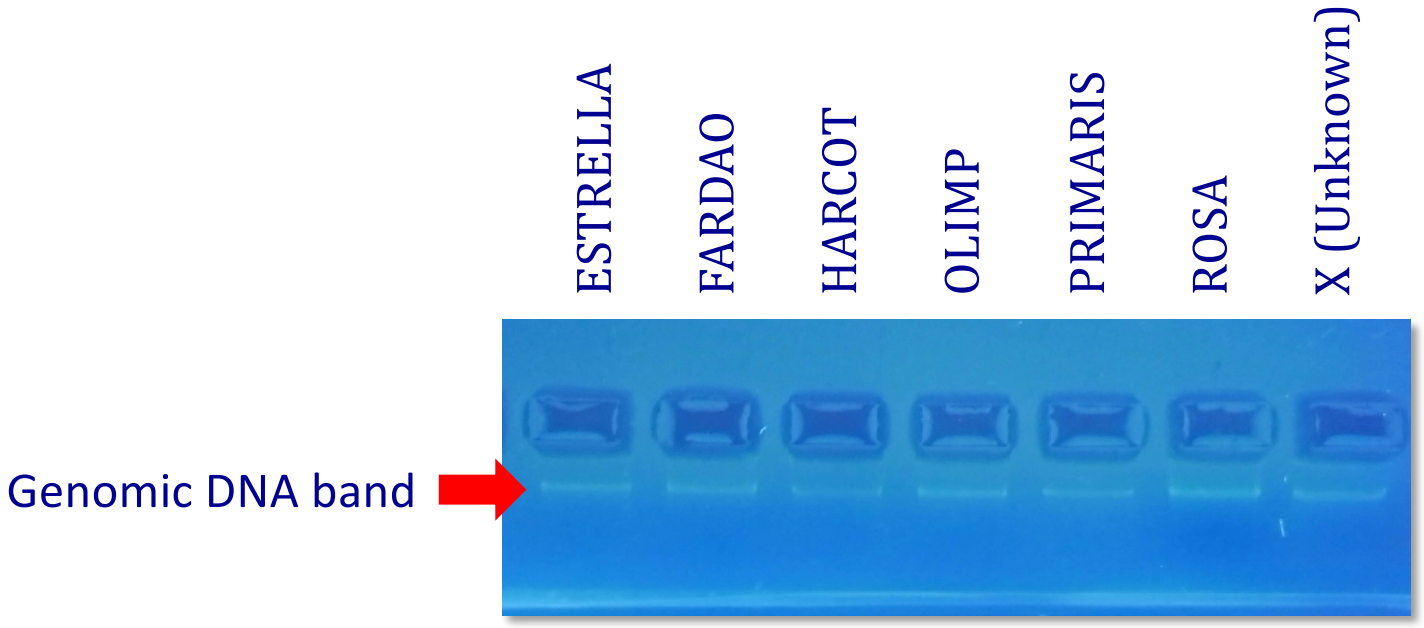
First lab activity
(DNA extraction from apricot leaves and agarose gel)

WE 26-05-2021

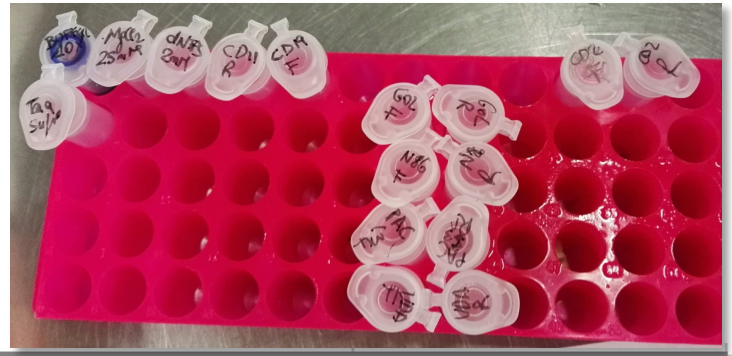


Second lab activity
(PCR amplification of six microsatellite loci)

WE 26-05-2021

SAMPLES

ESTRELLA
FARDAO
HARCOT
OLIMP
PRIMARIS
ROSA
X (Unknown)



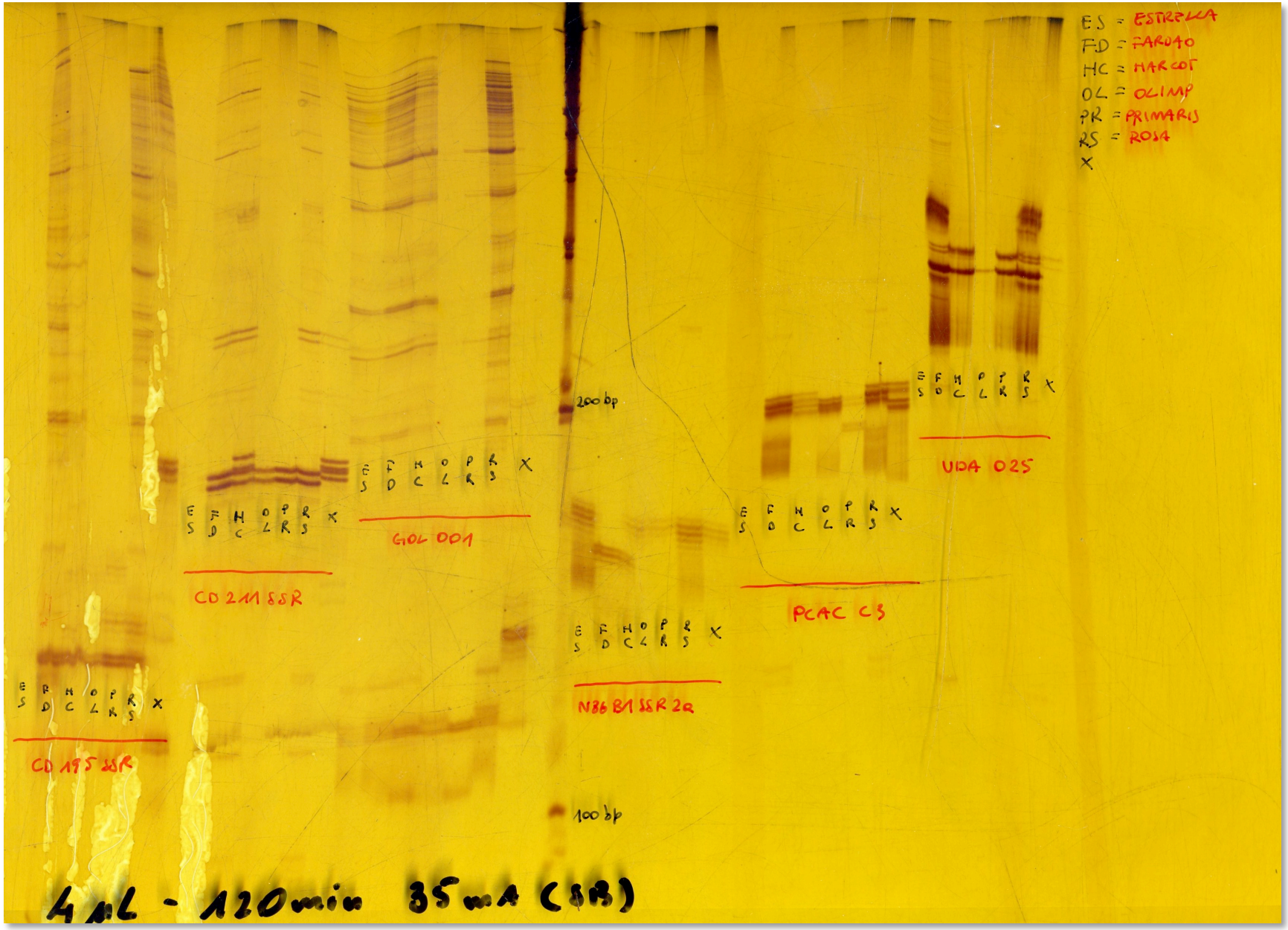
SSR name	LG	T°C ann	T°C PCR	Allele size LxS	PAGE run HxR	PAGE buffer HxR	Provider	Primer Forward	Primer Reverse
CD195SSR	L1	57	50	190	150 min 30 mA	SB	Sicard et al.	CCTCTGATGTATTATCTTTCTGGC	TTTCAACAGCTCCAAATTCAC
CD211SSR	L1	57	50	141	40+140 min 30mA	SB	Sicard et al.	GAAGATCATGTTAGAGAATAGTGG	CAGGGGTGACTTGGAAACC
Gol001	L1		55	200	150 min 30 mA	SB	Badenes	TGCAATCGATGAAGATTTTGAC	TGGTCACCTTCCTTCATATCC
N86B11SSR2a	L1	57	50	271	140 min 58 W	TBE	Decroocq (Marandel et al.)	GCCAATTTAACGCCCGAAT	CTGGTTCCTATACCTGCATCA
PacC3	L1	56	56		120 min 30 mA	SB	Decroocq et al.	TGACTTGATCAGACTCGACA	TTGCATTTGCATTTACAATAGA
UDA-025	L1	56	55		30+120 min 30 mA	SB	Testolin et al.	TCGAGAAAGCTGCACTGGTA	AAAGCTGCTTATTCGTGTGTG

Apricot PCR	Stock	Units	Final concentration	ul for each sample	ul for nr. of samples
DNA	10	ng/uL	10	1	
10X buffer A	10	X	1	2	
MgCl2	25	mM	2,5	2	
dNTPs	2	mM	0,1	1	
Primer for	5	uM	0,25	1	
Primer Rev	5	uM	0,25	1	
Taq polymerase	5	U/uL	0,025	0,1	
H2O	-	-	-	11,9	

Program	Time	Temp	Cycles
1	05:00	94°	1x
2	00:30	94°	
2	01:30	57°	35x
2	00:30	72°	
4	05:00	72°	1x
5	∞	10°	/

Third lab activity
(polyacrylamide gel electrophoresis of PCR products)

WE 26-05-2021



Fourth lab activity

WE 26-05-2021

(some questions that could be addressed by this experiment)

Calculate the genetic distance matrix and the *Heterozygosity* (He) for the six SSR loci

Can you draw any hypothesis on the possible parental relationships between individuals?

