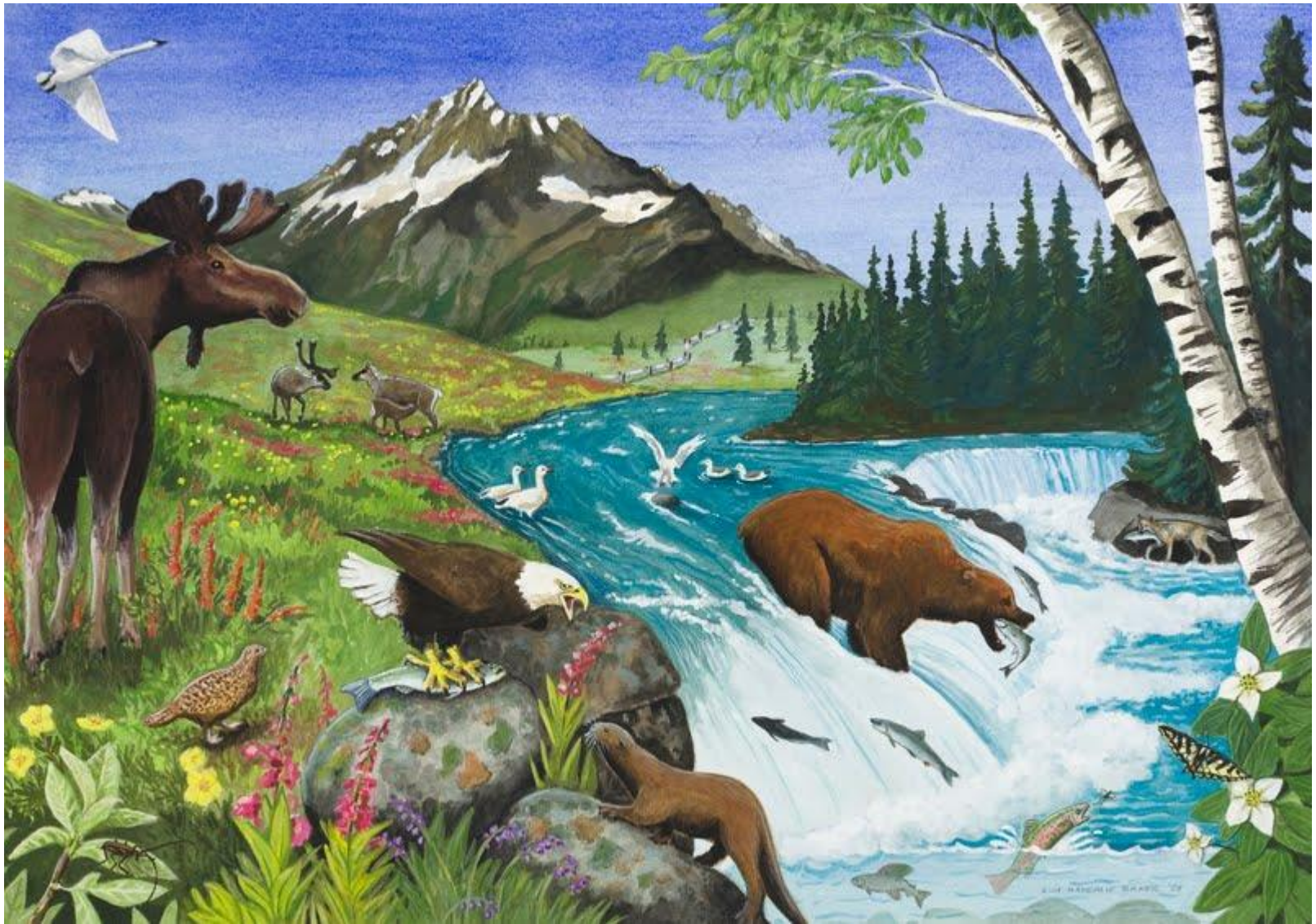
BIOL334 Computer Practical:
Analyse real genetic data for your Practical Report



Practical Report

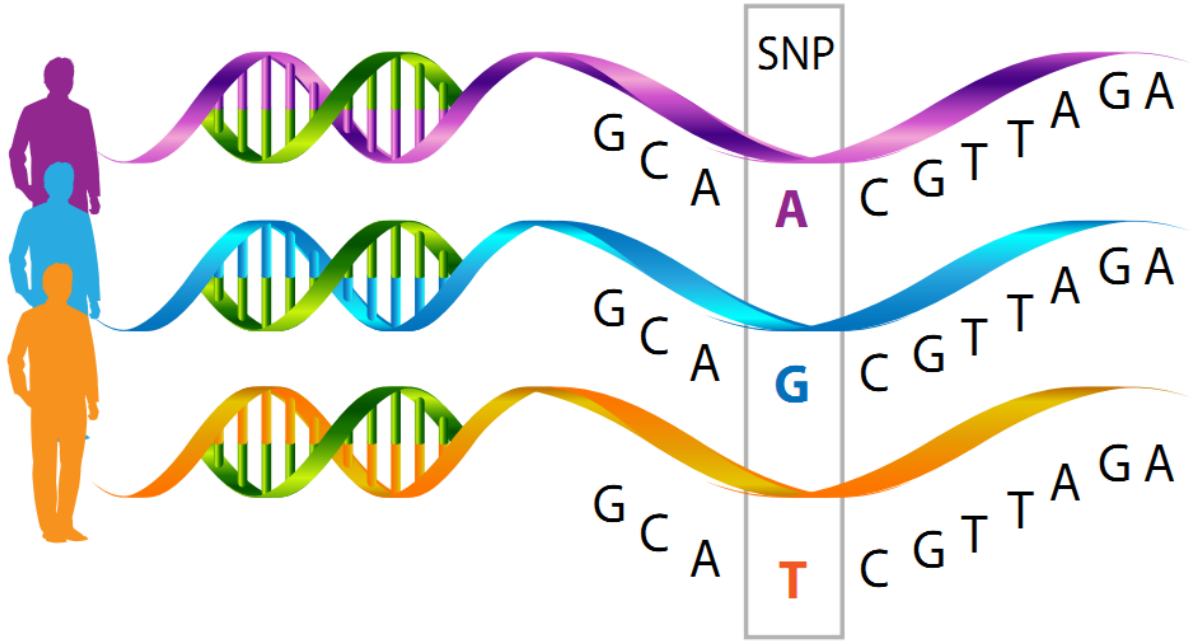
- Consult Unit Guide for Due Date
- Questions to be addressed are in these slides
- Rubric and instructions available online
  – under Wk 7 Tutorial on iLearn and in the Unit Guide

