

Starling-May18

Projects/Katarina Stuart/KStuart.Starling-Aug18/Sv4_Historic/Analysis/2021-10-21.RR_PopGen

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PopGen X Relatedness Analysyis

```
cd /srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/processing/align/bwa_aln_alignment
module load stacks/2.2
populations -P ./ -M ../historic_populations.txt --vcf -r 0.8 -p 2 --lnl_lim -15 --write_random_snp -t 8
```

cd /srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/processing/align/bwa_aln_alignment
OUTDIR=/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR
populations -P ./ -M ../historic_populations.txt -O \${OUTDIR}/ --vcf -r 0.8 -p 6 --lnl_lim -15 --write_random_snp -t 8
cd \$OUTDIR
vcftools --vcf populations.snps.vcf --maf 0.05 --minDP 5 --minGQ 15 --out bwaaln_filter_allsample_rr --recode
vcftools --vcf populations.snps.vcf --maf 0.05 --minDP 5 --minGQ 15 --hwe 0.001 --out bwaaln_filter_allsample_rr_hwe --recode

Population summary statistics (more detail in populations.sumstats_summary.tsv):

- aw: 14.479 samples per locus; pi: 0.12128; all/variant/polymorphic sites: 6548645/59983/37870; private alleles: 8577
 - hist: 9.1315 samples per locus; pi: 0.11409; all/variant/polymorphic sites: 1642141/9014/5577; private alleles: 2989
 - mv: 14.437 samples per locus; pi: 0.105; all/variant/polymorphic sites: 4777128/55609/23228; private alleles: 1244
 - mw: 14.415 samples per locus; pi: 0.11646; all/variant/polymorphic sites: 6478357/58182/32086; private alleles: 4401
 - nc: 14.485 samples per locus; pi: 0.11915; all/variant/polymorphic sites: 6139540/61130/34122; private alleles: 5321
 - or: 14.496 samples per locus; pi: 0.11238; all/variant/polymorphic sites: 6365745/60938/29065; private alleles: 2546
- with HWE filter:** kept 27284 out of a possible 64324 Sites
- without HWE filter:** After filtering, kept 25785 out of a possible 64324 Sites

Popgen filtering (with HWE):

cd /srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/processing/align/bwa_aln_alignment
populations -P ./ -M ../historic_populations.txt -O \${OUTDIR}/ --vcf -r 0.5 -p 2 --lnl_lim -15 --write_random_snp -t 2
cd \$OUTDIR
vcftools --vcf populations.snps.vcf --max-missing 0.5 --maf 0.025 --minDP 5 --minGQ 15 --hwe 0.001 --out bwaaln_filter_allsample_hwe_m50 --recode

After filtering, kept 23683 out of a possible 80450 Sites

cd /srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR

```
VCF=/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/outlier_analysis/bwaaln_allsample_selection_histSNPs.recode.vcf
```

```
vcftools --vcf ${VCF} --max-missing 0.9 --maf 0.025 --minDP 2 --minGQ 15 --out bwaaln_filter_allsample_rr --recode
```

```
vcftools --vcf ${VCF} --max-missing 0.9 --maf 0.025 --minDP 2 --minGQ 15 --hwe 0.001 --out bwaaln_filter_allsample_rr_hwe --recode
```

Relatedness

```
vcftools --gzvcf ../bwaaln_filter_allsample_hwe_m50.recode.vcf --relatedness2
```

```
cut -f1,2,7 out.relatedness2 > relatedness_short.txt
```

```
module load R/3.5.3
```

```
R
```

```
#install.packages("spaa")
```

```
library(spaa)
```

```
setwd("/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_summary/relatedness_m50")
```

```
relatedness <- read.table("relatedness_short.txt", sep="\t", header=T)
```

```
relatedness.dist <- list2dist(relatedness)
```

```
relatedness.dist.df <- as.data.frame(as.matrix(relatedness.dist))
```

```
write.csv(relatedness.dist.df,file = "relatedness_pairmatrix.csv")
```

