

VCFPOP V1.08 User Manual

Performing population genetics analyses based on NGS data for haploids, diploids, polyploids, aneuploids and mixed-ploidy populations.

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1 Quick start

1.1 A brief introduction

VCFPOP performs population genetics analyses based on the NGS genotype data (with the VCF and BCF formats), which can also support other genotype formats, such as GENEPOL (Rousset 2008) and STRUCTURE (Pritchard *et al.* 2000). Existing methods for population genetics analyses are restricted to diploids. VCFPOP extends these methods to include haploids, polyploids and aneuploids, and supports a maximum ploidy level of 10. For even ploidy levels, the genotypic frequencies are obtained under the double-reduction equilibrium (Huang *et al.* 2019).

The functions of VCFPOP consist of:

1. Variant information, individual, genotype and locus filters;
2. Differentiation, divergence, diversity, evolutionary parameters, inbreeding statistics for sliding windows;
3. Linkage-disequilibrium estimates between blocks;
4. Linkage-disequilibrium decay as distance;
5. Genome-wise association study;
6. Haplotype extraction from phased genotypes;
7. File format conversion;
8. Genetic diversity index estimation and genotypic equilibrium test;
9. Individual statistics estimation (inbreeding coefficient, kinship coefficient, heterozygosity);
10. Genetic differentiation index estimation and test;
11. Genetic distance estimation;
12. Analysis of molecular variances;
13. Population assignment;
14. Relatedness coefficient estimation;

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15. Kinship coefficient estimation;
16. Principal coordinate analysis;
17. Hierarchical clustering;
18. Bayesian clustering.

The pipeline of VCFPOP is shown in Figure 1 and an example of visualized results are shown in Figure 2.

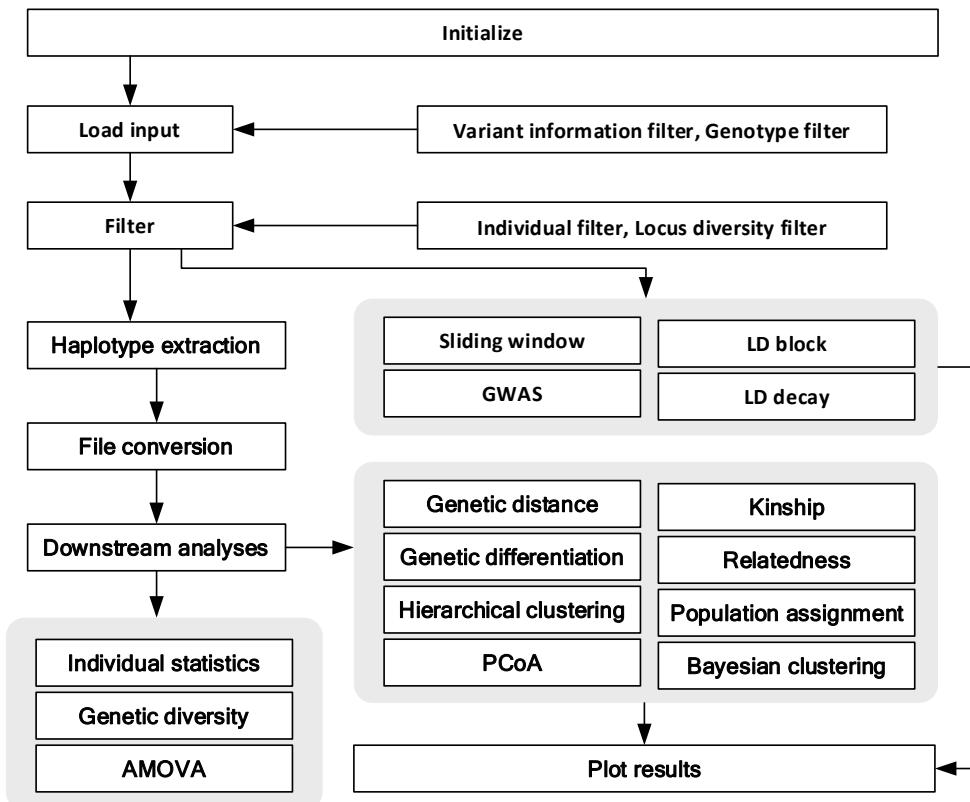


Figure 1. Pipeline of VCFPOP

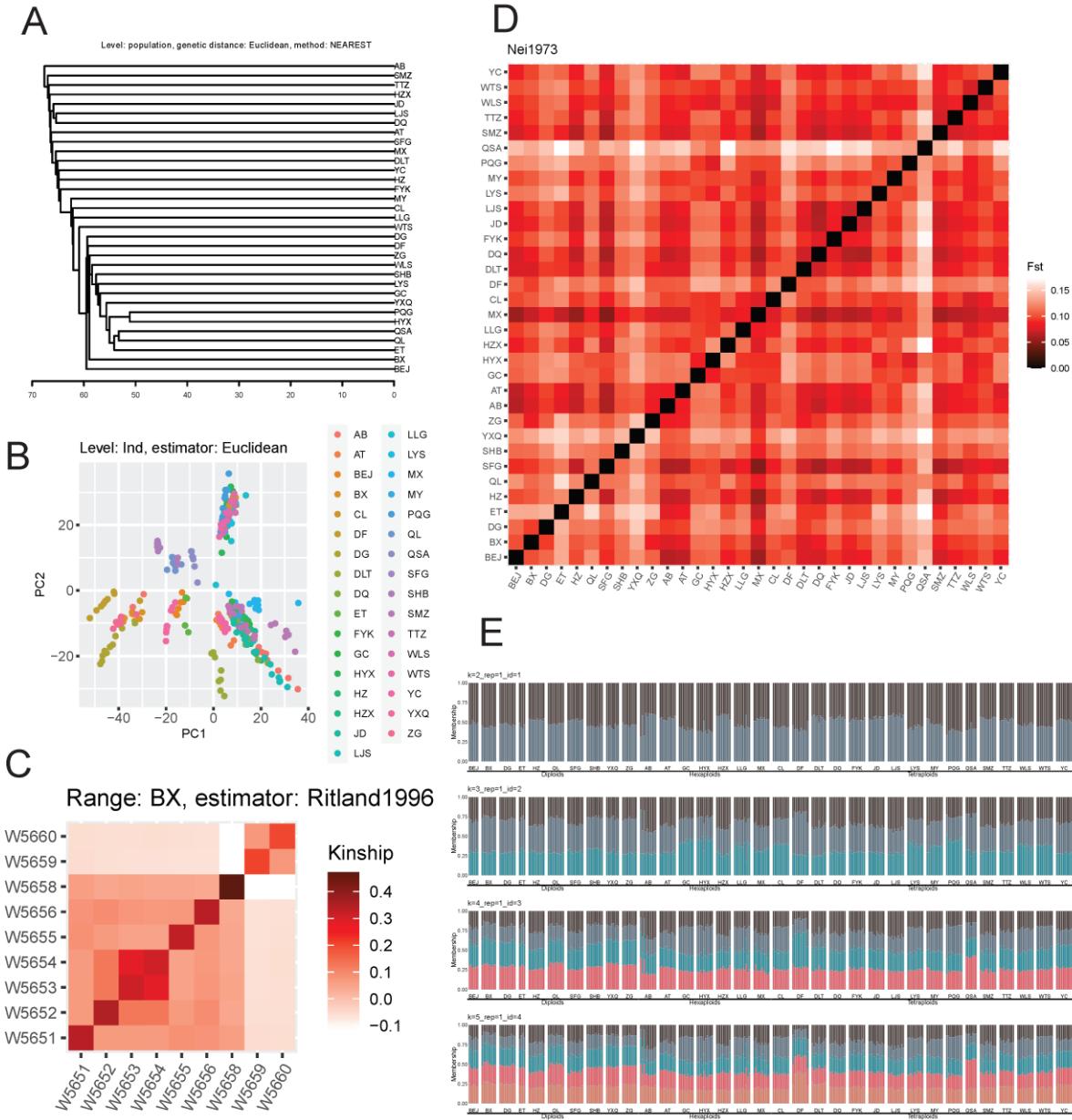


Figure 2. Example visualized results. A: Hierarchical clustering for populations; B: PCoA of individuals; C: kinship between individuals within a population; D: Nei's (1973) G_{ST} between populations; E: Admixture proportions of Bayesian clustering applying the filters and the ADMIX model.

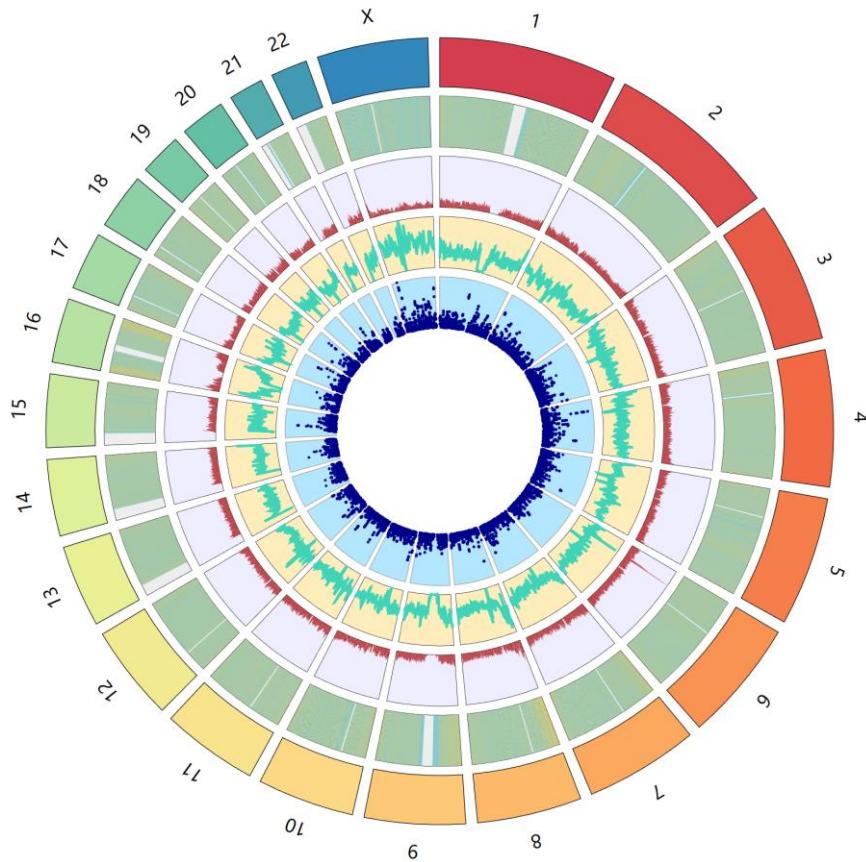


Figure 3. Example visualized results for sliding windows, which is generated from 1000 genome phase3 datasets. The circles from inner-most to outer-most were dot plot, line plot, bar plot and heatmap.

1.2 Run the example

There are some examples input files and parameters files. The following steps shows how to run examples. The times expense of analyses depends on the input file, for the polygene, human1G and willowherb1G dataset, it needs about 10 seconds, 3 minutes and 5 minutes, respectively.

Windows:

- (i) Rename one binary executables in the bin folder to 'vcfpop.exe', either

'vcfpop.msvc.exe or vcfpop.msvc clang.exe.

- (ii) Open the 'example' folder and double-click 'run.windows.bat'.
- (iii) Wait until finish, and the results are placed in 'polygene', 'human1G', and 'willowherb1G' folders in the 'example' folder. Press Ctrl+C to abort.

Linux or Mac OS X

- (i) Rename the correct version of binary executables in the 'bin' folder to 'vcfpop'.
- (ii) Open terminal and change to the 'example' folder
- (iii) Execute the following command to allow executable permissions for the binary executable.
`chmod 777 .../bin/vcfpop`
- (iv) Change to the example folder, copy and paste all lines (including the blank line at the end) in the 'run.linux.mac.txt':
`..../bin/vcfpop -p=par.polygene.txt`
`..../bin/vcfpop -p=par.human1G.txt`
`..../bin/vcfpop -p=par.willowherb1G.txt`
`# here is the blank line`
- (v) Wait until finish, and the results are placed in 'polygene', 'human1G', and 'willowherb1G' folders in the 'example' folder. Press Ctrl+C to abort.

1.3 Minimum steps to run

In order to run the software, the user should apply the following three steps:

- (iv) Setup the software. For Windows and Ubuntu platform, download the zip package and extract all the files to a folder. For other operation systems, users need to compile the executables. See Section [3.3](#) for details.
- (v) Prepare the input file. This can be VCF format V4 and BCF format V2 (Danecek *et al.* 2011), GENEPOL (Rousset 2008), SPAGEDI (Hardy & Vekemans 2002), CERVUS (Kalinowski *et al.*

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2007), ARLEQUIN (Excoffier & Lischer 2010), STRUCTURE (Pritchard *et al.* 2000), POLYGENE (Huang *et al.* 2020), POLYRELATEDNESS (Huang *et al.* 2015a), GENODIVE (Meirmans & Tienderen 2004) and PLINK (Purcell *et al.* 2007). All these input files can be compressed by GZIP with the extension *.gz.

- (vi) Use command line to run VCFPOP or write all parameters in a parameter file and use the following command to use the parameters

```
vcfpop -p=pars.txt
```

For parameters with spaces, use double-quotes to embrace them, e.g.,

```
-p="c:\my data\pars.txt" or "-p=c:\my data\pars.txt"
```

Because the command line does not allow line breaks, use #n as the escape character. An example of parameters and their descriptions are shown as follow:

-g_input=1M.vcf.gz	Input file is 1M.vcf.gz
-g_format=vcf	Input format is vcf
-g_output=1M.out	Output prefix is 1M.out
-g_decimal=6	Output real number by 6 decimal places
-g_nthread=4	Run vcfpop using 4 threads
-g_indtext="pop1:#1-#500#n pop2:#501-#1000#n pop3:#1001-#1500#n pop4:#1501-#2000#n pop5:#2001-#2504#n #REG#n reg1:#1-#2#n reg2:#3-#5"	Assign population structure, #n is not required in the parameter file pop1 contains #1 to #500 individuals in the input file pop2 contains #501 to #1000 individuals in the input file pop3 contains #1001 to #1500 individuals in the input file pop4 contains #1501 to #2000 individuals in the input file pop5 contains #2001 to #2504 individuals in the input file #REG#n reg1 contains pop1 and pop2 reg2 contains pop3 to pop5
-f	Enable filters
-f_type=snp	Use SNPs
-f_ptype=[0.5,1]	Use loci with a typed ratio in [0.5,1]
-f_bmaf=[0.1,0.5]	Use loci with a minor allele frequency in [0.1,0.5] (inapplicable for multi-allelic locus)
-haplotype	Enable haplotype extraction

-haplotype_variants=[2,3]	Use 2-3 adjacent variants as a locus
-haplotype_alleles=[2,999]	#alleles at this locus should in [2,999]
-xconvert	Disable file conversion
-convert_format=polyrelatedness	Convert into polyrelatedness format
-diversity	Enable diversity estimation
-diversity_level=pop,reg,tot, popXloc,regXloc,totXloc	Estimate diversity for each population, each region and the total population at all loci and at each locus
-diversity_model=rcs	Using RCS model to test the distribution of genotypes
-indstat	Enable individual statistics estimation
-indstat_ref=pop	Use current pop as the reference pop
-indstat_estimator=Ritland1996	Use Ritland 1996 kinship estimator
-indstat_model=prcs	Use PRCS model for genotypic frequencies
-indstat_locus=all,each	Estimate for all loci or for each locus
-indstat_type=hidx,lnpd,f,theta	Estimate heterozygosity index, multi- locus genotypic frequency, inbreeding coefficient, and kinship coefficient
-fst	Enable differentiation estimation
-fst_estimator=Nei1973	Use Nei 1973 estimator
-fst_level=popXtot,pop	Estimate differentiation among all pops and between any two pops
-fst_locus=all,each	Estimate and test differentiation for all loci or for each locus
-fst_test=allele	Testing the distribution of alleles
-fst_fmt=matrix	Output results using matrix format
-gdist	Enable genetic distance estimation
-gdist_level=pop	Estimate genetic distance between pops

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-gdist_estimator=Nei1972	Use Nei 1972 estimator
-gdist_fmt=matrix	Output results using matrix format
-amova	Enable AMOVA
-amova_method=homoploid	Use homoploid method
-amova_mutation=iam	Use IAM distance between alleles
-amova_ind=yes	Include individual as a level
-amova_test=no	Do not test each variance components
-amova_nperm=99	Use 99 permutations
-amova_printss=yes	Output Sum of Squares for each population
-popas	Enable population assignment
-popas_model=prcs	Use PRCS model for genotypic frequencies
-popas_level=pop,reg	Assign individual to a pop and a region
-popas_error=0.05	Use a mistype rate of 0.05
-relatedness	Enable relatedness estimation
-relatedness_range=pop	Estimate between inds in the same pop
-relatedness_fmt=matrix	Output results using matrix format
-relatedness_estimator=Huang 2014,Huang2015	Use Huang2014 and Huang2015 estimators
-kinship	Enable kinship estimation
-kinship_fmt=matrix,table	Output with matrix and table format
-kinship_range=total	Estimate between any two individuals
-kinship_estimator=Ritland1996, Loiselle1995,Weir1996, VanRaden2 008	Use three estimators
-pcoa	Enable PCoA
-pcoa_level=pop,ind	Ordinate individual or population
-pcoa_estimator=Euclidean	Use Euclidean genetic distance

		Enable hierarchical clustering
-cluster		Use nearest clustering method
-cluster_method=NEAREST		Use Cavalli-Sforza1967 genetic distance
-cluster_estimator=Cavalli-Sforza1967		Cluster populations
-cluster_level=pop		
		Enable Bayesian clustering
-structure		Admix model is not used
-structure_admix=no		Locpriori model is not used
-structure_locpriori=no		F model is not used
-structure_f=no		
		Do not update lambda
-structure_inferlambda=no		Use the same lambda for all clusters
-structure_difflambda=no		
		Use uniform prior distribution for alpha
-structure_uniformalpha=yes		Update alpha
-structure_inferalpha=yes		Use the same alpha for all clusters
-structure_diffalpha=no		Use different Fst for different clusters
-structure_singlef=no		
		Ranges of number of clusters
-structure_krange=[2,6]		Discard first 1000 iterations
-structure_nburnin=1000		Record for 10000 iterations
-structure_nreps=10000		Sampling interval to dememorize
-structure_nthinning=10		Number of independent runs for each K
-structure_nrungs=6		

- (vii) The population structure can be configured by -gindtext or -g_indtab options, where VCFPOP supports multi-level region definition. An example is as follows.

-g_indtab="ind1	pop1	reg1	Total
ind2	pop1	reg1	Total
ind3	pop1	reg1	Total
ind4	pop2	reg1	Total
ind5	pop2	reg1	Total
ind6	pop2	reg1	Total
ind7	pop3	reg2	Total
ind8	pop3	reg2	Total
ind9	pop3	reg2	Total
ind10	pop4	reg2	Total

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	ind11	pop4	reg2	Total
	ind12	pop4	reg2	Total"

- (viii) The results are saved in *.func.txt. Use a text editor to view the results or paste the results into a spread sheet.

2 System Requirement & Limitations

- CPU: x64 compatible
- OS: Windows, Linux and Mac OS X
- Memory: 8 Gb, more memory maybe required when large dataset is processed
- Hard drive: 4 Gb
- GPU: Nvidia 1050 or later (GPU acceleration is suggested only for large dataset >1GiB)

- Maximum ploidy level: 10 (support aneuploids)
- Maximum number of loci: 4294967295
- Maximum number of individuals: 4294967295
- Maximum number of populations: 4294967295
- Maximum number of regions: 4294967295
- Maximum number of alleles at each locus: 65535
- Maximum number of genotypes at each locus: 4194303

3 Download, Setup, Compile & Uninstall

3.1 Download

The precompiled binary executables (for Windows, Ubuntu and Mac OS X) and the source code can be download via <https://github.com/huangkang1987/vcfpop>.

3.2 Compilation

For Windows/Ubuntu/Mac OS X, the precompiled binary executables are provided. For other operation systems, the users can compile VCFPOP on their own. Note that the CPU should be either amd64 or arm64, where Appel M-Series CPU is arm64 compatible.

To compile the software on Linux, the following compiler or libraries are required.

- GCC <https://gcc.gnu.org>
- Clang <https://clang.llvm.org>
- NVCC <https://developer.nvidia.com/cuda-toolkit>

Where the version of GCC and Clang must be above 10 so as to use AVX-512 instructions and C++20 features. NVCC is used to compile `structureCUDA.cpp` and perform Bayesian Clustering by GPUs. If it is not required, `makefile.*.nocuda` can be used.

I will use Ubuntu command in the following as examples. The following tools are required: Make, CMake, Git.

```
sudo apt install make cmake git
```

The following libraries are required: `armadillo`, `openblas`, `omp`, `zlib`, `gcem` and `stats`. Because I have encountered several bugs when installing them from repositories using apt/brew, the latest versions for compilers, tools and libraries are suggested to be used. The commands to download the latest source code, to compile and to install them will be shown.

Zlib and OpenMP can be installed from repositories:

```
sudo apt install libomp-dev zlib1g-dev
```

For `armadillo`:

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```
git clone https://gitlab.com/conradsnicta/armadillo-code.git ./armadillo  
sudo cp armadillo/include /usr/local/ -r
```

For gcem:

```
git clone https://github.com/kthohr/gcem ./gcem  
sudo cp gcem/include /usr/local/ -r
```

For stats:

```
git clone https://github.com/kthohr/stats ./stats  
sudo cp stats/include /usr/local/ -r
```

For openblas (Mac OS X users can skip since they have framework Accelerate):

```
git clone https://github.com/xianyi/OpenBLAS.git ./OpenBLAS  
cd OpenBLAS  
make MAX_THREADS=64 NUM_THREADS=64 -j8  
sudo make PREFIX=/usr/local install
```

After that, use terminal to enter the `src` folder and run the command

```
make -j8 -f makefile.linux.gcc
```

where `makefile.linux.gcc` can be replaced with that suitable file for current operation system and compiler, and wait for a few minutes. It noteworthy that some modifications may be required in the file if there are errors. For example, change the path of the compilers, change the include path and library path of these libraries if they are not placed in the default path. The compilation requires a large amount of memory (8 Gib) to optimize these codes. After finish, run the following command

```
make install
```

to copy the compiled binary executable to the `bin` folder.

For Windows, by using the same method as Linux, the compilation can be performed under the following environments:

- Cygwin <https://www.cygwin.com>

- MinGW <http://www.mingw.org>
- MSYS2 <https://www.msys2.org>
- Windows Subsystem for Linux <https://ubuntu.com/desktop/wsl>

For Microsoft Visual Studio, the version should at least be 2019, and the LLVM-Clang extension should be installed. Double-click `makefile_msvc_clang.bat` and wait a few minutes. The program `vcfpop.msvc clang.exe` will be generated. Also, some modifications should be made in the `bat` file, for example, the install path of Visual Studio and CUDA.

3.3 Setup

To setup the software, please create a folder on your disk, and then extract the files to the folder.

The figure plotting function rely on R, please install R and

3.4 Launch and symbolic link

For Linux, open the command mode (bash). Then switch to the `bin` folder, and execute the command `./vcfpop`.

For Windows, press WINDOWS + R and run `cmd` to open the command mode. Then switch to the install path of VCFPOP, and execute the command `vcfpop.exe`.

The user can also launch VCFPOP in other directory by adding the install path before the command, such as the path `~/Desktop/vcfpop/bin/vcfpop` or `c:\vcfpop\bin\vcfpop.exe`.

For Linux, the symbolic link enables the user to run VCFPOP at any directory without adding the

install path, and the symbolic link can be added by:

```
ln -s -f vcfpop /usr/local/bin/vcfpop
```

For Windows, add the install path to the environment variable is the same as the symbolic link.

These can be done by the following procedure:

1. open the menu 'START', right click the icon 'This PC' and click 'Properties';
2. select 'Advanced system settings' to open the dialog box 'System properties';
3. click the button 'Environment Variables' to open the dialog box 'Environment Variables';
4. select the item 'PATH' in the top list of 'User Variable for XX', and then click the button 'Edit';
5. click the button 'New' and paste the install path into the dialog box, and then click 'OK' three times to exit.

3.5 Uninstall

To uninstall VCFPOP, simply delete the appropriate folder.

For Linux, if the symbolic link is created, the command '`rm -rf /usr/local/bin/vcfpop`' can be used to delete the symbolic link.

For Windows, to delete the environment variable, the former four steps in the previous section need to be conducted. Then select the install path, and click the button 'Delete'. Finally, click 'OK' three times.

3.6 R packages

The figures are plotting using R, the scripts to generate these figures are placed in `rscripts` folder,

`-g_rsciprt` can set the path of `rscript`. The following command can install R in Ubuntu:

```
sudo apt install r-base
```

The following R packages are required.

- `ggplot2`: <https://cran.r-project.org/package=ggplot2>
- `ggplotify`: <https://cran.r-project.org/package=ggplotify>
- `grid`: <https://cran.r-project.org/package=grid>
- `cowplot`: <https://cran.r-project.org/package=cowplot>
- `heatmaply`: <https://cran.r-project.org/package=heatmaply>
- `ggh4x`: <https://cran.r-project.org/package=ggh4x>
- `paletteer`: <https://cran.r-project.org/package=paletteer>
- `ape`: <https://cran.r-project.org/package=ape>
- `BioCircos`: <https://cran.r-project.org/package=BioCircos>
- `htmlwidgets`: <https://cran.r-project.org/package=htmlwidgets>
- `hash`: <https://cran.r-project.org/package=hash>
- `qqman`: <https://cran.r-project.org/package=qqman>
- `gtools`: <https://cran.r-project.org/package=gtools>

The scripts will automatically install libraries in Windows, while Linux users will need permissions to write to `/usr/local/lib/R/site-library`, so they are suggested to manually install these packages. First, run R as administrator by

```
sudo R
```

Run the following R commands to install packages

```
install.packages(c('ggplot2','ggplotify','grid','cowplot','heatmaply','ggh4x','paletteer','ape','BioCircos','htmlwidgets','hash','qqman','gtools'))
```

Finally, type `q()` to exit R.

4 Usage

4.1 Input file format

VCFPOP supports VCF format V4.x and BCF format V2.x (Danecek *et al.* 2011). Because VCFPOP does not use the information that precedes the header row ('#Chrom'), the obsolete or non-standard VCF/BCF files are also supported.

VCFPOP also supports some additional genotype formats but does not optimize their load speeds: GENEPOLY (Rousset 2008), SPAGEDI (Hardy & Vekemans 2002), CERVUS (Kalinowski *et al.* 2007), ARLEQUIN (Excoffier & Lischer 2010), STRUCTURE (Pritchard *et al.* 2000), POLYGENE (Huang *et al.* 2020), POLYRELATEDNESS (Huang *et al.* 2015a), GENODIVE (Meirmans & Tienderen 2004) and PLINK (Purcell *et al.* 2007). All these input files can be compressed by GZIP.

4.2 Command overviews

Use the command `vcfpop -h` to view the help information, or type the command `vcfpop -h -function_name` to view the detail information for specific functions. For example, the command `vcfpop -h -g` is able to show the detail command and description of general settings.

The names of the functions are listed below.

-g	General settings
-f	Filter for individual, locus, or genotype
-haplotype	Haplotype extraction
-convert	File conversion
-diversity	Genetic diversity indices
-indstat	Individual statistics
-fst	Genetic differentiation

-gdist	Genetic distance
-amova	Analysis of molecular variance
-popas	Population assignment
-relatedness	Relatedness coefficient estimation
-kinship	Kinship coefficient estimation
-pcoa	Principal coordinate analysis
-cluster	Hierarchical clustering
-structure	Bayesian clustering

To perform a specific function, use the command `vcfpop *-function_name -parameters*'. For example, the following command convert the input file into GENEPOP format:

```
vcfpop -convert -convert_format=genepop
```

The parameters are separated by spaces. If there are spaces inside a parameter (e.g., path), use double-quotes to embrace this parameter. Because there are too many parameters, it is troublesome to type them one by one in the console. Therefore, VCFPOP allows the user to write all parameters in a text file and use the command *'-p=parameter_file'* to run VCFPOP. For example, the command *'vcfpop -p=pars.txt'* uses all parameters configured in *'pars.txt'*. Parameters can be either in the command or in the parameter file, e.g., *'vcfpop -g_input=in.txt -p=pars.txt-g_format=vcf'*.

The line break can be used as the parameter separator in the parameter set file.

The data types used in parameters are:

integer	e.g., <i>-g_decimal=3</i>
real	e.g., <i>-popas_error=0</i>
string	e.g., <i>-g_scientific=yes</i>
integer range	e.g., <i>-haplotype_alleles=[2,10]</i>

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```
real range      e.g., -f_qual=[50,70]
```

Some parameters are optional. For example, if the filter `-f_qual` (used to exclude low quality variants) is not configured, it will not be applied. Some parameters have their default values. If they are not configured, the default values will be used. For example, the default option is `avx` for the parameter

```
-g_simd=none|sse|avx|avx512, integer, default:avx
```

For parameters with multiple selections, multiple values can be used and these values should be separated by commas. For this situation, the calculation will be performed for each value. For example, the following command will convert the input file into both the GENEPOP format and the SPAGEDI format:

```
vcfpop -convert -convert_format=genepop,spagedi
```

4.3 General settings

General settings consist of the input and output files, the input and output styles, the temporary directory, the definitions of population, region and group, and also include various behaviors in calculations, such as the number of threads, the amount of memory used, the CPU instructions, and the random number generator seed. The parameters are listed below.

```
-g_decimal=0~15, integer, default:5
```

Decimal places of output real numbers.

```
-g_scientific=yes|no, string, default:no
```

Use scientific notation to output real numbers.

```
-g_nthread=1~4096, integer, default:4
```

Number of threads used in calculation.

-g_simd=none|neon, integer, default:neon (For arm64 CPU)

SIMD (Single Instruction Multiple Data) instruction sets to accelerate float point or vector operations, where neon can handle 128 bits simultaneously, respectively.

