

# **SIMHYB 2: a software tool to explore and illustrate evolutionary forces in Population Genetics teaching and research**

Álvaro Soto de Viana<sup>1</sup>

David Rodríguez Martínez

Unai López de Heredia Larrea<sup>1</sup>

<sup>1</sup>GIE “Arboreto de Montes”

Dpto. Sistemas y Recursos Naturales.

ETSI Montes, Forestal y del Medio Natural

Universidad Politécnica de Madrid.

## **USER MANUAL**

<http://github.com/GGFHF/SimHyb2>

July, 2023

## **CONTENTS**

**Overview**

**Getting started**

**Simulation process**

**Input files and parameters**

Neutral loci allelic frequencies

Self-incompatibility loci

Specific classes and fitness coefficient

Fertility table

Fitness inheritance

Ageing

**Output files**

**References**

**How to cite this program**

## OVERVIEW

SIMHYB 2 is a Java-based software for the simulation of mixed hybridizing populations. The program is intended for the analysis of the effect of the different demographic, adaptive and reproductive factors on the evolution of these populations. Census size of each species, immigrants, number of intermediate specific classes, directional fertility among them and fitness coefficient of each class can be defined by the user. Inheritance of fitness and ageing effect are also taken into account. The software generates individuals of known pedigree, allowing their traceability throughout the generations. SIMHYB 2 yields for each simulated generation an output file easily convertible to inputs for GENALEX (Peakall & Smouse, 2012) or STRUCTURE (Pritchard *et al.*, 2000), some of the most popular softwares for population genetics and Bayesian analysis.

Initially, SIMHYB 2 was intended for hermaphrodite long-living plants, such as trees (so it considers features such as the presence of chloroplast, overlapping generations or the possibility of self-incompatibility processes), but it can be also applied for other organisms, taking into account certain considerations.

## GETTING STARTED

SIMHYB 2 is programmed in JAVA 16, and runs in any computer with an OS that allows for Java (<https://www.java.com/>), or OpenJDK (<http://openjdk.java.net/>): Linux/Unix, Microsoft Windows, Mac OS X, and other platforms, including cloud virtual desktops that operate in academic institutions to facilitate e-learning (Moser *et al.*, 2014). The application provides a user-friendly front-end for ease of use by students. To run SIMHYB 2 simply download and decompress the `simhyb2.rar` file. It includes the executable `simhyb2.jar`, the file `simhyb.properties` and an empty folder named `logs`, as well as the user manual and input examples. Be sure you have JAVA 16 (or a later version) installed in your OS. The `logs` folder, the file `simhyb.properties` and the executable `simhyb2.jar` must be located in the same path in your computer. No further installation is required, just execute `simhyb2.jar` and the simulation interface will open.

SIMHYB 2 can also be executed from the command line: move to the folder where `simhyb2.jar`, `simhyb.properties`, and the `logs` folder are located and just execute the command `java -jar "simhyb2.jar"`

If your computer has a Linux based OS of your computer, we recommend modifying the path of the input files by editing the following lines in the `simhyb.properties` file:

```
specificCategoryFile=/YOURPATH/spcat_fitness.txt  
alleleFrequenciesFile=/YOURPATH/allelefrequencies.csv  
immigrantAlleleFrequenciesFile=/YOURPATH/allelefrequenciesContinent.csv  
selfIncompatibilityLocusFile=/YOURPATH/selfincompatibility.csv  
fertilityTableFile=/YOURPATH/fertility.txt
```

Every time you launch a simulation, the file `simhyb.properties` will be overwritten with the parameters and instructions of the current simulation. It is therefore recommended to save this file together with the results for your own records. You don't have to worry about the content of the first copy of the file, included in `simhyb.rar` since it will be overwritten, but the executable needs to have this file and the `logs` folder to begin the simulations.

It is also recommended to place all the needed input files in the same directory where the executable is located.

## **SIMULATION PROCESS**

SIMHYB 2 simulates the evolution of a population of constant size with up to two different diploid genetic groups (we will refer to them as "species", for simplicity), which may hybridize, depending on the conditions defined by the user. The population consists of a fixed number of individuals, each of them identified by a vector including pedigree, a so-called "specific coefficient" (representing the expected contribution of each species to the individual genome), the complete genotype for a user-defined number of loci and other information.