Using output, remove one individuals from pairs of relatedness >0.2

Individuals to remove, placed in removed_realted_ind.txt

MONK17

MONK5

MONK2

MONK20

MONK27

NOR12

BPYY

PRBB

PRRR

PYRR

PYYY

```
vcftools --vcf ./bwaaln_filter_allsample_rr.recode.vcf --remove removed_realted_ind.txt --recode --out bwaaln_filter_allsample_rr_nofamily
```

```
vcftools --vcf ./bwaaln_filter_allsample_rr_hwe.recode.vcf --remove removed_realted_ind.txt --recode --out bwaaln_filter_allsample_rr_hwe_nofamily
```

```
vcftools --vcf bwaaln_filter_allsample_rr_hwe_nofamily.recode.vcf --out bwaaln_filter_allsample_rr_hwe_nofamily.plink --plink
```

```
plink --file bwaaln_filter_allsample_rr_hwe_nofamily.plink --make-bed --noweb --out bwaaln_filter_allsample_rr_hwe_nofamily
```

PCA of no family data:

```
module load R/3.5.3
```

```
R
```

```
library(RColorBrewer)
```

```
library(SNPRelate)
```

```
library(gdsfmt)
```

```
library(scales)
```

```
library(adegenet)
```

```
library(pegas)
```

```

library(ggplot2)
library(ape)
library(poppr)
library(rgl)
library(ggplot2)
library(grid)
library(gridExtra)

setwd("/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR")

snpgdsVCF2GDS(vcf.fn="/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR/bwaaln_filter_allsample_rr_hwe_nofamily.recode.vcf"
genofile1 <- snpgdsOpen("bwaaln_filter_allsample_rr_hwe_nofamily.recode.gds")

pca1 <- snpgdsPCA(gdsobj = genofile1,autosome.only=FALSE)
pc1.percent <- pca1$varprop*100
head(round(pc1.percent, 2))
pca_g1 <- data.frame(sample.id = pca1$sample.id,
                     EV1 = pca1$eigenvect[,1], # the first eigenvector
                     EV2 = pca1$eigenvect[,2], # the second eigenvector
                     EV3 = pca1$eigenvect[,3], # the second eigenvector
                     stringsAsFactors = FALSE)

head(pca_g1)
pca_g1

palette(brewer.pal(6,"Set2"))

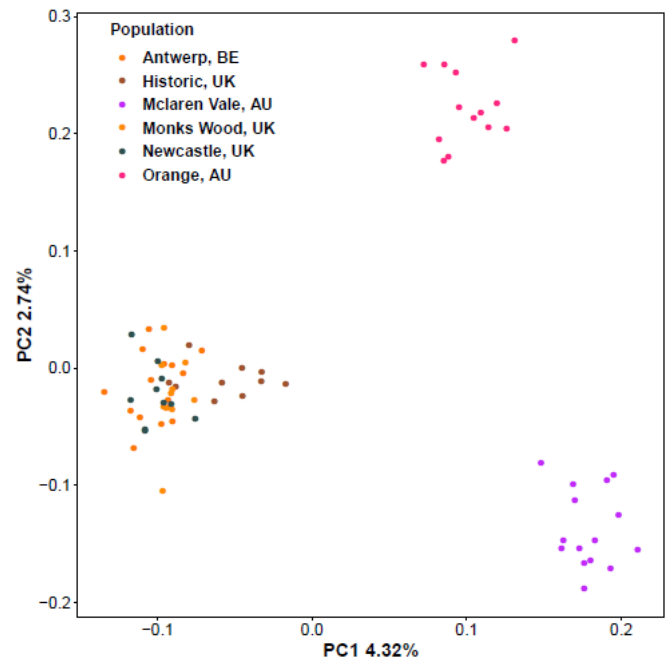
# add labels by population in correct order at vcf file
population <- as.factor(c(rep("Antwerp, BE",15), rep("Historic, UK",10),rep("McLaren Vale, AU",15),rep("Monks Wood, UK",10), rep("Newcastle, UK",10),rep
pca_g1 <- cbind(pca_g1,population)
pca_g1