-g_simd=none|sse|avx|avx512, integer, default:avx (For amd64 CPU)

SIMD (Single Instruction Multiple Data) instruction sets to accelerate float point or vector operations, where mmx, sse (using SSE1.0 to SSE4.2 instructions), avx (using AVX, AVX2 and FMA instructions) and avx512 (using AVX512 F & BW instructions) can handle 64, 128, 256, 512 bits simultaneously, respectively.

-g_gpu=none|cuda, integer, default:none

Use GPU to accelerate the calculation (Bayesian clustering, and Eigen-value decomposition in PCoA and GWAS), will override some SIMD accelerations.

-g_float=single|double, integer, default:double

Float point number precision, double use 64 bits and float use 32 bits, where float is faster but in lower precision.

-g_fastsingle=yes|no, string, default:yes

Use single precision float point number in calculation (otherwise convert into double precision float point number before calculation).

-g_seed=0~2147483647, integer, default:0

Random number generator seed, 0 denotes using system time as the seed.

-g_tmpdir=path, string, default:current_directory

Directory placing the temporary files.

-g_progress=10~100000, integer, default:80

The number of characters used for the progress bar.

-g_input=file_path, string

Input file. Multiple VCF/BCF files using 'l' and '&' as the column and row separators,

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respectively, e.g., var1-4ind1-3.vcf|var1-4ind4-6.vcf&var5-9ind1-3.vcf|var5-9ind4-6.vcf. The 'FORMAT' field in each file should be equal.

-g_format=vcf|bcf|genepop|spagedi|cervus|arlequin|structure|polygene|polyrelatedness|genodive|plink, string, default:vcf

Input file format. The population and region should be defined in **g_indtext**, and the within input file will not be used. For GENEPOLY format, VCFPOP do not support extra information; for CERVUS, at most one extra column (population or sex) is allowed; for SPAGEDI, multiple extra columns (population or coordinate) are allowed; for STRUCTURE, the number of extra columns can be specified; for PLINK, the locus names are load from *.map file if it exists.

-g_locusname= chr|pos|chr_pos|chr_ref_alt|pos_ref_alt|chr_pos_ref_alt, string, default:chr_pos

Locus name definition, only applicable for VCF/BCF input files.

-g_extracol=0~4096, integer, default:0

Regarding the number of extra columns between individuals and genotypes in the STRUCTURE input file, if there is a header row for locus, the number of extra columns can be automatically detected.

-g_output=file_path, string, default:vcfpop.out

Prefix of output files.

-g_indtext=text, string, optional

This assigns the population of each individual and region of each population (where regions can be nested to perform multi-level AMOVA). If not specified, all individuals are assigned to a default population. A population (or a region) name begins with an identifier and a colon, the individuals (or populations) of this population (or region) are separated by commas. The individuals, populations, and regions not included are assigned to a default population or a default region. '#REG' is used to separate the regions at different levels. In a command line, #n can be used as a line break, whilst in parameter files, line breaks can be used. For example:

-g_indtext="pop1:ind1,ind2,ind3

```

pop2:#4-#6
pop3:ind7,ind8,ind9
pop4:ind10,ind11,ind12
#REG
reg1:#1-#2
reg2:pop3,pop4
#REG
Total:reg1,reg2"

```

-g_indtab=text, string, optional

Tabular format of `-g_indtext`. In command line, `#n` can be used as line break, while in parameter files, line breaks can be used:

<code>-g_indtab="ind1</code>	<code>pop1</code>	<code>reg1</code>	<code>Total</code>
<code>ind2 pop1</code>	<code>reg1</code>	<code>Total</code>	
<code>ind3 pop1</code>	<code>reg1</code>	<code>Total</code>	
<code>ind4 pop2</code>	<code>reg1</code>	<code>Total</code>	
<code>ind5 pop2</code>	<code>reg1</code>	<code>Total</code>	
<code>ind6 pop2</code>	<code>reg1</code>	<code>Total</code>	
<code>ind7 pop3</code>	<code>reg2</code>	<code>Total</code>	
<code>ind8 pop3</code>	<code>reg2</code>	<code>Total</code>	
<code>ind9 pop3</code>	<code>reg2</code>	<code>Total</code>	
<code>ind10 pop4</code>	<code>reg2</code>	<code>Total</code>	
<code>ind11 pop4</code>	<code>reg2</code>	<code>Total</code>	
<code>ind12 pop4</code>	<code>reg2</code>	<code>Total</code> "	

-g_delimiter=comma|tab, string, default:tab

Column delimiter style, where a comma is used in the CSV format, and a tab is used in the text editors.

-g_linebreak=unix|win, string, default:win

Line break style, where '\n' is used for unix, and '\r\n' is used for windows.

-g_rscript=file_path, string, optional

Path of Rscript binary for plotting results. E.g., 'C:\Program Files\R\R-4.4.0\bin\x64\Rscript.exe'.

-g_benchmark=yes|no, string, default:no

Evaluates the performance of each SIMD instructions set from mmx to specified type before

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calculation.

-g_replot=yes|no, string, default:yes

Plots figures for previously calculated results instead of analyzing data.

-g_eval=yes|no, string, default:yes

Evaluates the time expense for each function.

-g_missingploidy=1|2|3|4|5|6|7|8|9|10, string, multiple selections, optional

If the VCF/BCF file is combined from various VCF/BCF files for mixed-ploidy populations, the missing data may be extended from the called genotypes at this variant and the ploidy levels are misassigned. This option assigns the ploidy levels of the missing genotypes to the minimum ploidy level of the individual and is applicable for VCF/BCF input files. The value of this option should be all possible ploidy levels in the samples, e.g., `-g_missingploidy=2,4,6`.

-g_maxlen=0~9999999999999999, integer, default:9999999999999999

Max length of uncompressed data loaded from each VCF/BCF file. This option is used to load a part of data from big input files.

4.4 Filter

A filter can exclude variants of low quality, the individuals with a poor genotyping ratio, the genotypes of low quality and the variants of low genetic diversity. This step is performed before haplotype extraction and file conversion. The parameters of filters are listed as follows.

-f

Enable filters.

Variant information filters: this filter is applied during loading variants.

-f_qual=[min_val,max_val], real range, optional

Range of variant quality. If multiple VCF/BCF files are used, the variant is filtered when at least one QUAL field is out of the range. Only applicable to VCF/BCF input files.

-f_type=snp|indel|both, string, optional

Type of variants used in calculations. Only applicable to VCF/BCF input files.

-f_original=yes|no, string, optional

Use original filter of VCF/BCF file. If multiple VCF/BCF files are used, the variant is filtered when at least one original filter is not a 'PASS'. Only applicable to VCF/BCF input files.

-f_chrprefix=*chromosome_prefix*, string array, optional

Include the variants with certain chromosome prefixes. Multiple prefixes are separated by commas. Only applicable to VCF/BCF input files.

-f_chrname=*chromosome_identifier*, string array, optional

Include the variants with certain chromosome names. Multiple chromosomes are separated by commas. Only applicable to VCF/BCF input files.

Genotype filters: this filter is applied during loading genotypes, and an excluded genotype will be set as a missing genotype.

-f_dp=[min_val,max_val], integer range, optional

Range of sequencing depth. Only applicable to VCF/BCF input files.

-f_gq=[min_val,max_val], integer range, optional

Range of genotype quality. Only applicable to VCF/BCF input files.

-f_ploidy=[min_val,max_val], integer range, optional

Range of ploidy level for genotypes.

Individual filters: this filter is applied after the file is loaded.

-f_itype=[min_val,max_val], real range, optional

Range of typed ratio of an individual.

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-f_iploidy=[min_val,max_val], integer range, optional

Range of ploidy level for individuals.

Variant diversity filters: this filter is applied after the individual filter. The diversity index of each variant will be calculated in a specific population or region.

-f_pop=pop_identifier, string, default:total

Target population or region used to calculate diversity and apply diversity filters.

-f_bmaf=[min_val,max_val], real range, optional

Range of frequencies of minor alleles for biallelic loci.

-f_k=[min_val,max_val], integer range, optional

Range of number of alleles.

-f_n=[min_val,max_val], integer range, optional

Range of number of typed individuals.

-f_ptype=[min_val,max_val], real range, optional

Range of typed ratio of a locus.

-f_pval=[min_val,max_val], real range, optional

Range of P values in equilibrium tests.

-f_model=rcs|prcs|ces|pes, string, default:rcs

Double-reduction model to calculate genotypic frequencies for polyploids.

-f_he=[min_val,max_val], real range, optional

Range of expected heterozygosity.

-f_ho=[min_val,max_val], real range, optional

Range of observed heterozygosity.

-f_pic=[min_val,max_val], real range, optional

Range of polymorphic information content.

-f_ae=[min_val,max_val], real range, optional

Range of effective number of alleles.

-f_I=[min_val,max_val], real range, optional

Range of Shannon's information index.

-f_windowsize=1~1000000000, integer, optional

Uses the most polymorphic variant in a window; only applicable to VCF/BCF input files.

-f_windowstat=bmaf|k|n|ptype|he|ho|pic|ae|I, string, default:he

The diversity statistic used to select the most polymorphic variant in a window; only applicable to VCF/BCF input files.

4.5 Sliding window

Sliding window estimates some genetic diversity, differentiation and selection statistics for each sliding window. This function can only be used for VCF/BCF input files and is performed before haplotype extraction.

This function can only be used for VCF/BCF input files and is performed before haplotype extraction.

-slide

Estimates statistics for each sliding window. Results are saved in `*.slide.txt`.

-slide_plot=yes|no, string, default:no

Draws a circle figure for the results of sliding window. Results are saved in `*.slide.html`.

This is interactive and can be converted into PDF file with web browsers and PDF printers.

-slide_plot_columns=1~100, integer array, default:1,2,3,4

The columns used to draw the circles from innermost to outermost, supporting a maximum of five columns. The elements are separated by commas.

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-slide_plot_styles=dot|bar|line|heat, string array, default:dot,bar,line,heat

The styles for the circles from innermost to outermost. The options are dot (scatter plot), bar (bar plot), line (line chart), and heat (heat map).

-slide_windowsize=1000~1000000000, integer, default:1000000

Sliding window size.

-slide_windowstep=1000~1000000000, integer, default:100000

Sliding window step size, smaller size yields more smooth curves.

-slide_minvariants=10~1000000000, integer, default:100

Minimum number of variants a sliding window should consist to perform further calculations.

**-slide_estimator=Nei1973|Weir1984|Hudson1992|Hedrick2005|Jost2008|Huang2021_an
eu|dxy|pi|thetaw|TajimaD|fis|ho|he|pic|ae|I, string, multiple selections, def
ault:Nei1973|dxy|pi|TajimaD|fis**

Estimates fst (differentiation among populations), dxy (absolute divergence), pi (nucleotide diversity), thetaw (Watterson's thetaW), TajimaD (Tajima's D), fis (inbreeding coefficient), and ho (mean observed heterozygosity), he (mean expected heterozygosity), pic (mean polymorphic information content), ae (mean effective number of alleles), and I (mean Shannon's information index) for each sliding window.

-slide_pop=pop_identifier, string, default:total

Estimates fst, dxy, pi, thetaw, tajimaD, fis, ho, he, pic, ae, I for a population, a region or in the total population. Note that fst and dxy are not calculated for a population.

The results are saved in `*.slide.txt`, which is consisting of a table showing the statistics of the sliding windows. An example is shown in Figure 4.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	Chrom	st	ed	#variants	Nei1973	Weir1994	Hudson	Hedrick	Jost2005	Huang2005	dxy	pi	theta_w	TajimaD	Fis	Ho	He	PIC	Ae	I
2	1	1	1000000	12553	0.02	0.03	0.03	0.03	0.03	0.03	43.12	381.18	1550.50	nan	0.09	0.03	0.03	0.03	1.05	0.05
3	1	100001	1100000	16503	0.02	0.03	0.03	0.03	0.03	0.03	58.67	519.39	2038.39	-3.21	0.09	0.03	0.03	0.03	1.05	0.06
4	1	200001	1200000	20655	0.02	0.03	0.03	0.03	0.03	0.03	71.21	645.33	2551.23	-3.22	0.10	0.03	0.03	0.03	1.05	0.06
5	1	300001	1300000	24976	0.02	0.03	0.03	0.03	0.03	0.03	85.62	755.59	3084.94	-3.25	0.11	0.03	0.03	0.03	1.04	0.05
6	1	400001	1400000	28933	0.02	0.03	0.03	0.03	0.03	0.03	104.56	912.26	3573.69	-3.21	0.12	0.03	0.03	0.03	1.05	0.06
7	1	500001	1500000	33786	0.02	0.03	0.03	0.03	0.03	0.03	121.01	1042.03	4173.12	-3.23	0.13	0.03	0.03	0.03	1.05	0.05
8	1	600001	1600000	36480	0.02	0.02	0.02	0.03	0.02	0.02	128.22	1137.82	4505.87	-3.22	0.13	0.03	0.03	0.03	1.05	0.05
9	1	700001	1700000	39633	0.02	0.02	0.02	0.03	0.02	0.02	145.56	1293.97	4895.32	-3.17	0.13	0.03	0.03	0.03	1.05	0.06
10	1	800001	1800000	40042	0.02	0.02	0.02	0.03	0.02	0.02	143.19	1275.52	4945.83	-3.20	0.13	0.03	0.03	0.03	1.05	0.06
11	1	900001	1900000	39587	0.01	0.01	0.01	0.01	0.01	0.01	146.31	1286.22	4889.63	-3.18	0.13	0.03	0.03	0.03	1.05	0.06
12	1	1000001	2000000	39635	0.01	0.01	0.01	0.01	0.01	0.01	145.03	1307.55	4895.56	-3.16	0.13	0.03	0.03	0.03	1.05	0.06
13	1	1100001	2100000	39878	0.01	0.01	0.01	0.01	0.01	0.01	142.30	1315.66	4925.58	-3.16	0.12	0.03	0.03	0.03	1.05	0.06
14	1	1200001	2200000	39142	0.01	0.01	0.01	0.01	0.01	0.01	136.22	1258.12	4834.67	-3.19	0.12	0.03	0.03	0.03	1.05	0.05
15	1	1300001	2300000	38788	0.01	0.01	0.01	0.01	0.01	0.01	132.84	1294.25	4790.94	-3.15	0.11	0.03	0.03	0.03	1.05	0.06
16	1	1400001	2400000	38735	0.02	0.02	0.02	0.02	0.02	0.02	121.17	1270.01	4784.40	-3.17	0.09	0.03	0.03	0.03	1.05	0.06
17	1	1500001	2500000	37885	0.02	0.02	0.02	0.02	0.02	0.02	112.44	1232.22	4679.41	-3.18	0.08	0.03	0.03	0.03	1.05	0.06
18	1	1600001	2600000	37363	0.02	0.03	0.03	0.03	0.03	0.03	109.78	1219.69	4614.93	-3.17	0.08	0.03	0.03	0.03	1.05	0.06
19	1	1700001	2700000	36474	0.02	0.03	0.03	0.03	0.03	0.03	96.80	1115.68	4505.13	-3.24	0.07	0.03	0.03	0.03	1.05	0.05
20	1	1800001	2800000	37291	0.03	0.04	0.03	0.04	0.04	0.04	98.32	1163.68	4606.04	-3.22	0.06	0.03	0.03	0.03	1.05	0.05
21	1	1900001	2900000	37957	0.03	0.03	0.03	0.04	0.04	0.03	96.51	1183.06	4688.30	-3.22	0.07	0.03	0.03	0.03	1.05	0.05
22	1	2000001	3000000	37460	0.03	0.03	0.03	0.04	0.04	0.03	92.96	1148.26	4626.91	-3.24	0.07	0.03	0.03	0.03	1.05	0.05
23	1	2100001	3100000	37293	0.03	0.03	0.03	0.04	0.04	0.03	90.73	1152.27	4606.29	-3.23	0.06	0.03	0.03	0.03	1.05	0.05

Figure 4. The results of sliding window.

4.6 Linkage disequilibrium decay

Linkage disequilibrium (LD) refers to the nonrandom associations of alleles at different loci. The rate of decay of LD is influenced by recombination rate and the number of generations of recombination. Therefore, investigating LD decay may reveal the population recombination history. For example, a slow LD decay pattern is correlated with the declined population size.

VCFPOP provides four LD measures, r^2 , D' , r_Δ^2 and Δ' . The former two are for phased genotypes and the latter two are based on Burrow's Δ and is designed for unphased genotypes. Their equations can be found in the methodology section.

This function can only be used for VCF/BCF input files and is performed before haplotype extraction.

-decay

Perform LD decay analysis. The results are saved in `*.decay.txt`.

-decay_plot=yes|no, string, default:no

Draws a scatter plot with the average estimate for each kind of estimate. Results are saved in

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```
*.decay.pdf.
```

-decay_chromosome=yes|no, string, default:no
Output results for each chromosome.

-decay_pair=yes|no, string, default:no
Output results for each pair of loci.

-decay_maxdist=10000~1000000000, integer, default:500000
Maximum distance between loci in the same contig (in bp).

-decay_nintervals=10~10000, integer, default:500
The number of intervals to average the LD estimates within intervals.

-decay_ratio=0.00000001~1, real, default:0.001
Sampling ratio for a pair of loci in the same contig. A large sampling ratio requires more time.

-decay_estimator=r2|r2Delta|D'|Delta', string, multiple selections, default:r2
Delta
Estimates the decay of r2, r2Delta, D', Delta' as a function of distance between loci (in bp).

-decay_pop=pop_identifier, string, default:total
Estimates the decay of r2, D', r2D, Delta' for a population, a region or in the total population.

The results are saved in *.decay.txt, including a table showing the weighted average of LD measures and their standard error. If -decay_pair is used, a second table showing the results of each loci pair is presented after the first table. The decay_plot.R draws results for each chromosome (if -decay_chromosome is used) and each estimate. The example results are shown in Figure 5.

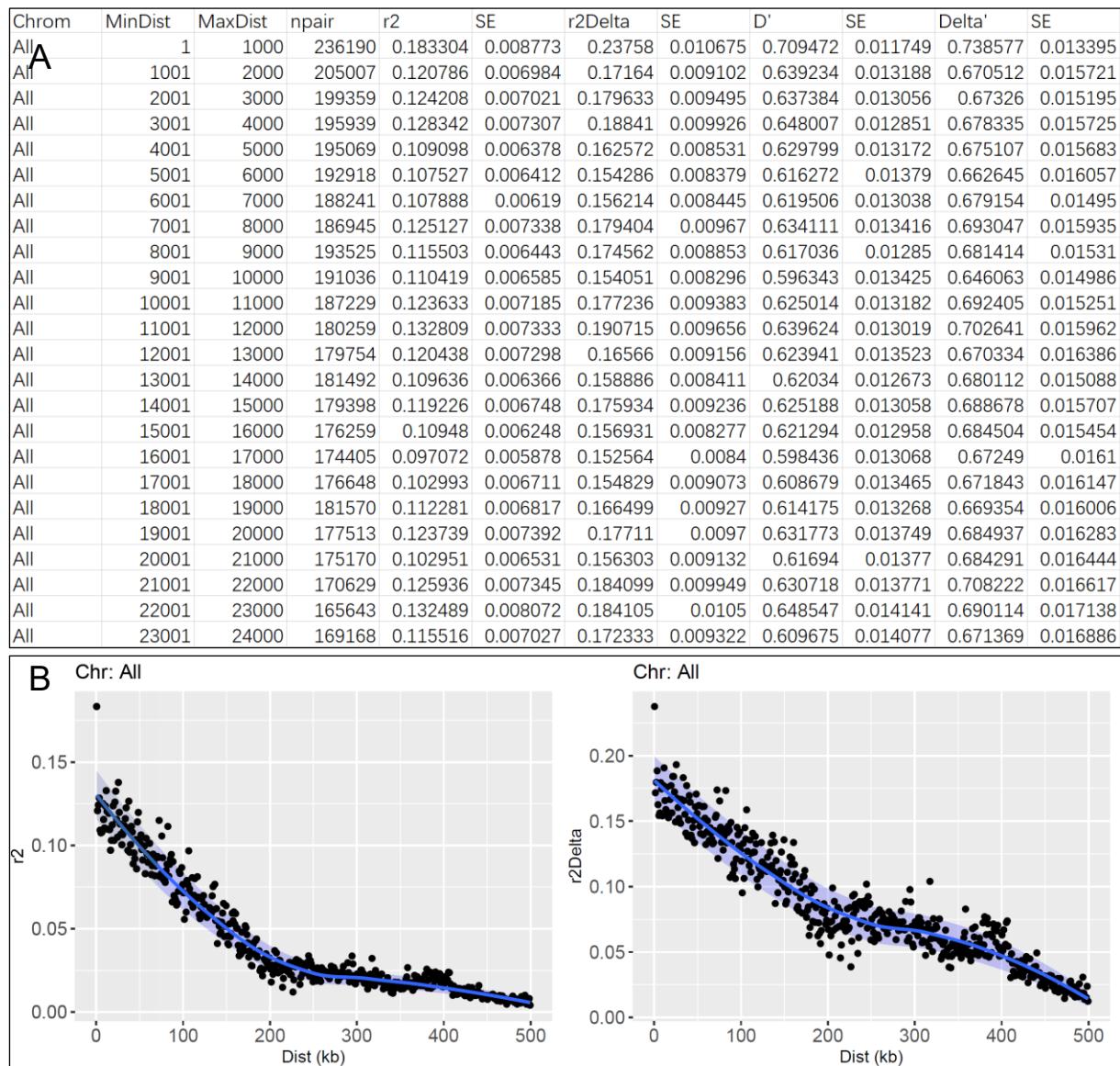


Figure 5. (A) The table showing the weighted average of LD measures and their standard error. (B) The visual results of LD decay, the points denote weighted average LD measures for each interval, and the blue line is the smooth LD measures and the blue area shows the 95% confidence interval of weighted average LD measures.

4.7 Linkage disequilibrium block

The LD block analysis partition each chromosome into several intervals and estimate the LD

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measures between intervals.

This function can only be used for VCF/BCF input files and is performed before haplotype extraction.

-block

Perform LD block analysis. The results are saved in `*.block.txt`.

`-block_plot=yes|no, string, default:no`

Draws a heat map for the LD blocks. Results are saved in `*.block.pdf`.

`-block_size=10000~10000000000, integer, default:1000000`

Size of each interval (in bp).

`-block_maxd=1~10000000000, integer, default:100`

Max distance between interval indices.

`-block_ratio=0.00000001~1, real, default:0.001`

Sampling ratio for a pair of loci. A large sampling ratio requires more time.

`-block_estimator=r2|r2Delta|D'|Delta', string, multiple selections, default:r2Delta`

The LD measures used.

`-block_pop=pop_identifier, string, default:total`

Perform LD block analysis for a population, a region or in the total population.

The results are saved in `*.block.txt`, which is very similar with the LD decay results. The figure results are saved in `*.block.pdf`. The example results are shown in Figure 6.

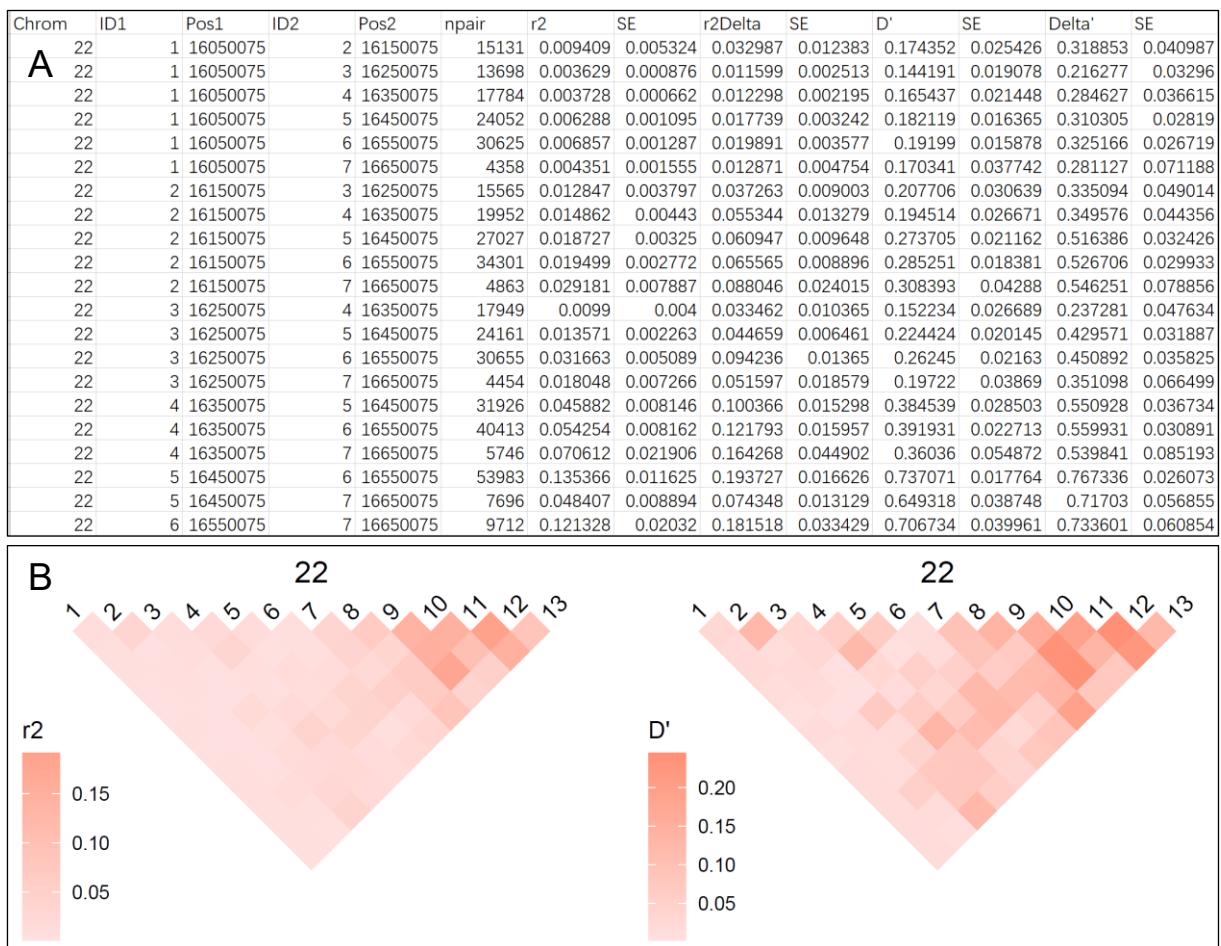


Figure 6. (A) The results of LD block showing the statistics between blocks. (B) The visual results of LD block showing the r^2 and D' between blocks.

4.8 Genome-wide association study

Genome-wide association study (GWAS) is a method to identify associations between genetic variants and traits across the entire genome, which applies a linear mixed-effect model and uses genotypes to explain the traits, with environmental variables as covariates and consider the genetic background as the random effect (whose covariance is proportional to the relatedness matrix). Compared with previous software, VCFPOP supports multi-allelic loci and multiple ploidies dataset, supports various relatedness estimators, and supports various data imputation methods.

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This function can only be used for VCF/BCF input files and is performed before haplotype extraction. To conduct the GWAS, an additional input CSV file is required, and its example is shown in Figure 7.

	A	B	C	D	E	F	G	H
1	Ind	Y1	Y2	X1	X2	X3	A	B
2	Ind	Normal	Normal	Numeric	Numeric	Numeric	Nominal	Nominal
3	HG00096	1.01541	0.09361	1.04729	-0.4452	0.28549	2	4
4	HG00097	-0.5325	-1.2265	-0.578	-0.1114	0.30061	1	2
5	HG00099	1.32541	2.3735	0.0081	0.03433	0.22993	2	1
6	HG00100	-1.0985	-1.8599	0.49284	-1.6571	0.14214	3	4
7	HG00101	-0.3895	0.88886	-0.5145	-1.1938	0.05903	2	6
8	HG00102	1.27159	-0.495	-0.2841	0.9657	0.33549	1	5
9	HG00103	-0.9531	-0.3719	0.38567	-0.0406	0.63422	4	3
10	HG00105	1.12872	-0.7606	-0.6301	0.77417	0.81209	5	4
11	HG00106	-1.1495	-0.2948	0.81615	-0.368	0.7359	2	1
12	HG00107	-0.5925	-0.4381	-0.1602	0.36453	0.05583	3	4
13	HG00108	-1.2257	1.13006	-1.1004	0.34272	0.39372	4	5
14	HG00109	-0.6104	-0.3106	0.79994	-0.4186	0.24306	5	3

Figure 7. The example of inputting CSV file. The first row saves the variable names, and the second row saves the variable types. Y1 and Y2 are two response variables, X1-X3 are numeric environmental variables, and A and B are nominal environmental variables.

The parameters are listed below.

-gwas

Perform GWAS. The results are saved in *.gwas.txt.

-gwas_plot=yes|no, string, default:no

Draw Manhattan plots. Results are saved in *.gwas.pdf.

-gwas_input=file_path, string

Phenotype and environmental covariates in CSV format. First row saves variable names (e.g., Ind Y1 Y2 X1 X2 A B), and second row saves variable types (e.g., Ind Norm Norm Numeric Numeric Nominal Nominal). Missing data are denoted by na or nan.

-gwas_pop=pop_identifier, string, default:total

Perform GWAS for a population, a region or in the total population.

```
-gwas_restimator=centered|standardlized|Lynch1999|Wang2002|Thomas2010|Li1993|
Queller1989|Huang2016A|Huang2016B|Milligan2003|Anderson2007|Huang2014|Huang2015|Ritland1996_modified|Loiselle1995_modified|Ritland1996|Loiselle1995|Weir1996|VanRaden2008, string, default=standardlized
```

Relatedness estimators: centered relatedness, standardlized relatedness Huang2014 and Huang2015 support ploidy level <= 8, Ritland1996, Loiselle1995, Weir1996 and VanRaden2008 estimators support ploidy level <= 10, and other estimators only support diploids. Milligan2003, Anderson2007 and Huang2015 are maximum-likelihood estimators, and other estimators are method-of-moment estimators. Unbiased Ritland1996 and Loiselle1995 relatedness estimates are converted from kinship coefficient by eqn (7) of Huang et al. (2015, Mol Ecol Resour).