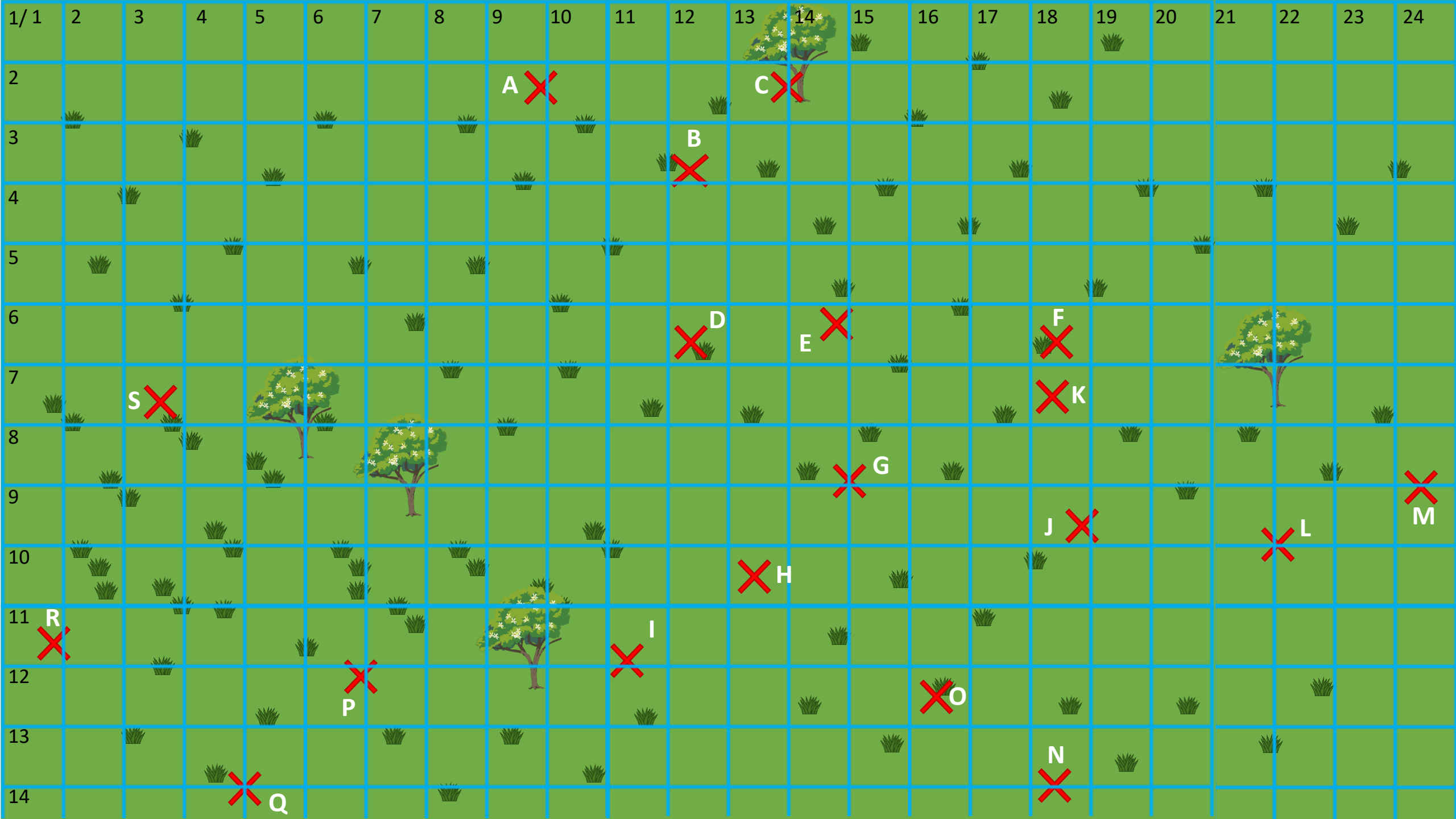
Make sure you save all of your results today!!!

The data:

- We have collected SNP data from 7 individuals at 19 locations

- We will assess:
  - Genetic summary statistics
  - Genetic structure
  - Spatial structure

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The Questions

1/ Is there any evidence for genetic structure among the sampling localities?

2/ Does genetic variation follow a pattern of isolation-by-distance?

3/ Are there environmental features that are implicated in patterns of genetic structure?

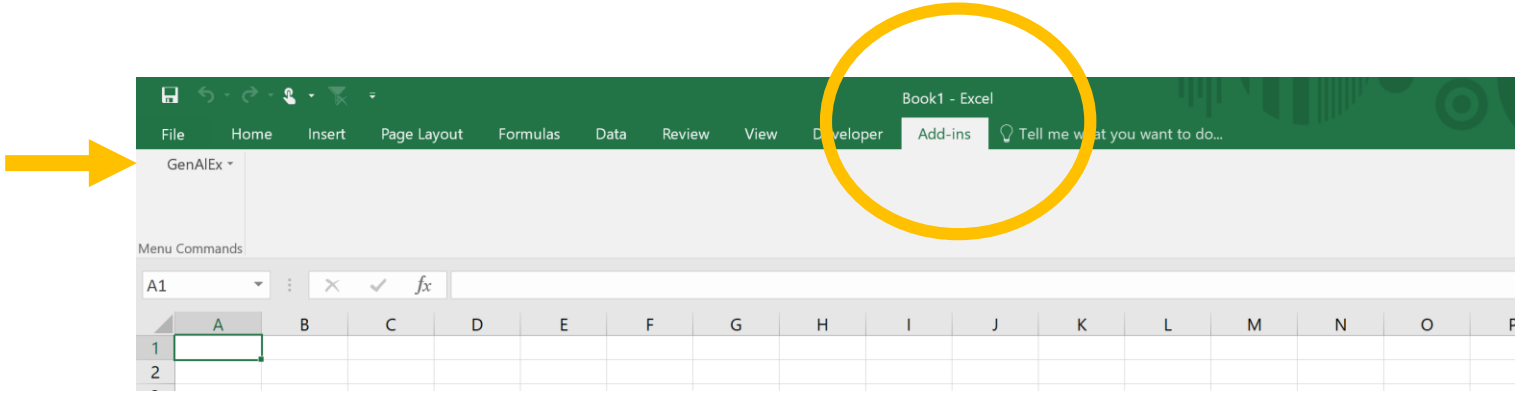
4/ Is there a risk of inbreeding?

Download Datasets and GenAlEx

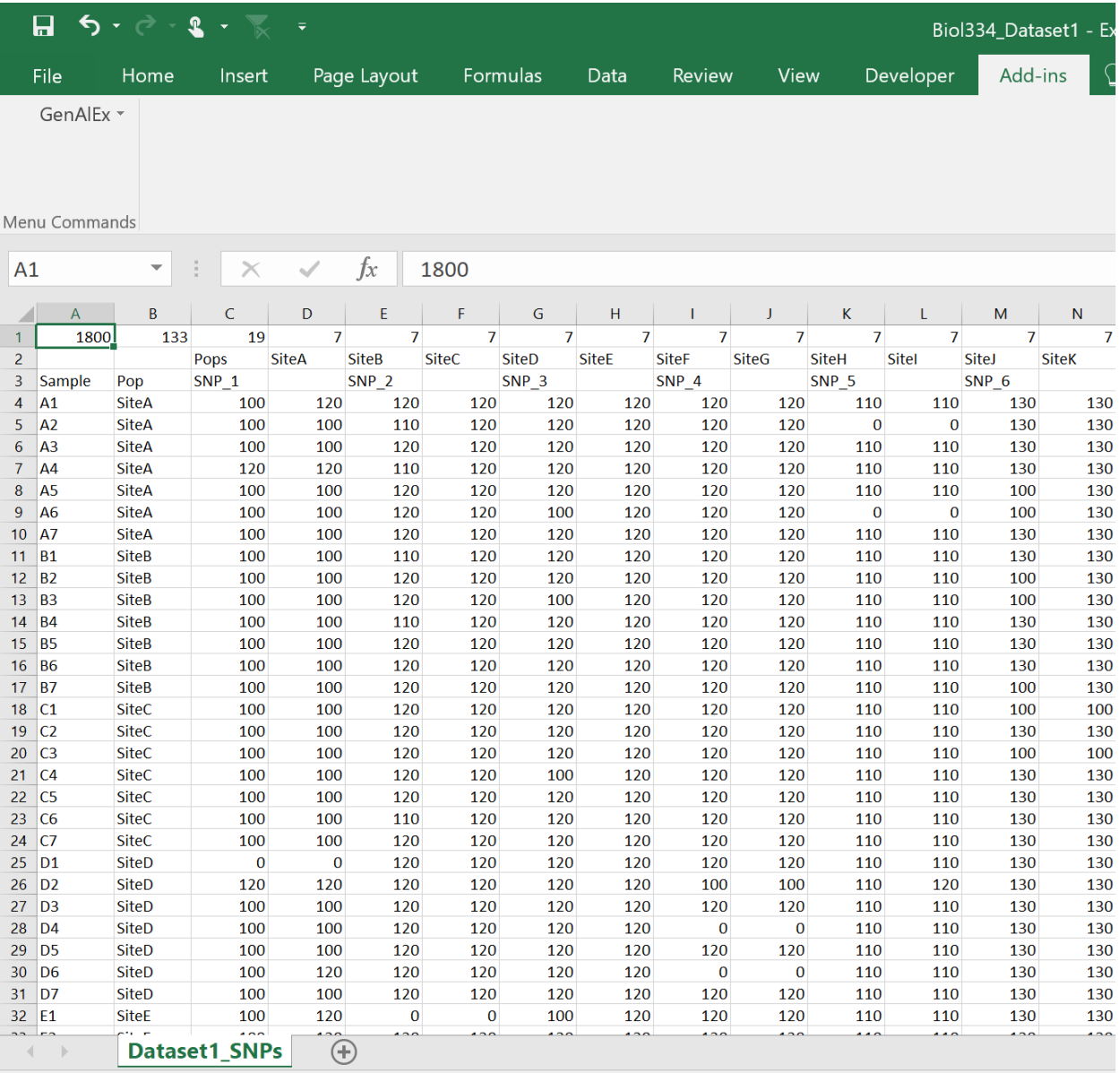
- We will be using GenAlEx – an add-in for Excel