Does the “X” individual show any similarity with the known accessions?

What is the degree of genetic similarity between the six accessions?

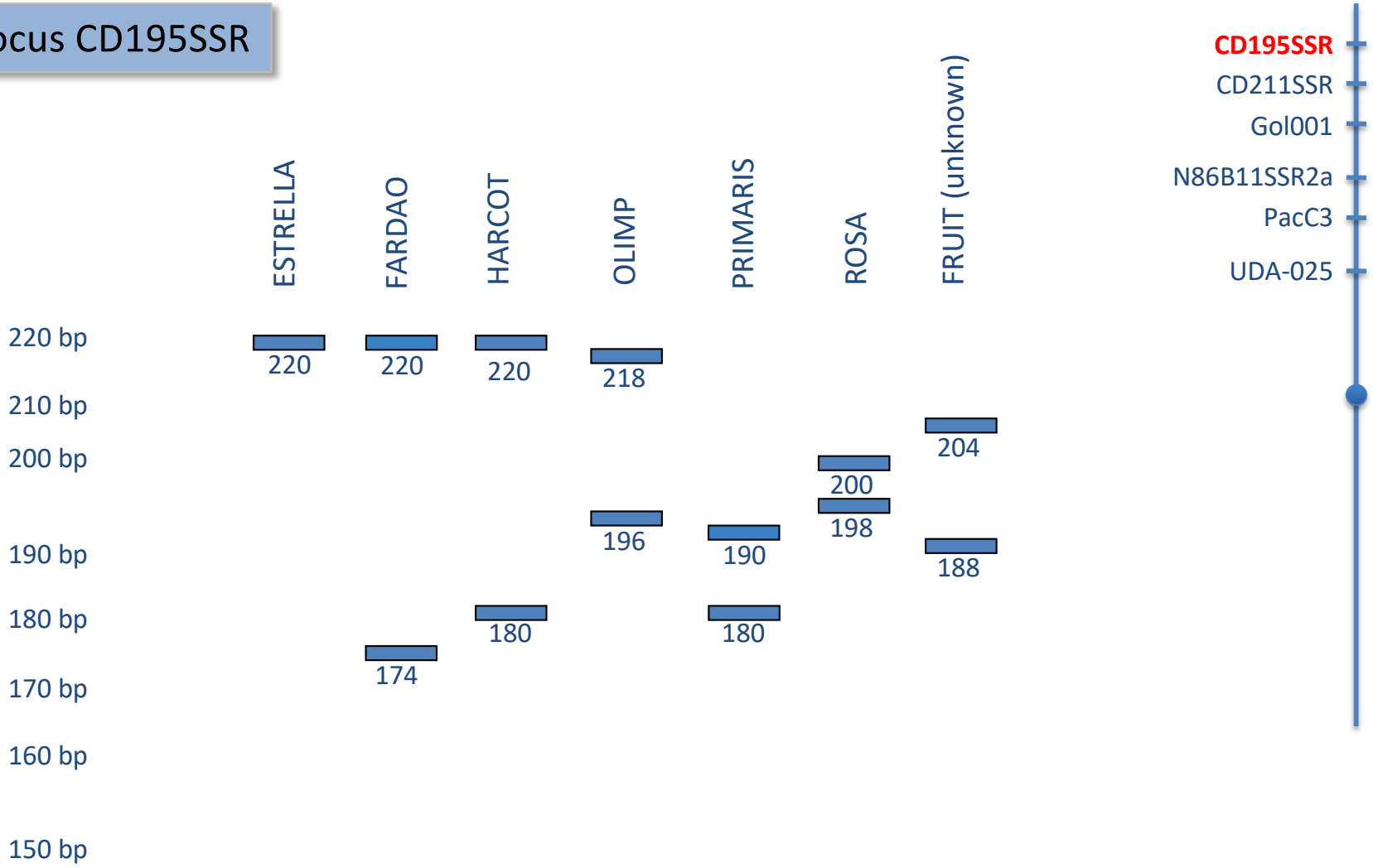
What can you tell about the possible resistance to PPV virus for these accessions?

Which accession(s) would you employ in a marker-assisted breeding (MAS) program targeting Sharka resistance?

Fourth lab activity
(data collection and analysis)

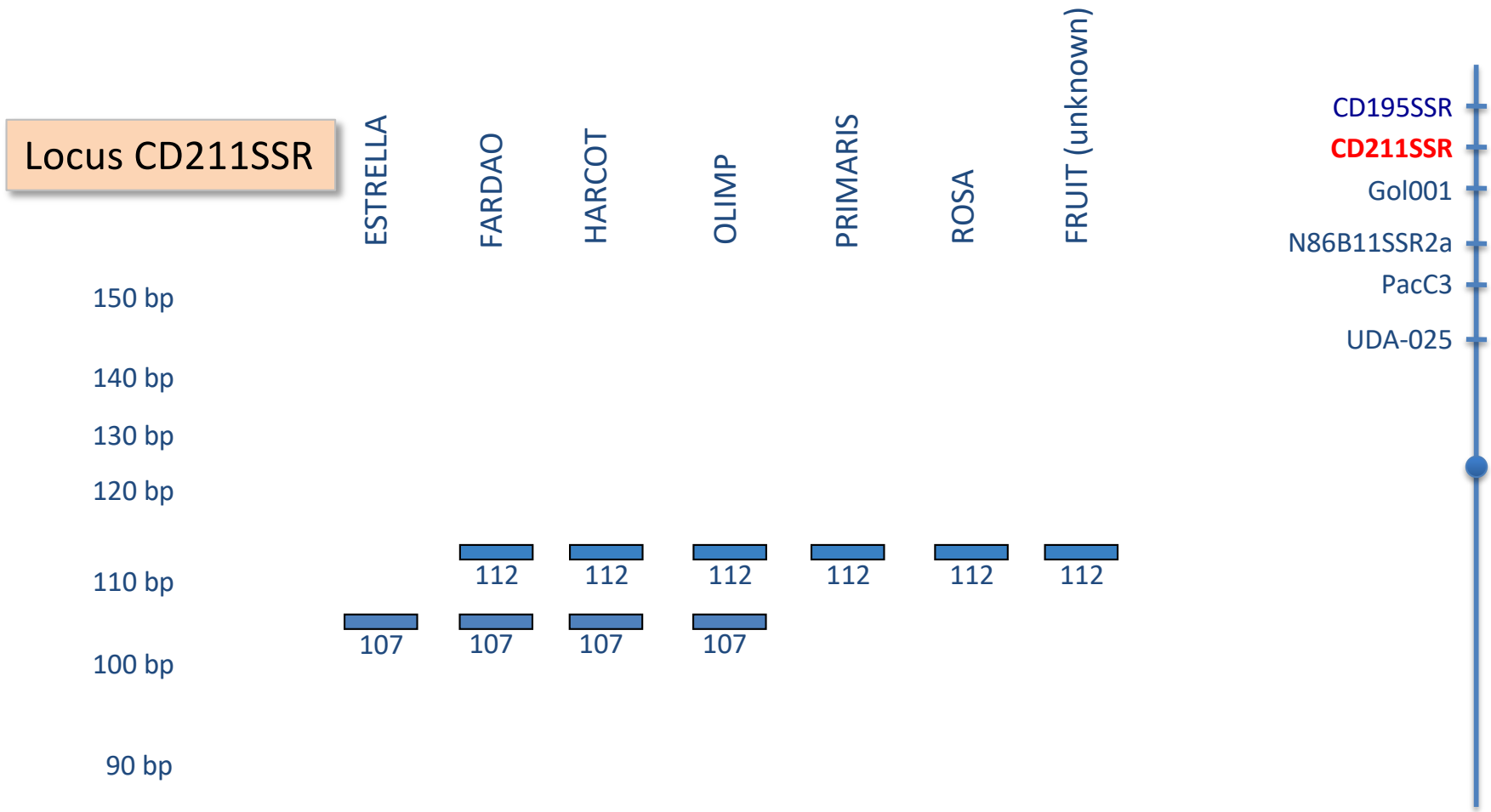
WE 26-05-2021

Locus CD195SSR



Fourth lab activity
(data collection and analysis)