```
-gwas_imputeG=mean|median|sample|norm|mvn|cmean|cmvn|knn|svd|discard, string,
default=svd
```

Method used to impute missing genotypes: mean: replace with mean; median: replace with median; sample: randomly sample from observed genotypes; norm: sample from $N(\text{mean}, \text{var})$ for each allele; mvn: sample from multivariate normal distribution MVN(Mu , Sigma) for each locus; cmean: replace with mean conditional on the observed genotyped of the same batch; cmvn: sample from multivariate normal distribution conditional on the observed genotyped of the same batch; knn: replace with mean of k-nearest neighbors; svd: perform SVD decomposition for the mean filled genotype matrix, then use the greatest singular value to reconstruct genotype matrix and replace the missing data; discard: discard missing genotypes (slow).

```
-gwas_imputeXY=mean|median|sample|norm|mvn|cmean|cmvn|knn|svd|discard, string,
default=svd
```

Method used to impute missing response and environmental variables: mean: replace with mean; median: replace with median; sample: randomly sample from observed genotypes; norm: sample from $N(\text{mean}, \text{var})$ for each allele; mvn: sample from multivariate normal distribution MVN(Mu , Sigma) for each locus; cmean: replace with mean conditional on the observed genotyped of the same batch; cmvn: sample from multivariate normal distribution

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conditional on the observed genotyped of the same batch; knn: replace with mean of k-nearest neighbors; svd: perform SVD decomposition for the mean filled genotype matrix, then use the greatest singular value to reconstruct genotype matrix and replace the missing data; discard: discard missing genotypes (slow).

`-gwas_nneighbor=1~100, integer, default:5`

Number of nearest neighbors used fill missing data.

`-gwas_nsvd=1~10, integer, default:1`

Number of singular values used to fill missing data.

`-gwas_batch=100~10000, integer, default:1000`

Number of loci used in a batch (e.g., estimate relatedness, fill missing data).

`-gwas_intercept=yes|no, string, default:no`

Use different intercepts for different ploidy levels.

`-gwas_slope=yes|no, string, default:no`

Use different slopes for different ploidy levels.

`-gwas_dosage=yes|no, string, default:no`

Use allele dosage as explanatory variables, otherwise use allele proportion instead.

`-gwas_test=Wald|LRT|Score, string, multiple selections, default:LRT`

The tests used in GWAS. Satterthwaite correction for d.f. is used for Wald test.

The results are saved in `*.gwas.txt`, which saves the results for each variant. The Manhattan plot is saved in `*.gwas.manhattan.pdf`, the quantile-quantile plot is saved in `*.gwas.qq.pdf`. The results table includes the Chromosome, Position, Locus, #Alleles, #Ploidy, sample size, degrees-of-freedom, Wald test results: lnL under the REML criterion, W static, $-\ln P$ and P ; LRT test: lnL under the ML criterion, LR static, $-\ln P$ and P ; Score test: lnL of the null model under the ML criterion, LM static, $-\ln P$ and P . The extreme small P (e.g., 10^{-1000}) may cause an under-flow, and

– $-\ln P$ can avoid this. The example results are shown in Figure 8.

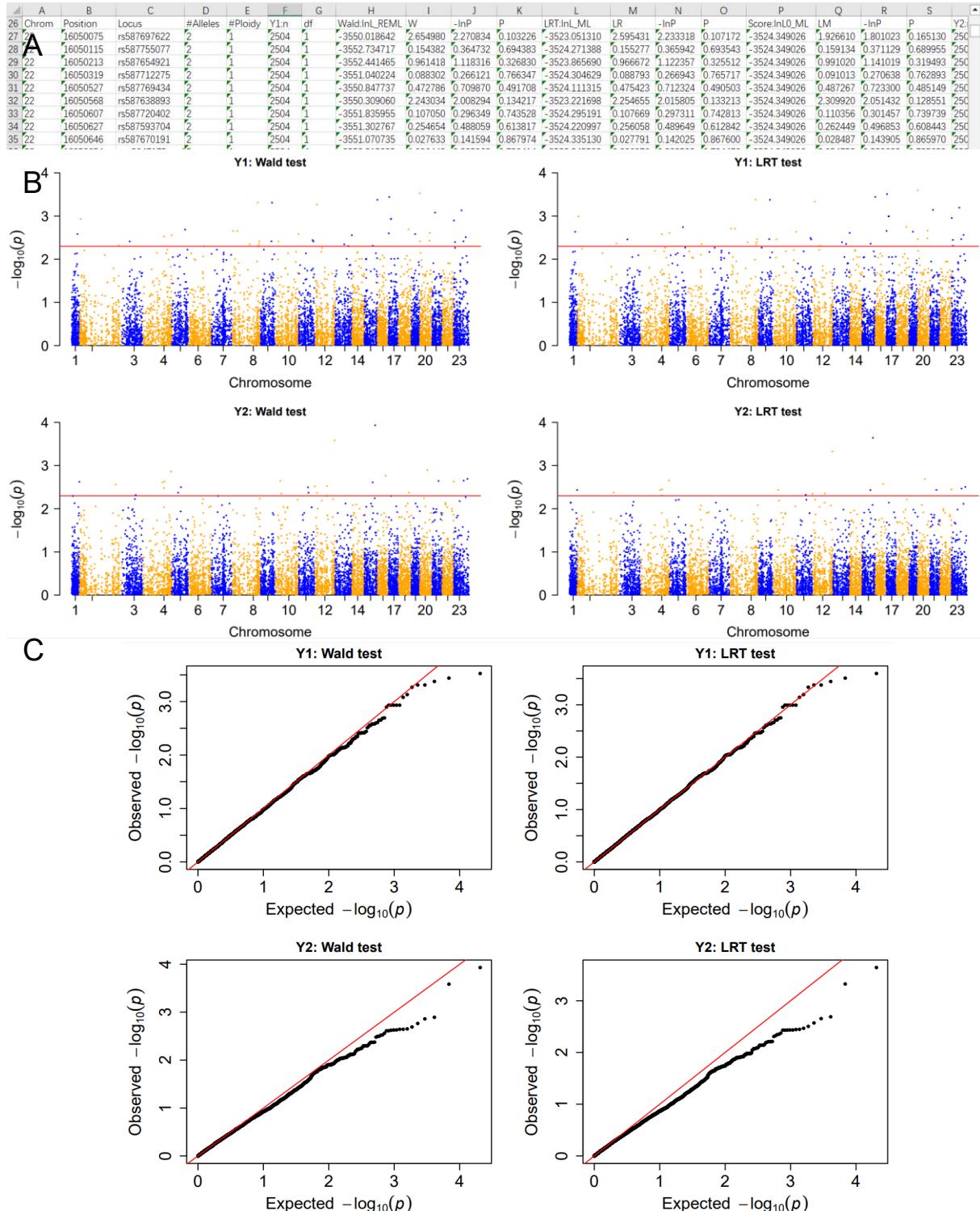


Figure 8. (A) The result table for GWAS. (B) The Manhattan plot for GWAS. (C) The QQ plot for GWAS.

4.9 Haplotype extraction

Haplotype extraction combines several adjacent variants into a single, highly polymorphic locus and is performed after the variants are filtered. The extracted haplotypes will be regarded as alleles, and the extracted locus can be further exported and further analysed. If there are any missing data for any combined variants of an individual, the genotype of the individual at this extracted locus is assigned to missing data.

-haplotype

Extracts haplotypes from phased genotypes, then use the haplotypes as alleles for further analysis. Note that all genotypes must be phased and only the variants genotyped in all individuals are used. The haplotype definitions are saved in `*.haplotype.txt`.

`-haplotype_ptype=[min_val,max_val], real range, default:[0.8,1]`

Range of genotype rate at extract loci.

`-haplotype_length=[min_val,max_val], integer range, default:[1,1000000]`

Range of haplotype size (in bp).

`-haplotype_variants=[min_val,max_val], integer range, default:[5,20]`

Range of number of variants in the haplotype.

`-haplotype_interval=0~1000000000, integer, default:0`

Minimum interval between extracted loci (in bp).

`-haplotype_alleles=[min_val,max_val], integer range, default:[2,65535]`

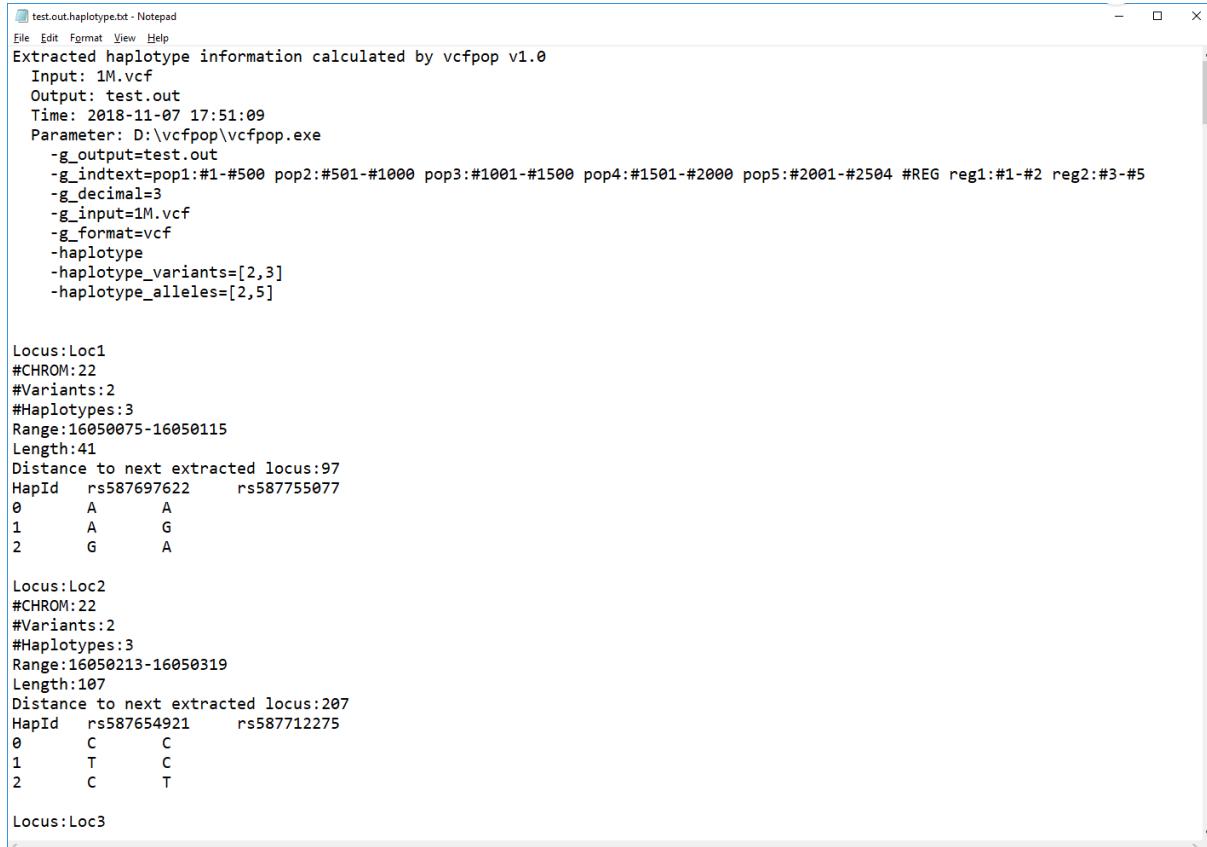
Range of number of alleles at the extracted locus.

`-haplotype_genotypes=[min_val,max_val], integer range, default:[2,65535]`

Range of number of genotypes at the extracted locus.

The results are saved in `*.haplotype.txt`, including a heading of parameters used, CHROM, the number of variants, the number of haplotypes (i.e., the number of alleles), range (from the first

position to the last position of variants), the length of haplotypes, the distance to the next extracted locus (i.e., the length of interval), the extracted haplotype and the corresponding alleles in each variant. An example is shown in Figure 9.



The screenshot shows a Notepad window titled "test.out.haplotype.txt - Notepad". The content of the file is as follows:

```

test.out.haplotype.txt - Notepad
File Edit Format View Help
Extracted haplotype information calculated by vcfpop v1.0
Input: 1M.vcf
Output: test.out
Time: 2018-11-07 17:51:09
Parameter: D:\vcfpop\vcfpop.exe
-g_output=test.out
-g_indtext=pop1:#1-#500 pop2:#501-#1000 pop3:#1001-#1500 pop4:#1501-#2000 pop5:#2001-#2504 #REG reg1:#1-#2 reg2:#3-#5
-g_decimal=3
-g_input=1M.vcf
-g_format=vcf
-haplotype
-haplotype_variants=[2,3]
-haplotype_alleles=[2,5]

Locus:Loc1
#CHROM:22
#Variants:2
#Haplotypes:3
Range:16050075-16050115
Length:41
Distance to next extracted locus:97
HapId rs587697622 rs587755077
0 A A
1 A G
2 G A

Locus:Loc2
#CHROM:22
#Variants:2
#Haplotypes:3
Range:16050213-16050319
Length:107
Distance to next extracted locus:207
HapId rs587654921 rs587712275
0 C C
1 T C
2 C T

Locus:Loc3
< >

```

Figure 9. Example output of haplotype extraction.

4.10 Conversion

This function is applied after haplotype extraction, and converts the genotype data into another file format, either GENEPOP (Rousset 2008), SPAGEDI (Hardy & Vekemans 2002), CERVUS (Kalinowski *et al.* 2007) , ARLEQUIN (Excoffier & Lischer 2010), STRUCTURE (Pritchard *et al.* 2000), POLYGENE (Huang *et al.* 2020), POLYRELATEDNESS (Huang *et al.* 2015a), GENODIVE (Meirmans & Tienderen 2004) or PLINK (PURCELL *ET AL.* 2007). The results are saved in *.convert.xxx.txt, whose parameter is

-convert

Converts filtered data (and extracted haplotype) into the input format of other software. The

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result is saved in `*.convert.genepop.txt`.

`-convert_format=genepop|spagedi|cervus|arlequin|structure|polygene|polyrelatedness|genodive|plink`, string, multiple selections, default:spagedi

Target format, where GENEPOP, CERVUS, ARLEQUIN and PLINK formats only support diploids.

`-convert_mode=disable|truncate|choose|split|shuffle`, string, default:disable

Converts polyploid genotype into diploid genotype: truncate (use first two alleles), choose (randomly sample two alleles without replacement), split (split one polyploid into floor(v/2) diploids), and shuffle (shuffle alleles and split). Haploid genotypes are considered as missing.

4.11 Genetic diversity indices

This function estimates the genetic diversity indices and performs the genotypic equilibrium test (e.g., the HWE test). For the polyploid data, VCFPOP employs a Fisher's G-test to perform the genotypic distribution test. However, it is noteworthy that this test is performed when the ploidy levels all individuals in the population/region are equal. The parameters related to the genetic diversity are as follows.

`-diversity`

Estimates the genetic diversity indices. Results are saved in `*.diversity.txt`.

`-diversity_level=pop|reg|tot|popXloc|regXloc|totXloc`, string, multiple selections, default:loc,pop

Output mean diversity across all loci in each population, each region or in the total population, or output diversity for each locus in each population, in each region or in the total population.

`-diversity_model=rcs|prcs|ces|pes`, string, multiple selections, default:rcs

Double-reduction model to calculate genotypic frequencies for polyploids.

The results are saved in `*.diversity.txt`. An example is shown in Figure 10.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
14	-diversity																			
15	-diversity_level=pop,reg,tot,popXloc,regXloc,totXloc																			
16	-diversity_model=hwe																			
17																				
18																				
19	Pop	Locus	Ploidy	k	n	#Hap	Ho	He	PIC	Ae	I	NE1P	NE2P	NEPP	NEID	NESID	Fis	G	d.f.	P-val
20	Total	(10)	4.000-4.04.000	120.000	480.000	0.696	0.702	0.648	3.358	1.284	0.040	0.003	0.000	0.000	0.000	0.008	-	-	-	
21																				
22	Pop	Locus	Ploidy	k	n	#Hap	Ho	He	PIC	Ae	I	NE1P	NE2P	NEPP	NEID	NESID	Fis	G	d.f.	P-val
23																				
24	Pop	Locus	Ploidy	k	n	#Hap	Ho	He	PIC	Ae	I	NE1P	NE2P	NEPP	NEID	NESID	Fis	G	d.f.	P-val
25	pop1	(10)	4.000-4.04.000	30.000	120.000	0.707	0.712	0.660	3.483	1.306	0.034	0.002	0.000	0.000	0.007	-	-	-	-	
26	pop2	(10)	4.000-4.04.000	30.000	120.000	0.693	0.691	0.634	3.241	1.254	0.047	0.004	0.000	0.000	0.000	-0.003	-	-	-	
27	pop3	(10)	4.000-4.04.000	30.000	120.000	0.694	0.693	0.638	3.263	1.267	0.044	0.003	0.000	0.000	0.000	-0.002	-	-	-	
28	pop4	(10)	4.000-4.04.000	30.000	120.000	0.692	0.696	0.641	3.299	1.272	0.043	0.003	0.000	0.000	0.000	0.007	-	-	-	
29																				
30	Pop	Locus	Ploidy	k	n	#Hap	Ho	He	PIC	Ae	I	NE1P	NE2P	NEPP	NEID	NESID	Fis	G	d.f.	P-val
31	Total	Loc1	4-4	4	120	480	0.686	0.700	0.646	3.337	1.280	0.726	0.558	0.385	0.254	0.436	0.020	-0.845	0	nan
32	Total	Loc2	4-4	4	120	480	0.689	0.697	0.643	3.298	1.275	0.729	0.561	0.387	0.255	0.438	0.011	-2.788	0	nan
33	Total	Loc3	4-4	4	120	480	0.688	0.704	0.652	3.383	1.292	0.721	0.551	0.376	0.245	0.433	0.024	1.203	0	nan
34	Total	Loc4	4-4	4	120	480	0.703	0.706	0.654	3.400	1.299	0.718	0.547	0.372	0.241	0.432	0.004	-6.104	0	nan
35	Total	Loc5	4-4	4	120	480	0.707	0.719	0.665	3.560	1.314	0.709	0.539	0.368	0.240	0.424	0.017	9.124	0	nan
36	Total	Loc5	4-4	4	120	480	0.669	0.691	0.635	3.233	1.259	0.736	0.569	0.397	0.262	0.442	0.031	4.286	0	nan
37	Total	Loc7	4-4	4	120	480	0.708	0.702	0.646	3.356	1.280	0.725	0.558	0.386	0.257	0.435	0.009	-4.250	0	nan
38	Total	Loc8	4-4	4	120	480	0.686	0.707	0.652	3.413	1.290	0.721	0.552	0.380	0.250	0.432	0.030	-0.946	0	nan
39	Total	Loc9	4-4	4	120	480	0.717	0.694	0.638	3.269	1.266	0.732	0.566	0.393	0.262	0.440	-0.033	13.770	0	nan
40	Total	Loc10	4-4	4	120	480	0.711	0.700	0.646	3.336	1.281	0.726	0.557	0.384	0.252	0.436	-0.016	2.304	0	nan

Figure 10. Example output of genetic diversity.

Here, the genetic diversity indices in the first five rows are divided into two parts. One part consists of k, n, #Hap, Ho, He, PIC, Ar and Fis, and the other part consists of NE1P, NE2P, NEPP, NEID and NESID. For the former (or the latter) part, the value of each index in a population is taken as the average (or the product) of those values across all locus.

The description of the table header is as follows (some modifications are made to be compatible with polyploids):

- Locus, number of loci (for the first five rows) or locus name (for the other rows);
- Ploidy, range of ploidy level;
- k, number of distinct alleles at this locus;
- n, total number of individuals genotyped at this locus;
- #Hap, number of haplotypes (or alleles copies);
- Ho, observed heterozygosity;
- He, expected heterozygosity;
- pi, nucleotide diversity;
- PIC, polymorphic information content;

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- A_e , effective number of alleles;
- I , Shannon's information index;
- $NE1P$, average probability of not excluding a candidate parent from the parentage of an arbitrary offspring, given only the genotype of the offspring;
- $NE2P$, average probability of not excluding a candidate parent from the parentage of an arbitrary offspring, given the genotype of the offspring and of the known parent of the opposite sex;
- $NEPP$, average probability of not excluding a candidate parent pair from the parentage of an arbitrary offspring, given only the genotype of the offspring;
- $NEID$, average probability that the genotypes at a single locus does not differ between two unrelated individuals;
- $NESID$, average probability that the genotypes at a single locus do not differ between two full siblings;
- F_{IS} , inbreeding coefficient;
- G , Fisher's G statistic of genotypic equilibrium test;
- d.f., degree of freedom of genotypic equilibrium test;
- P-val, significance of genotypic equilibrium test.

4.12 Individual statistics

This function calculates the individual statistics (e.g., inbreeding coefficient, heterozygosity, and likelihood, kinship coefficient). The parameters are as follows:

-indstat

Calculates individual statistics (e.g., inbreeding coefficient, heterozygosity, and etc). Results are saved in `*.indstat.txt`.

-indstat_type=hidx|lnp|f|theta, string, multiple selections, default:hidx,lng

Output statistics: heterozygosity index, natural logarithm of genotypic frequency, inbreeding coefficient and kinship coefficient.

-indstat_model=rcs|prcs|ces|pes, string, multiple selections, default:rcs

Double-reduction model to calculate genotypic frequencies for polyploids.

-indstat_estimator=Ritland1996|Loiselle1995|Weir1996|VanRaden2008, string, multiple selections, default:Ritland1996

Inbreeding coefficient and kinship coefficient (within an individual itself) estimators.

-indstat_ref=pop|reg|total, string, multiple selections, default:pop

Reference population: in the population, the region or the total population.

-indstat_locus=all|each, string, multiple selections, default:all

Output individual statistics for all loci or for each locus.

The results are saved in *.indstat.txt. An example is shown in Figure 11.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
14	-indstat														
15	-indstat_ref=pop														
16	-indstat_model=prcs														
17	-indstat_locus=all														
18	-indstat_type=hidx,lnl,f,theta														
19	-indstat_estimator=Ritland1996,Loiselle1995,Weir1996														
20															
21															
22							All loci								
23	Ind	Pop	#typed	#miss	Ploidy	#Hap	H-idx	InL_pop_p	F_pop_RI	F_pop_LO	F_pop_WE	Theta_pop	Theta_pop	Theta_pop	Theta_pop_WE
24	Ind1	pop1	10	0	4-4	40	1.000	-31.783	-0.234	-0.263	-0.404	0.075	0.053	-0.053	
25	Ind2	pop1	10	0	4-4	40	0.750	-31.606	-0.049	-0.024	-0.053	0.213	0.232	0.210	
26	Ind3	pop1	10	0	4-4	40	0.700	-35.653	0.166	0.118	0.017	0.374	0.339	0.263	
27	Ind4	pop1	10	0	4-4	40	0.717	-30.005	-0.043	-0.060	-0.006	0.218	0.205	0.246	
28	Ind5	pop1	10	0	4-4	40	0.750	-31.822	-0.052	-0.044	-0.053	0.211	0.217	0.210	
29	Ind6	pop1	10	0	4-4	40	0.683	-31.869	-0.004	0.004	0.041	0.247	0.253	0.281	
30	Ind7	pop1	10	0	4-4	40	0.617	-30.207	0.012	0.054	0.134	0.259	0.290	0.351	
31	Ind8	pop1	10	0	4-4	40	0.717	-30.996	-0.065	-0.046	-0.006	0.201	0.215	0.246	

Figure 11. Example output of individual statistics.

The description of table header is as follows:

- #typed, number of typed loci;
- #miss, number of missing genotypes;
- Ploidy, range of ploidy levels;

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- #Hap, total number of haplotypes (i.e., allele copies);
- H-idx, heterozygosity index, taken from the average of heterozygosity across all loci;
- lnL_pop_prcs, natural logarithm of likelihood of genotypes in the current population under the PRCS double-reduction equilibrium model;
- F_pop_RI, inbreeding coefficient, using the allele frequencies in the current population as a reference and estimated by the Ritland1996 estimator;
- Theta_pop_RI, kinship coefficient, using the allele frequencies in the current population as a reference and estimated by the Ritland1996 estimator.

4.13 Genetic differentiation

For the genetic differentiation analysis, several F_{ST} analogous indices are calculated, and the differentiation between/among populations or regions are tested. The differentiation tests are performed by Fisher's G-tests, in which one is based on the genotype distribution, and the other is based on the allele distribution. The parameters related to the genetic differentiation are listed below.

-fst

Estimates the genetic differentiation statistics. Results are saved in ***.fst.txt**.

-fst_plot=yes|no, string, default:no

Draws a heatmap for the results of the genetic differentiation estimation. Results are saved in ***.fst.pdf**.

-fst_level=regXtot|popXtot|popXreg|reg|pop, string, multiple selections, default:pop

Estimates the genetic differentiation among all regions, among all populations, among populations in each region, between any two regions, and between any two populations.

-fst_estimator=Nei1973|Weir1984|Hudson1992|Slatkin1995|Hedrick2005|Jost2008|Huang2021_homo|Huang2021_aneu, string, multiple selections, default:Nei1973

Genetic differentiation estimator, Nei1973 (Gst; Nei 1973, PNAS), Weir1984 (variance

decomposition method, Weir & Cockerham 1984, Evolution), Hudson1992 (mean difference method, Hudson et al. 1992, Genetics), Slatkin1995 (Rst, Slatkin 1995, Genetics, for non-VCF/BCF input file only), Hedrick2005 (G'st; Hedrick 2005, Evolution), Jost2008 (D; Jost 2008, Molecular Ecology), Huang2021 (variance decomposition method for polyploid or aneuploid, Integrative Zoology).

-fst_fmt=matrix|table, string, multiple selections, default:matrix

Output format.

-fst_locus=all|each, string, multiple selections, default:all

Calculates genetic differentiation and performs a test for all loci or for each locus.

-fst_test=genotype|allele, string, multiple selections, optional

Tests the significance of differentiation by Fisher's G-test based on genotype distributions or allele distributions.

The results are saved in ***.fst.txt**. An example is shown in Figure 12.

	A	B	C	D	E	F	G	H	I
14	-fst								
15	-fst_estimator=Nei1973								
16	-fst_level=popXtot,pop								
17	-fst_locus=all								
18	-fst_test=genotype								
19	-fst_fmt=matrix								
20									
21									
22	Locus	A	Among all	pop1	pop1	pop1	pop2	pop2	pop3
23		B		pop2	pop3	pop4	pop3	pop4	pop4
24	All loci	Nei1973	0.006	0.003	0.004	0.003	0.004	0.005	0.005
25	Genotype	103.228	40.301	39.051	50.986	28.089	37.836	31.812	
26	d.f.	90.000	30.000	30.000	30.000	30.000	30.000	30.000	31.000
27	P	0.161	0.099	0.125	0.010	0.566	0.154	0.426	
28									
29									
30	Nei1973	pop1	pop2	pop3	pop4				
31	pop1	0.000	0.003	0.004	0.003				
32	pop2	0.003	0.000	0.004	0.005				
33	pop3	0.004	0.004	0.000	0.005				
34	pop4	0.003	0.005	0.005	0.000				

Figure 12. Example output of genetic differentiation.

In the first table (Line 22-37), the header row shows four estimators and two Fisher's *G* statistics together with two degrees of freedom and two *P*-values (significances of differentiation by the

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Fisher's G -test); Line 24 shows various results related to the global F_{ST} among all populations; each of Lines 25-27 shows various results related to the differentiation test between a pair of populations. Next, the output format for Lines 30-34 is a matrix, where each element in this matrix is the F_{ST} estimate between a pair of populations for the Nei1973 estimator.

4.14 Genetic distance

This function calculates the genetic distance between individuals, populations or regions. Specifically, Reynolds *et al.*'s (1983) distance D and the Slatkin's (1995) linearized distance F_{ST} are obtained by transforming the corresponding F_{ST} estimators. The parameters of this function are listed below.

-gdist

Estimates the genetic distance. Results are saved in *.gdist.txt.

-gdist_plot=yes|no, string, default:no

Draws a heatmap for the results of genetic distance estimation. Results are saved in *.gdist.pdf.

-gdist_weightmissing=yes|no, string, default:yes

Use population/region allele frequency for missing data.

-gdist_level=ind|pop|reg, string, multiple selections, default:pop

Estimates the genetic distance between individuals, populations or regions.

**-gdist_estimator=Nei1972|Cavalli-Sforza1967|Reynolds1983|Nei1983|Euclidean|Gol
dstein1995|Nei1974|Roger1972|Slatkin_Nei1973|Slatkin_Weir1984|Slatkin_Hudson1
992|Slatkin_Slatkin1995|Slatkin_Hedrick2005|Slatkin_Jost2008|Slatkin_Huang202
1_homo|Slatkin_Huang2021_anneu|Reynolds_Nei1973|Reynolds_Weir1984|Reynolds_Hud
son1992|Reynolds_Slatkin1995|Reynolds_Hedrick2005|Reynolds_Jost2008|Reynolds_
Huang2021_homo|Reynolds_Huang2021_anneu, string, multiple selections, default:
Nei1972**

Genetic distance estimators: Nei1972 (Ds, Nei 1972, Am Nat), Cavalli-Sforza1967 (Cavalli-Sforza & Edwards 1967, Am J Human Genet), Reynolds1983 (thetaW, Reynolds et al. Genetics, 1983), Nei1983 (Da, Nei 1983, J Mol Evol), Euclidean, Goldstein1995 (dmu2, Goldstein 1995, PNAS), Nei1974 (Dm, Nei & Roychoudhury 1974, Am J Human Genet), Roger1972 (Rogers 1972, Studies in Genetics), the Slatkin's transform $d = Fst/(1-Fst)$ converts the range of Fst from [0,1] to [0, infinity), and the Reynolds's transformation $d = -\ln(1 - Fst)$.