```

```

theme <- theme(panel.background = element_blank(),panel.border=element_rect(fill=NA),panel.grid.major = element_blank(),panel.grid
pdf("PCA_bwaaln_filter_allsample_rr_nofamily_nofamily_12.pdf")
ggplot(pca_g1, aes(x=EV1,y=EV2,color=population)) +
geom_point() +
  xlab("PC1 4.32%") +
  ylab("PC2 2.74%") +
scale_color_manual(name="Population", values=c("#0073e6", "#cccccc", "#e6b800", "#7300e6", "#2F4F4F", "#cc0000")) +
  theme +
  theme(legend.justification=c(1,0), legend.position=c(0.45,0.70)) +
theme(legend.title = element_blank()) +
  theme(legend.text = element_text(colour="black", size=16,
face="bold")) +
  theme(legend.key = element_rect(fill = "white", color = NA)) +
  theme(axis.text=element_text(size=16),
axis.title=element_text(size=18,face="bold"))
dev.off()

```



Tree

```
cd /srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR

module load bioconductor/3.10

R

setwd("/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR")

library(qvalue)
library(SNPRelate)
library(dartR)
library(radiator)

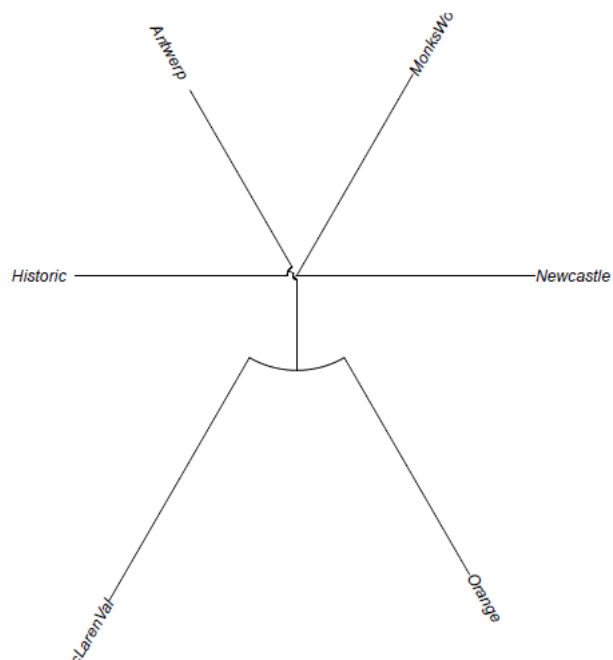
svulgaris <- genomic_converter(data = "/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR/bwaaln_filter_allsample_rr_hwe_nofamily.bed",

pops <- read.table("/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR/sample_names.txt", header=TRUE)

svulgaris$genlight$pop <- pops

pdf("Sv4_tree_bwaaln_filter_allsample_rr_hwe_nofamily.pdf")
gl.tree.nj(svulgaris$genlight, type="fan", labelsiz = 1)
dev.off()

gl.define.pop: Define a new population in a genlight {adegenet} object on... in dartR: Importing and Analysing SNP and Silicodart Data Generated by Gene
```