WE 26-05-2021



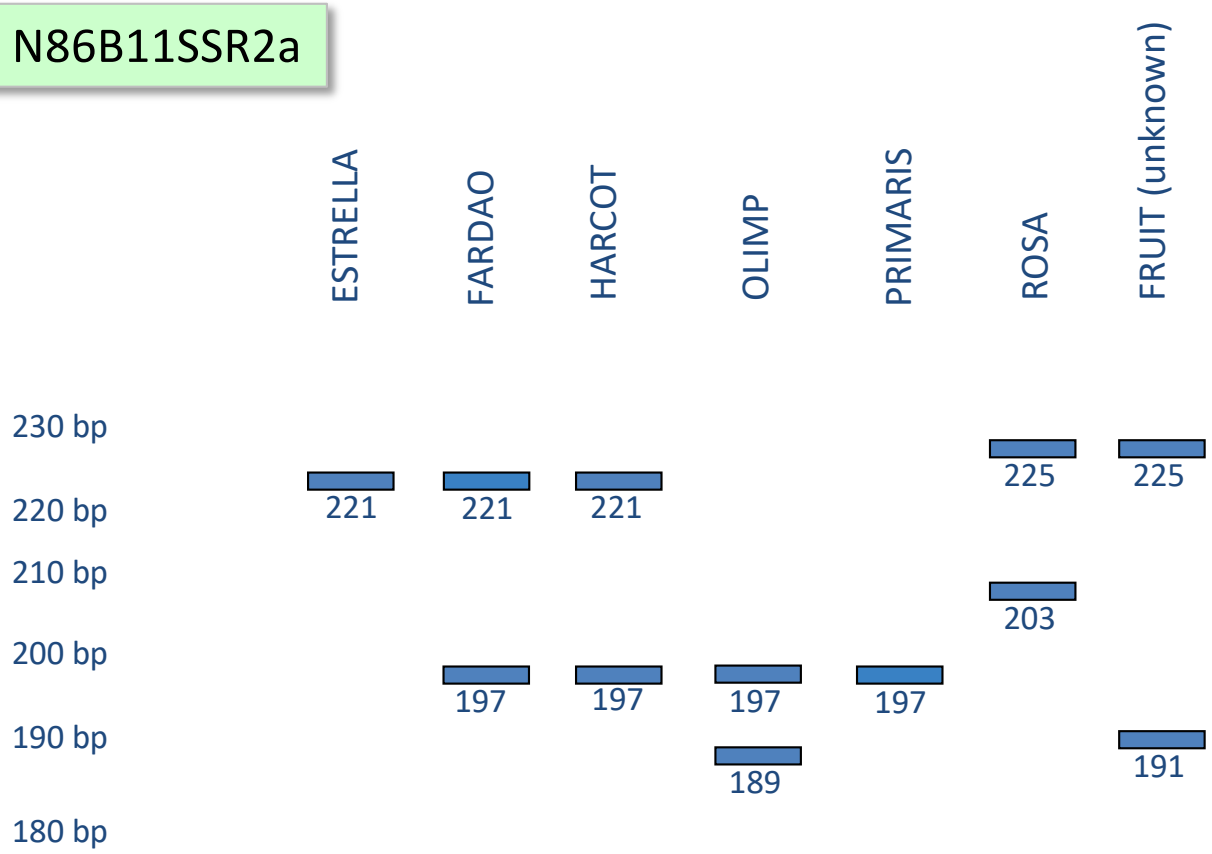
Fourth lab activity
(data collection and analysis)

WE 26-05-2021

Fourth lab activity
(data collection and analysis)

WE 26-05-2021

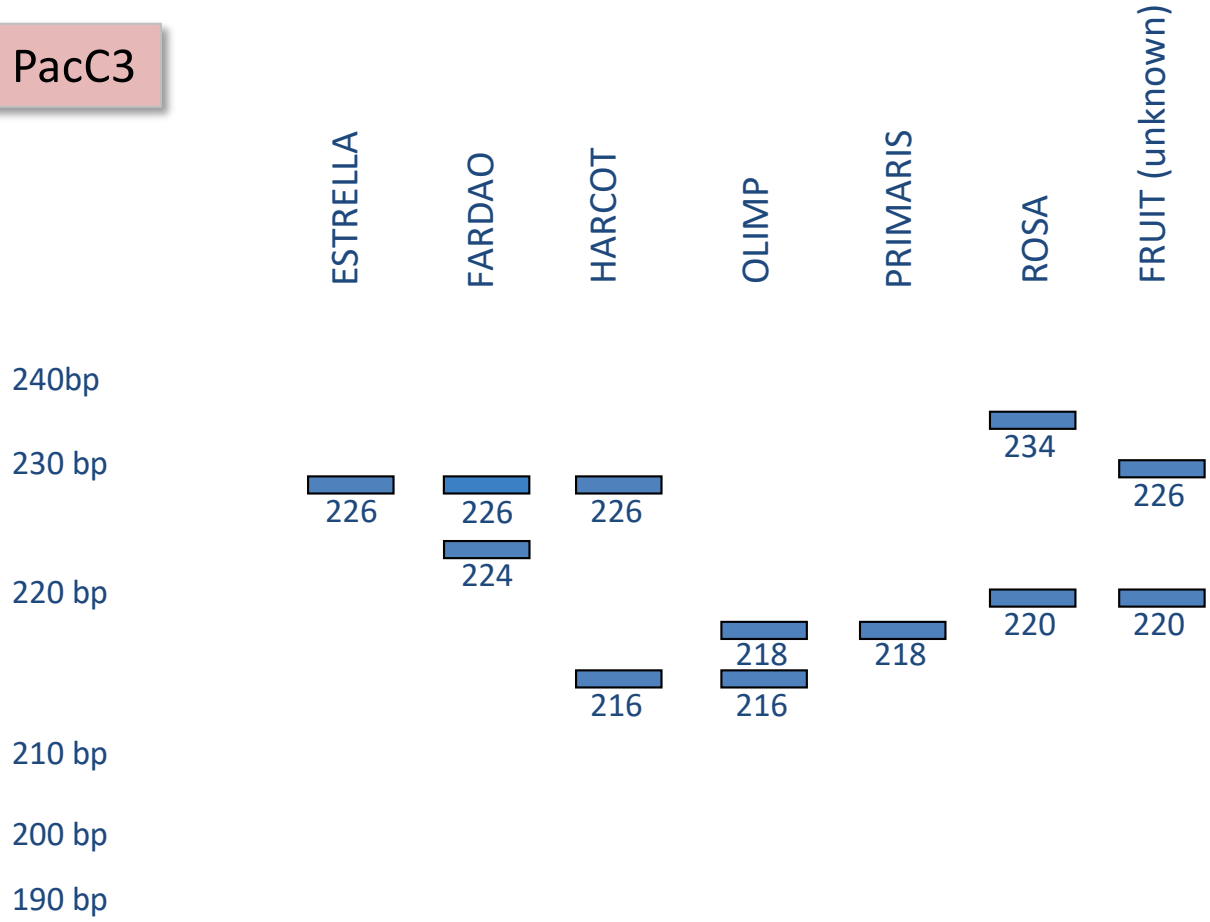
Locus N86B11SSR2a



Fourth lab activity
(data collection and analysis)

WE 26-05-2021

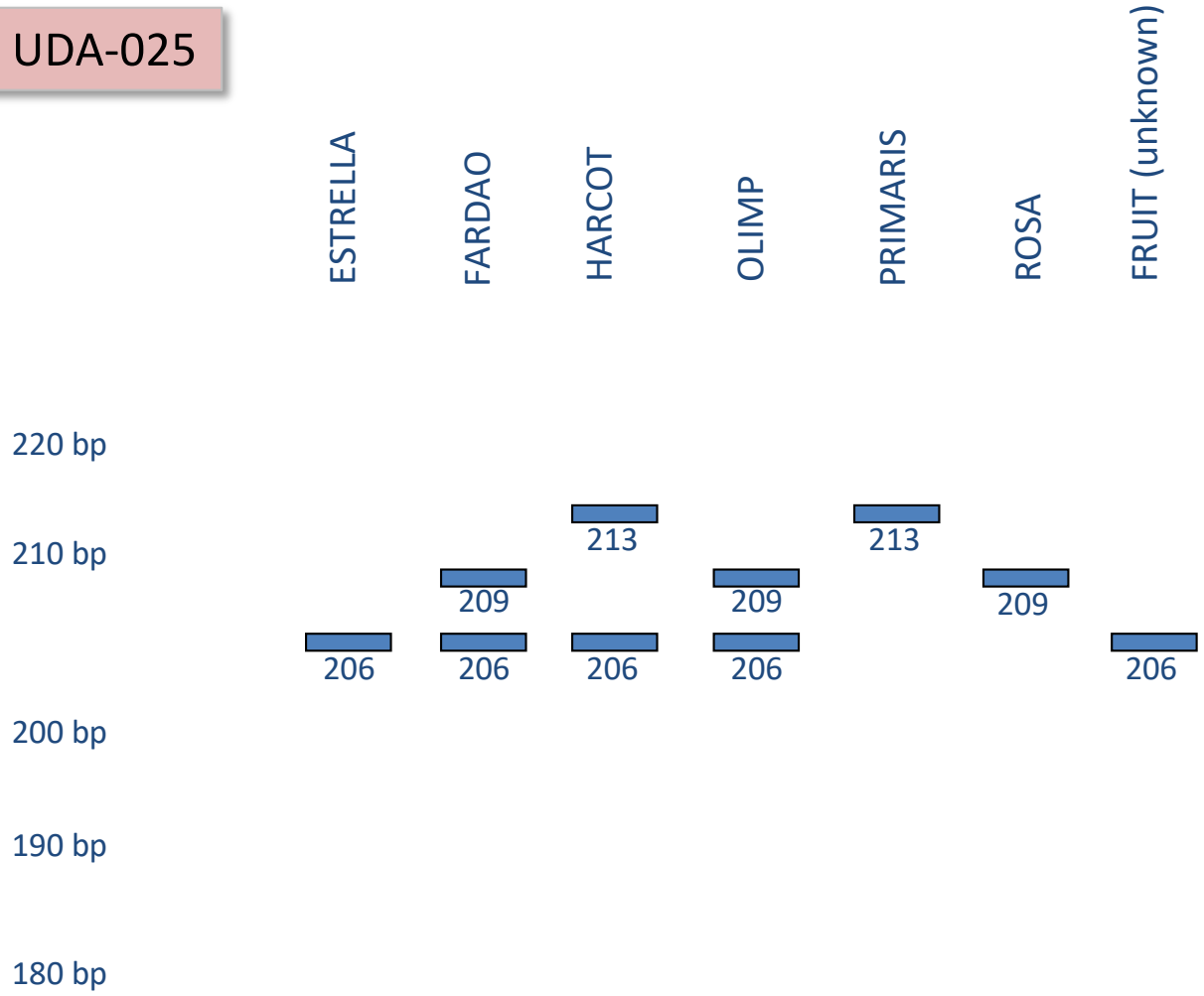
Locus PacC3



Fourth lab activity
(data collection and analysis)