### Initial population

The user can define the initial population size for each species, considering only adult individuals. The size of the global population will remain constant throughout the simulation. Genotypes of the individuals of the initial population are generated drafting alleles according to their frequencies in each species, from the input files described above. A chloroplast and a mitochondria are assigned to each species, and recorded for each individual. “Null” values are recorded as the parents of the initial population individuals. Individual fitness values are assigned randomly from the continuous interval  $(f_{sp} - \varepsilon_{sp}, f_{sp} + \varepsilon_{sp})$ , being  $f_{sp}$  the average value for the specific category and  $\varepsilon_{sp}$  a *within category variability* defined by the user.

### Reproductive events

Each cycle or generation includes five phases: 1) reproduction, 2) migration, 3) ageing, 4) selection, and 5) standardization. For **1) reproduction**, no spatial restriction is considered in the current version of SIMHYB 2, i.e., pollen from any individual (if applied to trees) can reach the flowers of any other tree with the same probability. The spatial positions of the individuals are not reckoned in SIMHYB 2 and, accordingly, there is no isolation by distance. The probability of obtaining a viable cross is firstly determined by the fertility coefficients defined in the input file `fertility.txt`, according to the specific classes of the mother tree and the pollen donor. After that, effective pollination is limited by self-incompatibility, if this option is selected. SIMHYB 2 considers gametophytic self-incompatibility driven by a single locus. This way, at pollination, one of the alleles at this locus from the pollen donor is randomly selected and compared with both alleles of the mother tree. After passing these barriers, the new individual will carry at each locus one of the alleles of each parent, randomly selected, and the chloroplast and mitochondria of the corresponding parent, as specified by the user. The species coefficient of the new individual will be the average of the parents’ values, so it becomes a precise estimation of the contribution of each

genetic pool (pure species) to the genome of the individual. A new fitness coefficient will be assigned to the new individual according to the following formula:

$$f = w_h f_h + w_{sp} f_{sp}$$

$$f_h = \frac{f_{mother} + f_{father}}{2} + \delta$$

where  $f_{sp}$  is the fitness coefficient corresponding to the specific class of the new individual,  $f_h$  is the fitness inherited from the parent trees;  $\delta$  is drafted from the interval  $(-\varepsilon_h, \varepsilon_h)$ , being  $\varepsilon_h$  the *within sibling variability* parameter, to include variability among full-sibs, and  $w_h$  and  $w_{sp}$  are weights, established by the user, so that  $w_h + w_{sp} = 1$ .

At this point, **2) immigration** can take place, according to a user-defined probability (between 0 and 1). The user also defines the number of individuals in the immigrant pool (they can be different number for each species). The current version of SIMHYB 2 only considers the *continent-island* migration model. Thus, the evolving population would be the *island*, which can incorporate immigrants from a *continent* whose allelic frequencies do not vary during the simulation. Immigrants are created in the same way that the initial population, but a .csv input file with the allelic frequencies for the *continent* population, with the same loci and alleles (it can be the same file as for the *island*).

**3) Ageing** affects each individual fitness every cycle, according to this formula:

$$f_t = f_{t-1} (1 - a)b + f_0 (1 - a)^t (1 - b)$$

where  $f_t$  is the fitness coefficient  $t$  cycles after birth,  $f_0$  is the initial fitness, at the time of birth,  $a$  is the ageing coefficient, and  $b$  is the linearity coefficient, which varies between 0 and 1.

**4) Selection** takes place at this moment: the  $N$  (population size, defined by the user) individuals with the highest fitness coefficients are selected and remain in the reproductive population, while the other individuals die. Finally, **5) standardization** is performed, so that fitness coefficients vary between 0 and 1 (this way, if high  $b$  and  $w_h$  have been selected, certain individuals can keep very high fitness values along the generations).

End of simulation

Three alternative criteria can be followed to finish the simulation: (1) a user-defined number of cycles; (2) the presence of individuals of a user-defined number of specific categories in the simulation; and (3) fixation of a chloroplast type in the population.





## INPUT FILES AND PARAMETERS

When `simhyb2.jar` is executed, and before starting the simulation, an interface window appears where the user must define the simulation parameters and input files (Figure 2).