- Download GenAlEx and the Datasets from iLearn
  - Located under Week 7 Tutorial in the folder “Computer Practical materials”
  - There will be 3 Excel files with genetic data (Datasets 1, 2a and 2b)

- Open Excel and then open GenAlEx
  - A tab called “add-ins” should appear in Excel, with GenAlEx within that tab



Open Dataset 1 – Get to know the data



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	1800	133	19	7	7	7	7	7	7	7	7	7	7	7
2			Pops	SiteA	SiteB	SiteC	SiteD	SiteE	SiteF	SiteG	SiteH	SiteI	SiteJ	SiteK
3	Sample	Pop	SNP_1		SNP_2		SNP_3		SNP_4		SNP_5		SNP_6	
4	A1	SiteA	100	120	120	120	120	120	120	120	110	110	130	130
5	A2	SiteA	100	100	110	120	120	120	120	120	0	0	130	130
6	A3	SiteA	100	100	120	120	120	120	120	120	110	110	130	130
7	A4	SiteA	120	120	110	120	120	120	120	120	110	110	130	130
8	A5	SiteA	100	100	120	120	120	120	120	120	110	110	100	130
9	A6	SiteA	100	100	120	120	100	120	120	120	0	0	100	130
10	A7	SiteA	100	100	120	120	120	120	120	120	110	110	130	130
11	B1	SiteB	100	100	110	120	120	120	120	120	110	110	130	130
12	B2	SiteB	100	100	120	120	120	120	120	120	110	110	100	130
13	B3	SiteB	100	100	120	120	100	120	120	120	110	110	100	130
14	B4	SiteB	100	100	110	120	120	120	120	120	110	110	130	130
15	B5	SiteB	100	100	120	120	120	120	120	120	110	110	130	130
16	B6	SiteB	100	100	120	120	120	120	120	120	110	110	130	130
17	B7	SiteB	100	100	120	120	120	120	120	120	110	110	100	130
18	C1	SiteC	100	100	120	120	120	120	120	120	110	110	100	100
19	C2	SiteC	100	100	120	120	120	120	120	120	110	110	130	130
20	C3	SiteC	100	100	120	120	120	120	120	120	110	110	100	100
21	C4	SiteC	100	100	120	120	100	120	120	120	110	110	130	130
22	C5	SiteC	100	100	120	120	120	120	120	120	110	110	130	130
23	C6	SiteC	100	100	110	120	120	120	120	120	110	110	130	130
24	C7	SiteC	100	100	120	120	120	120	120	120	110	110	130	130
25	D1	SiteD	0	0	120	120	120	120	120	120	110	110	130	130
26	D2	SiteD	120	120	120	120	120	120	100	100	110	120	130	130
27	D3	SiteD	100	100	120	120	120	120	120	120	110	110	130	130
28	D4	SiteD	100	100	120	120	120	120	0	0	110	110	130	130
29	D5	SiteD	100	100	120	120	120	120	120	120	110	110	130	130
30	D6	SiteD	100	120	120	120	120	120	0	0	110	110	130	130
31	D7	SiteD	100	100	120	120	120	120	120	120	110	110	130	130
32	E1	SiteE	100	120	0	0	100	120	120	120	110	110	130	130

Dataset1_SNPs

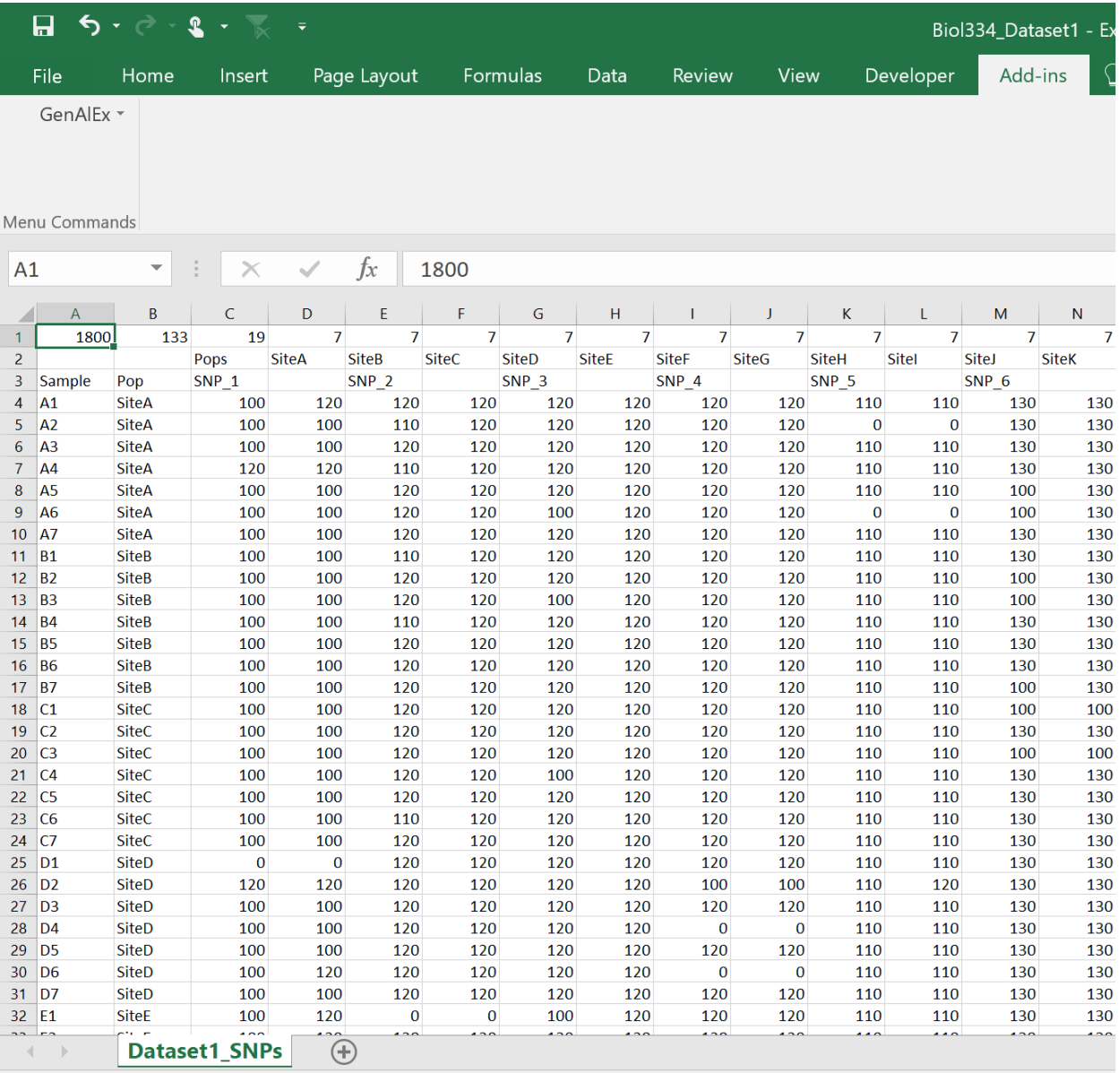
19 “Populations” (= “Sampling Sites”), named A → S