Admixture

```
cd /srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR
```

ALL

```
vcftools --vcf bwaaln_filter_allsample_rr_hwe_nofamily.recode.vcf --out bwaaln_filter_allsample_rr_hwe_nofamily.plink --plink
plink --file bwaaln_filter_allsample_rr_hwe_nofamily.plink --make-bed --noweb --out bwaaln_filter_allsample_rr_hwe_nofamily

mkdir admixture_nohwe_rr_all
cd admixture_nohwe_rr_all

for SEED in {1..25}
do
for K in 1 2 3 4 5 6 7 8;
do
echo working with k=$K seed=${SEED};
admixture -s ${SEED} -B -j16 --cv ../bwaaln_filter_allsample_rr_hwe_nofamily.bed $K | tee log${K}.${SEED}.all.out; done
mv bwaaln_filter_allsample_rr_hwe_nofamily.2.Q bwaaln_filter_allsample_rr_hwe_nofamily${SEED}.2.Q
mv bwaaln_filter_allsample_rr_hwe_nofamily.3.Q bwaaln_filter_allsample_rr_hwe_nofamily${SEED}.3.Q
done;

grep -h CV log*.all.out > x.log_all.txt

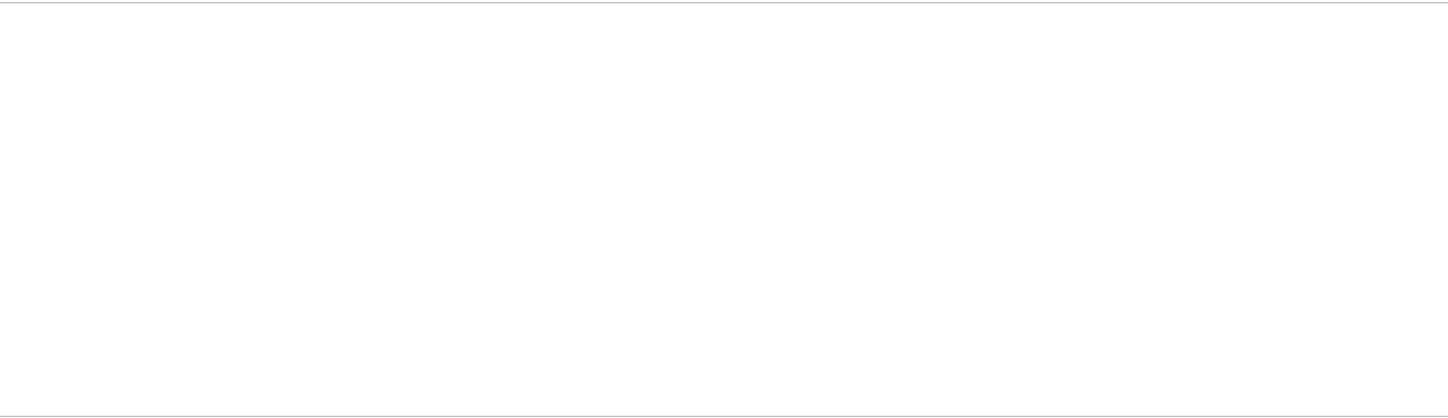
for i in {1..25}
do
cp bwaaln_filter_allsample_rr_hwe_nofamily${i}.2.Q bwaaln_filter_allsample_rr_hwe_nofamily${i}.2.meanQ
done

zip bwaaln_filter_allsample_rr_hwe_nofamily_K2_mean.zip bwaaln_filter_allsample_rr_hwe_nofamily*.2.meanQ
```

Colours: cornflowerblue, palevioletred

```
cp bwaaln_filter_allsample_rr_nofamily.2.Q bwaaln_filter_allsample_rr_nofamily.2.meanQ
```

```
python distruct2.3.py -K 2 --input=clumpak_average --output=clumpak_average --title="All Pops K=2" --popfile=distruct_pop_all_nofamily --poporder=distruct_pop_all_nofamily
```



UK HIST

```
cd /srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR

vcftools --vcf bwaaln_filter_allsample_rr_hwe_nofamily.recode.vcf --keep keepind_ukhist.txt --out bwaaln_filter_allsample_rr_hwe_nofamily_ukhist --recode

vcftools --vcf bwaaln_filter_allsample_rr_hwe_nofamily_ukhist.recode.vcf --out bwaaln_filter_allsample_rr_hwe_nofamily_ukhist.plink --plink

plink --file bwaaln_filter_allsample_rr_hwe_nofamily_ukhist.plink --make-bed --noweb --out bwaaln_filter_allsample_rr_hwe_nofamily_ukhist
```

```
mkdir admixture_nohwe_rr_ukhs
cd admixture_nohwe_rr_ukhs

for SEED in {1..25}
do
for K in 1 2 3 4 5 6 7 8;
do
echo working with k=$K seed=${SEED};
admixture -s ${SEED} -B -j16 --cv ../bwaaln_filter_allsample_rr_hwe_nofamily_ukhist.bed $K | tee log${K}.${SEED}.ukhs.out; done
mv bwaaln_filter_allsample_rr_hwe_nofamily_ukhist.2.Q bwaaln_filter_allsample_rr_hwe_nofamily_ukhist${SEED}.2.Q
mv bwaaln_filter_allsample_rr_hwe_nofamily_ukhist.3.Q bwaaln_filter_allsample_rr_hwe_nofamily_ukhist${SEED}.3.Q
done;
done

grep -h CV log*.ukhs.out > x.log_ukhs.txt

for i in {1..25}
do
cp bwaaln_filter_allsample_rr_hwe_nofamily_ukhist${i}.2.Q bwaaln_filter_allsample_rr_hwe_nofamily_ukhist${i}.2.meanQ
done

zip bwaaln_filter_allsample_rr_hwe_nofamily_ukhist_mean_K2.zip bwaaln_filter_allsample_rr_hwe_nofamily_ukhist*.2.meanQ
```