The screenshot shows the SimHyb2 interface window with the following sections and fields:

- Population size**
  - Species 1: [text box]
  - Species 2: [text box]
- Allele frequencies**
  - File: [text box]
  - Number of loci: [text box]
- Reproductive events**
  - Type: [dropdown menu showing 'Matings']
  - Number per cycle: [text box]
- Fertility table**
  - [text box]
- Self-incompatibility**
  - ☐
- Self-incompatibility locus file**
  - [text box]
- Plastidial inheritance**
  - [dropdown menu showing 'maternal']
- Mitochondrial inheritance**
  - [dropdown menu showing 'maternal']
- Immigrant population**
  - Probability of immigration per cycle: [text box]
  - Number of immigrants per cycle: [text box]
  - Species 1: [text box]
  - Species 2: [text box]
  - Allele frequencies of immigrant population: [text box]
- Fitness inheritance**
  - Specific category fitness table: [text box]
  - Within category variability: [text box] Sp. category weight: [text box]
  - Within sibling variability: [text box] Sibling weight: [text box]
  - Ageing coefficient: [text box] Ageing lineality: [text box]
- Output**
  - Output directory: [text box] Snapshot frequency: [text box]
- End of simulation**
  - Criterion: [dropdown menu showing '# reproductive cycles'] Number: [text box]

At the bottom right, there are two buttons: **Run** and **Cancel**.

**Figure 2.** The SIMHYB 2 interface. When the simulation is launched, after selecting the input files and parameters, a pop-up window announces the start of the simulation.

## Population size

The user can define the population size for each species, considering only adult individuals. The size of the global population will be fixed throughout the simulation.

## Neutral loci

The user must also provide an `allelefrequencies.csv` file with the loci and the allele frequencies of each species. The format is exemplified in Figure 3. The interface allows considering just a subsample of the loci in the simulations, using the same input file.

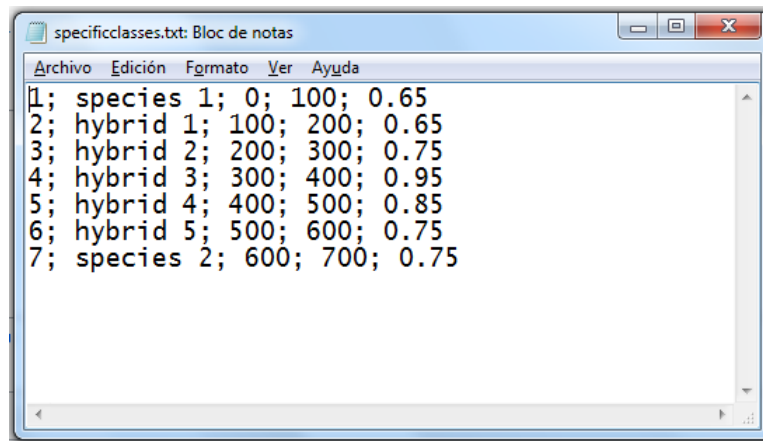
|    | A   | B       | C   | D       | E   | F       | G   | H       | I   | J       | K   | L       | M |
|----|-----|---------|-----|---------|-----|---------|-----|---------|-----|---------|-----|---------|---|
| 1  | 197 | 0.04348 | 209 | 0       | 206 | 0       | 197 | 0.02055 | 209 | 0.00685 | 206 | 0.02055 |   |
| 2  | 199 | 0.02174 | 219 | 0.85507 | 210 | 0       | 199 | 0       | 219 | 0.86986 | 210 | 0.0137  |   |
| 3  | 201 | 0.63768 | 223 | 0.14493 | 212 | 0.39855 | 201 | 0.67808 | 223 | 0.12329 | 212 | 0.62329 |   |
| 4  | 211 | 0.04348 | 0   | 0       | 214 | 0.2971  | 211 | 0       | 0   | 0       | 214 | 0.21918 |   |
| 5  | 213 | 0.05797 | 0   | 0       | 216 | 0.00725 | 213 | 0       | 0   | 0       | 216 | 0.00685 |   |
| 6  | 215 | 0.11594 | 0   | 0       | 218 | 0.02899 | 215 | 0.30137 | 0   | 0       | 218 | 0       |   |
| 7  | 221 | 0.07971 | 0   | 0       | 220 | 0.10145 | 221 | 0       | 0   | 0       | 220 | 0.0137  |   |
| 8  | 0   | 0       | 0   | 0       | 222 | 0.02899 | 0   | 0       | 0   | 0       | 222 | 0.0137  |   |
| 9  | 0   | 0       | 0   | 0       | 224 | 0.13768 | 0   | 0       | 0   | 0       | 224 | 0.03425 |   |
| 10 | 0   | 0       | 0   | 0       | 226 | 0       | 0   | 0       | 0   | 0       | 226 | 0.05479 |   |
| 11 |     |         |     |         |     |         |     |         |     |         |     |         |   |
| 12 |     |         |     |         |     |         |     |         |     |         |     |         |   |
| 13 |     |         |     |         |     |         |     |         |     |         |     |         |   |
| 14 |     |         |     |         |     |         |     |         |     |         |     |         |   |
| 15 |     |         |     |         |     |         |     |         |     |         |     |         |   |
| 16 |     |         |     |         |     |         |     |         |     |         |     |         |   |
| 17 |     |         |     |         |     |         |     |         |     |         |     |         |   |
| 18 |     |         |     |         |     |         |     |         |     |         |     |         |   |
| 19 |     |         |     |         |     |         |     |         |     |         |     |         |   |