WE 26-05-2021

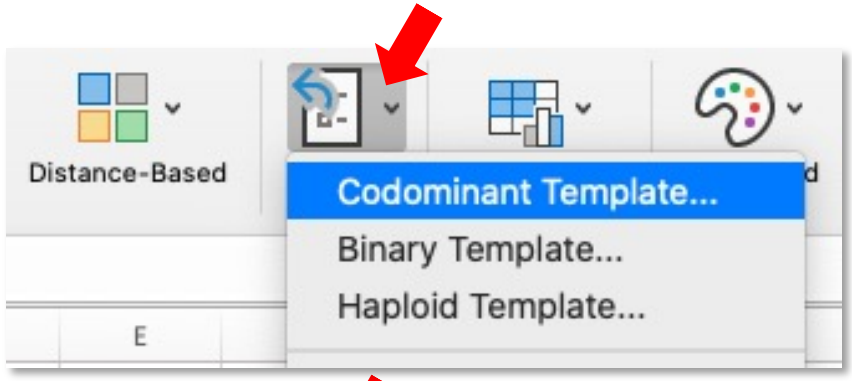
Locus UDA-025

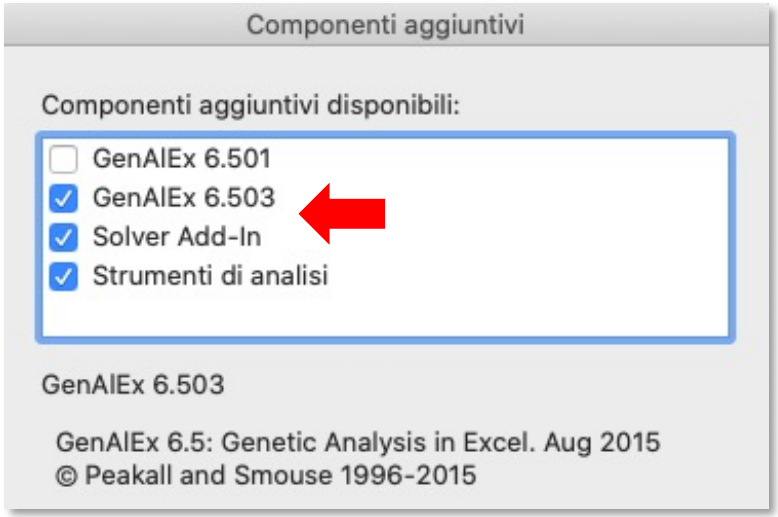


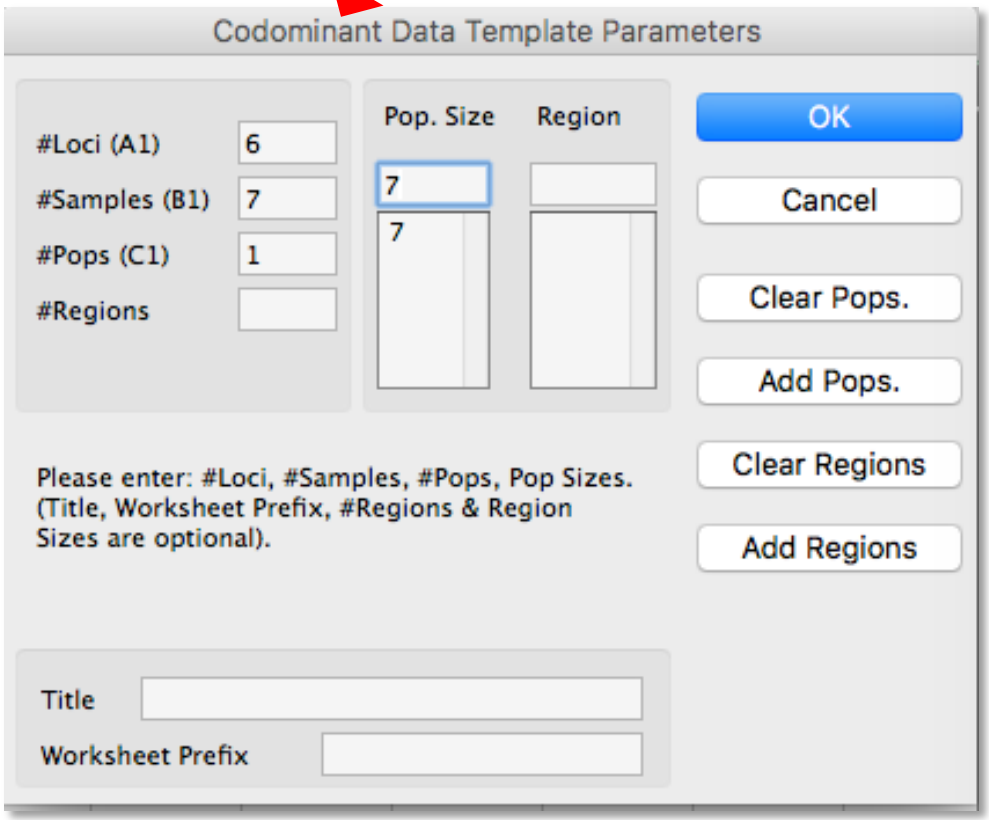
Fourth lab activity
(the GenAlEx software: preparing data)

WE 20-12-2023

GenAlEx
Genetic Analysis in Excel
6.5
©2006 to 2012





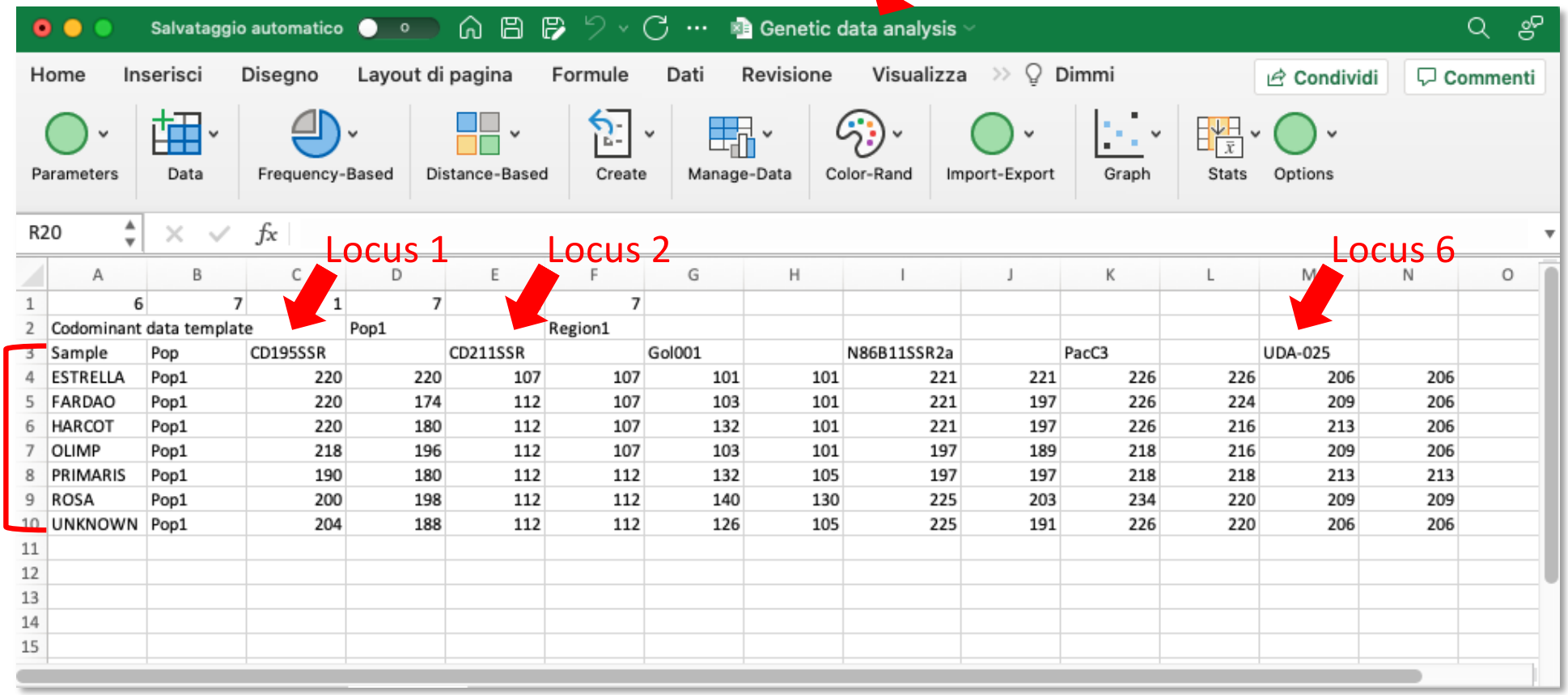


Fourth lab activity

WE 20-12-2023

(some questions that could be addressed by this experiment)