7 Samples (= “Individual animals”) per site, named with the Sample name and 1 → 7
= Total 133 samples

1800 SNPs (= “single nucleotide polymorphism”), sequenced for each individual:

100 = A
110 = C
120 = G
130 = T
0 = Missing data

Open Dataset 1 – Get to know the data



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	1800	133	19	7	7	7	7	7	7	7	7	7	7	7
2			Pops	SiteA	SiteB	SiteC	SiteD	SiteE	SiteF	SiteG	SiteH	SiteI	SiteJ	SiteK
3	Sample	Pop	SNP_1		SNP_2		SNP_3		SNP_4		SNP_5		SNP_6	
4	A1	SiteA	100	120	120	120	120	120	120	120	110	110	130	130
5	A2	SiteA	100	100	110	120	120	120	120	120	0	0	130	130
6	A3	SiteA	100	100	120	120	120	120	120	120	110	110	130	130
7	A4	SiteA	120	120	110	120	120	120	120	120	110	110	130	130
8	A5	SiteA	100	100	120	120	120	120	120	120	110	110	100	130
9	A6	SiteA	100	100	120	120	100	120	120	120	0	0	100	130
10	A7	SiteA	100	100	120	120	120	120	120	120	110	110	130	130
11	B1	SiteB	100	100	110	120	120	120	120	120	110	110	130	130
12	B2	SiteB	100	100	120	120	120	120	120	120	110	110	100	130
13	B3	SiteB	100	100	120	120	100	120	120	120	110	110	100	130
14	B4	SiteB	100	100	110	120	120	120	120	120	110	110	130	130
15	B5	SiteB	100	100	120	120	120	120	120	120	110	110	130	130
16	B6	SiteB	100	100	120	120	120	120	120	120	110	110	130	130
17	B7	SiteB	100	100	120	120	120	120	120	120	110	110	100	130
18	C1	SiteC	100	100	120	120	120	120	120	120	110	110	100	100
19	C2	SiteC	100	100	120	120	120	120	120	120	110	110	130	130
20	C3	SiteC	100	100	120	120	120	120	120	120	110	110	100	100
21	C4	SiteC	100	100	120	120	100	120	120	120	110	110	130	130
22	C5	SiteC	100	100	120	120	120	120	120	120	110	110	130	130
23	C6	SiteC	100	100	110	120	120	120	120	120	110	110	130	130
24	C7	SiteC	100	100	120	120	120	120	120	120	110	110	130	130
25	D1	SiteD	0	0	120	120	120	120	120	120	110	110	130	130
26	D2	SiteD	120	120	120	120	120	120	100	100	110	120	130	130
27	D3	SiteD	100	100	120	120	120	120	120	120	110	110	130	130
28	D4	SiteD	100	100	120	120	120	120	0	0	110	110	130	130
29	D5	SiteD	100	100	120	120	120	120	120	120	110	110	130	130
30	D6	SiteD	100	120	120	120	120	120	0	0	110	110	130	130
31	D7	SiteD	100	100	120	120	120	120	120	120	110	110	130	130
32	E1	SiteE	100	120	0	0	100	120	120	120	110	110	130	130

Dataset1_SNPs

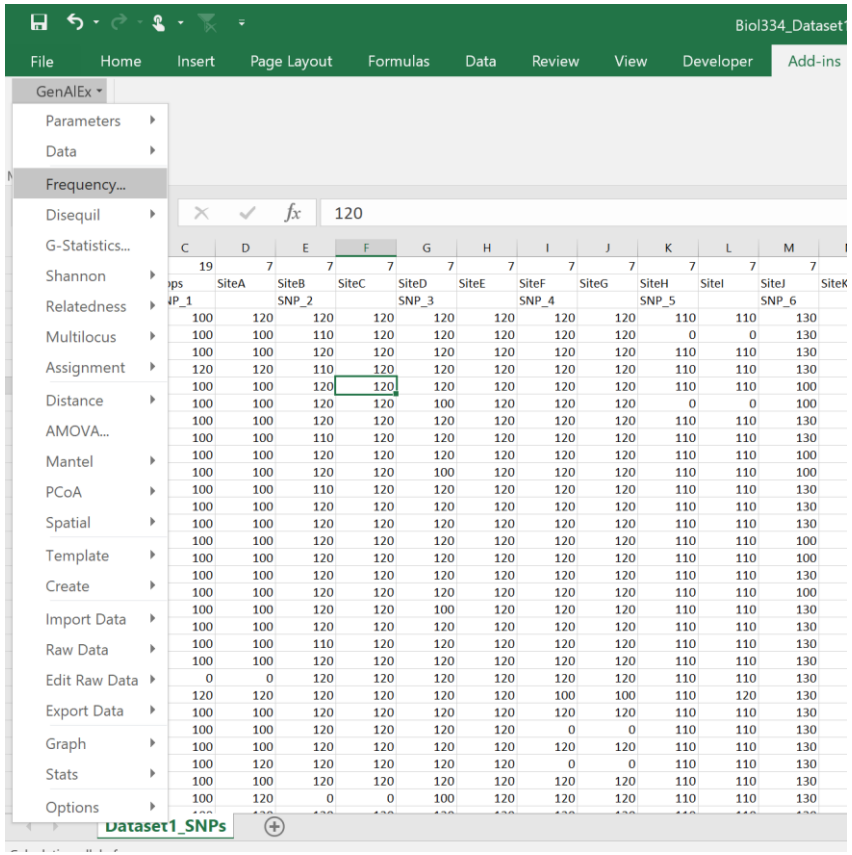
Data for each individual are in one row

Data for each SNP locus are in two columns,
= one column for each allele at that locus!

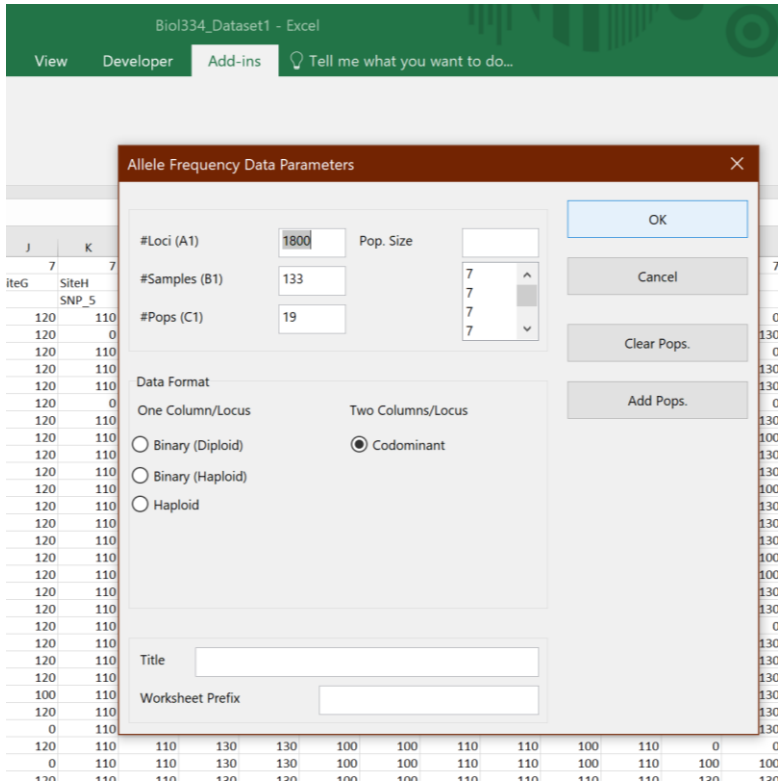
So individual E1:
Heterozygous for SNP 1 (A/G) and SNP 3 (A/G)
Has missing data for SNP 2
Homozygous for SNP 4 (G/G)

Calculate Genetic Summary Statistics:

Click **GenAlEx**, then **Frequency**



In the pop-up window, check that the numbers for Loci, Samples and Pops are correct. Check the **Codominant** data format and click **OK**