```
-gdist_fmt=matrix|table, string, multiple selections, default:matrix
```

Output format.

The results are saved *.gdist.txt. An example is shown in Figure 13.

	A	B	C	D	E
14	-gdist				
15	-gdist_level=pop				
16	-gdist_estimator=Nei1972,Euclidean				
17	-gdist_fmt=matrix,table				
18					
19					
20	A	B	Nei1972	Euclidean	
21	pop1	pop1	0.000	0.000	
22	pop1	pop2	0.012	0.274	
23	pop1	pop3	0.017	0.320	
24	pop1	pop4	0.016	0.307	
25	pop2	pop2	0.000	0.000	
26	pop2	pop3	0.020	0.351	
27	pop2	pop4	0.022	0.362	
28	pop3	pop3	0.000	0.000	
29	pop3	pop4	0.021	0.360	
30	pop4	pop4	0.000	0.000	
31					
32	Nei1972	pop1	pop2	pop3	pop4
33	pop1	0.000	0.012	0.017	0.016
34	pop2	0.012	0.000	0.020	0.022
35	pop3	0.017	0.020	0.000	0.021
36	pop4	0.016	0.022	0.021	0.000

Figure 13. Example output of genetic distance.

4.15 Analysis of molecular variance

The analysis of molecular variance (AMOVA) partitions the genetic variance into several hierarchies and tests the significance of each variance component. The procedure of AMOVA

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follows Excoffier *et al.* (1992) and Weir & Cockerham (1984), but some modifications are made so as to accommodate polyploids and aneuploids. The parameters related to the AMOVA are listed below.

-amova

Performs analysis of molecular variance. Results are saved in ***.amova.txt**.

-amova_method=homoploid|aneuploid|likelihood, string, multiple selections, default:homoploid

The homoploid method requires that all individuals are homoploids, and performs AMOVA and tests by extracting and permuting the dummy haplotypes. The aneuploid method supports aneuploids and permutes the alleles at each locus. The likelihood method also supports aneuploids, and uses the maximum-likelihood estimator to estimate F-statistics (Fis, Fic, Fit).

-amova_mutation=iam|smm, string, multiple selections, default:iam

Allele mutation model, iam denotes infinity alleles model (Fst like, distance between alleles is a binary variable) and smm denotes stepwise mutation model (Rst like, distance between alleles is the absolute value of their difference in sizes). The smm model can only be applied for non-vcf input file and should use size as the allele identifier.

-amova_ind=yes|no, string, multiple selections, default:yes

Includes the individual level during AMOVA.

-amova_trunc=yes|no, string, default:no

Truncates negative variance component estimates to zero.

-amova_test=yes|no, string, default:yes

Evaluates the significance of each variance component and F-statistics (Fis, Fic, Fit, Fsc, Fst, Fct).

-amova_nperm=99~99999999, integer, default:9999

Number of permutations.

`-amova_pseudo=0~9999, integer, default:50\n");`

Number of pseudo-permutations for the aneuploid method. Zero-value disables the pseudo-permutation.

`-amova_printss=yes|no, string, default:no`

Prints SS within individuals, populations and regions.

The results are saved in `*.amova.txt`. An example is shown in Figure 14.

	A	B	C	D	E	F
14	-amova					
15	-amova_method=anisoploid					
16	-amova_mutation=iam					
17	-amova_ind=yes					
18	-amova_test=yes					
19	-amova_nperm=999					
20	-amova_printss=no					
21						
22						
23	AMOVA Summary, method: anisoploid, mutation model: IAM, ind-level=yes					
24	Source	d.f.	SS	MS	Var	Percentage
25	Within Individual	3600	1253.500	0.348	0.348	99.000
26	Among Individual	1160	421.600	0.363	0.004	1.084
27	Among Population	30	9.831	0.328	0.000	-0.085
28	Total	4790	1684.931	0.352	0.352	100.000
29						
30	F-statistics					
31	Statistics	Value	Permute N	Permute V	Pr(rand>ol)	Pr(rand=obs)
32	FIS	0.011	-0.001	0.000	0.036	0.002
33	FIT	0.010	0.000	0.000	0.074	0.000
34	FST	-0.001	0.000	0.000	0.736	0.005

Figure 14. Example output of AMOVA.

The first table (Lines 23-28) shows the AMOVA summary, and the results of the following four sources are shown in Line 25 to Line 28 in turn: within individuals, among individuals, among populations, among regions and the total population. The second table (Lines 32-34) shows *F*-statistics. The meanings of column headers in Line 24 are as follows.

- D.f., degrees of freedom;
- SS, sum of squares;

4 Usage

- MS, mean squares, i.e., SS/d.f.;
- Var, Variance component.

The meanings of column headers in Line 31 are as follows.

- Permute Mean, Mean of permuted F -statistics;
- Permute Var, Variance of permuted F -statistics;
- Pr(rand>obs), Probability that permuted F -statistics is greater than the original value;
- Pr(rand=obs), Probability that permuted F -statistics is equal to the original value, note that if the difference is smaller than 10^{-7} , then they are considered equal.

4.16 Population assignment

Population assignment assigns each individual to the population/region with the maximum likelihood, and facilitates the identification of the natal population of each individual. The likelihood is the product of genotype frequencies across loci using the allele frequencies of the target population/region (Paetkau *et al.* 2004). The parameters related to the population assignment are listed below:

-popas

Assigns individuals to their natal population according to their genotypic frequencies in each population. Results are saved in `*.popas.txt`.

-popas_plot=yes|no, string, default:no

Draws a barplot for the results of population assignment. Results are saved in `*.popas.pdf`.

-popas_model=rcs|prcs|ces|pes, string, multiple selections, default:rcs

Double-reduction model to calculate genotypic frequencies for polyploids.

-popas_level=pop|reg, string, multiple selections, default:pop

Assigns individuals to populations or regions.

-popas_error=0~0.2, real, default:0.01

Mistype rate, used to avoid the probability of being zero.

The results are saved in *.`.popas.txt`. An example is shown in the Figure 15.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
14	-popas													
15	-popas_model=prcs													
16	-popas_level=pop,reg													
17	-popas_error=0.05													
18														
19														
20	Ind	Pop	#typed	#miss	Ploidy	#Hap	assign_	InL_pop1	InL_pop2	InL_pop3	InL_pop4	assign_reg	InL_Total	prcs
21	Ind1	pop1	10	0	4 - 4	40	pop1	-31.85	-34.714	-33.548	-33.419	Total	-32.894	
22	Ind2	pop1	10	0	4 - 4	40	pop2	-31.619	-31.207	-31.504	-32.51	Total	-31.475	
23	Ind3	pop1	10	0	4 - 4	40	pop1	-35.714	-39.213	-37.239	-37.617	Total	-37.119	
24	Ind4	pop1	10	0	4 - 4	40	pop3	-30.048	-31.974	-29.674	-31.564	Total	-30.52	
25	Ind5	pop1	10	0	4 - 4	40	pop1	-31.856	-33.475	-32.252	-32.73	Total	-32.237	
26	Ind6	pop1	10	0	4 - 4	40	pop1	-31.898	-32.315	-33.333	-32.682	Total	-32.217	
27	Ind7	pop1	10	0	4 - 4	40	pop4	-30.225	-30.146	-31.371	-30.112	Total	-30.219	
28	Ind8	pop1	10	0	4 - 4	40	pop4	-31.015	-31.858	-31.487	-30.806	Total	-30.959	
29	Ind9	pop1	10	0	4 - 4	40	pop2	-30.077	-29.083	-29.85	-30.163	Total	-29.595	
30	Ind10	pop1	10	0	4 - 4	40	pop1	-32.977	-34.492	-33.634	-34.693	Total	-33.583	

Figure 15. Example output of population assignment.

Where each identifier in the column `assign_pop_prcts` is an assigned population under the PRCS double-reduction model, and each value in the subsequent columns is the natural logarithm of the multi-locus genotypic frequency with the hypothesis that the individual originates from a target population.

4.17 Kinship coefficient

The kinship coefficient (also known as the coancestry coefficient) is the probability that two alleles, one randomly drawn from each individual with replacement, are identical-by-descent. VCFPOP provides three method-of-moment estimators to estimate the kinship coefficient between individuals. The parameters related to kinship coefficients are listed in the following.

`-kinship`

4 Usage

Estimates kinship coefficient between individuals. Results are saved in *.kinship.txt.

-kinship_plot=yes|no, string, default:no

Draws a heatmap for the results of kinship estimation. Results are saved in *.kinship.pdf.

-kinship_range=pop|reg|total, string, multiple selections, default:total

Estimates the kinship coefficient between members within the same population, the same region or the total population.

-kinship_fmt=matrix|table, string, multiple selections, default:matrix

Output format.

-kinship_estimator=Ritland1996|Loiselle1995|Weir1996|VanRaden2008, string, multiple selections, default:Ritland1996

Kinship estimators. Supports a maximum level of ploidy of 10.

The results are saved in *.kinship.txt, with the same format as the file of previous section. An example is shown in Figure 16.

	A	B	C	D	E	F	G	H	I	J
14	-kinship									
15	-kinship_fmt=matrix,table									
16	-kinship_range=total									
17	-kinship_estimator=Ritland1996									
18										
19										
20	Total									
21	A	pop	regL1	B	pop	regL1	AB_typed	A_typed	B_typed	Ritland1996
22	Ind1	pop1	reg1	Ind1	pop1	reg1	10	10	10	0.099
23	Ind1	pop1	reg1	Ind2	pop1	reg1	10	10	10	0.003
24	Ind1	pop1	reg1	Ind3	pop1	reg1	10	10	10	0.079
25	Ind1	pop1	reg1	Ind4	pop1	reg1	10	10	10	0.004
26	Ind1	pop1	reg1	Ind5	pop1	reg1	10	10	10	0.023
7282										
7283	Total									
7284	Ritland199	Ind1	Ind2	Ind3	Ind4	Ind5	Ind6	Ind7	Ind8	Ind9
7285	Ind1	0.099	0.003	0.079	0.004	0.023	0.018	-0.033	0.004	-0.055
7286	Ind2	0.003	0.211	0.066	0.022	-0.01	-0.037	-0.006	-0.062	-0.045
7287	Ind3	0.079	0.066	0.472	-0.006	-0.077	0.031	-0.003	0.006	-0.004
7288	Ind4	0.004	0.022	-0.006	0.242	0.012	-0.018	0.048	-0.011	-0.038
7289	Ind5	0.023	-0.01	-0.077	0.012	0.231	0.077	-0.051	0.033	-0.081

Figure 16. Example output of kinship coefficient.

4.18 Relatedness coefficient

Relatedness is the correlation between the means of allele frequencies between individuals (correlation definition), or the probability that an allele sampled from one individual is identical-by-descent to one of the alleles from the other individual (IBD definition). For diploids, VCFPOP provides nine relatedness estimators (Anderson & Weir 2007; Huang *et al.* 2016; Li *et al.* 1993; Lynch & Ritland 1999; Milligan 2003; Queller & Goodnight 1989; Thomas 2010; Wang 2002).

For polyploids and aneuploids, VCFPOP provides two relatedness estimators, in which one is a method-of-moment estimator (Huang *et al.* 2014), and the other is a maximum-likelihood estimator (Huang *et al.* 2015a). The maximum ploidy level supported is eight for Huang *et al.* (2014) and Huang *et al.* (2015a), or ten for Ritland (1996), Loiselle *et al.* (1995), Weir (1996) and VanRaden (2008). Huang *et al.* (2014) is designed for multi-allelic locus with number of alleles greater than ploidy level. Otherwise, a singular matrix problem may be encountered. For two individuals with distinct ploidy levels, the relatedness coefficient between them from the higher ploidy individual to the lower ploidy individual can also be calculated (see Huang *et al.* 2015b). There are two methods (one is the original version and the other is the modified version) to convert the kinship coefficients to the relatedness coefficients: (i) original: $\hat{r}_{HL} = v_{\min} \hat{\theta}_{xy}$ (eqn4, Huang *et al.* 2015b), or (ii) modified:

$$\hat{r}_{HL} = \frac{v_{\min}}{v_{\min} + v_{\max}} \hat{\theta}_{xy} \left(\frac{1}{\hat{\theta}_{xx}} + \frac{1}{\hat{\theta}_{yy}} \right) \text{ (eqn7, Huang } et al. 2015b).$$

The parameters associated with the relatedness coefficients are listed below.

-relatedness

Estimates pairwise relatedness between individuals. Results are saved in `*.relatedness.txt`.

-relatedness_plot=yes|no, string, default:no

Draws a heatmap for the results of relatedness estimation. Results are saved in `*.relatedness.pdf`.

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-relatedness_range=pop|reg|total, string, multiple selections, default:total

Estimates pairwise relatedness between members within the same population, the same region or the total population.

-relatedness_fmt=matrix|table, string, multiple selections, default:matrix

Output format.

-relatedness_estimator=Lynch1999|Wang2002|Thomas2010|Li1993|Queller1989|Huang2016A|Huang2016B|Milligan2003|Anderson2007|Huang2014|Huang2015|Ritland1996_modified|Loiselle1995_modified|Ritland1996|Loiselle1995|Weir1996|VanRaden2008, string, multiple selections, default=Huang2014

Relatedness estimators: Huang2014 and Huang2015 support ploidy level <= 8, Ritland1996, Loiselle1995, Weir1996 and VanRaden2008 estimators support ploidy level <= 10, and other estimators only support diploids. Milligan2003, Anderson2007 and Huang2015 are maximum-likelihood estimators, and other estimators are method-of-moment estimators. Unbiased Ritland1996 and Loiselle1995 relatedness estimates are converted from kinship coefficient by eqn (7) of Huang et al. (2015, Mol Ecol Resour).

The results are saved in *.relatedness.txt. The table format results come first, then the matrix format results. An example is shown in Figure 17.

	A	B	C	D	E	F	G	H	I
14	-relatedness								
15	-relatedness_range=pop								
16	-relatedness_fmt=matrix,table								
17	-relatedness_estimator=Huang2015								
18									
19									
20	pop1								
21	A	pop	B	pop	AB_typed	A_typed	B_typed	Huang2015	
22	Ind1	pop1	Ind1	pop1	10	10	10	1.000	
23	Ind1	pop1	Ind2	pop1	10	10	10	0.101	
24	Ind1	pop1	Ind3	pop1	10	10	10	0.250	
25	Ind1	pop1	Ind4	pop1	10	10	10	0.250	
26	Ind1	pop1	Ind5	pop1	10	10	10	0.228	
487									
488	pop1								
489	Huang2015	Ind1	Ind2	Ind3	Ind4	Ind5	Ind6	Ind7	Ind8
490	Ind1	1.000	0.101	0.250	0.250	0.228	0.250	0.000	0.121
491	Ind2	0.101	1.000	0.250	0.088	0.006	0.000	0.000	0.000
492	Ind3	0.250	0.250	1.000	0.000	0.000	0.250	0.000	0.000
493	Ind4	0.250	0.088	0.000	1.000	0.000	0.000	0.330	0.000
494	Ind5	0.228	0.006	0.000	0.000	1.000	0.250	0.000	0.310
495	Ind6	0.250	0.000	0.250	0.000	0.250	1.000	0.181	0.250
496	Ind7	0.000	0.000	0.000	0.330	0.000	0.181	1.000	0.063
497	Ind8	0.121	0.000	0.000	0.000	0.310	0.250	0.063	1.000

Figure 17. Example output of relatedness coefficient.

Where each value in the column AB_typed (A_typed or B_typed) is the number of loci genotyped in the individuals A and B (the individual A or the individual B), and each value in the column Thomas2010 is the relatedness estimate.

4.19 Principal coordinates analysis

The principal coordinates analysis (PCoA) is an ordination technique to coordinate individuals into a multi-dimensional space by their dissimilarity to visualize the data, reduce the dimension of data with the least loss of information, and eliminate the correlation among coordinates. This technique is performed based on the genetic distances. The results using Euclidean distance are equivalent to principal components analysis (PCA). The parameters related to the PCoA are listed as follows.

-pcoa

Performs a principal coordinate analysis for individuals, populations or regions. Results are saved in *.pcoa.txt.

-pcoa_plot=yes|no, string, default:no

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Draws a scatter plot for the results of principal coordinate analysis. Results are saved in *.pcoa.pdf.

-pcoa_level=ind|pop|reg, string, multiple selections, default:ind

Ordinate individuals, populations or regions.

-pcoa_dim=1~4096, default:3

Number of dimensions to output.

-pcoa_estimator=Nei1972|Cavalli-Sforza1967|Reynolds1983|Nei1983|Euclidean|Goldstein1995|Nei1974|Roger1972|Slatkin_Nei1973|Slatkin_Weir1984|Slatkin_Hudson1992|Slatkin_Slatkin1995|Slatkin_Hedrick2005|Slatkin_Jost2008|Slatkin_Huang2021_homo|Slatkin_Huang2021_aneu|Reynolds_Nei1973|Reynolds_Weir1984|Reynolds_Hudson1992|Reynolds_Slatkin1995|Reynolds_Hedrick2005|Reynolds_Jost2008|Reynolds_Huang2021_homo|Reynolds_Huang2021_aneu, string, multiple selections, default:Nei1972

Genetic distance estimators. Results of Euclidean distance are equivalent to PCA.

The results are saved in *.pcoa.txt. An example is shown in Figure 18.

	A	B	C	D
14	-pcoa			
15	-pcoa_level=pop			
16	-pcoa_estimator=Euclidean			
17				
18				
19	Euclidean			
20	Total variance	0.055		
21	Variance	0.022	0.021	0.011
22	Pop	PC1	PC2	PC3
23	pop1	0.023	0.059	-0.152
24	pop2	0.137	0.121	0.090
25	pop3	0.052	-0.212	0.016
26	pop4	-0.211	0.032	0.046

Figure 18. Example output of relatedness coefficient.

Row 19 shows the total variance, and Row 20 shows the variance in each extracted principal coordinates. Rows 22-26 form a table showing the results of a PCoA by using the Nei1972 estimator of genetic distances, in which PC1, PC2 and PC3 are three principal coordinates of an individual,

and each eigenvalue is the variance of the corresponding principal coordinates. Using a figure-plotting software, these individuals (populations or regions) can be shown in a 2D or 3D scatter-plot.

4.20 Hierarchical clustering

VCFPOP can also perform the hierarchical clustering based on the genetic distances. The parameters related to the hierarchical clustering are listed as follows.

-cluster

Perform hierarchical clustering for individuals, populations or regions. Results are saved in `*.cluster.txt` in standard tree format.

-cluster_plot=yes|no, string, default:no

Draws a dendrogram for the results of hierarchical clustering. Results are saved in `*.cluster.pdf`.

-cluster_level=ind|pop|reg, string, multiple selections, default:ind

Level of object in clustering: individuals, populations or regions.

-cluster_method=NEAREST|FURTHEST|UPGMA|WPGMA|UPGMC|WPGMC|WARD, string, multiple selections, default:UPGMA

Clustering methods.

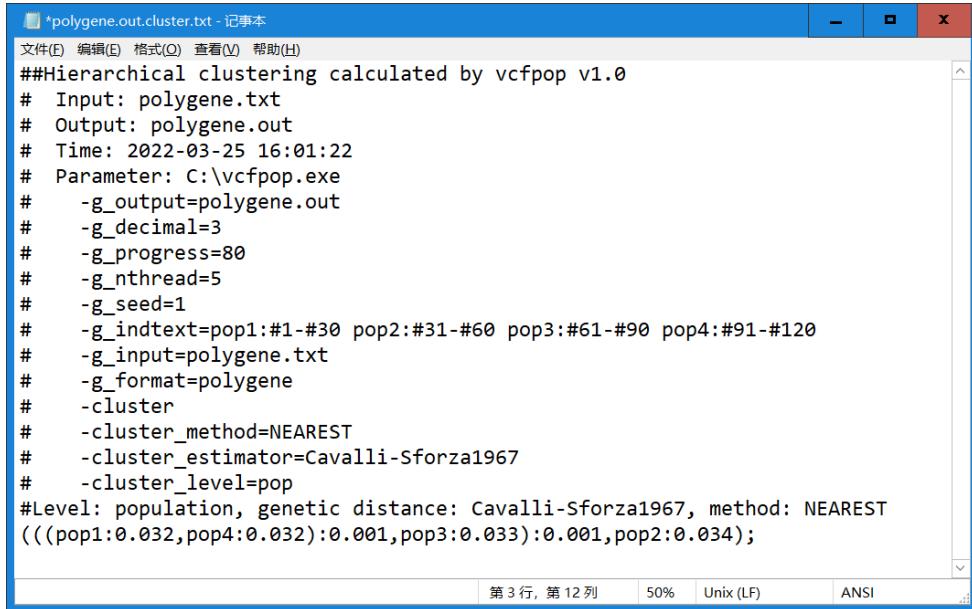
-cluster_estimator=Nei1972|Cavalli-Sforza1967|Reynolds1983|Nei1983|Euclidean|Goldstein1995|Nei1974|Roger1972|Slatkin_Nei1973|Slatkin_Weir1984|Slatkin_Hudson1992|Slatkin_Slatkin1995|Slatkin_Hedrick2005|Slatkin_Jost2008|Slatkin_Huang2021_homo|Slatkin_Huang2021_anneu|Reynolds_Nei1973|Reynolds_Weir1984|Reynolds_Hudson1992|Reynolds_Slatkin1995|Reynolds_Hedrick2005|Reynolds_Jost2008|Reynolds_Huang2021_homo|Reynolds_Huang2021_anneu, string, multiple selections, default:Nei1972

Genetic distance estimators.

The results are saved in `*.cluster.txt` in the standard tree format. Such a file can be loaded by

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other tree viewing software. An example of tree files is shown in Figure 19.



```
*polygene.out.cluster.txt - 记事本
文件(F) 编辑(E) 格式(Q) 查看(V) 帮助(H)
##Hierarchical clustering calculated by vcfpop v1.0
# Input: polygene.txt
# Output: polygene.out
# Time: 2022-03-25 16:01:22
# Parameter: C:\vcfpop.exe
#   -g_output=polygene.out
#   -g_decimal=3
#   -g_progress=80
#   -g_nthread=5
#   -g_seed=1
#   -g_inddtext=pop1:#1-#30 pop2:#31-#60 pop3:#61-#90 pop4:#91-#120
#   -g_input=polygene.txt
#   -g_format=polygene
#   -cluster
#   -cluster_method=NEAREST
#   -cluster_estimator=Cavalli-Sforza1967
#   -cluster_level=pop
#Level: population, genetic distance: Cavalli-Sforza1967, method: NEAREST
((pop1:0.032,pop4:0.032):0.001,pop3:0.033):0.001,pop2:0.034);
```

Figure 19. Example output of hierarchical clustering.

4.21 Bayesian clustering

Bayesian clustering estimates ancestral proportions of each individual by the Markov Chain Monte Carlo (MCMC) method. Following STRUCTURE (Pritchard *et al.* 2000), three models are included: ADMIXTURE (Pritchard *et al.* 2000), LOCPRIORI (Hubisz *et al.* 2009) and F model (Falush *et al.* 2003). The parameters related to Bayesian clustering are listed below.

-structure

Perform Bayesian clustering. Results are saved in ***.structure.txt** and ***.structure.k=? .rep=?_id=? .txt**. The former is the summary and the latter is the result of each run.

-structure_nstream=1~32

Number of tasks simultaneously run, each task uses $\text{ceil}(g_nthread / \text{structure_nstream})$ CPU threads.

-structure_plot=yes|no, string, default:no

Draws barplots for the results of Bayesian clustering. Results are saved in *.structure.pdf.

-structure_writelnl=yes|no, string, default:no

Write log likelihood for each run. Results are saved in *.lnl.txt.

Models:

In the non-ADMXTURE model, each individual is assumed to only originate from one cluster, whilst in the ADMIXTURE model, each allele copy within an individual is assumed to originate from one cluster. The LOCPRIORI model takes advantage of the sample population of each individual to help infer weak population structure. The F model assumes that the allele frequencies in each cluster are correlated with those in the ancestral cluster. The parameters used to configure these models are as follows.

-structure_admix=yes|no, string, default:no

ADMX model assumes each allele copy at each locus within the same individual can be drawn from the different clusters. Otherwise, all allele copies within the same individual are drawn from a cluster in each iteration.

-structure_locpriori=yes|no, string, default:no

LOCPRIORI model uses the sample population to cluster individuals.

-structure_f=yes|no, string, default:no

F model assumes the allele frequencies in each cluster are correlated with that in the ancestral population.

MCMC parameters:

Configures the parameters used for the MCMC algorithm.

-structure_krange=[min_val,max_val], integer range, default:[1,5]

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Range of K (number of clusters).

`-structure_nburnin=0~10000000, integer, default:1000`

Number of burn-in cycles.

`-structure_nreps=0~10000000, integer, default:10000`

Number of iterations after burn-in.

`-structure_nthinning=1~10000, integer, default:10`

Sampling interval to dememorize.

`-structure_nrungs=1~1000, integer, default:1`

Number of independent runs for each value of K.

`-structure_nadmburnin=0~10000000, integer, default:500`

Number of admixture burn-in cycles. This parameter is used for the non-ADMIXTURE and non-LOCPRIORI models, which generates a proper initial state to prevent the Markov chain to be blocked in the local maxima.

Misc:

λ is the Dirichlet parameter used to update the allele frequencies in each cluster. The value of λ can be updated during iterations if the option `-structure_inferlambda=yes` is used. The two options `-structure_stdlambda` and `-structure_maxlambda` configure the generation of a new λ . The option `-structure_difflambda=yes` allow using a different λ in different clusters. The parameters related to allele frequency and admixture burnin are as follows:

`-structure_lambda=0~10000, real, default:1`

The initial value of lambda.

`-structure_inferlambda=yes|no, string, default:no`

Updated lambda in each iteration.

`-structure_stdlambda=0~10000, real, default:0.3`

Standard deviation of new lambda.

-structure_maxlambda=0~10000, real, default:10

Maximum of new lambda.

-structure_difflambda=yes|no, string, default:no

Use separate lambda for each cluster.

-structure_diversity=yes|no, default:no

Output diversity parameters for each cluster.

ADMIX:

In the ADMIXTURE model (Pritchard *et al.* 2000), α is the Dirichlet parameter and is used to update the admixture proportions of individuals, whose initial value is configured by **-structure_alpha**. The value of α will be updated according to **-structure_stdalpha** or **-structure_maxalpha** if **-structure_inferalpha=yes** is used. The *a priori* distribution of α is either a uniform distribution or a gamma distribution, which is used to evaluate the new value of α . The parameters related to the ADMIXTURE model are as follows:

-structure_alpha=0~10000, real, default:1

The initial alpha, the priori Dirichlet parameter of admixture proportions Q.

-structure_inferalpha=yes|no, string, default:yes

Update alpha in ADMIX model.

-structure_diffalpha=yes|no, string, default:no

Use separate alpha for each cluster.

-structure_uniformalpha=yes|no, string, default:yes

Priori distribution for alpha, yes for uniform distribution and no for gamma distribution.

-structure_stdalpha=0~10000, real, default:0.025

Standard deviation of uniform priori distribution of alpha.

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-structure_maxalpha=0~10000, real, default:10

Maximum of uniform priori distribution of alpha.

-structure_alphaapriori=0~10000, real, default:0.05

One gamma priori distribution parameter.

-structure_alphaapriorb=0~10000, real, default:0.001

The other gamma priori distribution parameter.

-structure_metrofreq=0~1000000, integer, default:10

Frequency of Metropolis-Hastings update of admixture proportions Q, set 0 to disable Metropolis-Hastings update.

LOCPRIORI:

In the LOC PRIORI model, a weak population structure can be inferred under the assistance of sample group information. There are three parameters r , η and γ in this mode. The parameter r is used to estimate the informativeness of the data for the sampling location. The parameter η and γ are used in the non-ADMIIXTURE model to reflect the relative proportions of individuals assigned to a cluster. The values of η or γ is updated by drawing a new value from the uniform distribution $\eta \pm \text{eps}(\eta)$ or $\gamma \pm \text{eps}(\gamma)$. For the ADMIXTURE model, LOC PRIORI model adds one additional Dirichlet parameter, called the local α , and the original α is called the global α to distinguish from the local α . The global α and the local α are updated by drawing two new values from two normal distributions, respectively. The parameters related to the LOC PRIORI model are listed as follows:

-structure_r=0~10000, real, default:1

Initial value of r, where r evaluates the informativeness of data for the sampling location.

-structure_maxr=0~10000, real, default:20

Maximum of new r.

-structure_epsr=0~10000, real, default:0.1

Max step value of new r.

-structure_epseta=0~10000, real, default:0.025

Max step value of new eta for the non-ADMIIXTURE model, where eta reflects the relative proportion of individuals assigned to a cluster.

-structure_epsgamma=0~10000, real, default:0.025

Max step value of new gamma for the non-ADMIIXTURE model, where gamma reflects the relative proportion of individuals sampled from a location and assigned to a cluster.

FMODEL:

In the F model (Falush *et al.* 2003), the parameter F is a measure analogous to the Wright's F_{ST} , which is evaluated by the correlation between the allele frequencies in a cluster. In the update of F , the new value of F is drawn from the normal distribution $N(F, \text{std}^2(F))$, and is evaluated according to the priori gamma distribution $\Gamma(A, B)$. The frequencies of alleles in the ancestral cluster are also updated.

-structure_pmeanf=0~10000, real, default:0.01

Priori mean F, where F is the amount of drift from the ancestral population to the cluster k in the F model.

-structure_pstdf=0~10000, real, default:0.05

Priori standard deviation of F.

-structure_std़f=0~10000, real, default:0.05

Standard deviation of new F.

-structure_singlef=yes|no, string, default:no

Use the same F in all clusters.

The results are saved in `*.structure.txt` and `*.structure.k=5.r=1.txt`. The former is a summary and the latter is the result of each run. An example of summary file is shown in Figure

20.

ID	k	rep	Mean	lnL	Var	lnL	lnP(D)
1	2	1	-6132.644		119.283	-6192.285	
2	2	2	-6131.247		112.744	-6187.619	
3	2	3	-6129.806		99.679	-6179.645	
4	2	4	-6128.718		91.318	-6174.377	
5	3	1	-6125.633		188.282	-6219.774	
6	3	2	-6112.228		213.989	-6219.222	
7	3	3	-6115.024		158.782	-6194.415	
8	3	4	-6110.154		299.864	-6260.086	
9	4	1	-6097.419		269.300	-6232.069	

Figure 20. Example output of Bayesian clustering.

The out data consist of the mean and variance of natural logarithms of likelihoods, the $\ln P(D)$ and the estimated parameters used in a specified model, where the estimated parameters are as follows: α for the ADMIXTURE model; r , η and γ for the LOCPRIORI model; r , the global α and the local α for the LOCPRIORI + ADMIXTURE model. Moreover, for the F model, the value of F for each cluster or the value of the global F is also included in the output. The next output consists of four tables: (i) proportion of membership of each pre-defined population in each cluster; (ii) inferred ancestry of individuals; (iii) allele-frequency divergence among populations (net nucleotide distance); and (iv) estimated heterozygosity in each cluster (if the option `-structure_diversity=yes` is used, otherwise the results will not be outputted).

An example of results of a run is shown in the following.