**Figure 3.** An example of the `allelefrequencies.csv` file, opened in Windows Excel. Each loci (just three, in this example) is represented by two columns. The first one includes the allele names and the second one its frequencies in the species. All the loci for the first species are presented in consecutive columns and then, in the same order, they appear for the other species. Please notice the columns do not have headings.

## Specific classes

The user can define as many intermediate specific classes as desired, intermediate between the two pure species included in the original population, and can define the limits of each class, according to the so called “species coefficient” in a `spsat_fitness.txt` file. In this file, each row corresponds to a specific class, defined by a number, a name for the class, its boundaries

in terms of the species coefficient, and the fitness coefficient corresponding to the specific class ( $f_{sp}$ ). The route must be specified in the line “Specific category fitness table”, in the interface. See an example in Figure 4.



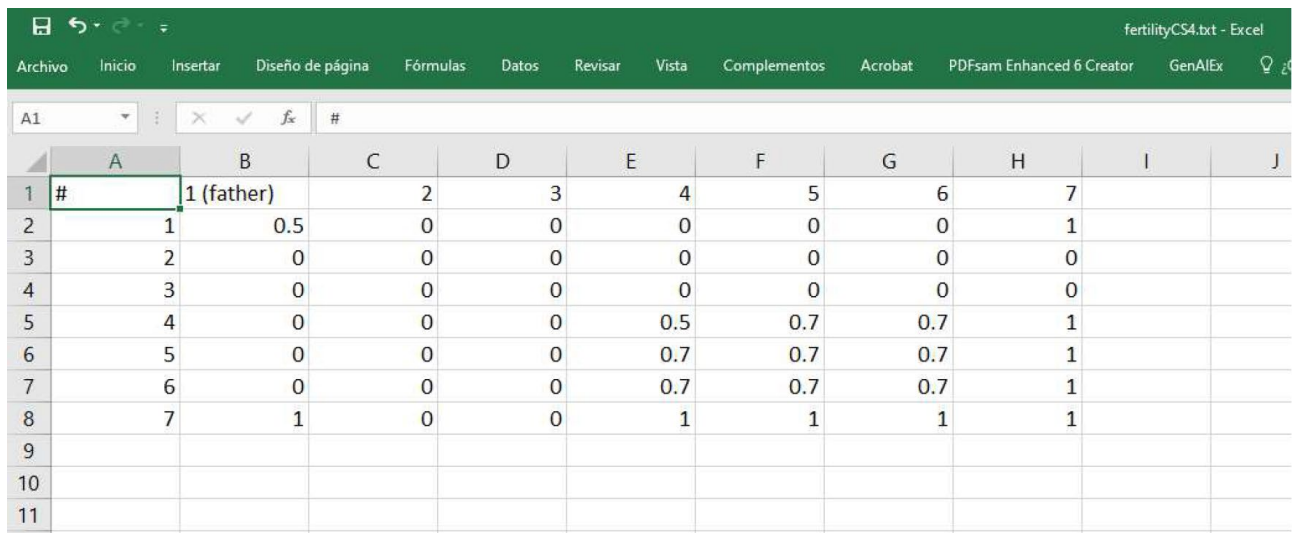
**Figure 4.** An example of the `spcat_fitness.txt` file, opened here in Windows Notepad. In each row, the first figure is the identification of the specific category, followed by its description; the next two numbers correspond to the category boundaries, according to the specific coefficient; the last number is the fitness coefficient associated to the specific category. In this example, seven categories are defined, two corresponding to the parental species (1 and 7) and with 5 intermediate, hybrid categories.

