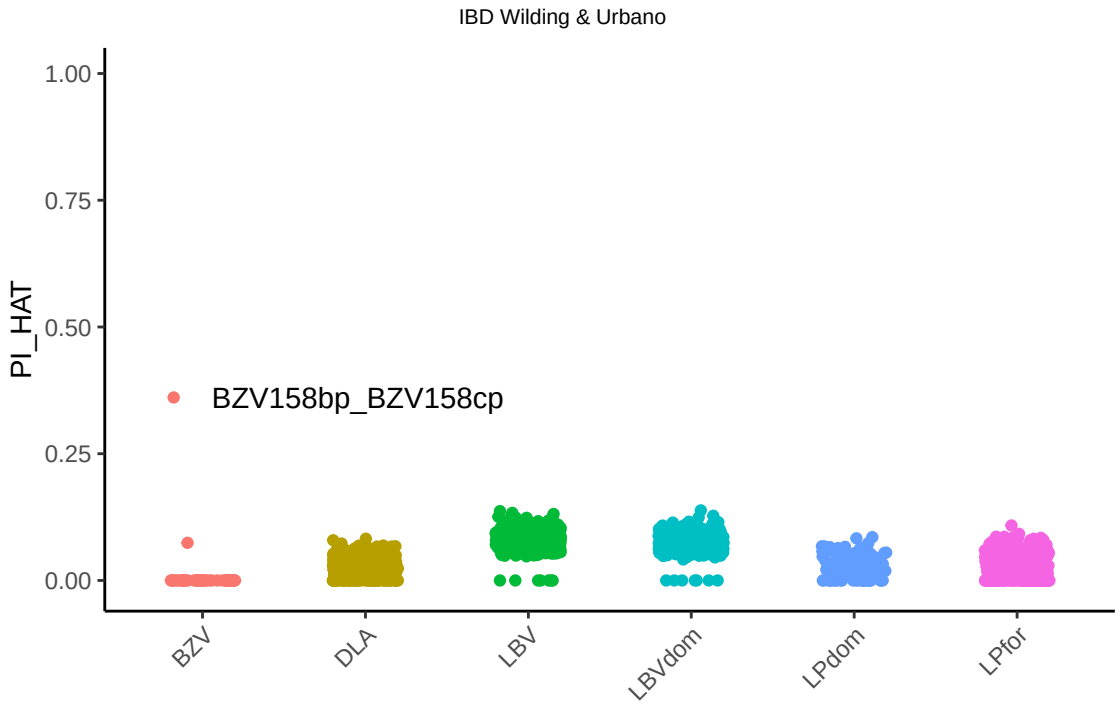
1. AG1000g



2. Wilding



3. Wilding & Urbano

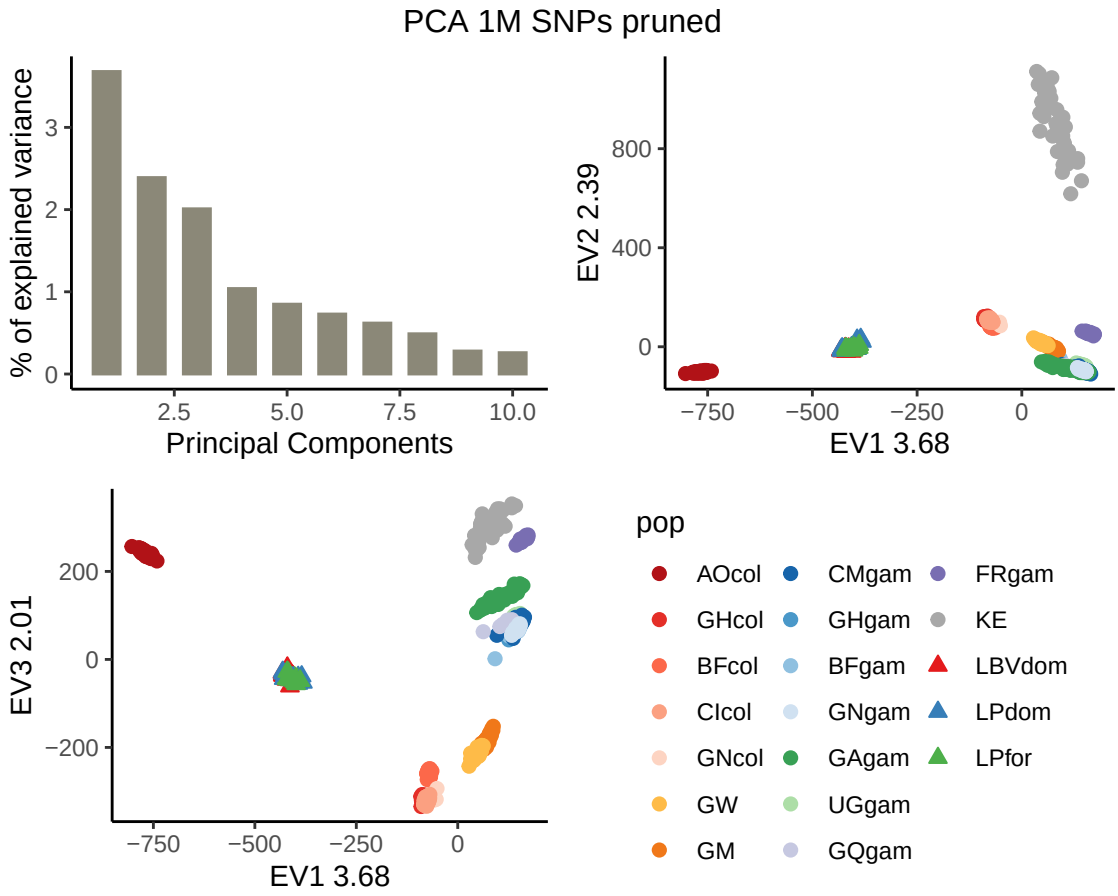


- Conclusion: Most sample pairs have low IBD value, meaning they are not related.

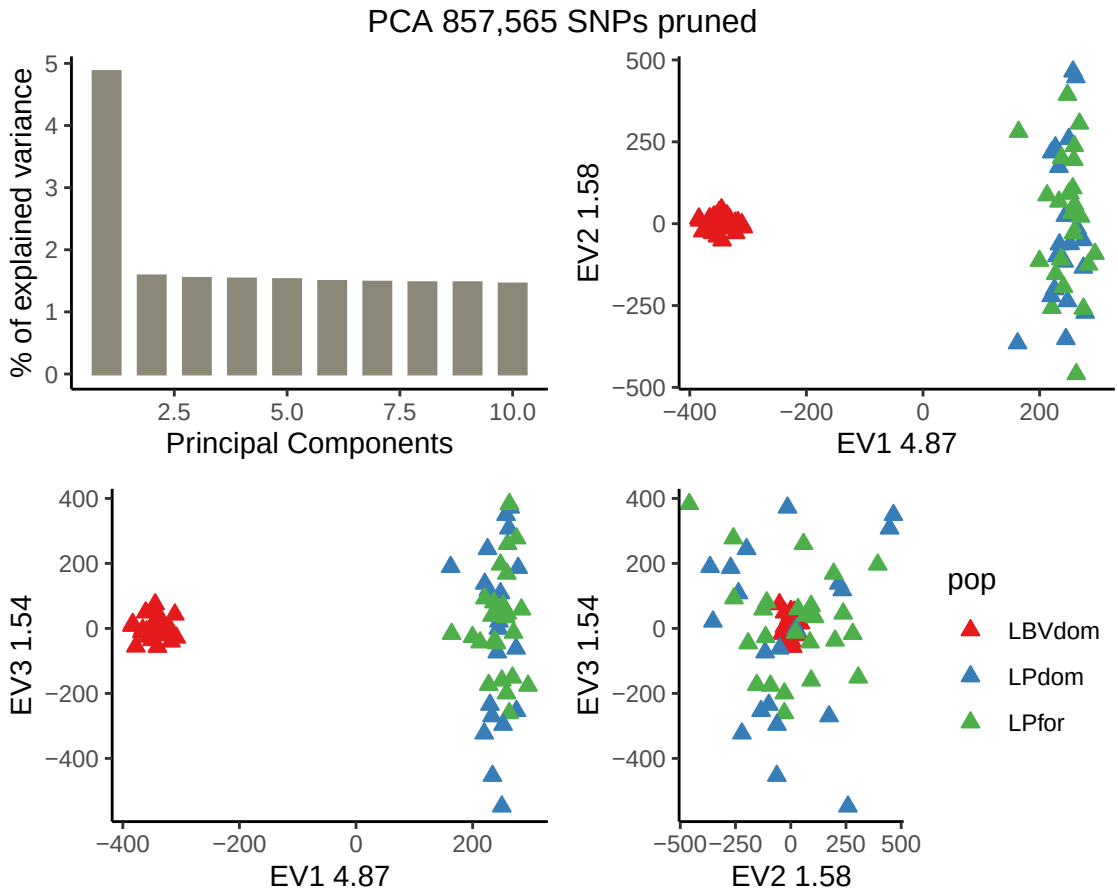
PCA

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

1. AG1000G and Wilding


PCA 1M SNPs pruned
% of explained variance
Principal Components
EV2 2.39
EV1 3.68
EV3 2.01
EV1 3.68
pop
AOcol
GHcol
BFcol
CIcol
GNcol
GW
GM
CMgam
GHgam
BFgam
GNgam
GAgam
UGgam
GQgam
FRgam
KE
LBVdom
LPdom
LPfor

2. Wilding



- 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

Stat descriptives

Deomgraphic history:

Investigate recent changes in population size over time.

SNP phasing

SNP polarization

Polarize alleles using as outgroup Anophele Merus and Anophele

IBDne

Infer recent population history (200-500 last generation). Determine change from La Lope village vs forest
Dataset: use data from IBDseq

Stairway plot

Datase: Use polarize alleles

MSMC

Dataset: Use phased SNPs