```

Parameters:
Seed=313261304
Model=NOADM,LOC,F
Number of cluster=4
Replicate=1
Run id=1
Mean value of ln likelihood=-9841.448123
Variance of ln likelihood=2628.160010
Estimated Ln Prob of Data=-11155.528129

Mean value of r=4.325618
Mean value of global eta
    Cluster
      1       2       3       4
    0.292969  0.148320  0.222200  0.336511
Mean value of local gamma for each location
    Cluster
Pop     1       2       3       4
DefPop  0.327675  0.196635  0.228096  0.247593
pop1    0.344244  0.060885  0.298596  0.296274
pop2    0.249133  0.191707  0.069353  0.489806
pop4    0.434370  0.039163  0.170569  0.355898
Mean value of Fst
    Cluster
      1       2       3       4
    0.660690  0.803997  0.668524  0.726568

Proportion of membership of each pre-defined population in each of the 4
clusters
    Cluster
Pop     1       2       3       4
DefPop  0.346233  0.179909  0.235900  0.237958
pop1    0.360000  0.000000  0.320000  0.320000
pop2    0.320000  0.120000  0.040000  0.520000
pop4    0.560000  0.000000  0.160000  0.280000

Inferred ancestry of individuals
    Cluster
Ind     Pop      1       2       3       4
HG00096 pop1    0.845900  0.082100  0.065500  0.006500
HG00097 pop1    0.000000  0.000000  1.000000  0.000000
HG00099 pop1    0.008800  0.000200  0.986400  0.004600
...
Allele-frequency divergence among pops (Net nucleotide distance)
    Cluster
Cluster 1       2       3       4
1        0.000000  0.001296  0.012116  0.009320
2        0.001296  0.000000  0.012108  0.006990
3        0.012116  0.012108  0.000000  0.014451
4        0.009320  0.006990  0.014451  0.000000

```

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5.1 Sliding window

Assuming the sliding window size is W and the target sliding window is consisting of K polymorphic variants in the current population. F_{ST} is estimated with the same method as genetic differentiation with the K variants.

Absolute divergence, d_{xy} , is defined as the average number of nucleotide differences per site between two sequences sampled from different populations. The value of d_{xy} is calculated by

$$d_{xy} = \sum_{k=1}^K \frac{D_k}{N_k}.$$

Where D_k is the number of nucleotide differences between two populations, or among all populations at k^{th} variant, and N_k is the total number of pairwise comparisons between two populations at k^{th} variant.

Nucleotide diversity, π , is defined as the expected number of nucleotide differences between two random sequences sampled without replacement. The value of π is calculated by

$$\pi = \sum_{k=1}^K \frac{n_k}{n_k - 1} \cdot H_{e,k}.$$

Where n_k is the number of sequences (i.e., haplotypes) at the k^{th} polymorphic variant, and $H_{e,k}$ is the expected heterozygosity at the k^{th} variant. $\frac{n_k}{n_k - 1}$ converts $H_{e,k}$ into π_k because $H_{e,k}$ is sampling with replacement, while π_k is sampling without replacement.

Watterson's θ_w estimates the population mutation rate of target sliding window. It is defined as

$$\theta_w = \frac{K}{a_n}.$$

Where a_n is the $(n - 1)^{\text{th}}$ harmonic number and is equal to $\sum_{i=1}^{n-1} 1/i$. However, different variants have different sample size, we use the following correction:

$$\theta_w = \sum_k \frac{1}{a_{n,k}}.$$

Tajima's D, D , measures the difference between the number of variants and the average number of pairwise differences in a sample of sequences. Which is calculated by

$$D = \frac{d}{\sqrt{\widehat{\text{Var}}(d)}} = \frac{\pi - \theta_w}{\sqrt{\widehat{\text{Var}}(\pi - \theta_w)}}.$$

Where $\widehat{\text{Var}}(\pi - \theta_w) = e_1 K + e_2 K(K - 1)$ and

$$\begin{aligned} a_1 &= \sum_{i=1}^{n-1} 1/i, & a_2 &= \sum_{i=1}^{n-1} 1/i^2, \\ b_1 &= \frac{n+1}{3(n-1)}, & b_2 &= \frac{2(n^2+n+3)}{9n(n-1)}, \\ c_1 &= b_1 - \frac{1}{a_1}, & c_2 &= b_2 - \frac{n+2}{a_1 n} + \frac{a_2}{a_1^2}, \\ e_1 &= \frac{c_1}{a_1}, & e_2 &= \frac{c_2}{a_1^2 + a_2}. \end{aligned}$$

For varying sample sizes,

$$\widehat{\text{Var}}(\pi - \theta_w) = \sum_{i=1}^K \frac{c_{1i}}{a_{1i}} + \sum_{i \neq j} \frac{\sqrt{c_{2i}c_{2j}}}{\sqrt{a_{2i}a_{2j}} + a_{1i}a_{1j}}.$$

5.2 LD decay and LD block

VCFPOP provides four LD measures, r^2 , D' , r_Δ^2 and Δ' . Where the former two can only be applied for phased genotypes, and latter two can be used for either phased or unphased genotypes.

Squared correlation coefficient, r^2 , is the squared Pearson's correlation coefficient between the allelic values at two loci for phased genotypic data in a population. Which is calculated by

$$r^2 = \frac{\sum_A \sum_B D_{AB}^2}{\sum_A \sum_B Q_{AB}}.$$

The two summation operators enumerate the alleles (denoted by A and B) in the two loci, D_{AB} and

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Q_{AB} are respectively calculated by

$$D_{AB} = P_{AB} - P_A P_B \quad \text{and} \quad Q_{AB} = P_A(1 - P_A)P_B(1 - P_B).$$

Where P_{AB} is the frequency of haplotype AB , and P_A and P_B are respectively the allele frequency of A and B .

Normalized linkage disequilibrium coefficient, D' , is calculated by

$$D' = \frac{\sum_A \sum_B |D_{AB}|}{\sum_A \sum_B |D_{\max,AB}|}.$$

Where

$$D_{\max,AB} = \begin{cases} \max\{-P_A P_B, -(1 - P_A)(1 - P_B)\}, & \text{if } D \leq 0 \\ \min\{P_A(1 - P_B), P_B(1 - P_A)\}, & \text{if } D > 0. \end{cases}$$

Squared correlation coefficient based on Burrow's Δ , r_Δ^2 , is the squared Pearson's correlation coefficient between the alleles at two loci for phased or unphased genotypic data. Which is calculated by

$$r_\Delta^2 = \frac{\sum_A \sum_B \Delta_{AB}^2}{\sum_A \sum_B R_{AB}}.$$

Which the first summation operator enumerates all loci pairs in the sliding window, and the second and third summation operator enumerate the alleles (denoted by A and B) at the two loci (l_1 and l_2), Δ_{AB} and R_{AB} are respectively calculated by

$$\Delta_{AB} = \left(\sum_{i=1}^v \sum_{j=1}^v \frac{ij}{v} G_{ij}^{AB} \right) - v P_A P_B,$$

$$R_{AB} = [P_A + (v - 1)P_{AA} - vP_A^2][P_B + (v - 1)P_{BB} - vP_B^2].$$

Where G_{ij}^{AB} is the frequency two-locus genotype that containing exactly i copies of allele A and j copies of allele B , P_{AA} and P_{BB} are the probabilities of sampling two copies of A and B within the same individual without replacement.

Normalized linkage disequilibrium coefficient based on Burrow's Delta, Δ' , is calculated by

$$\Delta' = \frac{\sum_A \sum_B |\Delta_{AB}|}{\sum_A \sum_B |\Delta_{\max,AB}|}.$$

Where

$$\Delta_{\max,AB} = \begin{cases} (v - 1)[P_{AX}P_B/(1 - P_A)] - vP_AP_B & \text{if } \Delta_{AB} \leq 0, P_A + P_B \leq 1 \\ \lambda + (v - 1)[P_{AA}\lambda/P_A + P_{AX}] - vP_AP_B, & \text{if } \Delta_{AB} \leq 0, P_A + P_B > 1 \\ P_A + (v - 1)[P_{AA} + P_{AX}(P_B - P_A)/(1 - P_A)] - vP_AP_B, & \text{if } \Delta_{AB} > 0, P_A \leq P_B \\ P_B + (v - 1)[P_{AA}P_B/P_A] - vP_AP_B, & \text{if } \Delta_{AB} > 0, P_A > P_B. \end{cases}$$

Where $\lambda = P_A + P_B - 1$, $P_{AX} = 1 - P_{AA}$.

For varying ploidy levels among individuals, because Δ_{AB} is defined as $P_s^{AB} + (v - 1)P_d^{AB} - vP_AP_B$, where P_s^{AB} is the probability that the alleles in the same haplotype are A and B , P_d^{AB} is the probability that alleles in different haplotypes within the same individual are A and B . We expand Δ_{AB} as

$$\Delta_{AB} = \frac{\sum_i^n \sum_a^{\nu_i} \sum_b^{\nu_i} B_{iab}^{AB}}{\sum_i \nu_i} - \tilde{v}P_AP_B.$$

Where ν_i is the ploidy level of individual i , B_{iab}^{AB} equal one if the a^{th} haplotype of individual i contains allele A and the b^{th} haplotype of individual i contains allele B , otherwise zero. Because there are $\sum_i \nu_i^2$ haplotype pairs that B_{iab}^{AB} indicates and $\sum_i \nu_i$ are the same haplotype, therefore we define the adjusted ploidy level $\tilde{v} = \sum_i \nu_i^2 / \sum_i \nu_i$ to allow the above equation consistent. \hat{R}_{AB} and $\Delta_{\max,AB}$ should be accordingly revised by replacing v as \tilde{v} .

Weighing procedure

The LD decay curve is the LD measures as a function of physical distance (in bp). The dot for an interval is the curve is the weighted average of LD measures across all allele pairs within this interval. Similarly, in LD block analysis, the LD measures are weighted averaged across all allele pairs between intervals.

All these LD measures can be expressed by

$$X = \frac{A}{B}.$$

Let V_1 be $\sum_i B_i$ and V_2 be $\sum_i B_i^2$, the weighted average is given by

$$\bar{X}^* = \frac{\sum_i A_i}{\sum_i B_i} = \frac{\sum_i B_i X_i}{V_1} = \sum_i w'_i X_i.$$

Where the normalized weight $w'_i = B_i/V_1$. Assuming X_i are independently drawn from the same distribution with a variance σ^2 , then

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$$\text{Var}(\bar{X}^*) = \sum_i w_i'^2 \text{Var}(X_i) = \sigma^2 V_2 / V_1^2.$$

Therefore, the standard error of \bar{X}^* can be estimated by

$$\text{SE}(\bar{X}^*) = \sqrt{s_W^2 \sum_i w_i'^2} = \sqrt{s_W^2 V_2 / V_1^2}.$$

Where the reliability-based weighted sample variance is calculated by

$$s_W^2 = \frac{\sum_i w_i' X_i^2 - \bar{X}^{*2}}{1 - V_2/V_1^2}.$$

The 95% confidence interval is calculated by $\bar{X}^* \pm 1.96\text{SE}(\bar{X}^*)$.

5.3 Genome-wide association study

The linear mixed-effect model of GWAS is

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{b} + \boldsymbol{\epsilon},$$

where \mathbf{Y} is a $n \times 1$ trait matrix, \mathbf{X} is the design marices of fixed effects, $\boldsymbol{\beta}$ is the fixed-effect matrix, \mathbf{b} is a $n \times 1$ random effect matrix and $\mathbf{b} \sim \text{MVN}(\mathbf{0}, r^2 \sigma^2 \mathbf{R})$, $\boldsymbol{\epsilon}$ is the random error and $\boldsymbol{\epsilon} \sim \text{MVN}(\mathbf{0}, \sigma^2 \mathbf{I})$, and \mathbf{R} is a $n \times n$ relatedness matrix.

Specifically, \mathbf{X} is consisting of three submatrices:

$$\mathbf{X} = [\mathbf{X}_E \ \mathbf{X}_I \ \mathbf{X}_G],$$

where \mathbf{X}_E is the environmental variables, \mathbf{X}_I is originally $\mathbf{1}$ to pass the intercept and \mathbf{X}_G is originally the column vector saving the dosage of reference allele at target locus. Similarly, $\boldsymbol{\beta}$ is also consisting of three submatrices which representing the effects of environmental variables, intercept and the effects of genotypes, i.e., $\boldsymbol{\beta}^T = [\boldsymbol{\beta}_E^T \ \boldsymbol{\beta}_I^T \ \boldsymbol{\beta}_G^T]$.

According to the above model, \mathbf{Y} is accord with a multivariate normal distribution

$$\mathbf{Y} \sim \text{MVN}(\mathbf{X}\boldsymbol{\beta}, \mathbf{D}), \quad \mathbf{D} = r^2 \sigma^2 \mathbf{R} + \sigma^2 \mathbf{I}.$$

The likelihood is

$$L(\boldsymbol{\beta}, \sigma^2, r^2) = f(\mathbf{Y} | \boldsymbol{\beta}, \sigma^2, r^2) = (2\pi)^{-n/2} |\mathbf{D}|^{-1/2} \exp \left[-\frac{1}{2} (\mathbf{Y} - \mathbf{X}\boldsymbol{\beta})^T \mathbf{D}^{-1} (\mathbf{Y} - \mathbf{X}\boldsymbol{\beta}) \right].$$

Where $f(\cdot)$ denotes the probability density function. The model is solved by optimizing the likelihood under maximum-likelihood (ML) criterion or restricted maximum-likelihood (REML) criterion. The ML criterion is the same as above equation, while the REML criterion integrates β over \mathbb{R}^k assuming the probability density function $f(\beta) = 1$.

$$L_{\text{REML}}(\sigma^2, r^2) = \int_{\beta \in \mathbb{R}^k} f(\beta) f(\mathbf{Y}|\beta, \sigma^2, r^2) d\alpha_\beta.$$

To reduced the optimization dimension, β and σ^2 can be profiled out, which still maximized the likelihoods. The profiled σ^2 and β can be obtained from r^2 by

$$\beta_{\text{profiled}} = \mathbf{E}_{0,1}^{-1} \mathbf{F}_{0,1} \quad \text{and} \quad \sigma_{\text{profiled}}^2 = \begin{cases} \frac{1}{n} (\mathbf{J}_{0,1} - \mathbf{F}_{0,1}^T \mathbf{E}_{0,1}^{-1} \mathbf{F}_{0,1}), & \text{for ML criterion,} \\ \frac{1}{n-k} (\mathbf{J}_{0,1} - \mathbf{F}_{0,1}^T \mathbf{E}_{0,1}^{-1} \mathbf{F}_{0,1}) & \text{for REML criterion.} \end{cases}$$

The profiled log-likelihoods are:

$$\log L_{\text{ML}}(r^2) = -\frac{1}{2} \left\{ n \log 2\pi + n \log \left[\frac{1}{n} (\mathbf{J}_{0,1} - \mathbf{F}_{0,1}^T \mathbf{E}_{0,1}^{-1} \mathbf{F}_{0,1}) \right] + \log |\mathbf{V}_r| + n \right\},$$

$$\log L_{\text{REML}}(r^2) = -\frac{1}{2} \left\{ (n-k) \log 2\pi + (n-k) \log \left[\frac{1}{n-k} (\mathbf{J}_{0,1} - \mathbf{F}_{0,1}^T \mathbf{E}_{0,1}^{-1} \mathbf{F}_{0,1}) \right] + \log |\mathbf{V}_r| + \log |\mathbf{E}_{0,1}| + (n-k) \right\}.$$

Where

$$\begin{aligned} \mathbf{V}_r &= r^2 \mathbf{V} + \mathbf{I}, & \mathbf{E}_{i,j} &= \mathbf{X}^T \mathbf{U} \mathbf{V}^i \mathbf{V}_r^{-j} \mathbf{U}^T \mathbf{X}, \\ \mathbf{F}_{i,j} &= \mathbf{X}^T \mathbf{U} \mathbf{V}^i \mathbf{V}_r^{-j} \mathbf{U}^T \mathbf{Y}, & \mathbf{J}_{i,j} &= \mathbf{Y}^T \mathbf{U} \mathbf{V}^i \mathbf{V}_r^{-j} \mathbf{U}^T \mathbf{Y}, \end{aligned}$$

and \mathbf{U} and \mathbf{V} are respectively the eigen-vectors and eigen-values of \mathbf{R} .

VCFPOP made several modications to accommodate multi-allelic loci and multiple ploidies.

1. \mathbf{X}_G becomes the proportion of alleles when `-gwas_dosage=no` is used, this assumes different ploidy have the same increase in trait value from the homozygote of alternative allele to the homozygote of reference allele, for example: $p_{AAAA} - p_{aaaa} = p_{AA} - p_{aa}$, where p_* is the expected trait value of genotype *. Otherwise, `-gwas_dosage=yes` assumes the per-allele-copy effect is the same among different ploidies, e.g., $p_{AAAA} - p_{aaaa} = 2(p_{AA} - p_{aa})$.
2. \mathbf{X}_G becomes a $n \times (J-1)$ matrix at multi-allelic loci, whose j -th column is the dosage (or proportion) of j -th allele.
3. Different ploidies can use different slopes by using `-gwas_slope=yes`. This is done by assigning \mathbf{X}_I as the binary ploidy matrix \mathbf{B} , whose ij -th element \mathbf{B} is one if individual i is

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ploidy j at target locus.

$$\mathbf{X}_I = \mathbf{1}_{n \times 1} \otimes \mathbf{B}.$$

The operator $\mathbf{A} \otimes \mathbf{B}$ combines the Hadamard products of between columns of \mathbf{A} and \mathbf{B} .

4. Different ploidies can use different intercepts by using `-gwas_slope=yes`. This is done by assigning \mathbf{X}_G as the binary ploidy matrix.

$$\mathbf{X}_G = \mathbf{G} \otimes \mathbf{B}.$$

5. The tests used are also extended to the case of multi-allelic and multiple ploidies.

The null hypothesis of these tests are the effect of genotypes $\beta_G = \mathbf{0}$. The likelihood ratio test (LRT) fits the model with \mathbf{X}_G (full model) and without \mathbf{X}_G (null model), the twice difference in the optimized likelihoods (under ML criterion) between the two models is the $\Delta\text{Deviance}$ static,

$$\Delta\text{Deviance} = 2 \cdot (\log L_{\text{ML},\text{full}} - \log L_{\text{ML},\text{null}}),$$

The rationale of LRT test is that the likelihood of full model will not greatly improved compared with the null model under null hypothesis, and $\Delta\text{Deviance}$ is asymptotically accord with a χ^2 distribution with d.f. being the number of columns of \mathbf{X}_G .

The score test fits the the null model, then extend the slope $\hat{\beta}_{\text{null}}$ with the assumption $\hat{\beta}_G = \mathbf{0}$:

$$\hat{\beta}_{\text{score}} = \begin{bmatrix} \hat{\beta}_{\text{null}} \\ \mathbf{0} \end{bmatrix}.$$

Then calculates the gradient vector of the full log-likelihood function of the full modell with respect to β at $\hat{\beta}_{\text{score}}$:

$$\mathbf{G}_{\text{score}} = \frac{\partial \log L(\beta, \sigma^2, r^2)}{\partial \beta} \Big|_{\beta=\hat{\beta}_{\text{score}}} = \frac{1}{\sigma^2} (\mathbf{F}_{0,1} - \mathbf{E}_{0,1} \hat{\beta}_{\text{score}}),$$

The rationale is $\mathbf{G} \sim \text{MVN}(\mathbf{0}, \mathbf{V})$ under null hypothesis, the normalizd \mathbf{G} (by $\mathbf{V}^{-1/2} \mathbf{G}$) is accord with $\text{MVN}(\mathbf{0}, \mathbf{I})$, then the sum of squares of the elements of normalized \mathbf{G} is accord with a χ^2 distribution. \mathbf{V} can be estimated by Outer product of gradients (OPG), Hessian and Sandwich estimators, but only the P value from Hessian estimator is uniformlly distributed under null hypothesis. The Hessian estimator of the covariance matrix of $\hat{\beta}_{\text{score}}$ is

$$\hat{\mathbf{V}}_{\text{score}} = \mathbf{X}^T \left[\mathbf{X} \circ \left(\frac{\mathbf{1}}{\sigma^2 + \sigma^2 r^2 \text{diag}(\mathbf{R})} \mathbf{1}_{1 \times k} \right) \right].$$

The Lagrange Multiplier (LM) statistic is then

$$\text{LM} = \mathbf{G}_{\text{score}}^T \widehat{\mathbf{V}}_{\text{score}}^{-1} \mathbf{G}_{\text{score}},$$

and LM is asymptotically accord with a χ^2 distribution.

The Wald test fit the model by the REML criterion, the rationale is $\widehat{\boldsymbol{\beta}} \sim \text{MVN}(\boldsymbol{\beta}, \mathbf{C})$, such that $\widehat{\boldsymbol{\beta}}_G$ is accord with a marginal distribution of $\widehat{\boldsymbol{\beta}}$, denoted by $\text{MVN}(\boldsymbol{\beta}_G, \mathbf{C}_G)$. Under null hypothesis, the normalized $\widehat{\boldsymbol{\beta}}_G$ (by $\mathbf{C}_G^{-1/2} \widehat{\boldsymbol{\beta}}_G$) accord with $\text{MVN}(\mathbf{0}, \mathbf{I})$, and the sum of squares of the normalized $\widehat{\boldsymbol{\beta}}_G$ is accord with a χ^2 distribution. \mathbf{C} can be estimated from the inverse of Fisher's information matrix:

$$\widehat{\mathbf{C}} = \left[-\frac{\partial^2 \log L(\boldsymbol{\beta}, \sigma^2, r^2)}{\partial \boldsymbol{\beta} \partial \boldsymbol{\beta}^T} \Big|_{\boldsymbol{\beta}=\widehat{\boldsymbol{\beta}}_{\text{score}}} \right]^{-1} = \sigma^2 \mathbf{E}_{0,1}^{-1}.$$

$\widehat{\mathbf{C}}_G^{-1}$ is a submatrix of $\widehat{\mathbf{C}}$ and the W -static is calculated by

$$W = \widehat{\boldsymbol{\beta}}_G^T \widehat{\mathbf{C}}_G^{-1} \widehat{\boldsymbol{\beta}}_G,$$

which is asymptotically accord with a χ^2 distribution.

5.4 Haplotype extraction

There are some preparations to be conducted before the haplotypes are extracted: (i) exclude individuals with varying ploidy level in the same chromosome (or contig); (ii) sort the loci according to their chromosomes and positions; and (iii) during loading genotypes, the unphased genotypes are set to ‘missing genotype.’

In the following description, we will use the ordinal numbers of variants in a chromosome to be their identifiers, and we let the variant i be ahead of the variant j (i.e., $i \leq j$). The procedures of our search algorithm for each chromosome are described as follows.

1. Set $i = 1$ and $j = 1$ in the beginning.
2. If the value of j exceeds the number of variants in this chromosome or contig, then terminate this algorithm.

3. Calculate the values of several parameters (the length of each haplotype, the number of variants, the number of alleles and the number of genotypes, the rate of missing data).
4. If any parameter exceeds the upper bound then increase i and go to step 2.
5. If any parameter exceeds the lower bound then increase j and go to step 2.
6. Combine all variants between the variants i and j , and extract the haplotypes.
7. Set i and j to the next applicable variant according to `-haplotype_interval`, and go to Step 2.

5.5 Genetic diversity indices

The definitions of genetic diversity indices and their calculating formulas are as follows.

- H_o , the observed heterozygosity, which is extended to polysomic inheritance. The H_o for an individual at a locus is defined as the probability of randomly choosing two non-IBS (identical-by-state) alleles within this individual at this locus without replacement; the H_o for a group (a population, a region or the total population) at a locus is calculated by

$$H_o = \frac{\text{\#non-IBS allele pairs (without replacement) within individuals}}{\text{\#allele pairs (without replacement) within individuals}}.$$

The above equation can be extended to calculate H_o at multiple loci, for example,

$$H_o = \frac{\sum_l \text{\#non-IBS allele pairs (without replacement) within individuals at locus } l}{\sum_l \text{\#allele pairs (without replacement) within individuals at locus } l}.$$

This is equivalent to calculate the weighted average of H_o across loci with the weight being $\#Hap(\#Hap - 1)$.

- He , the expected heterozygosity, the same as the disomic inheritance, whose value at a locus is $1 - \sum_j P_j^2$, where J is the number of alleles at this locus, and P_j is the frequency of the j^{th} allele A_j at this locus. The He for a group at a locus is calculated by

$$H_E = \frac{\text{\#non-IBS allele pairs (with replacement) within groups}}{\text{\#allele pairs (with replacement) within groups}}.$$

The above equation can be extended to calculate H_E at multiple loci, and is equivalent to calculate the weighted average of H_E across loci with the weight being $\#Hap^2$.

- π , the nucleotide diversity, defined as the expected number of nucleotide differences between two random sequences sampled without replacement. The π for a group at a locus is calculated by

$$\pi = \frac{\text{#non-IBS allele pairs (without replacement) within groups}}{\text{#allele pairs (without replacement) within groups}}.$$

The above equation can be extended to calculate π at multiple loci, and is equivalent to calculate the weighted average of π across loci with the weight being $\#Hap(\#Hap - 1)$.

- PIC, the polymorphic information content, the same as the disomic inheritance, whose value at a locus is $2 \sum_{i=1}^J \sum_{j=i+1}^J P_i P_j (1 - P_i P_j)$. PIC is weighted by $\#Hap^2$ across loci.
- Ae, the effective number of alleles, like the disomic inheritance, whose value at a locus is $1 / \sum_j P_j^2$. Ae is weighted by $\#Hap^2$ across loci.
- I, Shannon's Information Index, like the disomic inheritance, whose value at a locus is $-\sum_j P_j \ln P_j$. I is weighted by $\#Hap^2$ across loci.
- NE1P, the average probability of not excluding a candidate parent from the parentage of an arbitrary offspring, given only the genotype of this offspring. The same as the disomic inheritance, whose value at a locus is $4a_2 - 2a_2^2 - 4a_3 + 3a_4$, where $a_i = \sum_j P_j^i$, $i = 1, 2, 3, 4$. The NE1P at multiple loci is the product of NE1P across loci.
- NE2P, the average probability of not excluding a candidate parent from the parentage of an arbitrary offspring, given the genotype of this offspring and of the known parent of the opposite sex. The same as the disomic inheritance, whose value at a locus is $2a_2^2 + 2a_2 - a_3 - 3a_2 a_3 + 3a_5 - 2a_4$. The NE2P at multiple loci is the product of NE2P across loci.
- NEPP, the average probability of not excluding a candidate parent pair from the parentage of an arbitrary offspring, given only the genotype of this offspring. The same as the disomic inheritance, whose value at a locus is $4a_5 - 4a_4 + 3a_6 + 8a_2^2 - 8a_2 a_3 - 2a_3^2$. The NEPP at multiple loci is the product of NEPP across loci.
- NEI, the average probability that the genotypes at a single locus do not differ between two unrelated individuals. This is the same as the disomic inheritance, whose value is $4a_2^2 - 3a_4$. The NEI at multiple loci is the product of NEI across loci.
- NESI, the average probability that the genotypes at a single locus do not differ between two

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full siblings. This is the same as the disomic inheritance, whose value is $\frac{1}{4} + \frac{1}{2}a_2 + \frac{1}{2}a_2^2 - \frac{1}{4}a_4$.

The NESI at multiple loci is the product of NESI across loci.

- F_{IS} , the inbreeding coefficient, which is defined as the probability of sampling two identical-by-descent (IBD) alleles from an individual without replacement, whose value at this locus is calculated by $1 - H_O/H_E$.

Fisher's G -test is used to test the genotypic distribution. The null hypothesis is that this distribution accords with the expected frequencies under a specific double-reduction model (e.g., RCS, PRCS, CES or PES). Here, if the ploidy level at a locus varies, Fisher's G -test will not be performed.

Assuming there are J alleles at the target locus, and the ploidy level is v . Then the number of possible genotypes at this locus is $\binom{v+J-1}{v}$. Because the numbers of allele copies are fixed, the distribution of genotypes is subject to the following J constraints:

$$f_j = \sum_i v \Pr(A_j | G_i), \quad j = 1, 2, \dots, J,$$

where $\Pr(A_j | G_i)$ is the frequency of j^{th} allele A_j in the genotype G_i of individual i . Therefore, the degrees-of-freedom are $\binom{v+J-1}{v} - J$. If the expected occurrence of any genotype is less than 5, then two minor alleles are collapsed and treated as one allele.

The expected occurrence of each genotype can be calculated under a specific double-reduction model, such as the model of RCS, PRCS, CES or PES (Huang *et al.* 2019). Fisher's G statistic is then calculated by

$$G = \sum_i 2O_i \ln(O_i/E_i),$$

where O_i and E_i are the values of the observed and the expected occurrences of the genotype G_i . Finally, the P -value can be obtained by the right-tail probability of the Chi-squared distribution.

Note that from v1.06, all statistics (excluding the number of alleles and number of individual

genotyped) are set to zero for monomorphic loci and loci with none individuals, and these loci are not involved in calculating the multi-locus genetic diversity indices.

5.6 Individual statistics

In diploids, the heterozygosity-index (H-index) for a genotype is a binary variable, whose value is equal to one for a heterozygote, or equal to zero for a homozygote. In polyploids, the H-index for a genotype G is defined as the probability of randomly sampling two different IBS alleles within G without replacement. For example, the H-index for the tetraploid genotype $AABB$ is $2/3$. The H-index for an individual is defined as the arithmetic mean of H-indices for the genotypes of this individual across all loci.