### Fertility

Fertility within and between specific classes takes into account the pollination direction. Figure 5 shows an example of the `fertility.txt` file. Each row corresponds to a specific class, acting as mother tree, while each column corresponds to a specific class acting as pollen donor.

### Organelles inheritance

Chloroplast and mitochondria can be inherited, independently, from the mother or from the father, according to user specifications.



|    | A | B          | C | D | E   | F   | G   | H | I | J |
|----|---|------------|---|---|-----|-----|-----|---|---|---|
| 1  | # | 1 (father) | 2 | 3 | 4   | 5   | 6   | 7 |   |   |
| 2  | 1 | 0.5        | 0 | 0 | 0   | 0   | 0   | 1 |   |   |
| 3  | 2 | 0          | 0 | 0 | 0   | 0   | 0   | 0 |   |   |
| 4  | 3 | 0          | 0 | 0 | 0   | 0   | 0   | 0 |   |   |
| 5  | 4 | 0          | 0 | 0 | 0.5 | 0.7 | 0.7 | 1 |   |   |
| 6  | 5 | 0          | 0 | 0 | 0.7 | 0.7 | 0.7 | 1 |   |   |
| 7  | 6 | 0          | 0 | 0 | 0.7 | 0.7 | 0.7 | 1 |   |   |
| 8  | 7 | 1          | 0 | 0 | 1   | 1   | 1   | 1 |   |   |
| 9  |   |            |   |   |     |     |     |   |   |   |
| 10 |   |            |   |   |     |     |     |   |   |   |
| 11 |   |            |   |   |     |     |     |   |   |   |

**Figure 5.** An example of the fertility.txt file, opened here in Windows Excel. This example corresponds to 7 specific classes (two parental species, 1 and 7, and 5 intermediate, hybrid categories). In columns, pollen donors, and in rows, mother trees. Please notice asymmetric fertility is allowed.

### Self-incompatibility

The user can select the option of considering self-incompatibility in the simulations. The current version of SIMHYB 2 only takes into account gametophytic self-incompatibility. Figure 6 shows an example of the `selfincompatibility.csv` file.

### End of simulation

The user can select to finish the simulation when one of the chloroplasts is completely replaced by the other one, when a specified number of specific categories remain in the population or after a desired number of cycles. When the simulation finishes, a pop-up window appears.

|    | A       | B         | C         | D | E | F |
|----|---------|-----------|-----------|---|---|---|
| 1  | #Allele | Freq Sp 1 | Freq Sp 2 |   |   |   |
| 2  | SI1     | 0.15      | 0         |   |   |   |
| 3  | SI2     | 0.17      | 0         |   |   |   |
| 4  | SI3     | 0.08      | 0         |   |   |   |
| 5  | SI4     | 0.01      | 0         |   |   |   |
| 6  | SI5     | 0.14      | 0         |   |   |   |
| 7  | SI6     | 0.06      | 0         |   |   |   |
| 8  | SI7     | 0.03      | 0.09      |   |   |   |
| 9  | SI8     | 0.06      | 0.04      |   |   |   |
| 10 | SI9     | 0.05      | 0.1       |   |   |   |
| 11 | SI10    | 0.02      | 0.13      |   |   |   |
| 12 | SI11    | 0.04      | 0.08      |   |   |   |
| 13 | SI12    | 0.12      | 0.02      |   |   |   |
| 14 | SI13    | 0.07      | 0.06      |   |   |   |
| 15 | SI14    | 0         | 0.08      |   |   |   |
| 16 | SI15    | 0         | 0.1       |   |   |   |
| 17 | SI16    | 0         | 0.05      |   |   |   |
| 18 | SI17    | 0         | 0.08      |   |   |   |
| 19 | SI18    | 0         | 0.09      |   |   |   |
| 20 | SI19    | 0         | 0.04      |   |   |   |
| 21 | SI20    | 0         | 0.04      |   |   |   |
| 22 |         |           |           |   |   |   |
| 23 |         |           |           |   |   |   |
| 24 |         |           |           |   |   |   |
| 25 |         |           |           |   |   |   |
| 26 |         |           |           |   |   |   |