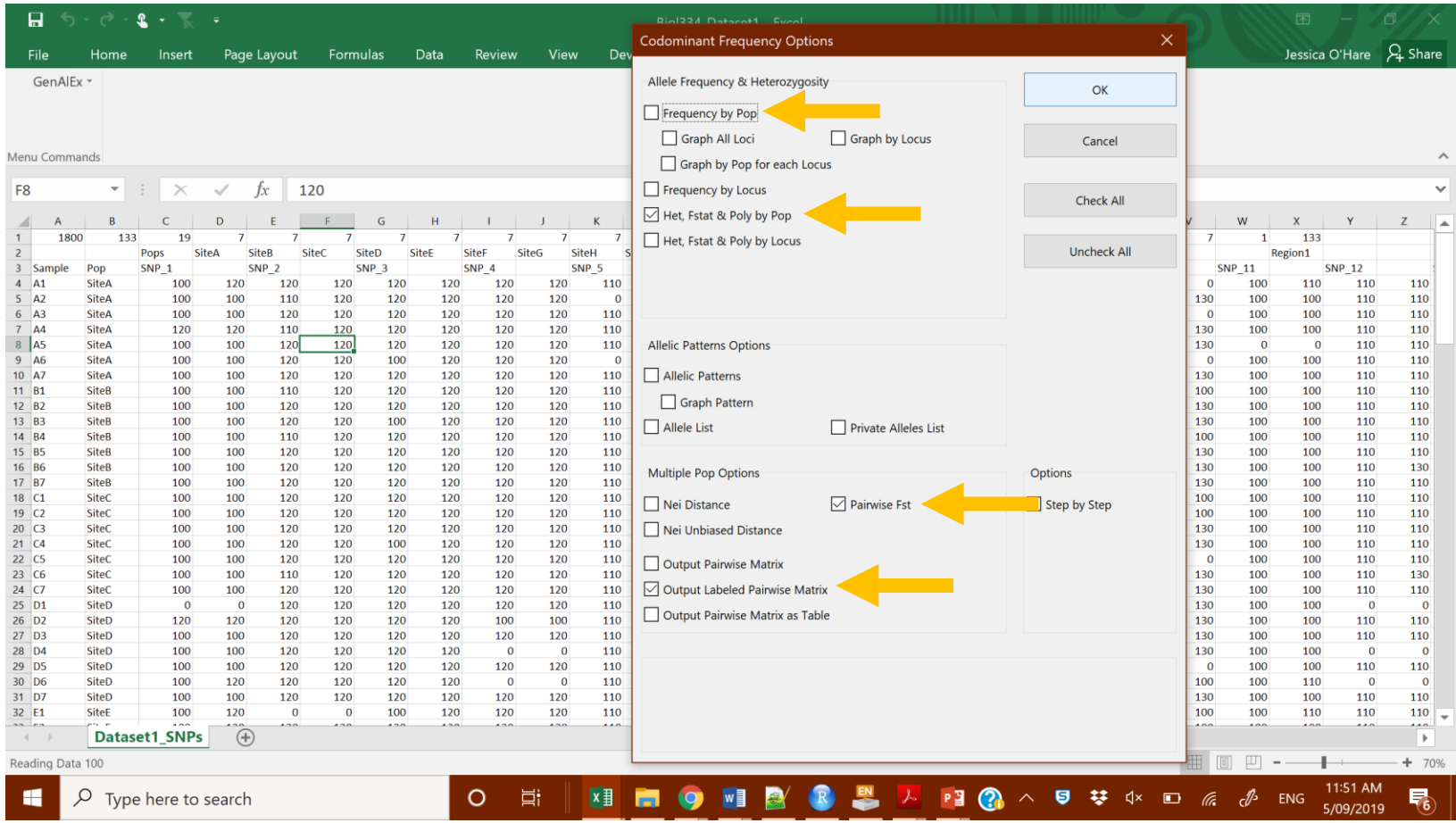
Calculate Genetic Summary Statistics:

In the pop-up window:

UN-check **Frequency by Pop**

Check:
Het, Fstat & Poly by Pop
Pairwise Fst
Output Labelled Pairwise Matrix

Then click **OK**



You will get two results sheets:
HFP = Het, Fstat & Poly by Pop
FstL = Labeled Pairwise Fst Matrix

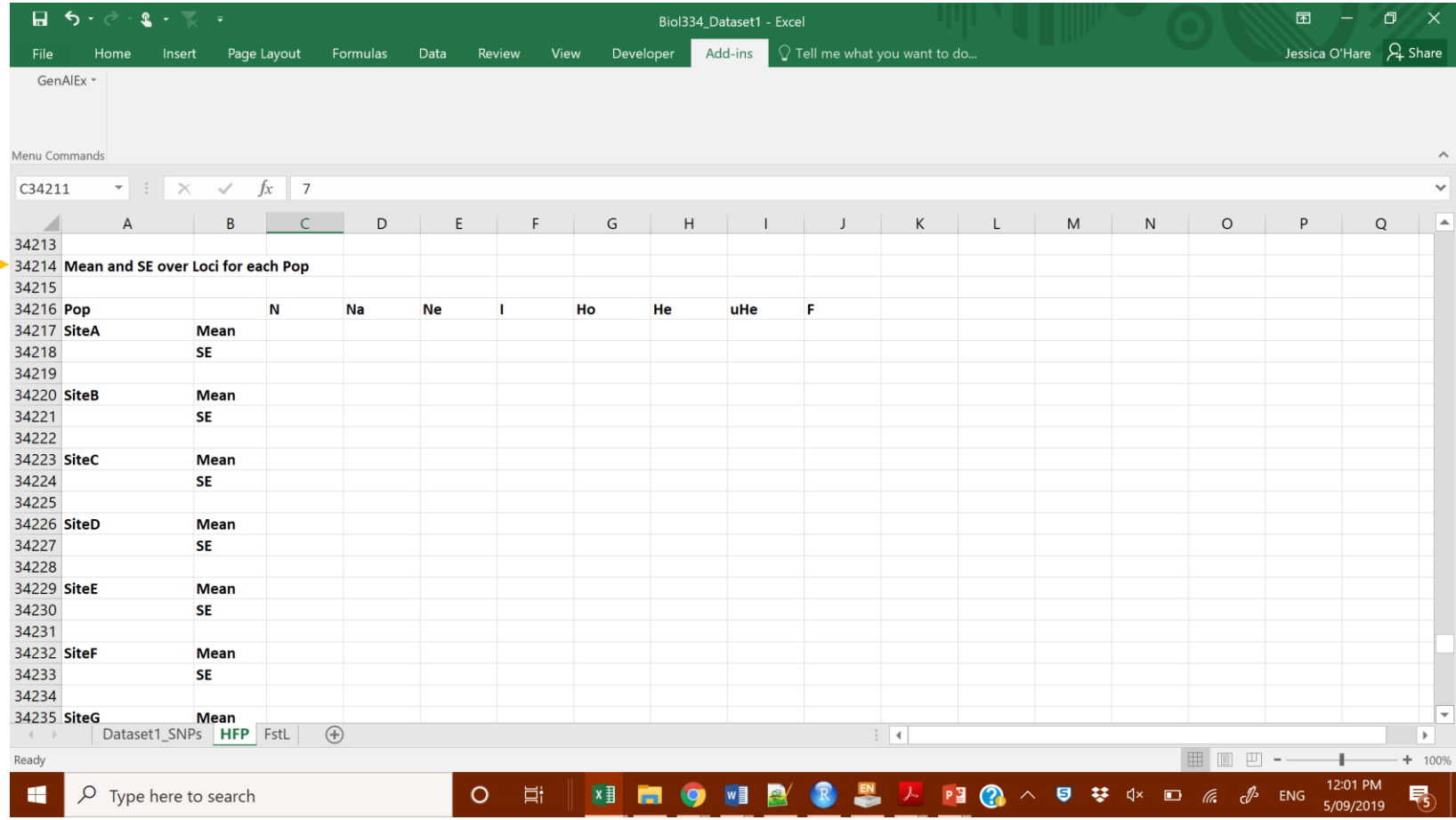
Be patient – the calculations will take a few minutes!!!

HFP – Het, Fstat, Poly by Pop

This sheet has info for each locus within each pop

At the end, there is a table with an overall mean per pop

(starting at row ~34214)



HFP – Het, Fstat, Poly by Pop

N = No. of samples with data

Na = No. of Different Alleles

Ne = No. of Effective Alleles = 1 / (Sum pi^2)

I = Shannon's Information Index
= -1* Sum (pi * Ln (pi))

Ho = Observed Heterozygosity = No. of Hets / N

He = Expected Heterozygosity = 1 - Sum pi^2

uHe = Unbiased Expected Heterozygosity
= (2N / (2N-1)) * He

F = Fixation Index = (He - Ho) / He = 1 - (Ho / He)

Where pi is the frequency of the ith allele for the population & Sum pi^2 is the sum of the squared population allele frequencies.