The multi-locus genotypic frequency of an individual is the product of frequencies of genotypes of this individual in this target population across all loci, symbolically

$$\Pr(G) = \prod_{l=1}^L \Pr(G_l),$$

where L is the number of loci.

The inbreeding coefficient of an individual is defined as the probability of sampling two IBD alleles from this individual without replacement, denoted by f . The kinship coefficient within an individual itself is defined as the probability of sampling two IBD alleles from this individual with replacement, denoted by θ . According to these definitions, the following relationship holds:

$$f = \frac{v\theta - 1}{v - 1},$$

where v is the ploidy level of the individual. Therefore, the inbreeding coefficient f can be converted from the kinship coefficient θ so long as θ is estimated.

VCFPOP provides three method-of-moment estimators to estimate the kinship coefficient θ :

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- Ritland's (1996) estimator

$$\hat{\theta}_{\text{RI}} = \frac{\sum_l [(\sum_j P_{alj}P_{blj}/P_{lj}) - 1]}{\sum_l (J_l - 1)};$$

- Loiselle's (1995) estimator

$$\hat{\theta}_{\text{LO}} = \frac{\sum_l \sum_j (P_{alj} - P_{lj})(P_{blj} - P_{lj})}{\sum_l \sum_j P_{lj}(1 - P_{lj})};$$

- Weir's (1996) estimator

$$\hat{\theta}_{\text{WE}} = \frac{\sum_l \sum_j (P_{alj}P_{blj} - P_{lj}^2)}{L - \sum_l \sum_j P_{lj}^2},$$

where J_l is the number of alleles at locus l , A_{lj} is the j^{th} allele at locus l , P_{alj} (or P_{blj}) is the frequency of A_{lj} in the individual a (or b), and P_{lj} is the frequency of A_{lj} in the reference population.

- VanRaden (2008) estimator

$$\hat{\theta}_{\text{VR}} = \frac{\sum_l (P_{al} - P_l)(P_{bl} - P_l)}{\sum_l (P_l - P_l^2)},$$

where P_{al} (or P_{bl}) is the frequency of major allele in the individual a (or b), and P_l is the frequency of major allele in the reference population.

5.7 Genetic differentiation

The F_{ST} estimators are Nei's (1973) G_{ST} , Weir & Cockerham's (1984) θ , Hudson *et al.*'s (1992) F_{ST} , Slatkin's (1995) R_{ST} , Hedrick's (2005) G'_{ST} , Jost's (2008) D and Huang *et al.*'s (2021) estimators. Their formulas are listed as follows.

- Nei's (1973) G_{ST} estimator is calculated by

$$G_{ST} = \frac{\sum_l^L (H_{Tl} - H_{Sl})}{\sum_l^L H_{Tl}},$$

where L is the number of loci, H_{Tl} is the expected heterozygosity in the total population at locus l , and H_{Sl} is the weighted average of expected heterozygosities in all populations at locus l , with the number of haplotypes in each population as a weight, whose expressions are as follows:

$$H_{Tl} = 1 - \sum_j^{J_l} P_{lj}^2,$$

$$H_{Sl} = \frac{\sum_p v_p (1 - \sum_j^{J_l} P_{plj}^2)}{v_t},$$

in which J_l is the number of alleles at locus l , p is taken from all populations, P_{lj} (or P_{plj}) is the frequency of allele A_{lj} in the total population (or the population p), v_p (or v_t) is the number of haplotypes in the population p (or the total population). Note that any monomorphic loci are excluded from the calculation.

- Weir & Cockerham's (1984) θ can only be used for diploids, and is calculated by

$$\theta = \frac{\sum_l^L \sum_j^{J_l} a_{lj}}{\sum_l^L \sum_j^{J_l} (a_{lj} + b_{lj} + c_{lj})},$$

where a_{lj} , b_{lj} and c_{lj} are respectively the variance components among populations, among individuals and within individuals for the allele A_{lj} , whose expressions are

$$a_{lj} = \frac{\bar{n}_l}{n_{cl}} \left\{ s_{lj}^2 - \frac{1}{\bar{n}_l - 1} \left[\bar{P}_{lj}(1 - \bar{P}_{lj}) - \frac{r-1}{r} s_{lj}^2 - \frac{\bar{h}_{lj}}{4} \right] \right\},$$

$$b_{lj} = \frac{\bar{n}_l}{\bar{n}_l - 1} \left[\bar{P}_{lj}(1 - \bar{P}_{lj}) - \frac{r-1}{r} s_{lj}^2 - \frac{2\bar{n}_l - 1}{4\bar{n}_l} \bar{h}_{lj} \right],$$

$$c_{lj} = \bar{h}_{lj}/2,$$

in which the symbol r denotes the number of populations, and the other symbols are explained as follows:

\bar{n}_l is the average sample size per population at locus l , i.e., $\bar{n}_l = \frac{\sum_p n_{pl}}{r}$, in which p is taken

from all populations, n_{pl} is the sample size of population p at locus l ;

n_{cl} is an intermediate variable, whose expression is $n_{cl} = \frac{r\bar{n}_l - \sum_p n_{pl}^2/r\bar{n}_l}{r-1}$;

\bar{P}_{lj} is the weighted average of frequencies of A_{lj} , i.e., $\bar{P}_{lj} = \frac{\sum_p n_{pl} P_{plj}}{\sum_p n_{pl}}$;

s_{lj}^2 is the sampling variance of frequencies of A_{lj} , i.e., $s_{lj}^2 = \frac{r(\bar{P}^2_{lj} - \bar{P}_{lj}^2)}{r-1}$, in which \bar{P}^2_{lj} is the

weighted average of frequency squares of A_{lj} , i.e., $\bar{P}^2_{lj} = \frac{\sum_p n_{pl} P_{plj}^2}{\sum_p n_{pl}}$,

\bar{h}_{lj} is the average heterozygosity for A_{lj} , i.e., $\bar{h}_{lj} = 2(\bar{P}_{lj} - \bar{P}^2_{lj})$.

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- Hudson *et al.*'s (1992) F_{ST} estimator is calculated by

$$F_{ST} = \frac{\sum_l (H_{bl} - H_{wl})}{\sum_l H_{bl}},$$

where H_{wl} is the average squared IAM distances between allele copies taken from the same population at locus l , symbolically:

$$H_{wl} = \frac{\sum_p \sum_{j_1 < j_2} v_{pl}^2 P_{plj_1} P_{plj_2} {}^{IAM}d_{lj_1lj_2}^2}{\sum_p v_{pl}(v_{pl} - 1)/2},$$

and H_{bl} is the average squared IAM distances between allele copies taken from two different populations at locus l , symbolically

$$H_{bl} = \frac{\sum_{j_1 < j_2} v_{tl}^2 P_{lj_1} P_{lj_2} {}^{IAM}d_{lj_1lj_2}^2 - \sum_p \sum_{j_1 < j_2} v_{pl}^2 P_{plj_1} P_{plj_2} {}^{IAM}d_{lj_1lj_2}^2}{v_{tl}(v_{tl} - 1)/2 - \sum_p v_{pl}(v_{pl} - 1)/2}.$$

- Slatkin's (1995) estimator R_{ST} is calculated by

$$R_{ST} = \frac{\sum_l (S_{tl} - S_{wl})}{\sum_l S_{tl}},$$

where S_{tl} is the average squared SMM distances within the total population at locus l , symbolically

$$S_{tl} = \frac{\sum_{j_1 < j_2} v_{tl}^2 P_{lj_1} P_{lj_2} {}^{SMM}d_{lj_1lj_2}^2}{v_{tl}(v_{tl} - 1)/2},$$

and S_{wl} is the average sum of squares of the SMM distances within each population, symbolically

$$S_{wl} = \frac{\sum_p \sum_{j_1 < j_2} v_{pl}^2 P_{plj_1} P_{plj_2} {}^{SMM}d_{lj_1lj_2}^2}{\sum_p v_{pl}(v_{pl} - 1)/2},$$

in v_{tl} (or v_{pl}) is the number of allele copies at the l^{th} locus and in the total population (or in the p^{th} population), P_{lj_1} and P_{lj_2} (or P_{plj_1} and P_{plj_2}) are respectively the frequencies of alleles A_{lj_1} and A_{lj_2} in the total population (or in the p^{th} population), and ${}^{SMM}d_{lj_1lj_2}$ is the SMM distance between the alleles A_{lj_1} and A_{lj_2} .

- Hedrick's (2005) estimator G'_{ST} is the standardization of Nei's (1973) estimator G_{ST} , obtained by dividing the theoretical maximum of G_{ST} , whose expression is

$$G'_{ST} = \frac{\sum_l^L (H_{Tl} - H_{Sl})(S - 1 + H_{Sl})}{(S - 1) \sum_l^L (1 - H_{Sl}) H_{Tl}},$$

where S is the number of populations.

- Jost's (2008) estimator D is calculated by

$$D = \frac{S \sum_l^L (H_{Tl} - H_{Sl})}{(S - 1) \sum_l^L (1 - H_{Sl})}.$$

- Huang *et al.* (2021) homo and aneu estimators are based on AMOVA, where

$$F_{ST} = \frac{\hat{\sigma}_{AP}^2}{\hat{\sigma}_{AP}^2 + \hat{\sigma}_{AI}^2 + \hat{\sigma}_{WI}^2},$$

The variance components for homoploid and aneuploid models are calculated by different weighting scheme, see Section [5.9](#) for detail.

The test of genetic differentiation is performed by Fisher's G -test. This test can be applied to either multiple loci or a single locus. For multiple loci, the values of the statistic G (or the values of the degree of freedom d.f.) will be summed.

5.8 Genetic distance

VCFPOP can calculate various genetic distances between individuals, populations, and regions. These measures are all based on allele frequencies. The following items show these measures together with the corresponding references and calculation methods.

- Nei's (1972) standard genetic distance D_S . This measure assumes that the genetic differences are caused by both mutation and genetic drift. If the mutation rate is constant, the distance D_S between the populations x and y is proportional to divergence time, whose calculating formula is

$$D_S = -\ln \frac{J_{xy}}{\sqrt{J_x J_y}},$$

where $J_x = \sum_l^L \sum_j^{J_l} P_{xlj} / L$, $J_y = \sum_l^L \sum_j^{J_l} P_{ylj} / L$, $P_{xy} = \sum_l^L \sum_j^{J_l} P_{xlj} P_{ylj} / L$ (J_l is the number of

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alleles at locus l), and P_{xlj} and P_{ylj} are the frequencies of the j^{th} alleles in the individuals/populations/regions x and y at locus l , respectively.

- Cavalli-Sforza's (1967b) chord distance D_{CH} . This measure assumes that genetic differences arise only due to the genetic drift. One major advantage of this measure is that the populations are represented in a hypersphere, the scale of which is each gene being converted as one unit. The distance D_{CH} in a hyperdimensional sphere is given by

$$D_{CH} = \frac{2}{\pi} \sqrt{2 \left(1 - \frac{1}{L} \sum_l^L \sum_j^{J_l} \sqrt{P_{xlj} P_{ylj}} \right)}.$$

- Reynolds *et al.*'s (1983) distance θ_w . This measure assumes that genetic differences occur only due to genetic drift without any mutations. This measure is used to estimate the kinship coefficient θ which provides a measure of the genetic divergence, where the expression of θ is

$$\theta_w = \sqrt{\frac{\sum_l^L \sum_j^{J_l} (P_{xlj} - P_{ylj})^2}{2 \sum_l^L \left(1 - \sum_j^{J_l} P_{xlj} P_{ylj} \right)}}.$$

- Nei's (1983) distance D_A . This measure assumes that genetic differences arise due to both mutation and genetic drift. It is known that such a measure gives more reliable population trees than other measures, particularly for microsatellite DNA data. The distance D_A is calculated by

$$D_A = 1 - \frac{1}{L} \sum_l^L \sum_j^{J_l} \sqrt{P_{xlj} P_{ylj}}.$$

- Euclidean distance D_{EU} . This is a usual measure based on Euclidean space, where the frequencies of alleles in a population at a locus form a vector in this space. The distance D_{EU} is calculated by

$$D_{EU} = \sqrt{\sum_l^L \sum_j^{J_l} (P_{xlj} - P_{ylj})^2}.$$

- Goldstein's (1995) distance $(\delta\mu)^2$. This measure was developed based on a stepwise

mutation model (SMM), used specifically for the microsatellite markers, the formula of which is:

$$(\delta\mu)^2 = \frac{1}{L} \sum_l^L (\mu_{xl} - \mu_{yl})^2,$$

where μ_{xl} (or μ_{yl}) is the average allele size in the population x (or y) at locus l .

- Nei's (1974) minimum genetic distance D_M . This measure assumes that the genetic differences arise due to mutation and genetic drift, the formula being:

$$D_M = \frac{J_x + J_y}{2} - J_{xy},$$

where the meanings of J_x , J_y and J_{xy} are as indicated in the first item of this section.

- Roger's (1972) distance D_R . This measure is closely related to the Euclidean distance D_{EU} , both of which have the relation that $D_R = D_{EU}/\sqrt{2}$, namely

$$D_R = \frac{1}{\sqrt{2}L} \sum_l^L \sqrt{\sum_j^{J_l} (P_{xlj} - P_{ylj})^2}.$$

- Reynolds *et al.*'s (1983) distance D_{RA} . This measure is defined as the divergence time. If two diploid populations of a constant size N diverged t generations ago, then the divergence time is $t/2N$. In this scenario, Wright's F_{ST} between these two populations can be expressed as

$F_{ST} = 1 - \left(1 - \frac{1}{2N}\right)^t$. Because $1 - \left(1 - \frac{1}{2N}\right)^t \approx 1 - e^{-t/2N}$, it is easy to derive that $t/2N \approx -\ln(1 - F_{ST})$. Therefore, the distance D_{RA} can be calculated as:

$$D_{RA} = -\ln(1 - F_{ST}).$$

- Slatkin's (1995) linearized distance D_{Sl} . This measure is also defined as the divergence time. Slatkin considered a simple demographic model, in which two diploid populations of size N diverged at τ generations ago from a population of identical size, and have remained isolated ever since, without exchanging any migrants. The divergence time is thus $\tau/2N$. On the other hand, because Wright's F_{ST} can be expressed as $F_{ST} = \tau/(\tau + 2N)$ in this model, it is easy to derive that $\tau/2N = F_{ST}/(1 - F_{ST})$. Therefore, the distance D_{Sl} can be calculated as:

$$D_{Sl} = F_{ST}/(1 - F_{ST}).$$

5.9 Analysis of molecular variance

Homoploid method

The homoploid method follows that of GENALEX (Peakall & Smouse 2006). In this method, all loci are treated as one dummy locus, and the j^{th} haplotype of an individual at this dummy locus consists of the j^{th} allele copies within this individual across all loci. In this method, the missing data of each individual are weighted according to the allele frequencies in the sampling population of this individual.

The square of the genetic distance $d_{hh'}$ between two haplotypes h and h' is the sum of squares of either the IAM distances or the SMM distances of allele pairs across all loci, which is:

$$d_{hh'}^2 = \sum_l d_{A_{hl} A_{h'l}}^2$$

where A_{hl} and $A_{h'l}$ are a pair of allele copies at locus l with the former in h and the latter in h' . Note that the SMM distance can only be applied for non-VCF/BCF input files, and the identifier of each allele must be its size.

We use the symbol SS_{WI} to denote the sum $\sum_{i \in \mathbf{I}} \bar{S}_i$, where \mathbf{I} is the collection of all individuals, and \bar{S}_i is the arithmetic mean of the squares of genetic distances between haplotypes within the individual i . For convenience, we call roughly SS_{WI} as the sum of squares of haplotype distances within individuals. Similarly, the symbol SS_{WP} (SS_{WR} or SS_{TOT}) is roughly called the sum of squares of haplotype distances within populations (regions or the total population), denoted by:

$$\text{SS}_{\text{WI}} = \frac{1}{2} \sum_{i \in \mathbf{I}} \sum_{h, h' \in i} \frac{d_{hh'}^2}{v_i},$$

$$\text{SS}_{\text{WP}} = \frac{1}{2} \sum_{p \in \mathbf{P}} \sum_{h, h' \in p} \frac{d_{hh'}^2}{v_p},$$

$$SS_{WR} = \frac{1}{2} \sum_{r \in R} \sum_{h,h' \in r} \frac{d_{hh'}^2}{v_r},$$

$$SS_{TOT} = \frac{1}{2} \sum_{h,h'} \frac{d_{hh'}^2}{v_t},$$

where v_i , v_p , v_r and v_t are the numbers of haplotypes within the individual i , the population p , the region r and the total population in turn, and \mathbf{P} and \mathbf{R} are the collection of all populations and all regions, respectively.

The degrees-of-freedom and the sum of squares at each hierarchy level are listed in the next table.

Source	d.f.	SS
Within individuals	$N_H - N_I$	SS_{WI}
Within populations	$N_H - N_P$	SS_{WP}
Within regions	$N_H - N_R$	SS_{WR}
Total population	$N_H - 1$	SS_{TOT}

Here, N_H , N_I , N_P and N_R are the total number of haplotypes, individuals, populations and regions, respectively.

The expressions of expected SS at each hierarchy level are listed in the following equations:

$$\begin{aligned} E(SS_{WI}) &= \sigma_{WI}^2(N_H - N_I), \\ E(SS_{WP}) &= \sigma_{WI}^2(N_H - N_P) + \sigma_{AI}^2 \left(N_H - \sum_{p \in P} \sum_{i \in p} \frac{v_i^2}{v_p} \right), \\ E(SS_{WR}) &= \sigma_{WI}^2(N_H - N_R) + \sigma_{AI}^2 \left(N_H - \sum_{r \in R} \sum_{i \in r} \frac{v_i^2}{v_r} \right) + \sigma_{AP}^2 \left(N_H - \sum_{r \in R} \sum_{p \in r} \frac{v_p^2}{v_r} \right), \\ E(SS_{TOT}) &= \sigma_{WI}^2(N_H - 1) + \sigma_{AI}^2 \left(N_H - \sum_{i \in I} \frac{v_i^2}{v_t} \right) + \sigma_{AP}^2 \left(N_H - \sum_{p \in P} \frac{v_p^2}{v_t} \right) + \sigma_{AR}^2 \left(N_H - \sum_{r \in R} \frac{v_r^2}{v_t} \right), \end{aligned}$$

where σ_{WI}^2 is the variance of genetic distances between the dummy haplotypes within all individuals, and σ_{AI}^2 (σ_{AP}^2 or σ_{AR}^2) is the variance of genetic distances between the dummy haplotypes among all individuals (among all populations or among all regions).

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If we regard these variances as unknowns, the above expressions form a linear equation set. Therefore, we can obtain the values of these variances by solving this equation set. It is worth noting that because this variance estimator is unbiased, the estimated value of a variance may be negative when the true value of this variance is close to zero.

Imitating the above method, for any positive integer M , we can extend the AMOVA to M hierarchies, i.e., the relations between the expected SS_i and the variance component σ_i ($i = 1, 2, \dots, M$) can be expressed as follows:

$$E(SS_i) = \sum_{j=1}^i \sigma_j^2 \left(|V_M| - \sum_{V_i} \sum_{V_{j-1} \in V_i} \frac{|V_{j-1}|^2}{|V_i|} \right), \quad i = 1, 2, \dots, M,$$

where V_M denotes the vessel of highest hierarchy, i.e., the total population; when i ranges from 0 to M , the corresponding vessels represent in turn an allele, an individual, a population, a region I (of the third hierarchy), a region II (of the fourth hierarchy), and so on; similarly, the mobile subscript V_i is taken from all vessels of the i^{th} hierarchy. Moreover, if the within individual hierarchy is ignored, then V_1 denotes a population, V_2 denotes a region I, and etc.

The above related expressions can be expressed as the form of matrices as follows:

$$\mathbf{S} = \mathbf{C}\boldsymbol{\Sigma},$$

where $\mathbf{S} = [E(SS_1), E(SS_2), \dots, E(SS_M)]^T$, $\boldsymbol{\Sigma} = [\sigma_1^2, \sigma_2^2, \dots, \sigma_M^2]^T$, and the coefficient matrix \mathbf{C} is a lower triangular matrix of type $M \times M$, whose ij^{th} element C_{ij} is

$$C_{ij} = \begin{cases} |V_M| - \sum_{V_i} \sum_{V_{j-1} \in V_i} \frac{|V_{j-1}|^2}{|V_i|} & \text{if } i \geq j, \\ 0 & \text{if } i < j. \end{cases}$$

A method-of-moment estimation of variance components is given by $\hat{\boldsymbol{\Sigma}} = \mathbf{C}^{-1}\hat{\mathbf{S}}$. After that, the F -statistics can be solved by

$$\hat{F}_{ij} = 1 - \frac{\sum_{k=1}^i \hat{\sigma}_k^2}{\sum_{k=1}^j \hat{\sigma}_k^2}, \quad 1 \leq i < j \leq M.$$

Following Excoffier *et al.* (1992), the differentiation test is performed for each F -statistic independently. The null hypothesis is that each F -statistic (e.g., F_{ij}) is zero, which is equivalent to that the hierarchy j is real but hierarchy i is artificial. To obtain the null distribution of \hat{F}_{ij} , we randomly permute hierarchy $i - 1$ within hierarchy j to generate the new datasets. For each generated dataset, the \hat{F}_{ij} is estimated by the same procedures. Similarly, the probability that \hat{F}_{ij} is greater than the original value is used as a single-tailed P-value.

Aneuploid method

The aneuploid method is similar to the locus-by-locus AMOVA in ARLEQUIN (Excoffier & Lischer 2010), where the SS and equations of E(SS) are calculated at each locus and summed across loci. This method does not extract the dummy haplotypes from each individual, and supports aneuploids. This method also supports both the IAM and the SMM distances between a pair of alleles at the same locus without any additional restrictions.

Following the homoploid method, the matrices \mathbf{C}_l and $\hat{\mathbf{S}}_l$ at each locus can be obtained, and VCFPOP sums these matrices across loci, and next solves the linear equation set to obtain the variance components Σ . If these variances have been obtained, the F -statistics can be calculated by using the relational expressions described above. Moreover, the hierarchies at each locus will be permuted independently in the test for F -statistics. Note that because any missing data are not considered in the aneuploid method, the two values of N_{Al} (N_{Il} , N_{Pl} or N_{Rl}) before and after a permutation may not be identical.

Because this method permutes vessels for each locus computation is slow. VCFPOP uses a pseudo-permutation method to solve this problem, which first performs a small number of permutations (e.g., 100) for each locus, then subsamples one permutation at each locus to generate results for each permutation. The number of permutations is suggested to be greater than 100.

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Maximum-likelihood method

A reverse procedure is used, in which the F -statistics are estimated first, and then the variance components and other statistics are solved from the estimated F -statistics. Due to the constraint $(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$, all F -statistics can be obtained from $F_{12}, F_{13}, \dots, F_{1M}$.

The global likelihood for individuals at the i^{th} hierarchy is the product of frequencies of all phenotypes conditional on \mathbf{p}_{V_il} and F_{1i} , symbolically

$$\mathcal{L}_i = \prod_{V_i} \prod_{l=1}^L \prod_{j=1}^{N_{V_i}} \Pr(\mathcal{P}_{V_ilj} | \mathbf{p}_{V_il}, F_{1i}), \quad i = 2, 3, \dots, M,$$

where V_i is taken from all vessels of the i^{th} hierarchy, N_{V_i} is the number of individuals in V_i , \mathcal{P}_{V_ilj} is the phenotype of j^{th} individual in V_i and at the l^{th} locus, \mathbf{p}_{V_il} is the vector consisting of the frequencies of all alleles in V_i and at the l^{th} locus, $\Pr(\mathcal{P}_{V_ilj} | \mathbf{p}_{V_il}, F_{1i})$ is the frequency of \mathcal{P}_{V_ilj} conditional on \mathbf{p}_{V_il} and F_{1i} , that is

$$\Pr(\mathcal{P}_{V_ilj} | \mathbf{p}_{V_il}, F_{1i}) = \sum_{G \in \mathcal{P}_{V_ilj}} \Pr(G | \mathbf{p}_{V_il}, F_{1i}).$$

Under the RCS model, if the inbreeding is considered, $\Pr(G | \mathbf{p}_{V_il}, F_{1i})$ is calculated by

$$\Pr(G | \mathbf{p}_{V_il}, F_{1i}) = \binom{|V_1|}{c_1, c_2, \dots, c_{J_l}} \prod_{j=1}^{J_l} \prod_{k=0}^{c_j-1} (\alpha_{1ij} + k) / \prod_{k'=0}^{|V_1|-1} (\alpha_{1i} + k'),$$

where c_j in the multinomial coefficient is the number of the j^{th} allele copies in G ($j = 1, 2, \dots, J_l$), $\alpha_{1i} = 1/F_{1i} - 1$ and $\alpha_{1ij} = \alpha_{1i} P_{V_ilj}$ (P_{V_ilj} is the j^{th} element in \mathbf{p}_{V_il}). Because the true value of \mathbf{p}_{V_il} is unavailable, the estimated $\hat{\mathbf{p}}_{V_il}$ is used as \mathbf{p}_{V_il} in the calculating process. A down-hill simplex algorithm (Nelder & Mead 1965) can be used to find the optimal F_{1i} ($i = 2, 3, \dots, M$).

The variance components can be solved from the F -statistics using the following additional constraint:

$$E(SS_M) = \sum_{j=1}^M \sigma_j^2 \left(|V_M| - \sum_{V_{j-1} \in V_M} \frac{|V_{j-1}|^2}{|V_i|} \right).$$

The SS_M under IAM model can be obtained from the allele frequencies in the total population V_M , that is

$$SS_M = |V_M| \sum_{1 \leq i < j \leq J} P_{Mi} P_{Mj} d_{A_i A_j}^2,$$

where P_{Mi} (or P_{Mj}) is the frequency of A_i (or A_j) in V_M . The permutation test is the same as the homoploid method, but the F_l . (e.g., F_{IS} , F_{IC} and F_{IT}) are not tested because the haplotype is not permuted if there are aneuploids.

5.10 Population assignment

For the population assignment, the likelihood \mathcal{L} of an individual for a target population is the probability of observing the genotypic data of this individual under the hypothesis that this individual originated from the target population. Such a probability is defined as the product of the probabilities of observing the genotypic data of this individual across all loci under the above hypothesis, symbolically $\mathcal{L} = \prod_l \Pr(G_l)$, in which G_l is the genotype at locus l . A higher likelihood implies that the hypothesis is more reliable, i.e., the individual is more likely to originate from the target population.

Each individual is assigned to the population with the highest likelihood. The difference between the highest and the second highest common logarithm of likelihoods of an individual for all target populations is called the LOD score of this individual. Such a LOD score can be used to evaluate the accuracy of highest likelihood, and the greater the LOD score, the higher the accuracy of highest likelihood. For example, if the LOD score is up to 3, it means that $\mathcal{L}_{\text{most}}$ is 1000 times of $\mathcal{L}_{\text{second}}$, where $\mathcal{L}_{\text{most}}$ (or $\mathcal{L}_{\text{second}}$) is the likelihood of this individual for the most (or the second most) possible target population.

The likelihood of an individual for a target population will be equal to zero if this individual carries any alleles absent in the target population. Moreover, the individual will be excluded from its true

population if at least one of its genotypes is mistyped (e.g. false allele, Taberlet *et al.* 1996).

In VCFPOP, the mistyping rate e is added into the calculation of genotypic frequency, where e is the probability of a genotype being mistyped. When a genotype is mistyped, the observed genotype is randomly drawn according to the genotypic frequency in the total population. Therefore, the posterior probability $\Pr(G)$ that a given genotype G is observed from the population p is

$$\Pr(G) = \Pr(G|p)(1 - e) + [1 - \Pr(G|p)] e \Pr(G|t),$$

where $\Pr(G|p)$ (or $\Pr(G|t)$) is the frequency of G in the population p (or the total population).

5.11 Kinship coefficient

There are two kinds of kinship coefficients. One is within an individual, denoted by θ , whose definition has been given in Section [5.6](#). The other is between two individuals, which is defined as the probability of sampling two IBD alleles from these two individuals (each sampled from one individual), still denoted by θ .

For these two kinds of kinship coefficients, their estimators have the same calculating formulas. VCFPOP provides three estimators of method-of-moment to estimate these two kinds of kinship coefficients, and their calculating formulas have been listed in Section [5.6](#). After a kinship coefficient is calculated, it can be converted as either an inbreeding coefficient (see Section [5.6](#)) or a relatedness coefficient (see Section [5.12](#)).

5.12 Relatedness coefficient

The relatedness coefficient r is the correlation of allele frequencies between two individuals. The relatedness estimators can be roughly divided into two categories: one is the estimators of method-of-moment, and the other is the estimators of maximum-likelihood. The former category of

estimators is unbiased, while the latter has a slight RMSE (the root mean square error).

In diploids, if the inbreeding is absent, the relatedness coefficient r can be expressed as

$$r = \phi/2 + \Delta,$$