Working file

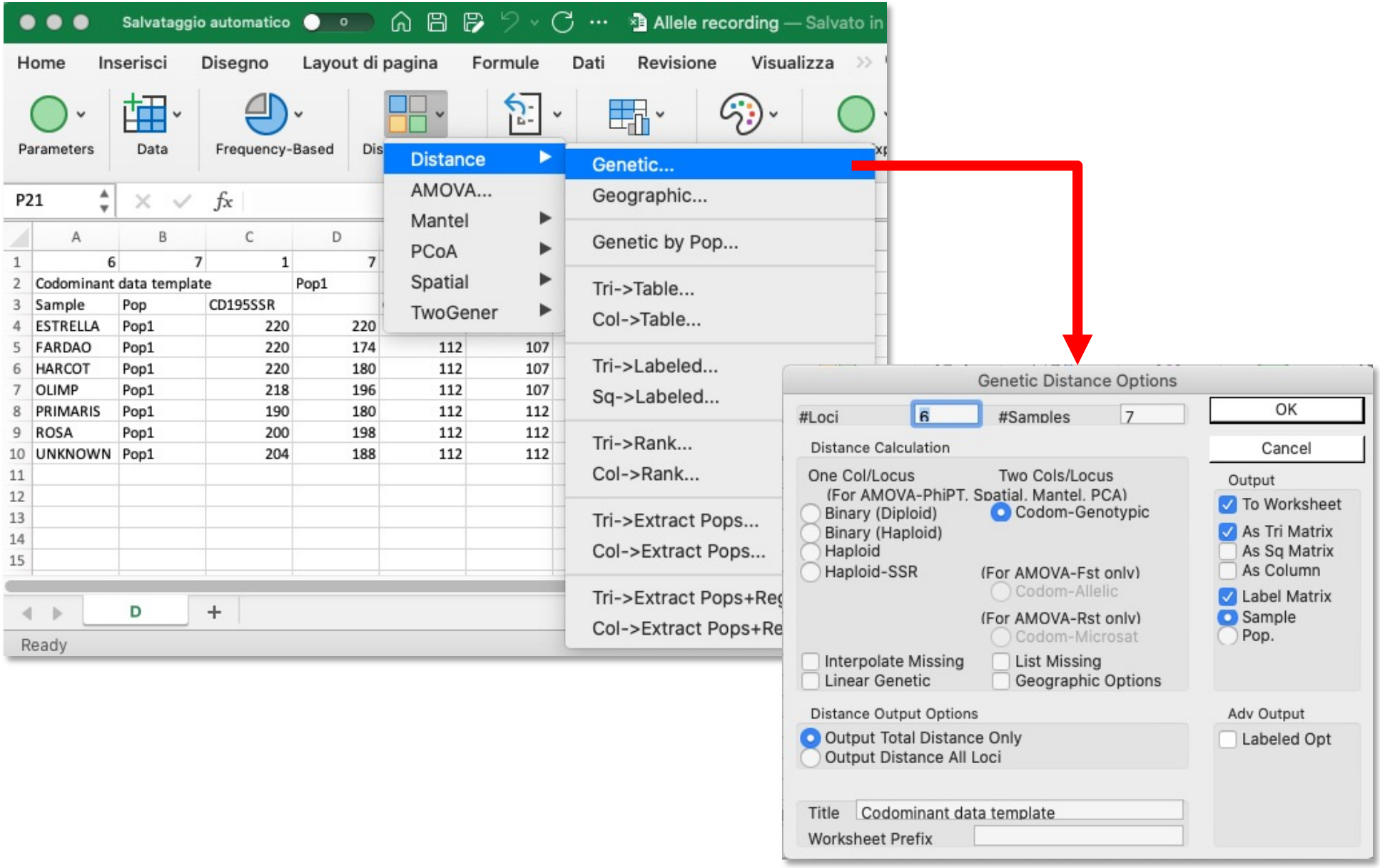


	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	6	7	1	7		7									
2	Codominant data template			Pop1		Region1									
3	Sample	Pop	CD195SSR		CD211SSR		Gol001		N86B11SSR2a		PacC3		UDA-025		
4	ESTRELLA	Pop1	220	220	107	107	101	101	221	221	226	226	206	206	
5	FARDAO	Pop1	220	174	112	107	103	101	221	197	226	224	209	206	
6	HARCOT	Pop1	220	180	112	107	132	101	221	197	226	216	213	206	
7	OLIMP	Pop1	218	196	112	107	103	101	197	189	218	216	209	206	
8	PRIMARIS	Pop1	190	180	112	112	132	105	197	197	218	218	213	213	
9	ROSA	Pop1	200	198	112	112	140	130	225	203	234	220	209	209	
10	UNKNOWN	Pop1	204	188	112	112	126	105	225	191	226	220	206	206	
11															
12															
13															
14															
15															

Fourth lab activity

WE 20-12-2023

(some questions that could be addressed by this experiment)



Fourth lab activity (GenAlEx Tutorials)

WE 20-12-2023

Welcome Download Tutorials Export Citation Authors

GenAlEx Tutorials

An Overview of Topics

We are pleased to offer a series of self-paced tutorials on population genetic analysis that employ hand calculations and exercises within GenAlEx. These are drawn in part from the graduate workshops that we have offered (jointly and independently), around the world. Click the links below to download any tutorials of interest. In 2012, Tutorials 1-6 were revised to bring them up to date with the new features of GenAlEx 6.5. The newer Trouble Shooting Tutorial is strongly recommended for all users. It provides helpful tips for solving some of the issues that may prevent some data sets from running.

Tutorial 1 (zip 6.9 mb)

An Introduction to Frequency-Based Population Genetic Analysis: scoring genetic markers, Allele Frequency, Heterozygosity, F-statistics, Nei Genetic Distance, Shannon Diversity Indices and Chi-square tests for Hardy-Weinberg Equilibrium

Tutorial 2 (zip 1.3 mb)

Genetic Distance and AMOVA: Haploid, Codominant and Binary Genetic Distance, AMOVA and F-statistics

Tutorial 3 (zip 4.5 mb)

Spatial Genetic Analysis: Principal Coordinate Analysis (PCoA), Mantel Tests for Matrix Correspondence and Spatial Autocorrelation Analysis

Fourth lab activity
(formatting the total distance matrix)

WE 20-12-2023

	A	B	C	D	E	F	G	H	I	J
1	1	7	1	7	1	7				
2	Codominant	D	Codominant	Pop1						
3	ESTRELLA	FARDAO	HARCOT	OLIMP	PRIMARIS	ROSA	UNKNOWN			
4	0							ESTRELLA		
5	6	0						FARDAO		
6	6	4	0					HARCOT		
7	12	5	6	0				OLIMP		
8	22	12	8	10	0			PRIMARIS		
9	20	10	12	10	14	0		ROSA		
10	14	9	9	10	13	10	0	UNKNOWN		
11										
12										
13										
14										
15										

GD D +

	A	B	C	D	E	F	G	H	I	J
1	ESTRELLA	0								
2	FARDAO	6	0							
3	HARCOT	6	4	0						
4	OLIMP	12	5	6	0					
5	PRIMARIS	22	12	8	10	0				
6	ROSA	20	10	12	10	14	0			
7	UNKNOWN	14	9	9	10	13	10	0		
8										
9										
10										
11										
12										

GD D +

Fourth lab activity
(formatting the total distance matrix)

WE 20-12-2023

	A	B	C	D	E	F	G	H	I	J
1	ESTRELLA	0								
2	FARDAO	6	0							
3	HARCOT	6	4	0						
4	OLIMP	12	5	6	0					
5	PRIMARIS	22	12	8	10	0				
6	ROSA	20	10	12	10	14	0			
7	UNKNOWN	14	9	9	10	13	10	0		
8										
9										
10										
11										
12										

GD D +

Add "7"