Can you see any major differences between pops?

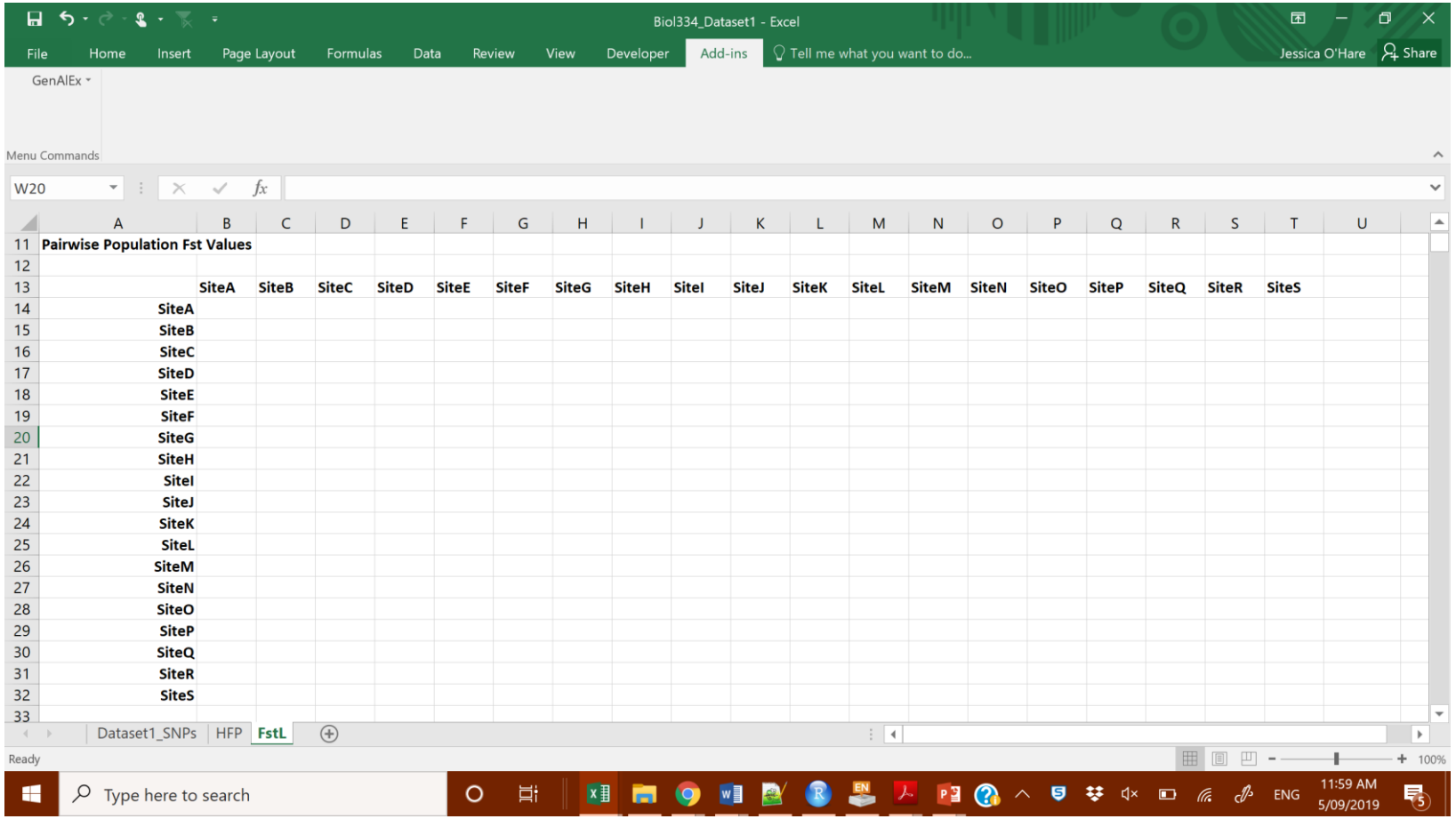
A	B	C	D	E	F	G	H	I	J
Pop		**N**	**Na**	**Ne**	**I**	**Ho**	**He**	**uHe**	**F**
SiteA	**Mean**	6.876	1.732	1.389	0.360	0.213	0.236	0.254	0.072
	SE	0.009	0.010	0.008	0.006	0.005	0.004	0.005	0.010
SiteB	**Mean**	6.878	1.733	1.400	0.368	0.214	0.242	0.261	0.088
	SE	0.010	0.010	0.008	0.006	0.005	0.004	0.005	0.010
SiteC	**Mean**	6.912	1.745	1.401	0.370	0.215	0.242	0.261	0.081
	SE	0.008	0.010	0.008	0.006	0.005	0.004	0.005	0.010
SiteD	**Mean**	6.944	1.719	1.401	0.364	0.265	0.240	0.259	-0.090
	SE	0.006	0.011	0.008	0.006	0.006	0.004	0.005	0.010
SiteE	**Mean**	6.958	1.686	1.413	0.361	0.287	0.241	0.260	-0.150
	SE	0.005	0.011	0.009	0.006	0.007	0.005	0.005	0.010
SiteF	**Mean**	6.901	1.780	1.440	0.397	0.266	0.262	0.283	-0.019
	SE	0.008	0.010	0.008	0.006	0.005	0.004	0.005	0.009
SiteG	**Mean**	6.924	1.844	1.448	0.414	0.267	0.271	0.292	0.001
	SE	0.007	0.009	0.008	0.005	0.005	0.004	0.004	0.009
SiteH	**Mean**	6.808	1.790	1.436	0.398	0.260	0.262	0.283	0.001
	SE	0.012	0.010	0.008	0.006	0.005	0.004	0.004	0.010
SiteI	**Mean**	6.920	1.787	1.431	0.396	0.256	0.261	0.281	0.006
	SE	0.007	0.010	0.008	0.006	0.005	0.004	0.004	0.009
SiteJ	**Mean**	6.926	1.836	1.446	0.413	0.270	0.270	0.292	-0.011
	SE	0.007	0.009	0.008	0.005	0.005	0.004	0.004	0.009
SiteK	**Mean**	6.876	1.813	1.441	0.406	0.257	0.266	0.287	0.017
	SE	0.009	0.009	0.008	0.006	0.005	0.004	0.004	0.009
SiteL	**Mean**	6.929	1.809	1.439	0.404	0.262	0.265	0.286	-0.003
	SE	0.007	0.009	0.008	0.006	0.005	0.004	0.004	0.009
SiteM	**Mean**	6.932	1.743	1.412	0.374	0.248	0.247	0.266	-0.012
	SE	0.007	0.010	0.008	0.006	0.005	0.004	0.005	0.009
SiteN	**Mean**	6.932	1.792	1.432	0.396	0.259	0.260	0.281	-0.005
	SE	0.007	0.010	0.008	0.006	0.005	0.004	0.004	0.009
SiteO	**Mean**	6.923	1.752	1.411	0.375	0.259	0.247	0.266	-0.047
	SE	0.007	0.010	0.008	0.006	0.006	0.004	0.005	0.009
SiteP	**Mean**	6.938	1.816	1.444	0.407	0.268	0.267	0.288	-0.013
	SE	0.006	0.009	0.008	0.006	0.005	0.004	0.004	0.009
SiteQ	**Mean**	6.921	1.784	1.413	0.384	0.261	0.251	0.270	-0.044
	SE	0.007	0.010	0.008	0.006	0.005	0.004	0.004	0.009
SiteR	**Mean**	6.901	1.673	1.390	0.349	0.253	0.232	0.250	-0.081
	SE	0.009	0.011	0.009	0.006	0.006	0.005	0.005	0.010
SiteS	**Mean**	6.936	1.741	1.425	0.382	0.263	0.253	0.272	-0.037
	SE	0.006	0.010	0.008	0.006	0.006	0.004	0.005	0.009

FstL = Pairwise FST matrix

This sheet has a matrix for FST between each possible pair of pops

(with zero along the diagonal, where it is a comparison of each pop with itself)