where ϕ is the probability that two individuals share one pair of IBD alleles, called the two-gene coefficient, and Δ is the probability that two individuals share two pairs of IBD alleles, called the four-gene coefficient. Therefore, in the absence of inbreeding, the relatedness coefficient r can be converted from ϕ and Δ by using the above relational expression.

In the absence of inbreeding, the calculating formula of each relatedness estimator of method- of-moment is a linear equation set with ϕ and Δ as the unknowns. VCFPOP provides several such estimators, whose calculating formulas are listed below.

- Lynch & Ritland's (1999) estimator: if G_x is a homozygote, it can be calculated by

$$\begin{bmatrix} \Pr(G_y = A_i A_i | G_x = A_i A_i) \\ \Pr(G_y = A_i A_x | G_x = A_i A_i) \end{bmatrix} = \begin{bmatrix} p_i^2 \\ 2p_i p_x \end{bmatrix} + \begin{bmatrix} 1 - p_i^2 & p_i - p_i^2 \\ -2p_i p_x & p_x - 2p_i p_x \end{bmatrix} \begin{bmatrix} \Delta \\ \phi \end{bmatrix};$$

if G_x is a heterozygote, it can be calculated by

$$\begin{bmatrix} \Pr(G_y = A_i A_i | G_x = A_i A_j) \\ \Pr(G_y = A_j A_j | G_x = A_i A_j) \\ \Pr(G_y = A_i A_j | G_x = A_i A_j) \\ \Pr(G_y = A_i A_x | G_x = A_i A_j) \\ \Pr(G_y = A_j A_x | G_x = A_i A_j) \end{bmatrix} = \begin{bmatrix} p_i^2 \\ p_j^2 \\ 2p_i p_j \\ 2p_i p_x \\ 2p_j p_x \end{bmatrix} + \begin{bmatrix} -p_i^2 & \frac{1}{2}p_i - p_i^2 \\ -p_j^2 & \frac{1}{2}p_j - p_j^2 \\ 1 - 2p_i p_j & \frac{1}{2}p_i + \frac{1}{2}p_j - 2p_i p_j \\ -2p_i p_x & \frac{1}{2}p_x - 2p_i p_x \\ -2p_j p_x & \frac{1}{2}p_x - 2p_j p_x \end{bmatrix} \begin{bmatrix} \Delta \\ \phi \end{bmatrix},$$

where A_x is an allele not appearing in G_x , p_i (or p_j) is the frequency of allele A_i (or A_j), and $p_x = 1 - p_i$ if $G_x = A_i A_i$, or $p_x = 1 - p_i - p_j$ if $G_x = A_i A_j$ ($i \neq j$).

- Wang's (2002) estimator is calculated by

$$\begin{bmatrix} \Pr(S = 1) \\ \Pr(S = 3/4) \\ \Pr(S = 1/2) \end{bmatrix} = \begin{bmatrix} \lambda_1 \\ \lambda_2 \\ \lambda_3 \end{bmatrix} + \begin{bmatrix} 1 - \lambda_1 & a_2 - \lambda_1 \\ -\lambda_2 & 2a_2 - 2a^3 - \lambda_2 \\ -\lambda_3 & 1 - 3a_2 + 2a_3 - \lambda_3 \end{bmatrix} \begin{bmatrix} \Delta \\ \phi \end{bmatrix},$$

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where $\lambda_1 = 2a_2^2 - a_4$, $\lambda_2 = 4a_3 - 4a_4$, $\lambda_3 = 4a_2 - 4a_2^2 - 8a_3 + 8a_4$, $a_k = \sum_j p_j^k$ ($k = 1, 2, 3, 4$), and S is the similarity index of the allelic pattern of G_x and G_y , i.e.,

$$S = \begin{cases} 1 & \text{if } G_x = A_iA_i, G_y = A_iA_i \text{ or if } G_x = A_iA_j, G_y = A_iA_j, \\ 3/4 & \text{if } G_x = A_iA_i, G_y = A_iA_j, \\ 1/2 & \text{if } G_x = A_iA_j, G_y = A_iA_k, \\ 0 & \text{otherwise,} \end{cases}$$

in which i, j and k are different with each other.

- Thomas's (2010) estimator is calculated by

$$\begin{bmatrix} \Pr(S = 1) \\ \Pr(S = 3/4 \text{ or } 1/2) \end{bmatrix} = \begin{bmatrix} \lambda_1 \\ \lambda_2 \end{bmatrix} + \begin{bmatrix} 1 - \lambda_1 & a_2 - \lambda_1 \\ -\lambda_2 & 1 - a_2 - \lambda_2 \end{bmatrix} \begin{bmatrix} \Delta \\ \phi \end{bmatrix},$$

where $\lambda_1 = 2a_2^2 - a_4$, $\lambda_2 = 4a_2 - 4a_2^2 - 4a_3 + 4a_4$.

- Huang *et al.* (2016a) estimator: if G_x is homozygous, it can be calculated by

$$\begin{bmatrix} E(S) \\ E(S^2) \end{bmatrix} = \begin{bmatrix} 1 & \frac{3}{4} & \frac{1}{2} \\ 1 & \frac{9}{16} & \frac{1}{4} \end{bmatrix} \left(\begin{bmatrix} p_i^2 \\ 2p_i p_x \\ 0 \end{bmatrix} + \begin{bmatrix} 1 - p_i^2 & p_i - p_i^2 \\ -2p_i p_x & p_x - 2p_i p_x \\ 0 & 0 \end{bmatrix} \begin{bmatrix} \Delta \\ \phi \end{bmatrix} \right);$$

if G_x is heterozygous, it can be calculated by

$$\begin{bmatrix} E(S) \\ E(S^2) \end{bmatrix} = \begin{bmatrix} 1 & \frac{3}{4} & \frac{1}{2} \\ 1 & \frac{9}{16} & \frac{1}{4} \end{bmatrix} \left(\begin{bmatrix} 2p_i p_j \\ p_i^2 + p_j^2 \\ 2p_x(p_i + p_j) \end{bmatrix} + \begin{bmatrix} 1 - 2p_i p_j & \frac{1}{2}(p_i + p_j) - 2p_i p_j \\ -p_i^2 - p_j^2 & \frac{1}{2}(p_i + p_j) - p_i^2 - p_j^2 \\ -2p_x(p_i + p_j) & p_x - 2p_x(p_i + p_j) \end{bmatrix} \begin{bmatrix} \Delta \\ \phi \end{bmatrix} \right).$$

- Huang *et al.* (2016b) estimator is calculated by

$$\begin{bmatrix} E(S) \\ E(S^2) \end{bmatrix} = \begin{bmatrix} 1 & \frac{3}{4} & \frac{1}{2} \\ 1 & \frac{9}{16} & \frac{1}{4} \end{bmatrix} \left(\begin{bmatrix} \lambda_1 \\ \lambda_2 \\ \lambda_3 \end{bmatrix} + \begin{bmatrix} 1 - \lambda_1 & a_2 - \lambda_1 \\ -\lambda_2 & 2a_2 - a_3 - \lambda_2 \\ -\lambda_3 & 1 - 3a_2 + 2a_3 - \lambda_3 \end{bmatrix} \begin{bmatrix} \Delta \\ \phi \end{bmatrix} \right),$$

Where $\lambda_1 = 2a_2^2 - a_4$, $\lambda_2 = 4a_3 - 4a_4$ and $\lambda_3 = 4a_2 - 4a_2^2 - 8a_3 + 8a_4$.

In the next two estimators, we omit the expressions of linear equation sets for simplicity.

- Queller & Goodnight's (1989) estimator is calculated by $\hat{r} = (\hat{r}_{xy} + \hat{r}_{yx})/2$, where

$$\hat{r}_{xy} = \frac{K_{ac} + K_{ad} + K_{bc} + K_{bd}}{2(1 + K_{ab} - p_a - p_b)},$$

$$\hat{r}_{yx} = \frac{K_{ac} + K_{ad} + K_{bc} + K_{bd}}{2(1 + K_{cd} - p_c - p_b)},$$

in which \hat{r}_{xy} (or \hat{r}_{yx}) is the estimated value with G_x (or G_y) as the reference individual, a and b are the two alleles in G_x , c and d are those in G_y , and $K_{a_1a_2}$ is a Kronecker operator, such that $K_{a_1a_2} = 1$ if $a_1 = a_2$, or $K_{a_1a_2} = 0$ if $a_1 \neq a_2$. Note that this estimator cannot be applied for a biallelic marker. That is because at least one of the denominators of the above fractions is equal to zero if G_x or G_y is heterozygous.

- Li *et al.*'s (1993) estimator is calculated by

$$r = \frac{S - S_0}{1 - S_0},$$

where $S_0 = \sum_j^J p_j^2 (2 - p_j)$.

For diploids, VCFPOP also provides two relatedness estimators of maximum likelihood: one is Milligan's (2003) estimator, and the other is Anderson & Weir's (2007) estimator. The likelihood $\mathcal{L}(G_x, G_y | \phi, \Delta)$ of observing the genotypic data of a pair of individuals G_x and G_y conditional on ϕ and Δ is defined in these two estimators, whose expression for all patterns of genotypic pairs is

$$\mathcal{L}(G_x, G_y | \phi, \Delta) = \begin{cases} p_i^2 \Delta + p_i^3 \phi + p_i^4 (1 - \phi - \Delta) & \text{if } G_x = A_i A_i, G_y = A_i A_i, \\ p_i^2 p_j^2 (1 - \phi - \Delta) & \text{if } G_x = A_i A_i, G_y = A_j A_j, \\ p_i^2 p_j \phi + 2p_i^3 p_j (1 - \phi - \Delta) & \text{if } G_x = A_i A_i, G_y = A_i A_j, \\ 2p_i^2 p_j p_k (1 - \phi - \Delta) & \text{if } G_x = A_i A_i, G_y = A_j A_k, \\ p_i^2 p_j \phi + 2p_i^3 p_j (1 - \phi - \Delta) & \text{if } G_x = A_i A_j, G_y = A_i A_i, \\ 2p_i^2 p_j p_k (1 - \phi - \Delta) & \text{if } G_x = A_j A_k, G_y = A_i A_i, \\ 2p_i p_j \Delta + p_i p_j (p_i + p_j) \phi + 4p_i^2 p_j^2 (1 - \phi - \Delta) & \text{if } G_x = A_i A_j, G_y = A_i A_j, \\ p_i p_j p_k \phi + 4p_i^2 p_j p_k (1 - \phi - \Delta) & \text{if } G_x = A_i A_j, G_y = A_i A_k, \\ 4p_i p_j p_k p_l (1 - \phi - \Delta) & \text{if } G_x = A_i A_j, G_y = A_k A_l. \end{cases}$$

A numerical algorithm (e.g., Nelder-Mead simplex algorithm) is used to search the optimal ordered

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couple (ϕ, Δ) that maximize this likelihood, and next the relatedness coefficient r will be converted from this ordered couple (ϕ, Δ) , i.e., $r = \phi/2 + \Delta$, which is the maximum likelihood estimate. For Anderson & Weir's (2007) estimator, the higher-order relatedness coefficients ϕ and Δ are subject to an additional constraint: $4\Delta(1 - \Delta) < \phi^2$.

For polyploids, VCFPOP provides two relatedness estimators (Huang *et al.* 2015a; Huang *et al.* 2014) and four kinship estimators (Loiselle *et al.* 1995; Ritland 1996; VanRaden 2008; Weir 1996).

The polyploid method-of-moment estimator is a modification of Huang *et al.*'s (2016a) estimator. In this estimator, all possible genotype patterns are enumerated (there are 5, 11 and 22 reference genotype modes for tetraploids, hexaploids and octoploids, respectively). The definition of similarity index S is modified as the number of alleles that are identical-by-state between two individuals, with each allele being counted only once. Therefore, there are $v + 1$ possible values of S , i.e., the range of S is $1, \frac{v-1}{v}, \frac{v-2}{v}, \dots, 0$, where v is the ploidy level. Let $E(S^k)$ be the expected value of the k^{th} moment of S , and let Δ_k be the probability that two individuals at any given locus share k pairs of IBD alleles, $k = 1, 2, \dots, v$. If we denote \mathbf{E} for the vector $[E(S), E(S^2), \dots, E(S^v)]^T$, and $\mathbf{\Delta}$ for the vector $[\Delta_1, \Delta_2, \dots, \Delta_v]^T$, then the relation between \mathbf{E} and $\mathbf{\Delta}$ can be expressed as

$$\mathbf{E} = \mathbf{A} + \mathbf{M}\mathbf{\Delta},$$

where \mathbf{A} is a column vector containing v elements, and \mathbf{M} is a square matrix with order v . Now, if we regard the elements in $\mathbf{\Delta}$ as unknowns, the above expression is a linear equation set, whose solution is $\hat{\mathbf{\Delta}} = \mathbf{M}^{-1}(\hat{\mathbf{E}} - \mathbf{A})$. For example, in tetraploids, if $G_x = A_i A_i A_i A_i$, then

$$\mathbf{A} = \begin{bmatrix} 1 & 0.75 & 0.5 & 0.25 & 0 \\ 1 & 0.75^2 & 0.5^2 & 0.25^2 & 0 \\ 1 & 0.75^3 & 0.5^3 & 0.25^3 & 0 \\ 1 & 0.75^4 & 0.5^4 & 0.25^4 & 0 \end{bmatrix} \begin{bmatrix} p_i^4 \\ 4p_i^3 p_x \\ 6p_i^2 p_x^2 \\ 4p_i p_x^3 \\ p_x^4 \end{bmatrix},$$

$$\mathbf{M} = \begin{bmatrix} 1 & 0.75 & 0.5 & 0.25 & 0 \\ 1 & 0.75^2 & 0.5^2 & 0.25^2 & 0 \\ 1 & 0.75^3 & 0.5^3 & 0.25^3 & 0 \\ 1 & 0.75^4 & 0.5^4 & 0.25^4 & 0 \end{bmatrix} \begin{bmatrix} p_i^3 - p_i^4 & p_i^2 - p_i^4 & p_i - p_i^4 & 1 - p_i^4 \\ (3p_i^2 - 4p_i^3)p_x & (2p_i - 4p_i^3)p_x & (1 - 4p_i^3)p_x & -4p_i^3 p_x \\ (3p_i - 6p_i^2)p_x^2 & (1 - 6p_i^2)p_x^2 & -6p_i^2 p_x^2 & -6p_i^2 p_x^2 \\ (1 - 4p_i)p_x^3 & -4p_i p_x^3 & -4p_i p_x^3 & -4p_i p_x^3 \\ -p_x^4 & -p_x^4 & -p_x^4 & -p_x^4 \end{bmatrix}$$

and so the equation set $\mathbf{E} = \mathbf{A} + \mathbf{M}\Delta$ becomes

$$\begin{aligned} \begin{bmatrix} E(S) \\ E(S^2) \\ E(S^3) \\ E(S^4) \end{bmatrix} &= \begin{bmatrix} 1 & 0.75 & 0.5 & 0.25 & 0 \\ 1 & 0.75^2 & 0.5^2 & 0.25^2 & 0 \\ 1 & 0.75^3 & 0.5^3 & 0.25^3 & 0 \\ 1 & 0.75^4 & 0.5^4 & 0.25^4 & 0 \end{bmatrix} \begin{bmatrix} p_i^4 \\ 4p_i^3 p_x \\ 6p_i^2 p_x^2 \\ 4p_i p_x^3 \\ p_x^4 \end{bmatrix} \\ &+ \begin{bmatrix} p_i^3 - p_i^4 & p_i^2 - p_i^4 & p_i - p_i^4 & 1 - p_i^4 \\ (3p_i^2 - 4p_i^3)p_x & (2p_i - 4p_i^3)p_x & (1 - 4p_i^3)p_x & -4p_i^3 p_x \\ (3p_i - 6p_i^2)p_x^2 & (1 - 6p_i^2)p_x^2 & -6p_i^2 p_x^2 & -6p_i^2 p_x^2 \\ (1 - 4p_i)p_x^3 & -4p_i p_x^3 & -4p_i p_x^3 & -4p_i p_x^3 \\ -p_x^4 & -p_x^4 & -p_x^4 & -p_x^4 \end{bmatrix} \begin{bmatrix} \Delta_1 \\ \Delta_2 \\ \Delta_3 \\ \Delta_4 \end{bmatrix}. \end{aligned}$$

The relatedness estimator of maximum-likelihood for polyploid is a modification of Milligan's (2003) estimator. In this estimator, all patterns of genotypic pairs between two individuals are enumerated. There are 9 patterns in diploids (see the expression of $\mathcal{L}(G_x, G_y | \phi, \Delta)$ mentioned above). There are 109, 1043, 8405 patterns in tetraploids, hexaploids and octoploids, respectively. For example, if $G_x = A_i A_i A_i A_i$ in tetraploids, then the expression of $\mathcal{L}(G_x, G_y | \phi, \Delta)$ for the first several patterns is as follows:

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$$\mathcal{L}(G_x, G_y | \phi, \Delta) = \begin{cases} p_i^4 \Delta_4 + p_i^5 \Delta_3 + p_i^6 \Delta_2 + p_i^7 \Delta_1 + p_i^8 \Delta_0 & \text{if } G_y = A_i A_t A_i A_i, \\ 4p_i^4 p_j^4 \Delta_0 & \text{if } G_y = A_j A_t A_j A_j, \\ p_i^4 p_j \Delta_3 + 2p_i^5 p_j \Delta_2 + 3p_i^6 p_j \Delta_1 + 4p_i^7 p_j \Delta_0 & \text{if } G_y = A_i A_t A_i A_j, \\ p_i^4 p_j^3 \Delta_1 + 4p_i^5 p_j^3 \Delta_0 & \text{if } G_y = A_t A_j A_j A_j, \\ 4p_i^6 p_j p_k^3 \Delta_0 & \text{if } G_y = A_j A_k A_k A_k, \\ p_i^4 p_j^2 \Delta_2 + 3p_i^5 p_j^2 \Delta_1 + 6p_i^6 p_j^2 \Delta_0 & \text{if } G_y = A_t A_t A_j A_j, \\ 6p_i^4 p_j^2 p_k^2 \Delta_0 & \text{if } G_y = A_j A_t A_k A_k, \\ 2p_i^4 p_j p_k \Delta_2 + 6p_i^5 p_j p_k \Delta_1 + 12p_i^6 p_j p_k \Delta_0 & \text{if } G_y = A_t A_t A_j A_k, \\ 3p_i^4 p_j^2 p_k \Delta_1 + 12p_i^5 p_j^2 p_k \Delta_0 & \text{if } G_y = A_t A_j A_j A_k, \\ 12p_i^4 p_j^2 p_k p_l \Delta_0 & \text{if } G_y = A_j A_t A_k A_l, \\ 6p_i^4 p_j p_k p_l \Delta_1 + 24p_i^5 p_j p_k p_l \Delta_0 & \text{if } G_y = A_t A_j A_k A_l, \\ 24p_i^4 p_j p_k p_l p_m \Delta_0 & \text{if } G_y = A_j A_t A_k A_l A_m, \\ \dots & \dots \end{cases}$$

For different ploidy levels, VCFPOP can be used to calculate the relatedness \hat{r}_{HL} from a higher ploidy individual to a lower ploidy individual (Huang *et al.* 2015b). If the ploidy levels of these two individuals are equal, then \hat{r}_{HL} is denoted by \hat{r} .

The relatedness coefficients can also be converted from the kinship coefficient except the above methods. There are two methods to achieve this conversion. One method is the original conversion, whose formula is

$$\hat{r}_{HL} = v_{\min} \hat{\theta},$$

where v_{\min} is the ploidy level of the lower ploidy individual, and $\hat{\theta}$ is the kinship coefficient between these two individuals. It is worth noting that this conversion can only be used for outbred populations, and \hat{r}_{HL} may be greater than one. The other method is provided by Huang *et al.* (2015a), whose converting formula is

$$\hat{r}_{HL} = \frac{v_{\min}}{v_{\min} + v_{\max}} \hat{\theta}_{xy} \left(\frac{1}{\hat{\theta}_{xx}} + \frac{1}{\hat{\theta}_{yy}} \right),$$

where $\hat{\theta}_{xy}$ is the kinship coefficient between two individuals, $\hat{\theta}_{xx}$ and $\hat{\theta}_{yy}$ are the kinship coefficients within the individuals x and y , respectively, and v_{\max} is the ploidy level of the higher

ploidy individual. The latter method can be used for either outbred or inbred populations, with the maximum of \hat{r}_{HL} being equal to 1. Such method can be applied to Ritland's (1996) and Loiselle *et al.*'s (1995) estimators, but not to Weir's (1996) and VaRaden's (2008) estimator because its $\hat{\theta}_{xx}$ may be zero or negative.

The relatedness coefficient of Weir's (1996) and VaRaden's (2008) estimators are

$$\begin{aligned}\hat{r}_{WE} &= v_{\min} \hat{\theta}_{WE}, \\ \hat{r}_{VR} &= \sqrt{v_{\min} v_{\max}} \hat{\theta}_{VR}.\end{aligned}$$

5.13 Principal coordinates analysis

Assume that \mathbf{D} is the genetic distance matrix $[d_{ij}]$ of order n , and \mathbf{A} is the matrix $[a_{ij}]$, in which $a_{ij} = -\frac{1}{2}d_{ij}^2$, $i, j = 1, 2, \dots, n$. Then the Gower's (1966) centered matrix \mathbf{G} can be calculated by centering the elements of \mathbf{A} , whose calculating formula is $\mathbf{G} = \mathbf{EAE}$, where \mathbf{E} is a square matrix of order n , whose diagonal elements are all $1 - 1/n$, and other elements are all $-1/n$.

Because the distance matrix \mathbf{D} is symmetric, so is \mathbf{G} . Therefore, there are exactly n real eigenvalues of \mathbf{G} , denoted by $\lambda_1, \lambda_2, \dots, \lambda_n$, and there are n orthonormal eigenvectors of \mathbf{G} , denoted by $\xi_1, \xi_2, \dots, \xi_n$, where each eigenvector is a column vector with n components. Let \mathbf{U} be the matrix $[\xi_1, \xi_2, \dots, \xi_n]$. Then \mathbf{U} is an orthogonal matrix. Assume that the corresponding eigenvalues of $\xi_1, \xi_2, \dots, \xi_n$ are $\lambda_1, \lambda_2, \dots, \lambda_n$ in turn, then \mathbf{G} can be decomposed as $\mathbf{G} = \mathbf{UVU}^T$, where $\mathbf{V} = \text{diag}(\lambda_1, \lambda_2, \dots, \lambda_n)$.

It is noteworthy that each eigenvalue λ_i represents variance in the data that is projected into the eigenvector ξ_i , and each variance is a non-negative number. Therefore, any negative eigenvalues should be excluded. It does not affect the hypothesis if the first m eigenvalues are whole non-negative eigenvalues of \mathbf{G} . If so, then the output matrix \mathbf{X} can be expressed as

$$\mathbf{X} = [\sqrt{\lambda_1} \xi_1, \sqrt{\lambda_2} \xi_2, \dots, \sqrt{\lambda_m} \xi_m].$$

Finally, if all eigenvalues of \mathbf{G} are negative, an output matrix will not be generated.

5.14 Hierarchical clustering

The hierarchical clustering analysis is based on the distance matrix \mathbf{D} of order n , where n is the number of clusters (assuming the identifiers of clusters are from 1 to n), and ‘distance’ has various indicators between two clusters, such as the genetic distance, phenotypic dissimilarity, geographical distance and so on. Initially, each individual, each population or each region is defined as a cluster, and the element d_{ij} in \mathbf{D} is the distance between the clusters i and j . We will repeatedly update the matrix \mathbf{D} . The updated procedure is described as follows.

Assume that the minimum non-diagonal element in \mathbf{D} is d_{ab} ($a \neq b$). Then, for the output dendrogram, the two nodes representing the clusters a and b are merged to the node with the coordinate $d_{ab}/2$. Second, update the elements in the a^{th} row and the a^{th} column of \mathbf{D} except for the diagonal element d_{aa} , and the updated elements are written as d'_{ac} and d'_{ca} ($c = 1, 2, \dots, n$ and $c \neq a$). Third, delete the b^{th} row and the b^{th} column of \mathbf{D} , such that the order of \mathbf{D} is reduced to $n - 1$. Such a procedure needs to be repeated $n - 1$ times, until the order of \mathbf{D} is reduced to 1. Noticing that each distance matrix is symmetric, the updated distances d'_{ac} and d'_{ca} should be equal.

There are several methods to calculate the updated distances d'_{ac} and d'_{ca} , which are listed below (N_a , N_b and N_c denote the numbers of the members of the clusters a , b and c in turn).

- Nearest

$$d'_{ac} = d'_{ca} = \min(d_{ac}, d_{bc}).$$

- Furthest

$$d'_{ac} = d'_{ca} = \max(d_{ac}, d_{bc}).$$

- UPGMA

$$d'_{ac} = d'_{ca} = \frac{N_a d_{ac} + N_b d_{bc}}{N_a + N_b}.$$

- UPGMC

$$d'_{ac} = d'_{ca} = \sqrt{\frac{N_a d_{ac}^2 + N_b d_{bc}^2}{N_a + N_b} - \frac{N_a N_b d_{ab}^2}{(N_a + N_b)^2}}.$$

- WPGMA

$$d'_{ac} = d'_{ca} = \frac{d_{ac} + d_{bc}}{2}.$$

- WPGMC

$$d'_{ac} = d'_{ca} = \sqrt{\frac{d_{ac}^2 + d_{bc}^2}{2} - \frac{d_{ab}^2}{4}}.$$

- WARD

$$d'_{ac} = d'_{ca} = \sqrt{\frac{(N_a + N_c)d_{ac}^2 + (N_b + N_c)d_{bc}^2 - N_c d_{ab}^2}{N_a + N_b + N_c}}.$$

5.15 Bayesian clustering

MCMC algorithm. Pritchard *et al.* (2000) used a Markov Chain Monte-Carlo (MCMC) algorithm with Gibbs sampling to infer population structure. In the MCMC algorithm, various parameters are repeatedly updated according to the allele frequencies in both each cluster and its individual originating clusters so as to obtain the clustering results. The state of this system can be described by **P**, **Q**, Z_i , Z_{ila} , r , α , λ , η , γ and F , where **P** and **Q** are the vectors consisting of some parameters, the meanings of these parameters are shown below. The state will be updated by iterations. Each new state is generated based on the current state. The sequence consisting of these states is called a Markov chain. In an iteration, all parameters listed above are updated in turn, with each being updated conditional on all other parameters. Such a process is called Gibbs sampling. The Markov chain starts from a randomly generated initial state, and the state after several iterations will become stable and independent to the initial state. This iteration period is the ‘burnin’. After that, this algorithm begins to record the number of times that an allele or an individual is assigned to each cluster. Such iterations are the ‘sampling period’. In order to prevent the results in two adjacent iterations being too similar, the state will be recorded at an interval called the ‘thinning interval’ to eliminate any autocorrelation. Some independent runs (with different random number

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generator seeds) can be performed to repeat the results or to avoid the Markov chain being blocked at local maxima. The number of independent runs is ‘number of runs’.

Vector P of allele frequencies. Let $A_{l1}, A_{l2}, \dots, A_{lJ_l}$ be all distinct alleles at locus l , and let P_{klj} be the frequency of the allele A_{lj} in the cluster k , $j = 1, 2, \dots, J_l$. Denote \mathbf{P} for the vector $[P_{kl1}, P_{kl2}, \dots, P_{kJ_l}]$, and \mathbf{P}' for the updated vector of \mathbf{P} . The elements $P'_{kl1}, P'_{kl2}, \dots, P'_{kJ_l}$ in \mathbf{P}' are randomly drawn from the Dirichlet distribution

$$\mathcal{D}(n_{kl1} + \lambda, n_{kl2} + \lambda, \dots, n_{kJ_l} + \lambda)$$

for the non-F model; or from the Dirichlet distribution

$$\mathcal{D}(n_{kl1} + \varepsilon_{l1}f_k, n_{kl2} + \varepsilon_{l2}f_k, \dots, n_{kJ_l} + \varepsilon_{lJ_l}f_k)$$

for the F model, where λ is the Dirichlet parameter of allele frequencies, n_{klj} is the number of copies of the allele A_{lj} that is assigned to the cluster k , ε_{lj} is the frequency of A_{lj} in the ancestral cluster, and $f_k = (1 - F_k)/F_k$. The F model together with the value F_k of parameter F will be described in the penultimate paragraph of this section. The parameter λ can be used to prevent the allele frequencies to be fixed at zero or one. When λ is large, it will result in the allele frequencies becoming more evenly distributed, and thus will reduce any differentiation among clusters, and slow the convergence of allele frequencies.

Dirichlet parameter λ of allele frequencies. If the option `-infer_lambda=yes` is checked, then λ will be updated by the Metropolis-Hastings approach. The updated value λ' is randomly drawn from the normal distribution $N(\lambda, \text{std}^2(\lambda))$. If λ' is below zero or above $\max(\lambda)$, it is rejected directly. Otherwise, it is accepted at the probability of $\min(1, E)$, where E is the probability of accepting a worse value of λ' , whose calculating formula is as follows:

$$E = \prod_l^L \prod_k^K \left[\frac{\Gamma(J_l\lambda')[\Gamma(\lambda)]^{J_l}}{\Gamma(J_l\lambda)[\Gamma(\lambda')]^{J_l}} \left(\prod_j^{J_l} P_{klj} \right)^{\lambda' - \lambda} \right]$$

for the non-F model, or

$$E = \prod_l^L \left[\frac{\Gamma(J_l \lambda') [\Gamma(\lambda)]^{J_l}}{\Gamma(J_l \lambda) [\Gamma(\lambda')]^{J_l}} \left(\prod_j^{J_l} \varepsilon_{lj} \right)^{\lambda' - \lambda} \right]$$

for the F model, in which K is the number of clusters, $\Gamma(\cdot)$ is the gamma function, and P_{klj} is an element in \mathbf{P} .

Moreover, if the option ‘Diff λ' is checked, this enables this algorithm to use different values of λ for different clusters. If the option ‘Diff λ' is checked, the updated value λ'_k of λ for the cluster k will be randomly drawn from the normal distribution $N(\lambda_k, \text{std}^2(\lambda))$, whose accepted probability E_k is as follows:

$$E_k = \prod_l^L \left[\frac{\Gamma(J_l \lambda'_k) [\Gamma(\lambda_k)]^{J_l}}{\Gamma(J_l \lambda_k) [\Gamma(\lambda'_k)]^{J_l}} \left(\prod_j^{J_l} P_{klj} \right)^{\lambda'_k - \lambda_k} \right], \quad k = 1, 2, \dots, K.$$

Originating clusters of individuals. For the non-ADMIXTURE model, it is assumed that an individual can only originate from one cluster, and all alleles in this individual are assigned to this cluster. Denote Z_i for the current originating cluster of the individual i . The updated cluster Z'_i of Z_i is randomly drawn from all K clusters, and the accepting probability $\Pr(Z'_i = k)$ that Z'_i is equal to the cluster k is

$$\Pr(Z'_i = k) = \frac{\prod_l^L \prod_a^{v_i} P_{kA_{la}}}{\sum_{k'}^K \prod_l^L \prod_a^{v_i} P_{k'A_{la}}}, \quad k = 1, 2, \dots, K,$$

where v_i is the ploidy level of the individual i , A_{la} is the a^{th} allele copy in the individual i at locus l , and $P_{kA_{la}}$ is the frequency of A_{la} in the cluster k . For the ADMIXTURE model, it is assumed that different alleles in the same individual can originate from different clusters. Let Z_{ila} be the current originating cluster of the allele copy A_{la} . Like the situation of non-admixture model, the updated cluster Z'_{ila} of Z_{ila} is randomly drawn from all K clusters. Using the elements in the vector \mathbf{Q} , the accepting probability that Z'_{ila} is equal to the cluster k is

$$\Pr(Z'_{ila} = k) = \frac{Q_{ik} P_{kA_{la}}}{\sum_{k'}^K Q_{ik'} P_{k'A_{la}}},$$

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where $l = 1, 2, \dots, L$, $a = 1, 2, \dots, v_i$, $k = 1, 2, \dots, K$ and the meanings of \mathbf{Q} together with its elements are explained in the next paragraph.

Vector \mathbf{Q} of admixture proportions. Assume that Q_{ik} denotes the admixture proportion of genome of the individual i that originates from the cluster k ($k = 1, 2, \dots, K$), and \mathbf{Q} denotes the vector $[Q_{i1}, Q_{i2}, \dots, Q_{iK}]$. As stated previously, \mathbf{Q} is used in the ADMIXTURE model (Pritchard *et al.* 2000) to update the originating cluster Z_{ila} . The elements in \mathbf{Q} are randomly drawn from the Dirichlet distribution

$$\mathcal{D}(m_{i1} + \alpha_1, m_{i2} + \alpha_2, \dots, m_{iK} + \alpha_K),$$

where m_{ik} is the number of allele copies at all loci and in the individual i that is assigned to the cluster k , and α_k is the value of Dirichlet parameter α for the cluster k ($k = 1, 2, \dots, K$). These alphas can be used to prevent the proportions becoming fixed. The higher the values of these alphas, the higher the mixture level. Additionally, the elements in \mathbf{Q} are also updated via a Metropolis-Hastings approach in the sense that the updated values $Q'_{i1}, Q'_{i2}, \dots, Q'_{iK}$ are randomly drawn from the Dirichlet distribution

$$\mathcal{D}(\alpha_1, \alpha_2, \dots, \alpha_K)$$

for the non- LOCPRIORI model, or from

$$\mathcal{D}(\alpha_{\text{local},s1}, \alpha_{\text{local},s2}, \dots, \alpha_{\text{local},sk})$$