Colours: indianred, darksalmon

```
cp bwaaln_filter_allsample_m50_nofamily_ukhist_S1.2.Q bwaaln_filter_allsample_m50_nofamily_ukhist_S1.2.meanQ
cp bwaaln_filter_allsample_m50_nofamily_ukhist_S1.3.Q bwaaln_filter_allsample_m50_nofamily_ukhist_S1.3.meanQ

python distruct2.3.py -K 2 --input=clumpak_average_ukhs --output=clumpak_average_ukhs --title="ukhist Pops K=2" --popfile=distruct_pop_ukhist_nofam
```

AU

```
cd /srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR
vcftools --vcf bwaaln_filter_allsample_rr_hwe_nofamily.recode.vcf --keep keepind_au.txt --out bwaaln_filter_allsample_rr_hwe_nofamily_au --recode
vcftools --vcf bwaaln_filter_allsample_rr_hwe_nofamily_au.recode.vcf --out bwaaln_filter_allsample_rr_hwe_nofamily_au.plink --plink
plink --file bwaaln_filter_allsample_rr_hwe_nofamily_au.plink --make-bed --noweb --out bwaaln_filter_allsample_rr_hwe_nofamily_au
```

```
mkdir admixture_nohwe_rr_au
cd admixture_nohwe_rr_au

for SEED in {1..25}
do
for K in 1 2 3 4 5 6 7 8;
do
echo working with k=$K seed=S${SEED};
admixture -s ${SEED} -B -j16 --cv ../bwaaln_filter_allsample_rr_hwe_nofamily_au.bed $K | tee log${K}.S${SEED}.au.out; done
mv bwaaln_filter_allsample_rr_hwe_nofamily_au.2.Q bwaaln_filter_allsample_rr_hwe_nofamily_au${SEED}.2.Q
mv bwaaln_filter_allsample_rr_hwe_nofamily_au.3.Q bwaaln_filter_allsample_rr_hwe_nofamily_au${SEED}.3.Q
done;
done

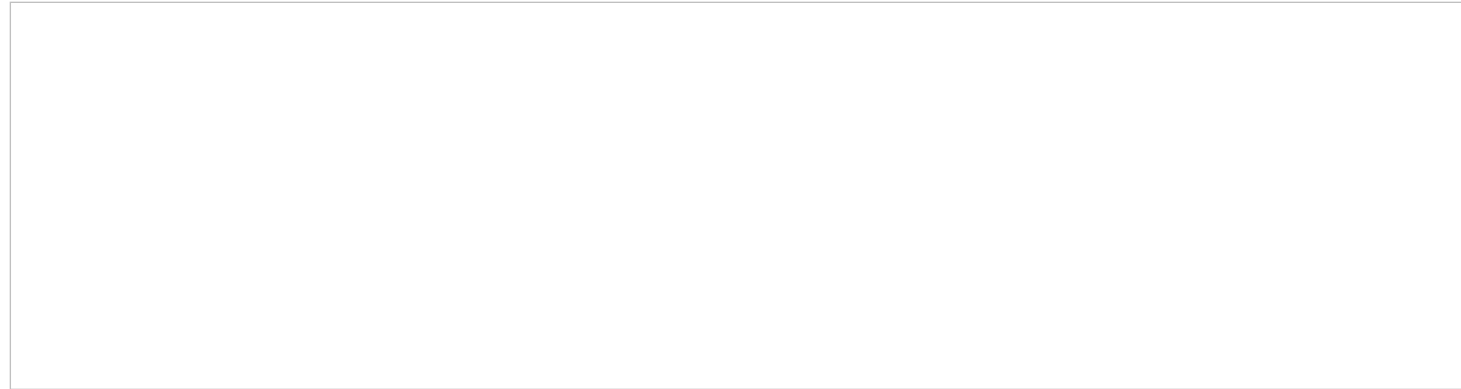
grep -h CV log*.au.out > x.log_au.txt

for i in {1..25}
do
cp bwaaln_filter_allsample_rr_hwe_nofamily_au${i}.2.Q bwaaln_filter_allsample_rr_hwe_nofamily_au${i}.2.meanQ
done

zip bwaaln_filter_allsample_rr_hwe_nofamily_au_mean_K2.zip bwaaln_filter_allsample_rr_hwe_nofamily_au*.2.meanQ
```