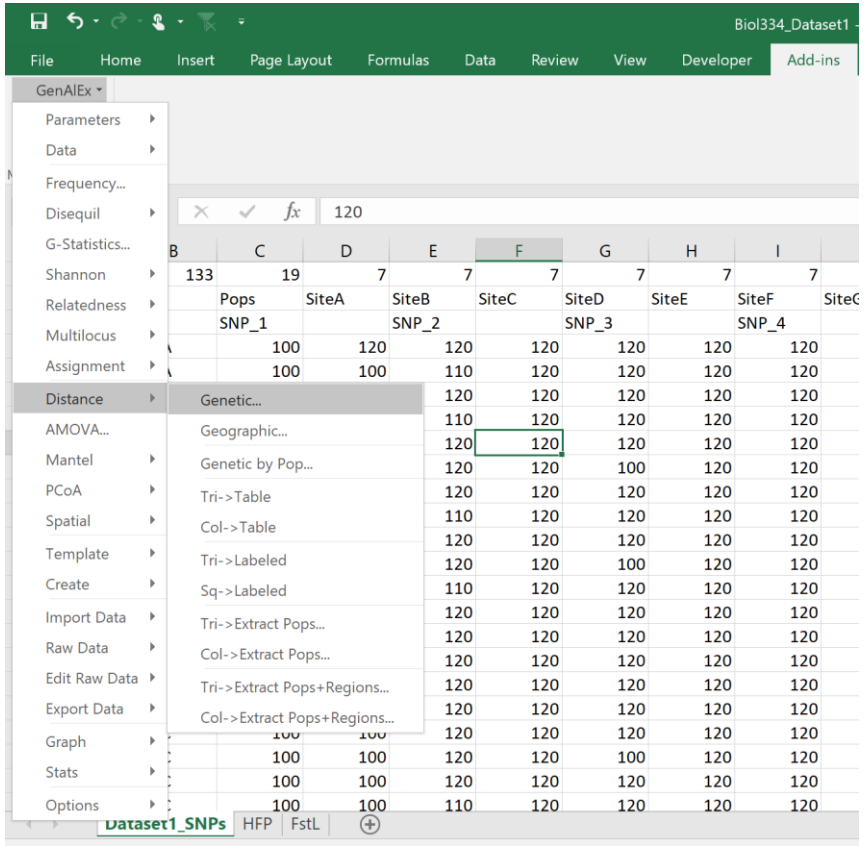
Can you see any patterns?



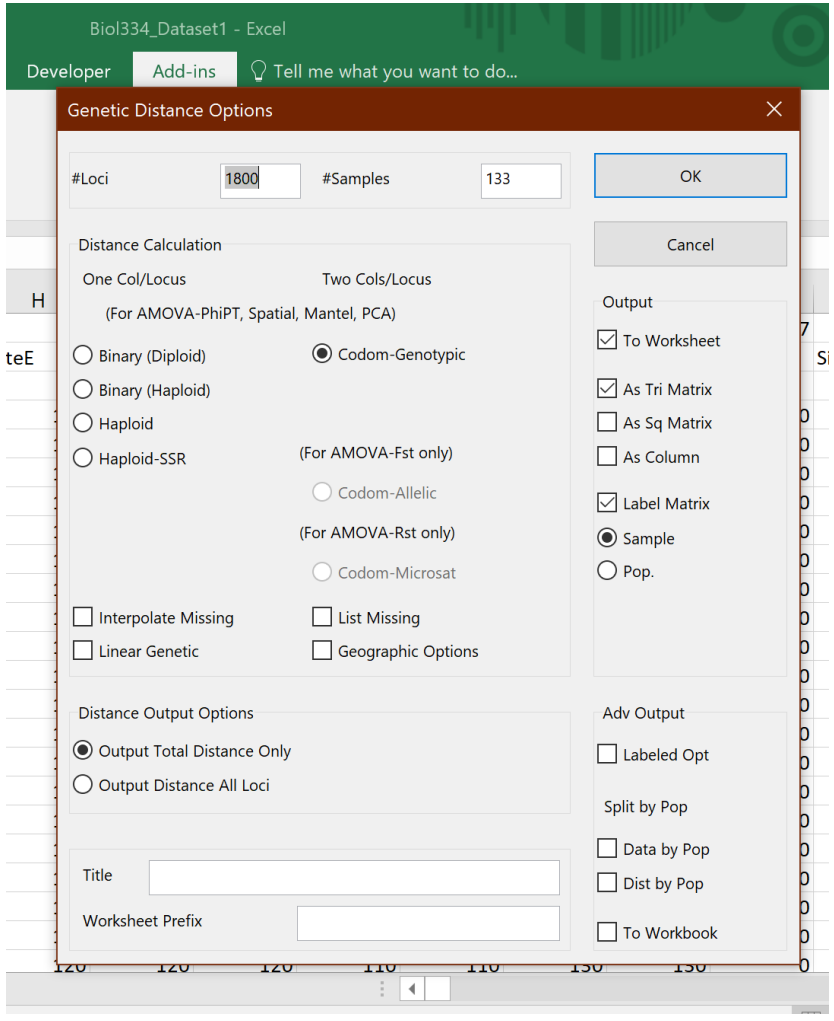
	SiteA	SiteB	SiteC	SiteD	SiteE	SiteF	SiteG	SiteH	SiteI	SiteJ	SiteK	SiteL	SiteM	SiteN	SiteO	SiteP	SiteQ	SiteR	SiteS	
SiteA	0.000																			**SiteA**
SiteB	0.050	0.000																		**SiteB**
SiteC	0.047	0.048	0.000																	**SiteC**
SiteD	0.135	0.132	0.130	0.000																**SiteD**
SiteE	0.135	0.133	0.132	0.053	0.000															**SiteE**
SiteF	0.111	0.108	0.105	0.043	0.058	0.000														**SiteF**
SiteG	0.101	0.100	0.098	0.050	0.067	0.039	0.000													**SiteG**
SiteH	0.110	0.109	0.106	0.044	0.046	0.041	0.039	0.000												**SiteH**
SiteI	0.107	0.105	0.102	0.056	0.046	0.048	0.044	0.034	0.000											**SiteI**
SiteJ	0.097	0.095	0.092	0.074	0.077	0.051	0.041	0.054	0.053	0.000										**SiteJ**
SiteK	0.103	0.103	0.102	0.064	0.063	0.048	0.042	0.045	0.045	0.038	0.000									**SiteK**
SiteL	0.102	0.099	0.096	0.068	0.067	0.052	0.043	0.047	0.042	0.039	0.043	0.000								**SiteL**
SiteM	0.105	0.104	0.102	0.090	0.080	0.070	0.063	0.059	0.047	0.057	0.060	0.048	0.000							**SiteM**
SiteN	0.104	0.104	0.100	0.088	0.098	0.070	0.061	0.069	0.075	0.061	0.067	0.067	0.080	0.000						**SiteN**
SiteO	0.115	0.112	0.108	0.100	0.113	0.084	0.072	0.086	0.085	0.073	0.078	0.075	0.090	0.052	0.000					**SiteO**
SiteP	0.103	0.100	0.098	0.082	0.097	0.067	0.053	0.070	0.072	0.056	0.066	0.062	0.079	0.055	0.053	0.000				**SiteP**
SiteQ	0.113	0.110	0.108	0.093	0.111	0.079	0.064	0.083	0.083	0.067	0.075	0.073	0.090	0.061	0.046	0.040	0.000			**SiteQ**
SiteR	0.136	0.135	0.132	0.101	0.118	0.091	0.076	0.095	0.095	0.083	0.093	0.086	0.109	0.094	0.091	0.057	0.065	0.000		**SiteR**
SiteS	0.115	0.114	0.109	0.091	0.108	0.076	0.062	0.081	0.083	0.069	0.077	0.072	0.091	0.070	0.066	0.043	0.048	0.046	0.000	**SiteS**
	SiteA	**SiteB**	**SiteC**	**SiteD**	**SiteE**	**SiteF**	**SiteG**	**SiteH**	**SiteI**	**SiteJ**	**SiteK**	**SiteL**	**SiteM**	**SiteN**	**SiteO**	**SiteP**	**SiteQ**	**SiteR**	**SiteS**	