for the LOCPRIORI model, where $\alpha_{\text{local},sk}$ is a local alpha that is used for sampling the individuals from both the location s and the cluster k ($k = 1, 2, \dots, K$). These updated values $Q'_{i1}, Q'_{i2}, \dots, Q'_{iK}$ are accepted at the probability of $\min(1, E)$, where

$$E = \prod_a^{v_i} \prod_l^L \frac{\sum_k^K Q'_{ik} P_{kA_{la}}}{\sum_k^K Q_{ik} P_{kA_{la}}}.$$

Such an update will improve the mixing when these alphas are small, and it will shuffle the individuals to prevent the Markov chain becoming blocked at local maxima.

Dirichlet parameter α for allele frequencies. This parameter will be updated by a Metropolis-Hastings approach if the option ‘Infer alpha’ is checked in the LOCPRIORI model or during the

admburnin period in the non-LOCPRIORI model. For the non-LOCPRIORI model, if the values of α for the whole clusters are assumed to be all equal, the updated value α' is randomly drawn from the normal distribution $N(\alpha, \text{std}^2(\alpha))$, and α' is accepted at the probability of $\min(1, E)$, where

$$E = \frac{\Pr(\alpha')}{\Pr(\alpha)} \prod_i^N \left[\frac{\Gamma(K\alpha')}{\Gamma(K\alpha)} \prod_k^K \left(\frac{\Gamma(\alpha)}{\Gamma(\alpha')} Q_{ik}^{\alpha'-\alpha} \right) \right],$$

in which N is the number of individuals, $\Pr(\alpha)$ is the priori probability of the parameter α , and $\frac{\Pr(\alpha')}{\Pr(\alpha)}$ is equal to 1 if α is assumed to be drawn from a uniform distribution, or equal to $\left(\frac{\alpha'}{\alpha}\right)^{A-1} \exp\left(\frac{\alpha-\alpha'}{B}\right)$ if α is assumed to be drawn from the gamma distribution $\Gamma(A, B)$. Moreover, if the values of α for the whole clusters are assumed to be not all equal, the updated value α'_k for the cluster k is randomly drawn from the normal distribution $N(\alpha_k, \text{std}^2(\alpha))$, and α'_k is accepted at the probability of $\min(1, E_k)$, where

$$E_k = \frac{\Pr(\alpha'_k)}{\Pr(\alpha_k)} \prod_i^N \frac{\Gamma(\alpha'_k - \alpha_k + \sum_{k'}^K \alpha'_{k'}) \Gamma(\alpha_k)}{\Gamma(\sum_{k'}^K \alpha'_{k'}) \Gamma(\alpha'_k)} Q_{ik}^{\alpha'_k - \alpha_k}, \quad k = 1, 2, \dots, K,$$

in which the meanings of $\Pr(\alpha_k)$ and $\frac{\Pr(\alpha'_k)}{\Pr(\alpha_k)}$ are similar to $\Pr(\alpha)$ and $\frac{\Pr(\alpha')}{\Pr(\alpha)}$, respectively. For the LOCPRIORI model, the parameter α will be updated by using an alternative approach (see the next paragraph for details).

LOCPRIORI model. In this model, the population information of individuals is used as *a priori* information to assist clustering, which is powerful when the population differentiation is relatively weak (Hubisz *et al.* 2009). For the non-ADMIXTURE model, the vectors $\boldsymbol{\eta}$ and $\boldsymbol{\gamma}_s$ are used, where the k^{th} element η_k in $\boldsymbol{\eta}$ is the priori probability of individuals assigned to the cluster k , and the k^{th} element γ_{sk} in $\boldsymbol{\gamma}_s$ is the priori probability of individuals sampled from the location s and assigned to the cluster k ($k = 1, 2, \dots, K$). For the ADMIXTURE model, the vectors $\boldsymbol{\alpha}$ and $\boldsymbol{\alpha}_{\text{local}}$ are used, which consist of the global alphas and the local alphas, respectively, where the k^{th} element α_k in $\boldsymbol{\alpha}$ (or $\alpha_{\text{local},sk}$ in $\boldsymbol{\alpha}_{\text{local}}$) reflects reflect the relative levels of admixture from each cluster over all individuals (individuals sampled from the location s), $k = 1, 2, \dots, K$. Moreover, the parameter r

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related to the informativeness of data parameterizes the extent to which the ancestral proportions at the locations of the sampled individuals can deviate from the overall proportion. If r is high ($\gg 1$), then the priori ancestry proportions across all locations are essentially the same (i.e., it is approximately proportional to η_k or α_k). In contrast, if r is near one or lower, the values of those γ_{sk} or of those $\alpha_{local,sk}$ may vary substantially at different locations, implying that the location data are more informative about ancestry.

Update of LOCpriori model + non-ADMIXTURE model. All data of parameters for LOCpriori model are updated by using a Metropolis-Hastings approach. The new value r' of the parameter r is randomly drawn from the uniform distribution $U(r - \text{eps}(r), r + \text{eps}(r))$, which is checked within the range [0,1], whose acceptance rate E for the non-ADMIXTURE model is

$$E = \prod_s^S \left[\frac{\Gamma(r')}{\Gamma(r)} \prod_k^K \left(\frac{\Gamma(r\eta_k)}{\Gamma(r'\eta_k)} \gamma_{sk}^{\eta_k(r'-r)} \right) \right],$$

where S is the number of sampling locations. Next, the update of the vector $\boldsymbol{\eta}$ (or $\boldsymbol{\gamma}_s$) only needs to update two elements in $\boldsymbol{\eta}$ (or in $\boldsymbol{\gamma}_s$). The updating procedures are as follows. First, randomly sample two elements from $\boldsymbol{\eta}$ (or $\boldsymbol{\gamma}_s$), denoted by η_a and η_b (or γ_{sk} and γ_{sl}). Second, randomly draw a difference variable d from the uniform distribution $U(0, \text{eps}(\eta))$ for $\boldsymbol{\eta}$, or from $U(0, \text{eps}(\gamma))$ for $\boldsymbol{\gamma}_s$. Finally, the updated values η'_a and η'_b for $\boldsymbol{\eta}$ are given by

$$\begin{aligned}\eta'_a &= \eta_a + d, \\ \eta'_b &= \eta_b - d.\end{aligned}$$

Now, if η'_a or η'_b is not in the acceptable range [0, 1], both are rejected, and the original η_a and η_b are regarded as the updated values; if ε'_{la} and ε'_{lb} are in the acceptable range [0, 1], both are accepted at the acceptance rate E , where

$$E = \prod_s^S \left[\frac{\Gamma(r\eta_a)\Gamma(r\eta_b)}{\Gamma(r\eta'_a)\Gamma(r\eta'_b)} \left(\frac{\gamma_{sa}}{\gamma_{sb}} \right)^{rd} \right].$$

Moreover, the updated values γ'_{sk} and γ'_{sl} for $\boldsymbol{\gamma}_s$ are given by

$$\gamma'_{sk} = \gamma_{sk} + d,$$

$$\gamma'_{sl} = \gamma_{sl} - d.$$

Like the situation of η , γ'_{sk} and γ'_{sl} are checked within the range [0,1], and both are accepted at the acceptance rate E_s , where

$$E_s = \left(\frac{\gamma'_{sk}}{\gamma_{sk}} \right)^{r\eta_k-1-N_{sk}} \left(\frac{\gamma'_{sl}}{\gamma_{sl}} \right)^{r\eta_l-1-N_{sl}}, \quad s = 1, 2, \dots, S,$$

in which N_{sk} (or N_{sl}) is the number of the individuals sampled from the location s and assigned to the cluster k (or l).

Update of LOCPRIORI model + ADMIXTURE model. For these two models, the new value r' of the parameter r is randomly drawn from the same uniform distribution as described in the preceding paragraph, whose acceptance rate E is

$$E = \prod_k^K \prod_s^S \left[\frac{r'^{r'\alpha_k}}{r^{r\alpha_k}} \frac{\Gamma(r\alpha_k)}{\Gamma(r'\alpha_k)} \alpha_{\text{local},sk}^{\alpha_k d} \exp(-d\alpha_{\text{local},sk}) \right],$$

where $d = r' - r$. Next, the k^{th} updated value α'_k of the global alphas is randomly drawn from the normal distribution $N(\alpha_k, \text{std}^2(\alpha))$, whose acceptance rate E_k is

$$E_k = \prod_s^S \left[\alpha_k^{r(\alpha'_k - \alpha_k)} \frac{\Gamma(\alpha_k r)}{\Gamma(\alpha'_k r)} r^{r(\alpha'_k - \alpha_k)} \right], \quad k = 1, 2, \dots, K.$$

This is followed by the k^{th} updated value $\alpha'_{\text{local},sk}$ of the local alphas, which is randomly drawn from the normal distribution $N(\alpha_{\text{local},sk}, \text{std}^2(\alpha))$, whose acceptance rate E_{sk} is

$$E_{sk} = \left(\frac{\alpha'_{\text{local},sk}}{\alpha_{\text{local},sk}} \right)^{r\alpha_k-1} \left[\frac{\Gamma(d + \sum_k^K \alpha_{\text{local},sk'}) \Gamma(\alpha_{\text{local},sk})}{\Gamma(\sum_k^K \alpha'_{\text{local},sk'}) \Gamma(\alpha'_{\text{local},sk})} \right]^{N_s} \left(\prod_i^{N_s} Q_{ik} \right)^d \exp(-rd),$$

where $d = \alpha'_{\text{local},sk} - \alpha_{\text{local},sk}$, and N_s is the number of individuals sampled from the location s , $s = 1, 2, \dots, S$, $k = 1, 2, \dots, K$.

F model. In this model, it is assumed that all K clusters have undergone the independent drift away from an ancestral cluster, and the allele frequencies in each cluster are correlated with those in the

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ancestral cluster. The measure F in this model is analogous to the Wright's F_{ST} , and different value of F can be used for each cluster. For the cluster k , the differentiation from the ancestral cluster is measured by F_k , where F_k is the value of F for the cluster k ($k = 1, 2, \dots, K$). The allele frequencies of the cluster k at locus l are drawn from the Dirichlet distribution

$$\mathcal{D}(\varepsilon_{l1}f_k, \varepsilon_{l2}f_k, \dots, \varepsilon_{lJ_l}f_k), \quad k = 1, 2, \dots, K,$$

where $f_k = (1 - F_k)/F_k$, and $\varepsilon_{l1}, \varepsilon_{l2}, \dots, \varepsilon_{lJ_l}$ are the frequencies of alleles in the ancestor cluster at locus l . The values of F are also updated. If different value F in each cluster is used, the updated value F'_k of F_k is randomly drawn from the normal distribution $N(F_k, \text{std}^2(F))$, which is accepted at the probability of $\min(1, E_k)$, where

$$E_k = \left(\frac{F'_k}{F_k}\right)^{\mu^2/\sigma^2-1} \exp\left(\frac{\mu(F_k - F'_k)}{\sigma^2}\right) \prod_l^L \frac{\Gamma(f'_k) \prod_j^{J_l} \Gamma(f_k \varepsilon_{lj}) P_{klj}^{f'_k \varepsilon_{lj}}}{\Gamma(f_k) \prod_j^{J_l} \Gamma(f'_k \varepsilon_{lj}) P_{klj}^{f_k \varepsilon_{lj}}}, \quad k = 1, 2, \dots, K,$$

in which μ and σ are respectively the priori mean and the priori standard deviation of F , P_{klj} is an element in \mathbf{P} , and $f'_k = (1 - F'_k)/F'_k$. Moreover, if the values of F for the all clusters are assumed to be all equal, the updated value F' of F will be randomly drawn from the normal distribution $N(F, \text{std}^2(F))$, whose acceptance rate E is

$$E = \left(\frac{F'}{F}\right)^{\mu^2/\sigma^2-1} \exp\left(\frac{\mu(F - F')}{\sigma^2}\right) \prod_k^K \prod_l^L \frac{\Gamma(f') \prod_j^{J_l} \Gamma(f \varepsilon_{lj}) P_{klj}^{f' \varepsilon_{lj}}}{\Gamma(f) \prod_j^{J_l} \Gamma(f' \varepsilon_{lj}) P_{klj}^{f \varepsilon_{lj}}}.$$

Updating the allele frequencies of ancestral clusters. Two approaches are available for selection to update the allele frequencies of the ancestral clusters, each of which is selected at the probability 0.5. For the first approach, the updated values $\varepsilon'_{l1}, \varepsilon'_{l2}, \dots, \varepsilon'_{lJ_l}$ of $\varepsilon_{l1}, \varepsilon_{l2}, \dots, \varepsilon_{lJ_l}$ are randomly drawn from the Dirichlet distribution

$$\mathcal{D}\left(\lambda + \sum_k^K P_{kl1} f_k, \lambda + \sum_k^K P_{kl2} f_k, \dots, \lambda + \sum_k^K P_{klJ_l} f_k\right), \quad l = 1, 2, \dots, L,$$

and the acceptance rate $E_{lj'}$ is

$$E_{lj'} = \prod_j^{J_l} \left[\left(\frac{\varepsilon_{lj}}{\varepsilon'_{lj}} \right)^{\sum_k^K P_{klj} f_k} \prod_k^K \frac{\Gamma(f_k \varepsilon_{lj})}{\Gamma(f_k \varepsilon'_{lj})} P_{klj}^{f(\varepsilon'_{lj} - \varepsilon_{lj})} \right], \quad l = 1, 2, \dots, L, j' = 1, 2, \dots, J_l.$$

For the second approach, this only needs to be updated to the frequencies of two randomly chosen alleles. Let ε_{la} and ε_{lb} be these two frequencies. Next, a difference variable d is drawn from the uniform distribution $U(0, N^{-1/2})$, where N is the number of individuals. Then the updated values ε'_{la} and ε'_{lb} are respectively

$$\varepsilon'_{la} = \varepsilon_{la} + d,$$

$$\varepsilon'_{lb} = \varepsilon_{lb} - d.$$

Now, if ε'_{la} or ε'_{lb} is not in the acceptable range $[0, 1]$, both are rejected, and the original ε_{la} and ε_{lb} are regarded as the updated values. If ε'_{la} and ε'_{lb} are in the acceptable range $[0, 1]$, both are accepted at the acceptance rate E , where

$$E = \left(\frac{\varepsilon'_{la} \varepsilon'_{lb}}{\varepsilon_{la} \varepsilon_{lb}} \right)^{\lambda-1} \prod_k^K \left[\frac{\Gamma(f_k \varepsilon_{la}) \Gamma(f_k \varepsilon_{lb})}{\Gamma(f_k \varepsilon'_{la}) \Gamma(f_k \varepsilon'_{lb})} \left(\frac{P_{kla}}{P_{klb}} \right)^{f_k d} \right].$$

5.16 Aneuploids and mixed-ploidy populations

Most functions in VCFPOP support aneuploids and mixed-ploidy populations.

Input format: Aneuploids or mixed-ploidy populations dataset can be VCF, BCF, SPAGEDI or POLYRELATEDNESS.

Haplotype extraction: This function supports aneuploids and mixed-ploidy populations, because it is performed for each chromosome separately.

Conversion: SPAGEDI and POLYRELATEDNESS format support aneuploids and mixed-ploidy populations; STRUCTURE supports mixed-ploidy populations, and POLYGENE format support homoploids, and ARLEQUIN, CERVUS and GENEPOLY only support diploids.

Genetic diversity indices: This function supports aneuploids and mixed-ploidy populations, except for the Fisher's G test is not performed for mixed-ploidy populations. The expected heterozygosity, polymorphic information content, effective number of alleles, Shannon's information index and exclusion probability are estimated from allele frequencies, and can all be directly applied for aneuploids and mixed-ploidy populations. The observed heterozygosity is

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defined as the frequency of non-IBS allele pairs within individuals. This is then averaged across individuals, weighted according to the number of allele pairs. The inbreeding coefficient is estimated by $F_{IS} = 1 - H_O/H_E$.

Individual statistics: This function supports aneuploids and mixed-ploidy populations. The heterozygosity index, inbreeding coefficient, kinship coefficients at all loci are the arithmetic means of those across loci, and multi-locus genotypic frequency is the product of genotypic frequencies across loci.

Genetic differentiation: This function supports aneuploids and mixed-ploidy populations. The differentiation estimators are based on allele frequencies (Hedrick 2005; Jost 2008; Nei 1973), *infinity allele model* (IAM) or *stepwise mutation model* (SMM) distances (Huang *et al.* 2021; Hudson *et al.* 1992; Slatkin 1995). These estimators support aneuploids and mixed-ploidy populations. Whereas Weir & Cockerham's (1984) estimator only support diploids.

Genetic distance: This function supports aneuploids and mixed-ploidy populations. These distances are based on allele frequencies (Cavalli-Sforza & Edwards 1967a; Nei 1972; Euclidean distance, Nei & Roychoudhury 1974; Nei *et al.* 1983; Reynolds *et al.* 1983; Rogers 1972), SMM distance (Goldstein *et al.* 1995), or genetic differentiation (Reynolds *et al.* 1983; Slatkin 1995).

Analysis of molecular variance: All estimators support mixed-ploidy populations. Homoplaid and likelihood estimators support homoploids, and the aneuploids estimator supports homoploids and aneuploids. Details can be found in Huang *et al.* (2021).

Population assignment: This function supports aneuploids and mixed-ploidy populations, because this is based on multi-locus genotypic frequencies.

Kinship coefficient: This function supports aneuploids and mixed-ploidy populations, because all three kinship estimators are estimated from the allele frequencies.

Relatedness coefficient: The native polyploid relatedness estimator and estimators and the three kinship-based relatedness estimator support aneuploids and mixed-ploidy populations. For mixed-ploidy populations, the two native polyploids estimators and the three kinship-based relatedness estimators can estimate the relatedness of higher ploidy individuals to lower ploidy individuals r_{HL} . Details can be found in Huang *et al.* (2015b). For aneuploids, the multi-locus

estimate is the weighted average of the single-locus estimates across loci for method-of-moment estimators, or the estimate with the maximum-likelihood for the maximum-likelihood estimator.

Bayesian clustering: This function supports aneuploids and mixed-ploidy populations. For the ADMIXTURE model, the originating cluster of each allele copy within an individual is independently drawn using the allele frequencies in different clusters. For the non-ADMXTURE model, all alleles of an individual are assumed to have originated from the same cluster. The originating cluster of an individual is randomly drawn using the product of the frequencies of all allele copies in this individual. The updates of the other parameters are also modified to accommodate aneuploids and mixed-ploidy population (see the Methodology section in the user manual).

5.17 Optimization

VCFPOP has been optimized for memory usage and calculation speed for large datasets. For the Intel SkyLake processors (used on server or workstation, released on 2017) as well as the CannonLake processors (used on PC and laptop, released on 2018), the AVX-512 instructions can be used to increase calculation speed. These processors are able to handle 512 bits data simultaneously. For Intel processors later than Haswell (released on 2013) and the AMD processors later than Excavator (released on 2015), the AVX2 instructions can be used to increase calculating speed. These processors are able to handle 256 bits data simultaneously. Both instruction sets can be freely switched without additional compilation.

For memory usage, bitwise storage is used to save the individual genotype indices. The memory usage for each genotype for SNPs is reduced from 4 bytes (e.g., `0/0 ') to 2 bits for VCF format (16-folds). Because there are some extra costs for the information related to the locus and individual as well as the population, the typical compression ratio is 14.5-fold (e.g., if the size of Chr22 of 1000 genome data is 10.45 GiB, and VCFPOP use 1.1 Gib memory after loading the file). This method is a trade-off between access speed and memory expense, which can access the genotype data at a high

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rate and only requires several bit manipulations together with the integer arithmetic instructions. Although some advanced compressions can achieve a compression ratio of 50-fold, the random access of genotype data requires a longer decompression time. Therefore, a typical laptop with a 16 GiB memory can load 100 GiB VCF files, and a regular workstation with 256 GiB memory can load 2 TiB VCF files.

Several methods are used in VCFPOP to optimize the calculation speed, which can fully exploit the potential of computers. These methods are listed below.

- Optimized algorithm (to accelerate the calculation);
- Advanced instruction set (e.g., AVX, FMA, AVX512, NEON);
- Reduce program branches (to reduce branch prediction failure);
- Loop unrolling (to increase the utility of ALUs and FPUs);
- Lock-free programming (to reduce access conflicts among threads);
- Virtual memory allocation (to avoid re-allocation and move of memory);
- Local memory management class (to allocate millions of small pieces of memory);
- Variable length array (to place local array on stack memory);
- Fast hash algorithm (to detect identical genotypes);
- Fast hash table (to access genotypes by either hash or index);
- Fast genotype iterator (to read and write the genotypes at a locus);
- Fast logarithm algorithm (to avoid float-point underflow);
- Memory cache (to avoid frequent disk I/O).

The loading speed of VCFPOP is also optimized, and uses a single thread to read the data from the disk and multiple threads to process the data. With a sample benchmark test of a 10.4 GiB uncompressed VCF file, VCFPOP can load data at 320 MiB/s and 560 MiB/s on a laptop (Intel i7-8750H CPU with 2.2GHz and 6 cores, 16 GiB memory, 256 Gib nvme SSD) and a workstation (Intel

Xeon E5-2696 V4 CPU with 2.2GHz and 44 cores, 64 GiB memory and 1 TiB nvme SSD), respectively.

Restricted by the additional decompression process, the loading speed is reduced to 255 and 460 MiB/s on the laptop and the workstation for the compressed format (`vcf.gz`), respectively.

5 Update history

2025/2/1 V1.08

- + Add VanRaden2008 kinship and relatedness estimator.
- + Add LD decay and LD block functions.
- + Add GWAS function.
- * Use CUDA to perform EVD and SVD.
- * Automatically set `-g_rscript`.
- * Set path of parameter file as working directory.

2023/5/9 V1.07

- + Output nucleotide diversity in the diversity output.
- + Add `-g_maxlen`, `-f_chrprefix` and `-f_chrname` options.
- * Statically link `glibc` and `cudart` for linux and mac, so the user do not need to recompile a `nocuda` version.
- * Call `Rscript` in a new thread to save time.
- * Fix a bug causing crash in calculating Tajima's D for GCC.
- * Fix a bug for obtaining executable path.
- * Fix a bug in plotting sliding window.
- * Fix a bug in `-f_pop` and `-slide_pop`.
- * Fix a bug in plotting sliding window.

5 Update history

- * Fix a bug in calculating Tajima's D for sliding windows.

2023/4/20 V1.06

- +Add `-convert_mode` options.
- + Add load from and conversion into PLINK format.
- + Add sliding window function.
- Remove `-f_region` option.
- * Set genetic diversity indices to NaN for locus with number of alleles below 2 (none individuals genotyped or monomorphic) and exclude them calculation of averaged indices.
- * Adjust average Fis from arithmetic average to weighted average.
- * Fix a bug in calculating number of alleles in populations without any individuals genotyped.
- * Fix a bug in calculating Fis in populations without any individuals genotyped.
- * Fix a bug in loading VCF files.
- * Fix a bug when call executable using soft symbolic link.
- * Fix a bug in plotting figures.
- * Fix a bug in haplotype extraction.
- * Fix a bug in loading VCF/BCF formats.
- * Fix a bug in converting SPAGEDI and GENODIVE files.

2022/11/11 V1.05

- * Optimize Bayesian clustering speed.
- * Ensure results consistent for different compilers and platforms.
- * Fix a bug in Bayesian clustering causing crash.
- * Fix a bug in Bayesian clustering cause float point underflow.
- * Fix a bug in AMOVA generates wrong permutation results.
- * Fix a bug in calculating Tajima's D .

- + Add GPU acceleration for Bayesian clustering `-g_gpu`.
- + Add single precision float-point number storage `-g_float`.
- + Add fast single precision float-point number calculation `-g_fastsingle`.
- + Add outputting structure log-likelihood function `-structure_writeln` and corresponding figure plotting function.
- Remove `-structure_decompress` because non-decompression is sufficient fast.

2022/9/6 V1.04

- * Fix a bug in creating haplotype.
- * Fix a bug in calculating hash.
- * Fix a bug in allocating virtual memory.

2022/8/25 V1.03

- * Optimize codes.
- * Optimize SIMD functions.
- * Optimize loading speed, now only read VCF/BCF files once.
- * Optimize memory expense during filtering.
- * Optimize file conversion speed.
- * Optimize calculation speed for Bayesian clustering.
- * Optimize calculation speed for AMOVA.
- * Optimize calculation speed for genetic distance estimation.
- * Optimize calculation speed for relatedness and kinship estimation.
- * Update locus name definition.
- * Fix a bug in creating haplotype.
- * Fix a bug in Bayesian clustering for individuals with no data.
- * Fix a bug in outputting SS in AMOVA.
- * Replace ‘aniso’ with ‘aneu’ in the codes and parameters.

5 Update history

- * Fix minploidy and maxploidy in the diversity output.
- * Change random number generator from XorShift96 to XorShift128+.
- + Add `-g_indtab`, `-amova_trunc`, `-g_locusname`, `-g_eval`, `-f_I`, `-f_windowsize`, `-f_windowstat` options.
- + Add `-structure_decompress` option to optimize Bayesian clustering speed.
- + Support arm64 CPU and NEON SIMD instructions.
- + Add load and conversion for GENODIVE.
- + Add figure plotting function using R script.
- Remove `-g_indfile` option.
- Disable output invalid filters parameters in the result file.
- Disable output `-g_indtest` and `-g_indtab` parameters in the result file.

2022/4/13 V1.02

- * Support C++20 standard.
- * Optimize calculation speed for genetic distance.
- * Use `shared_mutex` class to realize read/write lock.
- * Update linear algebra library to Eigen 3.4.0 and Spectra 1.0.1.

2021/4/12 V1.01

- * Fix a bug in calculating genetic distance using multiple threads.
- + Replace genetic distance with average value for pairs with missing data.

2021/3/31 V1.0

- * Reduce memory expense for locus and genotype
- * Optimize sequential genotype index accession speed
- * Reduce temporary file use
- + Add collapse allele function in testing genotype distribution

- + Add variable length arrays to optimize speed
- + Add `-haplotype_ptype` option
- Remove `-g_buffer` and `-g_memory` option and reduce memory during loading

2020/9/22 V0.9

- * Fix a bug in calculating hash values
- * Optimize AMOVA speed
- * Optimize memory expense
- * Optimize loading and calculation speed

2020/5/21 V0.8

- * Optimize AMOVA code
- + Add multi-level region definition
- + Modify source code to support LLVM/Clang
- + Add Huang (2021) Fst estimators

2019/12/8 V0.7

- * Optimize calculating speed
- * Optimize memory expense
- * Fix bugs
- * Optimize Bayesian clustering code
- * Optimize calculating speed
- + Add support for AVX-512 instructions

2018/12/8 V0.6

- + Add support for BCF format and .vcf.gz compression
- + Add support for other genotype formats

5 Update history

+ Add file format converter for other genotype formats

2018/9/6 V0.5

* Optimize loading speed

+ Add haplotype extraction function

+ Add population differentiation estimator

+ Add PCoA function

+ Add hierarchical clustering function

2018/6/19 V0.4

+ Add support for different ploidy level

+ Add Bayesian clustering function

2018/4/9 V0.3

+ Add relatedness coefficient estimators

+ Add AMOVA function

2018/2/19 V0.2

+ Add genotype distribution test

+ Add population differentiation estimator

+ Add genetic differentiation tests

+ Add individual H-index estimator

+ Add population assignment

+ Add genetic distance estimators

2018/2/6 V0.1

+ Loading from VCF files

- + Add population genetic diversity estimators
- + Add double-reduction model: RCS, PRCS, CES, PES
- + Add file format converter for GENEPOP and STRUCTURE

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