7-6.txt

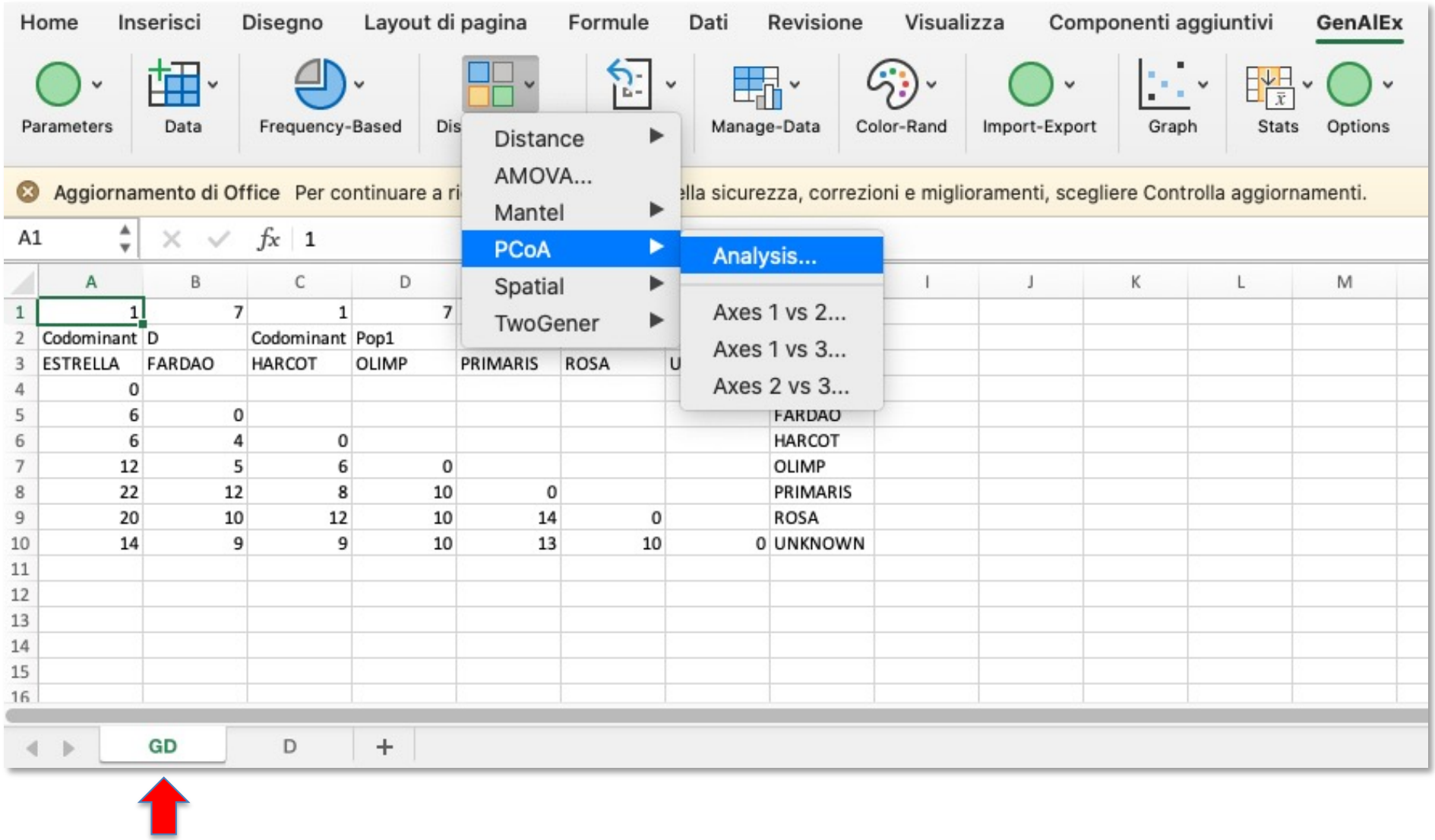
~/Documents/DIDATTICA/LAUREA SPECIALISTICA/Es.../Elaborazione dati esercitazione 2023 (Albicocco)/7-6....

```
1  7¬
2  ESTRELLA··0ΔΔ      Δ   Δ   Δ   Δ   ¿¬
3  FARDAO····6Δ0Δ     Δ   Δ   Δ   Δ   ¿¬
4  HARCOT····6Δ4Δ     0Δ  Δ   Δ   Δ   ¿¬
5  OLIMP·····12Δ      5Δ  6Δ  0Δ  Δ   Δ   ¿¬
6  PRIMARIS··22Δ      12Δ 8Δ  10Δ 0Δ  Δ   ¿¬
7  ROSA·····20Δ       10Δ 12Δ 10Δ 14Δ 0Δ  ¿¬
8  UNKNOWN···14Δ      9Δ  9Δ  10Δ 13Δ 10Δ 0¬
9
```

Ten characters

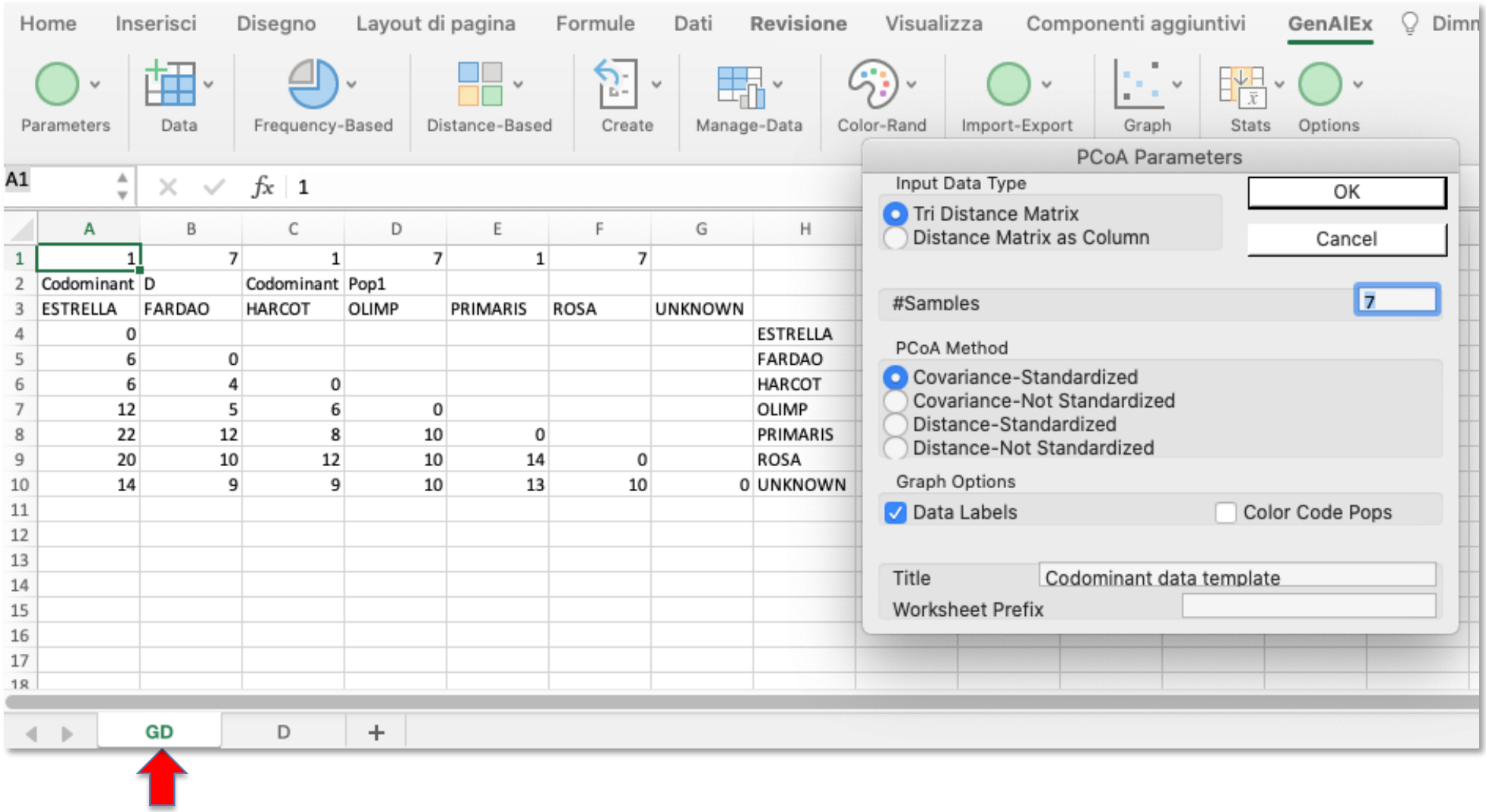
Fourth lab activity
(the GenAlEx software)
Principal Component Analysis