Colours: lightsteelblue, steelblue

```
cp bwaaln_filter_allsample_m50_nofamily_au_S1.2.Q bwaaln_filter_allsample_m50_nofamily_au_S1.2.meanQ
python distruct2.3.py -K 2 --input=clumpak_average_au --output=clumpak_average_au --title="au Pops K=2" --popfile=distruct_pop_au_nofamily --poporc
```

Distance Heatmaps

```
cd /srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR

module load bioconductor/3.10

R

setwd("/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR")

library(qvalue)
library(SNPRelate)
library(dartR)
library(radiator)

svulgaris <- genomic_converter(data = "/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR/bwaaln_filter_allsample_rr_hwe_nofamily.bed",

svulgaris <- genomic_converter(data = "bwaaln_filter_allsample_rr_hwe_nofamily.recode.vcf", strata = "sample_names.tsv", output = c("genind", "genlight"), filter.common.markers =

radiator::detect_genomic_format(data = "bwaaln_filter_allsample_rr_hwe_nofamily.recode.vcf")

svulgaris <- genomic_converter(data = "/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR/bwaaln_filter_allsample_rr_hwe_nofamily.recode

names(svulgaris)

svulgaris$genlight
```

```
library(StAMPP)

Dist <- stamppNeisD(svulgaris$genlight, pop = TRUE)

write.csv(Dist,'heatmap_dist.csv')
```

	[,1]	[,2]	[,3]	[,4]	[,5]	[,6]
Antwerp	0.000000	0.014445	0.023202	0.012841	0.014068	0.020350
Historic	0.014445	0.000000	0.024833	0.015568	0.016183	0.022116
McLarenVale	0.023202	0.024833	0.000000	0.024466	0.025526	0.019075
MonksWood	0.012841	0.015568	0.024466	0.000000	0.014691	0.022156
Newcastle	0.014068	0.016183	0.025526	0.014691	0.000000	0.022859
Orange	0.020350	0.022116	0.019075	0.022156	0.022859	0.000000

```
library(StAMPP)
```

```
Fst <- stamppFst(svulgaris$genlight, nboots=1, percent=95, nclusters=1)
write.csv(Fst,'heatmap_fst.csv')
```

```
starling.freq <- stamppConvert(svulgaris$genlight, "genlight")
```

```
starling.fst <- stamppFst(starling.freq, 100, 95, 1)
```

```
names(starling.fst)
```

```
starling.fst$Pvalues
```

```
> Fst
```

	Antwerp	Historic	McLarenVale	MonksWood	Newcastle	Orange
Antwerp	NA	NA	NA	NA	NA	NA
Historic	0.004414411	NA	NA	NA	NA	NA
McLarenVale	0.050759977	0.0441591276	NA	NA	NA	NA
MonksWood	0.004596349	-0.0002042719	0.04663636	NA	NA	NA
Newcastle	0.009223553	0.0024359418	0.05014725	0.00215079	NA	NA
Orange	0.038083996	0.0315725651	0.03405249	0.03565069	0.03803102	NA

```
> starling.fst$Pvalues
```

	Antwerp	Historic	McLarenVale	MonksWood	Newcastle	Orange
Antwerp	NA	NA	NA	NA	NA	NA
Historic	0.02	NA	NA	NA	NA	NA
McLarenVale	0.00	0.00	NA	NA	NA	NA
MonksWood	0.00	0.49	0	NA	NA	NA
Newcastle	0.00	0.11	0	0.1	NA	NA
Orange	0.00	0.00	0	0.0	0	NA

```
module load R/3.5.3
```

```
R
```

```
library(reshape2)
```

```
setwd("/srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_RR")
```

```
heatmapdata_dist <- read.csv("heatmap_dist.csv", header=TRUE)
```

```
melted_heatmapdata_dist <- melt(heatmapdata_dist)
```

```
head(melted_heatmapdata_dist)
```

```
melted_heatmapdata_dist$valueR <- round(melted_heatmapdata_dist$value, digits=3)
```

```
heatmapdata_fst <- read.csv("heatmap_fst.csv", header=TRUE)
```

```
melted_heatmapdata_fst <- melt(heatmapdata_fst)
```

```
melted_heatmapdata_fst$valueR <- round(melted_heatmapdata_fst$value, digits=3)
```

```
library(reshape2)
```

```
library(ggplot2)
```

```
pdf("Sv4_heatmap2.pdf")
```

```
ggplot(data = melted_heatmapdata_dist, aes(X, variable, fill = value))+ geom_tile(color = "white")+ scale_fill_gradien
```

```
ggplot(data = melted_heatmapdata_dist, aes(X, variable, fill = value))+ geom_tile(color = "white")+ scale_fill_gradien
```

```
ggplot(data = melted_heatmapdata_fst, aes(X, variable, fill = valueR))+ geom_tile(color = "white")+ scale_fill_gradier
```

```
ggplot(data = melted_heatmapdata_fst, aes(X, variable, fill = valueR))+ geom_tile(color = "white")+ scale_fill_gradier
```

```
dev.off()
```