**Figure 6.** An example of the selfincompatibility.csv file

## OUTPUT FILES

SIMHYB 2 provides two output files. The first one includes all the individuals in the population every X cycles, as a sort of “snapshot album” of the evolving population. The user can define the frequency of these “snapshots” and the output can be consulted while the simulation is still running. The second output file is available only when the simulation is finished, and includes all the individuals of the population in each cycle.

Output files are provided as .csv files. The first row register the headings of the first 10 columns, which include different information of each individual (see below). The second row includes the name of the nuclear, diploid loci, starting with the SI locus and followed by the neutral loci. Third

and successive rows include the virtual individuals. The first 10 positions include the following information: (1) Individual ID (integer); (2) Specific coefficient (numeric character); (3) Father individual ID (“null” value for individuals in the first generation and for immigrants); (4) Mother individual ID (“null” value for individuals in the first generation and for immigrants); (5) Chloroplast (A or B); (6) Mitochondria (A or B); (7) Generation (integer; cycle of the simulation); (8) Birth Generation (integer; cycle in which the individual is added to the population); (9) Death Generation (integer; cycle in which the individual is removed from population; “-1” for survivors); and (10) individual fitness value in that generation (number, between 0 and 1). The next positions register the diploid genotype of the individual, starting with the self-incompatibility locus (alphanumeric characters, two alleles) and neutral loci (alphanumeric characters, two alleles per locus) (Figure 7).

Therefore, this output file is easily convertible into the input file of different and widely used population genetics software tools. For instance, just modification of the heading rows and removing some of the first columns would be needed to get an input file for GENALEX (Peakall & Smouse, 2012) or NEWHYBRIDS (Anderson & Thompson, 2002). In a similar way, removing the first row is enough to get an input file for STRUCTURE (Pritchard *et al.*, 2000), where columns 3-10 or 3-12 can be labelled as “extra columns”. Of course, non-desired rows (according to the analysis to be performed), such as repeated individuals, present in the population for several cycles (where the only variation would be registered in the relative fitness value), must also be removed accordingly.