WE 20-12-2023



Fourth lab activity
(the GenAlEx software)
Principal Component Analysis

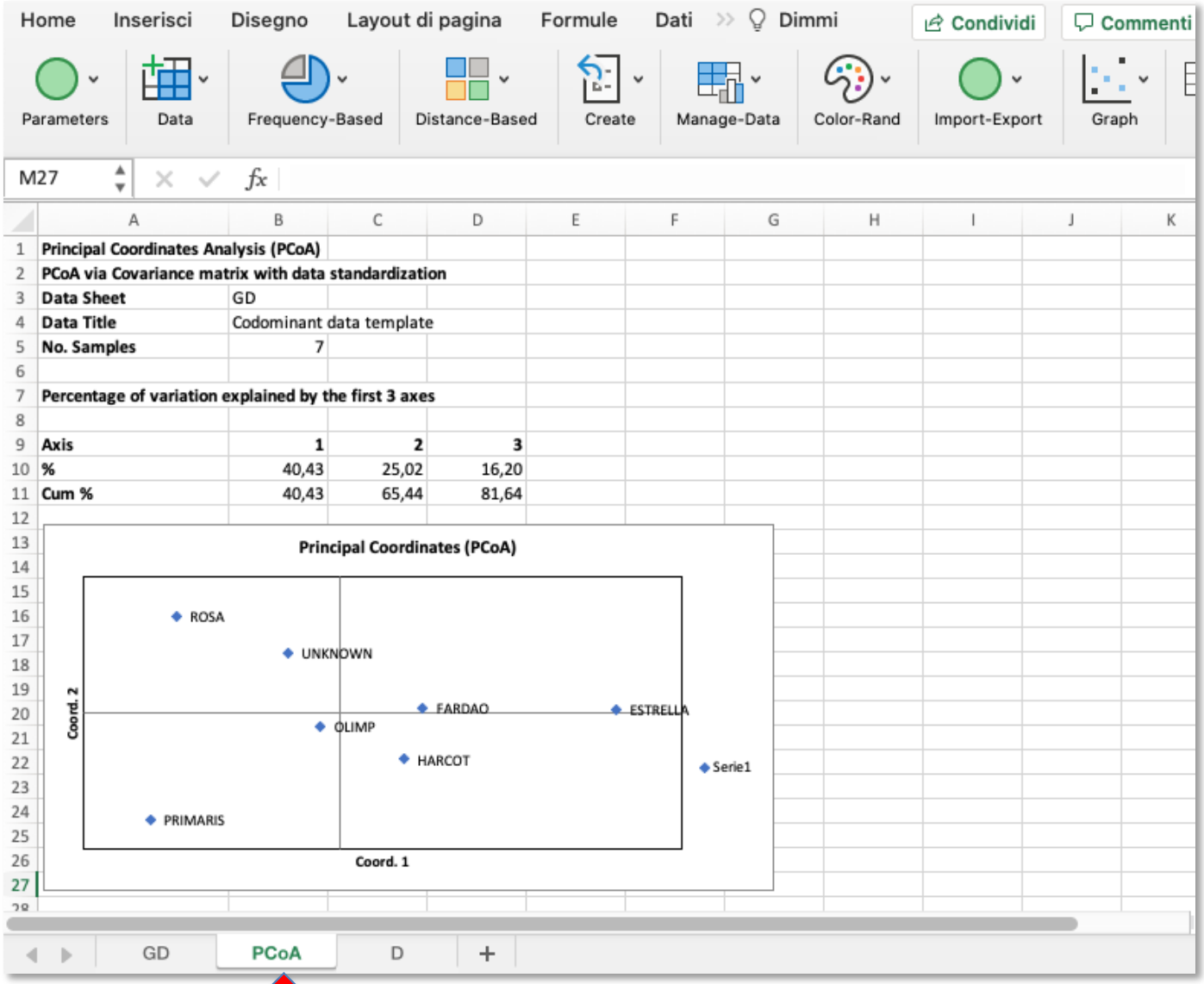
WE 20-12-2023



	A	B	C	D	E	F	G	H
1	1	7	1	7	1	7		
2	Codominant	D	Codominant	Pop1				
3	ESTRELLA	FARDAO	HARCOT	OLIMP	PRIMARIS	ROSA	UNKNOWN	
4	0							ESTRELLA
5	6	0						FARDAO
6	6	4	0					HARCOT
7	12	5	6	0				OLIMP
8	22	12	8	10	0			PRIMARIS
9	20	10	12	10	14	0		ROSA
10	14	9	9	10	13	10	0	UNKNOWN

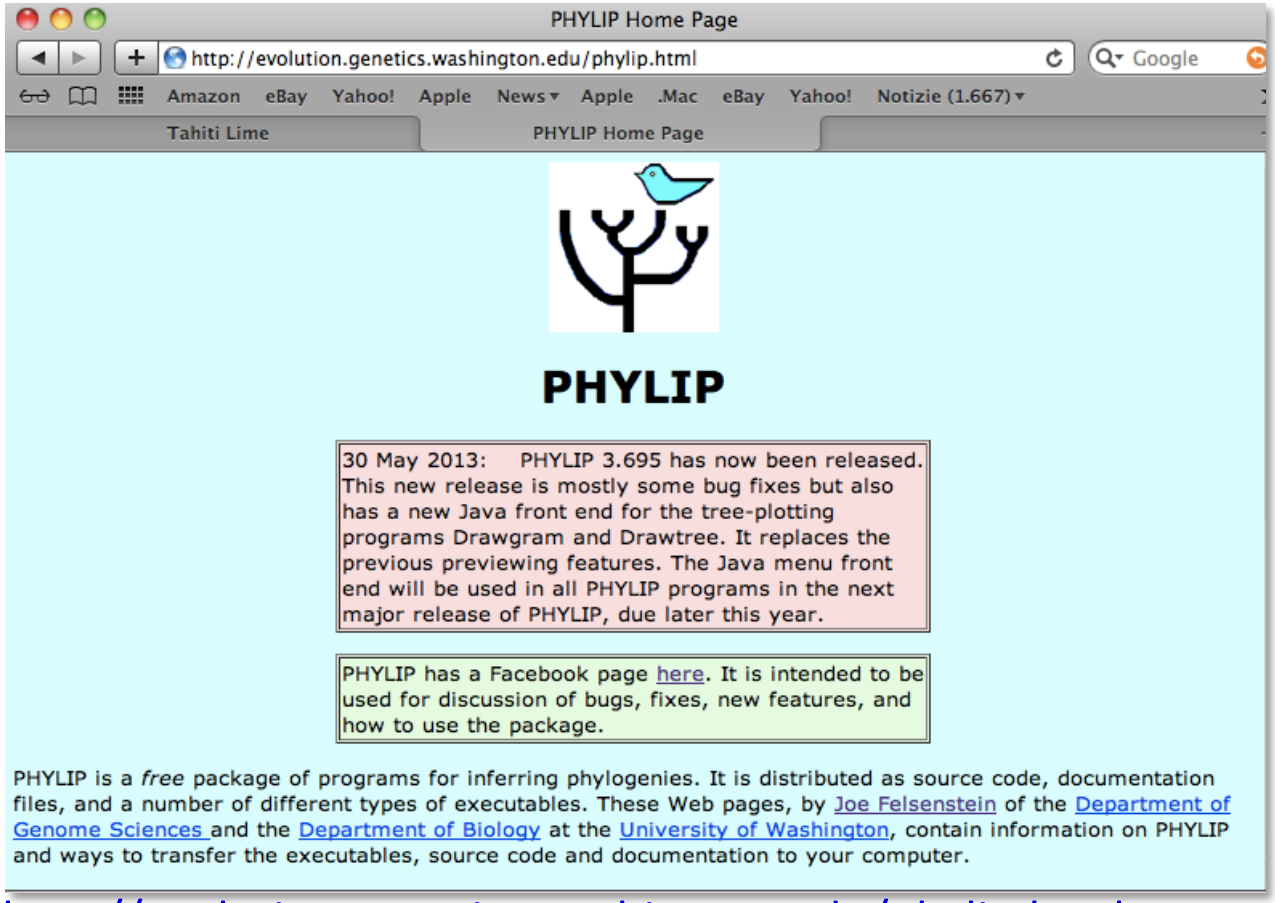
Fourth lab activity
(the GenAlEx software)
Principal Component Analysis

WE 20-12-2023



Fourth lab activity
(the PHYLIP package and the relative documentation)