FST

```
cd /srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/analysis/popgen_summary/relatedness_m50
```

```
populations -V bwaaln_filter_allsample_hwe_m50_nofamily.recode.vcf -M /srv/scratch/z5188231/KStuart.Starling-Aug18/Sv4_Historic/processing/align
```

Found 23683 SNP records in file 'bwaaln_filter_allsample_hwe_m50_nofamily.recode.vcf'. (Skipped 0 already filtered-out SNPs and 0 non-SNP records ; n

Removed 0 loci that did not pass sample/population constraints from 23683 loci.

Kept 23676 loci, composed of 23676 sites; 0 of those sites were filtered, 23661 variant sites remained.

23674 genomic sites, of which 2 were covered by multiple loci (0.0%).

Mean genotyped sites per locus: 1.00bp (stderr 0.00).

Population summary statistics (more detail in populations.sumstats_summary.tsv):

aw: 13.749 samples per locus; pi: 0.21756; all/variant/polymorphic sites: 23671/23656/19618; private alleles: 218

hist: 5.2734 samples per locus; pi: 0.19583; all/variant/polymorphic sites: 4983/4982/2724; private alleles: 5

mv: 13.24 samples per locus; pi: 0.21058; all/variant/polymorphic sites: 23653/23638/17081; private alleles: 152

mw: 8.6589 samples per locus; pi: 0.22118; all/variant/polymorphic sites: 23655/23640/17844; private alleles: 111

nc: 9.3786 samples per locus; pi: 0.22066; all/variant/polymorphic sites: 23669/23654/18236; private alleles: 173

or: 13.052 samples per locus; pi: 0.21559; all/variant/polymorphic sites: 23665/23650/18257; private alleles: 159

Population pair divergence statistics (more in populations.fst_summary.tsv and populations.phistats_summary.tsv):

aw-hist: mean Fst: 0.030688; mean Phi_st: -0.017604; mean Fst': 0.004114

aw-mv: mean Fst: 0.042079; mean Phi_st: 0.040506; mean Fst': 0.018818

aw-mw: mean Fst: 0.027795; mean Phi_st: 0.0062614; mean Fst': 0.0030077

aw-nc: mean Fst: 0.027347; mean Phi_st: 0.0077804; mean Fst': 0.0035807

aw-or: mean Fst: 0.0366; mean Phi_st: 0.030789; mean Fst': 0.014048

hist-mv: mean Fst: 0.049756; mean Phi_st: 0.03015; mean Fst': 0.019615

hist-mw: mean Fst: 0.039598; mean Phi_st: -0.016764; mean Fst': 0.0039998

hist-nc: mean Fst: 0.040686; mean Phi_st: -0.012226; mean Fst': 0.0049291

hist-or: mean Fst: 0.044364; mean Phi_st: 0.010644; mean Fst': 0.014166

mv-mw: mean Fst: 0.045676; mean Phi_st: 0.038531; mean Fst': 0.01771

mv-nc: mean Fst: 0.046063; mean Phi_st: 0.041261; mean Fst': 0.018929

mv-or: mean Fst: 0.035262; mean Phi_st: 0.027324; mean Fst': 0.01285

mw-nc: mean Fst: 0.032054; mean Phi_st: 0.0025108; mean Fst': 0.0017383

mw-or: mean Fst: 0.039727; mean Phi_st: 0.027767; mean Fst': 0.012899

nc-or: mean Fst: 0.039878; mean Phi_st: 0.030143; mean Fst': 0.013821