|     | A         | B         | C       | D       | E        | F        | G        | H         | I        | J       | K    | L    | M   | N   | O   | P   |
|-----|-----------|-----------|---------|---------|----------|----------|----------|-----------|----------|---------|------|------|-----|-----|-----|-----|
| 1   | Id        | Sp. Coef. | Father  | Mother  | Chloropl | Mitochor | Generati | Birth Ger | Death Ge | Fitness |      |      |     |     |     |     |
| 2   | Self-inco | locus 1   | locus 2 | locus 3 | locus 4  | locus 5  | locus 6  | locus 7   | locus 8  | locus 9 |      |      |     |     |     |     |
| 306 | 272       | 700       | 132     | 185     | B        | B        | 3        | 2         | 4        | 0.0678  | SI12 | SI2  | 197 | 197 | 203 | 207 |
| 307 | 262       | 525       | 137     | 128     | B        | B        | 3        | 2         | 4        | 0.07    | SI3  | SI1  | 197 | 215 | 207 | 211 |
| 308 | 269       | 700       | 167     | 128     | B        | B        | 3        | 2         | 4        | 0.1177  | SI2  | SI13 | 197 | 197 | 207 | 205 |
| 309 | 286       | 350       | 165     | 162     | B        | B        | 3        | 2         | 4        | 0.1413  | SI6  | SI10 | 197 | 197 | 219 | 219 |
| 310 | 297       | 525       | 172     | 167     | B        | B        | 3        | 2         | 4        | 0.1476  | SI9  | SI12 | 197 | 201 | 211 | 219 |
| 311 | 247       | 700       | 134     | 176     | B        | B        | 3        | 2         | 4        | 0.1838  | SI7  | SI13 | 197 | 197 | 203 | 207 |
| 312 | 210       | 700       | 170     | 139     | B        | B        | 3        | 2         | 4        | 0.1853  | SI3  | SI12 | 197 | 197 | 205 | 207 |
| 313 | 242       | 700       | 169     | 128     | B        | B        | 3        | 2         | 4        | 0.2024  | SI2  | SI13 | 197 | 197 | 205 | 205 |
| 314 | 338       | 350       | 159     | 144     | B        | B        | 3        | 3         | 4        | 0.2029  | SI5  | SI3  | 215 | 211 | 211 | 203 |
| 315 | 248       | 700       | 170     | 104     | B        | B        | 3        | 2         | 4        | 0.2031  | SI2  | SI3  | 197 | 197 | 203 | 207 |
| 316 | 305       | 525       | 107     | 168     | B        | B        | 3        | 3         | 4        | 0.2065  | SI2  | SI12 | 197 | 213 | 209 | 219 |
| 317 | 395       | 350       | 173     | 144     | B        | B        | 3        | 3         | 4        | 0.2279  | SI7  | SI3  | 215 | 201 | 219 | 219 |
| 318 | 399       | 525       | 173     | 287     | B        | B        | 3        | 3         | 4        | 0.2286  | SI5  | SI1  | 197 | 197 | 215 | 207 |
| 319 | 352       | 437       | 150     | 233     | B        | B        | 3        | 3         | 4        | 0.24    | SI8  | SI9  | 197 | 197 | 219 | 219 |
| 320 | 396       | 525       | 173     | 243     | B        | B        | 3        | 3         | 4        | 0.2415  | SI7  | SI12 | 197 | 197 | 203 | 207 |
| 321 | 288       | 350       | 192     | 193     | B        | B        | 3        | 2         | 4        | 0.2496  | SI11 | SI5  | 197 | 197 | 219 | 219 |
| 322 | 315       | 437       | 236     | 129     | B        | B        | 3        | 3         | 4        | 0.2791  | SI1  | SI12 | 215 | 197 | 203 | 207 |
| 323 | 118       | 700       | 93      | 56      | B        | B        | 3        | 1         | 4        | 0.2825  | SI2  | SI5  | 197 | 197 | 191 | 211 |
| 324 | 386       | 612       | 262     | 147     | B        | B        | 3        | 3         | 4        | 0.2871  | SI3  | SI1  | 197 | 197 | 207 | 207 |
| 325 | 356       | 525       | 179     | 243     | B        | B        | 3        | 3         | 4        | 0.2883  | SI2  | SI1  | 197 | 197 | 211 | 219 |
| 326 | 187       | 700       | 58      | 71      | B        | B        | 3        | 1         | 4        | 0.2891  | SI11 | SI2  | 197 | 197 | 203 | 211 |
| 327 | 202       | 525       | 173     | 199     | B        | B        | 3        | 2         | 4        | 0.2892  | SI7  | SI3  | 197 | 197 | 207 | 219 |
| 328 | 320       | 612       | 236     | 199     | B        | B        | 3        | 3         | 4        | 0.2935  | SI5  | SI3  | 197 | 201 | 201 | 207 |
| 329 | 254       | 525       | 123     | 159     | B        | B        | 3        | 2         | 4        | 0.3003  | SI2  | SI5  | 211 | 197 | 219 | 199 |

**Figure 7.** An example of SIMHYB 2 output file.

## REFERENCES

- Anderson EC, Thompson EA (2002). A model-based method for identifying species hybrids using multilocus genetic data. *Genetics* 160: 1217-1229. DOI: 10.1093/genetics/160.3.1217
- Peakall R, Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research. *Bioinformatics* 28: 2537-2539. DOI: 10.1093/bioinformatics/bts460
- Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. *Genetics* 155(2): 945-959. DOI: 10.1093/genetics/155.2.945

## HOW TO CITE THIS PROGRAM

Soto A, Rodríguez-Martínez D, López de Heredia U (2023) SIMHYB 2: a software tool to explore and illustrate evolutionary forces in Population Genetics teaching and research