WE 20-12-2023



http://evolution.genetics.washington.edu/phylip.html

A primer to phylogenetic analysis using the PHYLIP package

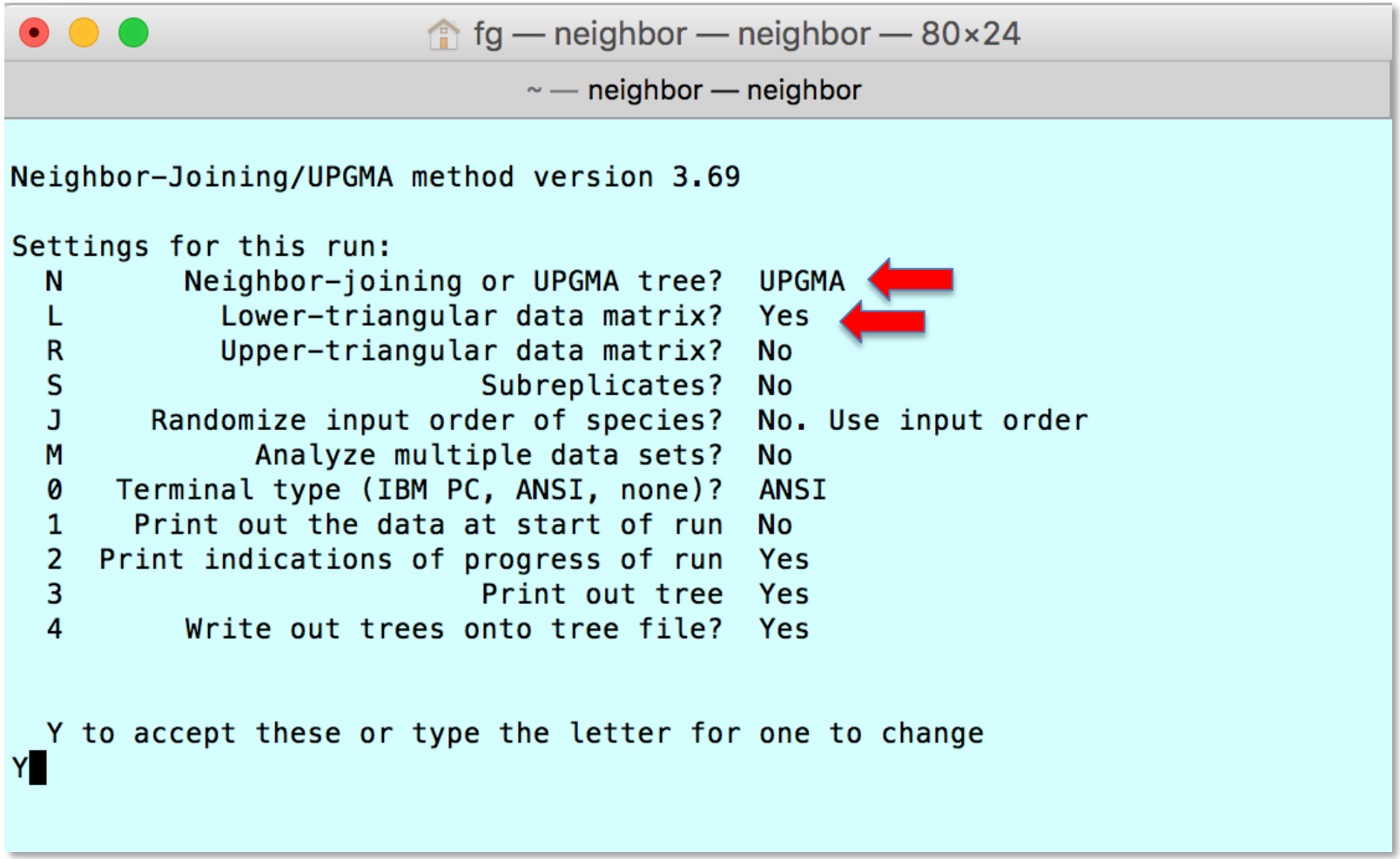
Jarno Tuimala - Fifth Edition

http://koti.mbnet.fi/tuimala/oppaat/phylip2.pdf

Fourth lab activity

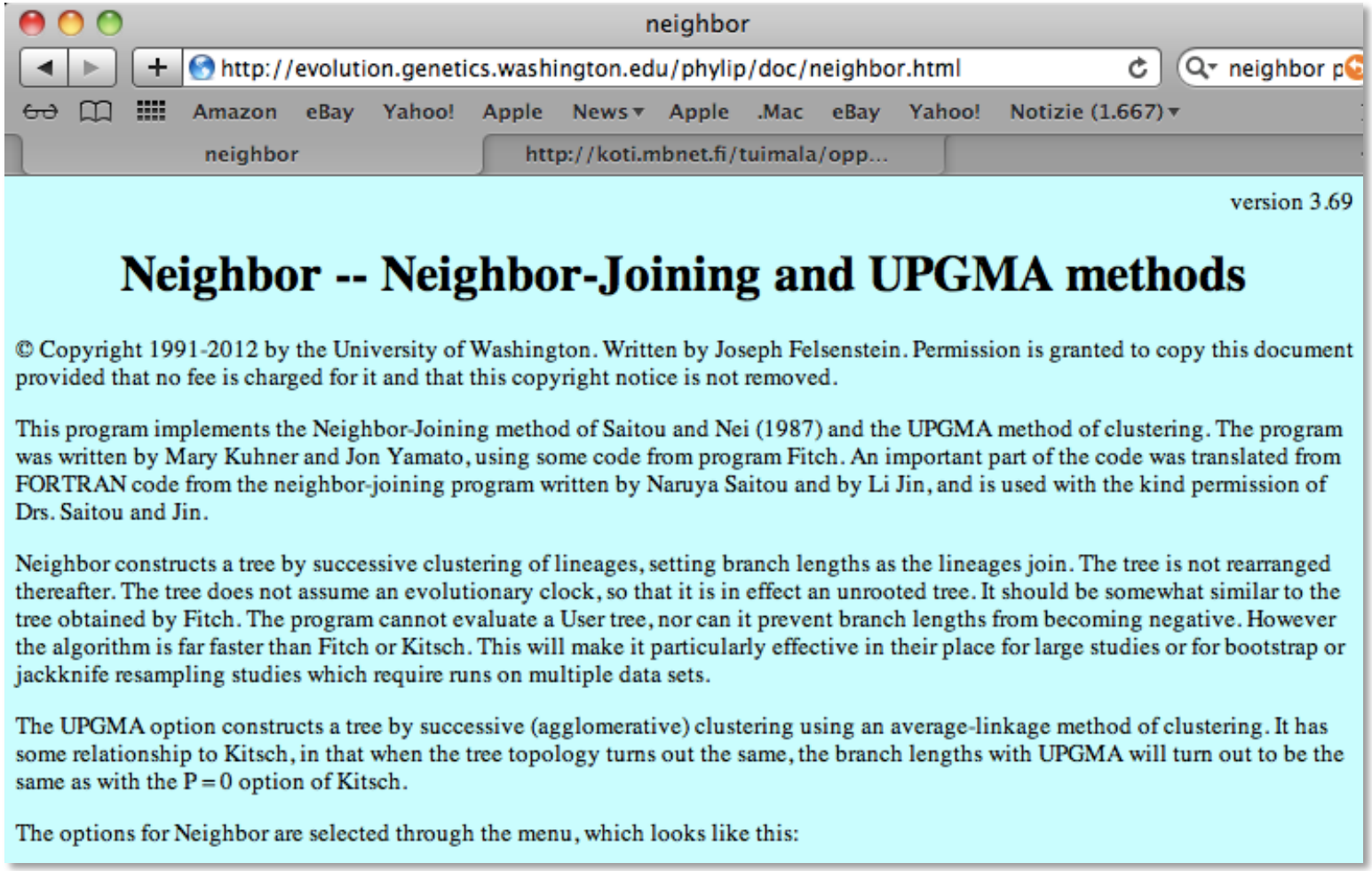
WE 20-12-2023

(the PHYLIP package and the relative documentation)



Fourth lab activity
(creating the dendrogram through “Neighbor” of the PHYLIP package)

WE 20-12-2023



http://evolution.genetics.washington.edu/phylip/doc/neighbor.html

Fourth lab activity
(creating the dendrogram through “Neighbor” of the PHYLIP package)

WE 20-12-2023

outfile_UPGMA

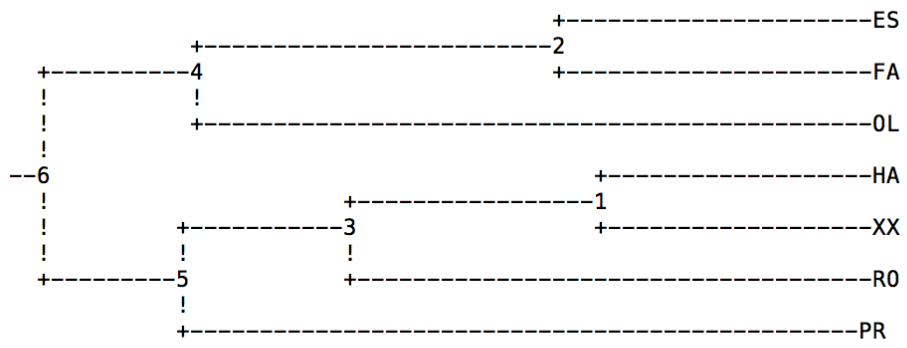
UPGMA

```
   7 Populations

Neighbor-Joining/UPGMA method version 3.69


 UPGMA method

 Negative branch lengths allowed
```



From	To	Length	Height
6	4	9.66667	9.66667
4	2	23.50000	33.16667
2	ES	21.00000	54.16667
2	FA	21.00000	54.16667
4	OL	44.50000	54.16667
6	5	9.00000	9.00000
5	3	10.66667	19.66667
3	1	16.50000	36.16667
1	HA	18.00000	54.16667
1	XX	18.00000	54.16667
3	RO	34.50000	54.16667
5	PR	45.16667	54.16667

outfile_NJ

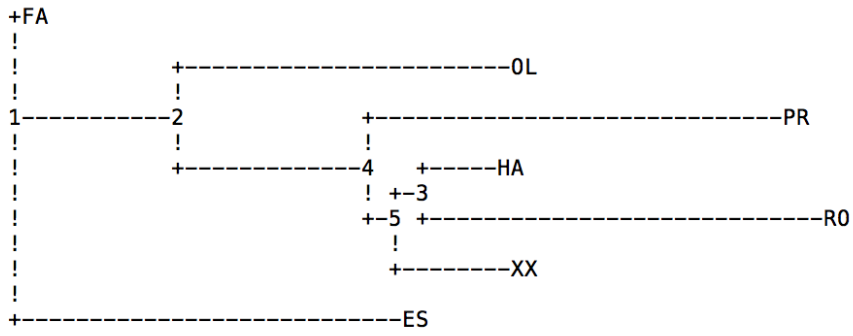
Neighbor Joining

```
   7 Populations

Neighbor-Joining/UPGMA method version 3.69


 Neighbor-joining method

 Negative branch lengths allowed
```




```
remember: this is an unrooted tree!
```

Between	And	Length
1	FA	-11.30000
1	2	22.06250
2	OL	45.93750
2	4	25.43750
4	PR	56.56250
4	5	2.93750
5	3	4.31250
3	HA	11.83333
3	RO	55.16667
5	XX	15.68750
1	ES	53.30000

outtree_UPGMA

```
(((ES:21.00000,FA:21.00000):23.50000,OL:44.50000):9.66667,
(((HA:18.00000,XX:18.00000):16.50000,RO:34.50000):10.66667,
PR:45.16667):9.00000);
```

outtree_NJ

```
(FA:-11.30000,(OL:45.93750,(PR:56.56250,((HA:11.83333,RO:55.16667):4.31250,
XX:15.68750):2.93750):25.43750):22.06250,ES:53.30000);
```

Fourth lab activity
(identifying the genomic regions of the SSR loci analyzed)

WE 20-12-2023

Using the sequences of Forward and Reverse primers for each SSR locus it is possible to identify their location and sequence in the reference genomes of *Prunus* species (i.e. Peach, Apricot, *Prunus mume*).
A comparison can be done with the SSR allele length of the genomic sequence(s) in the reference sequence.

Hint: use the *Phytozome* database that has proven effective at locating short primer sequences.