Genetic distance between individuals

Click **GenAlEx** > **Distance** > **Genetic**



In the pop-up window,
make sure the defaults match those shown here



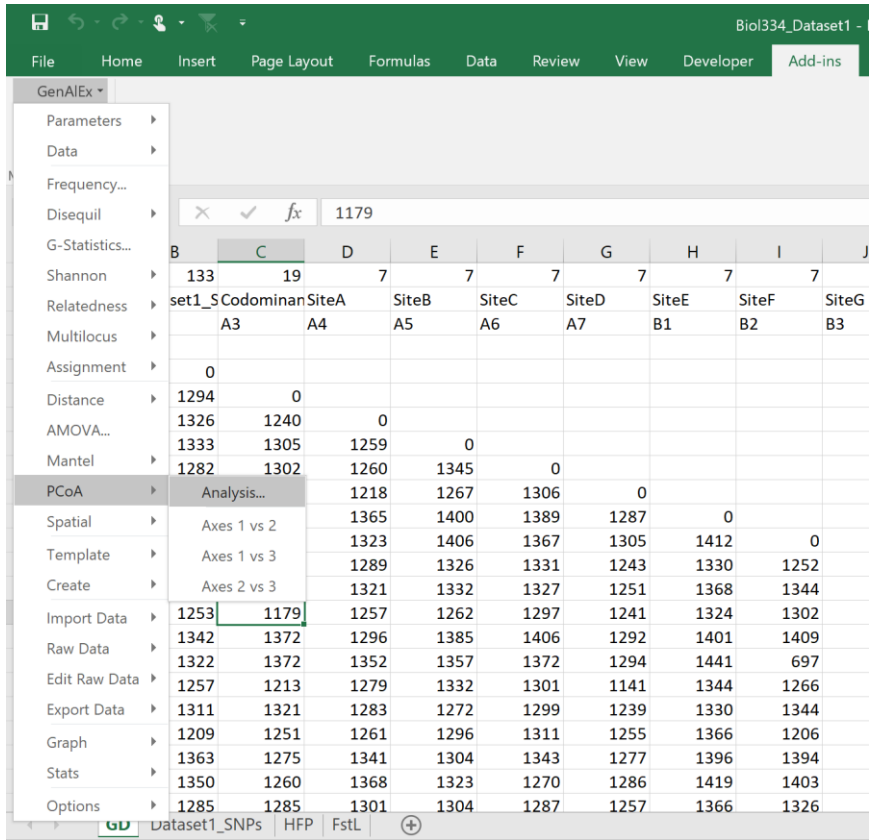
Click **OK**

You will get a sheet called **GD** (Genetic distance)

Genetic distance between individuals

Complete a Principal Coordinates Analysis (PCoA) using the Genetic Distance data

Within the GD sheet,
Click **Genalex** > **PCoA** > **Analysis**

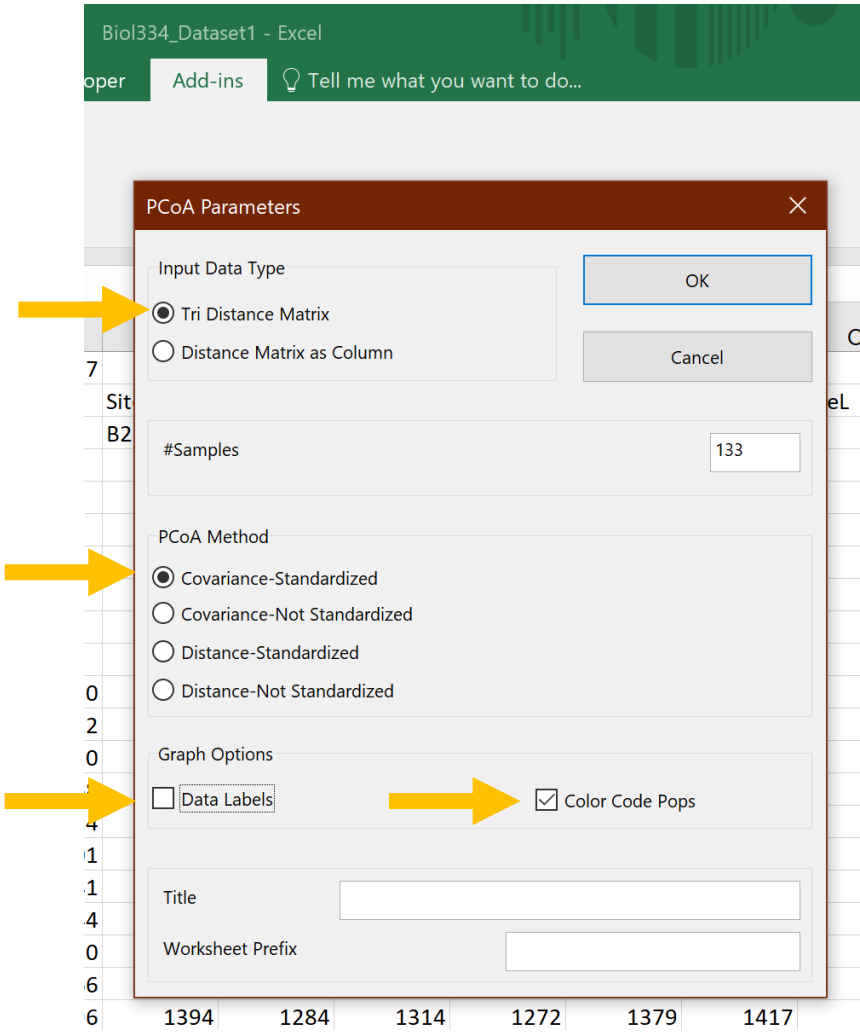


In the pop-up window, click:
Tri Distance Matrix
Covariance-Standardised
Color Code Pops

UN-check **Data Labels**

Click **OK**

You will get a new sheet called **PCoA** with a plot

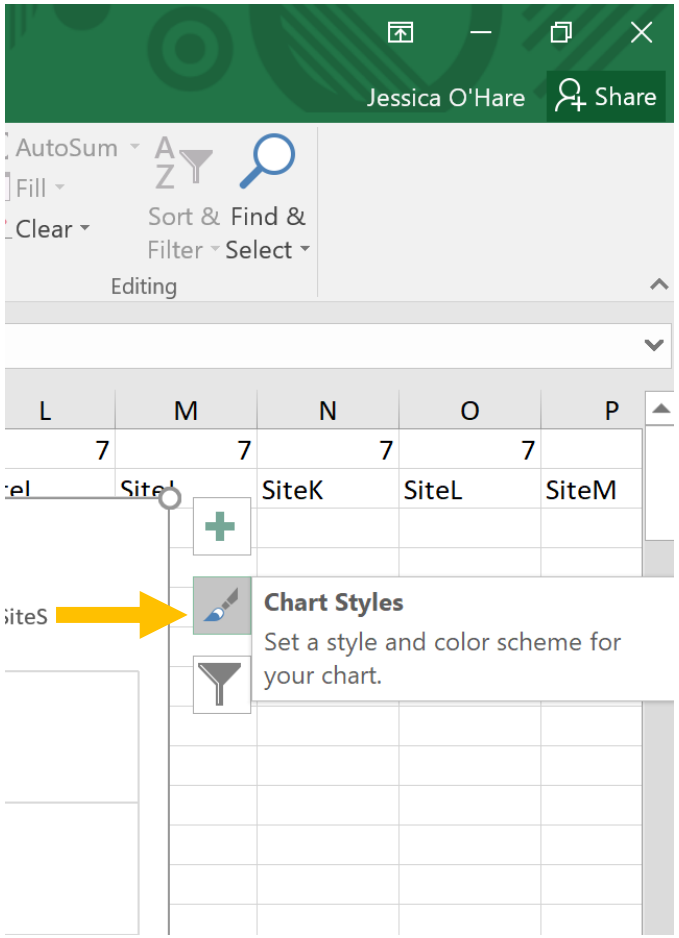


Genetic distance between individuals

In the PCoA, each dot represents an individual
The closer together the dots (individuals),
the more closely related they are

Make the PCoA plot bigger so that you can clearly see all the Pop category labels

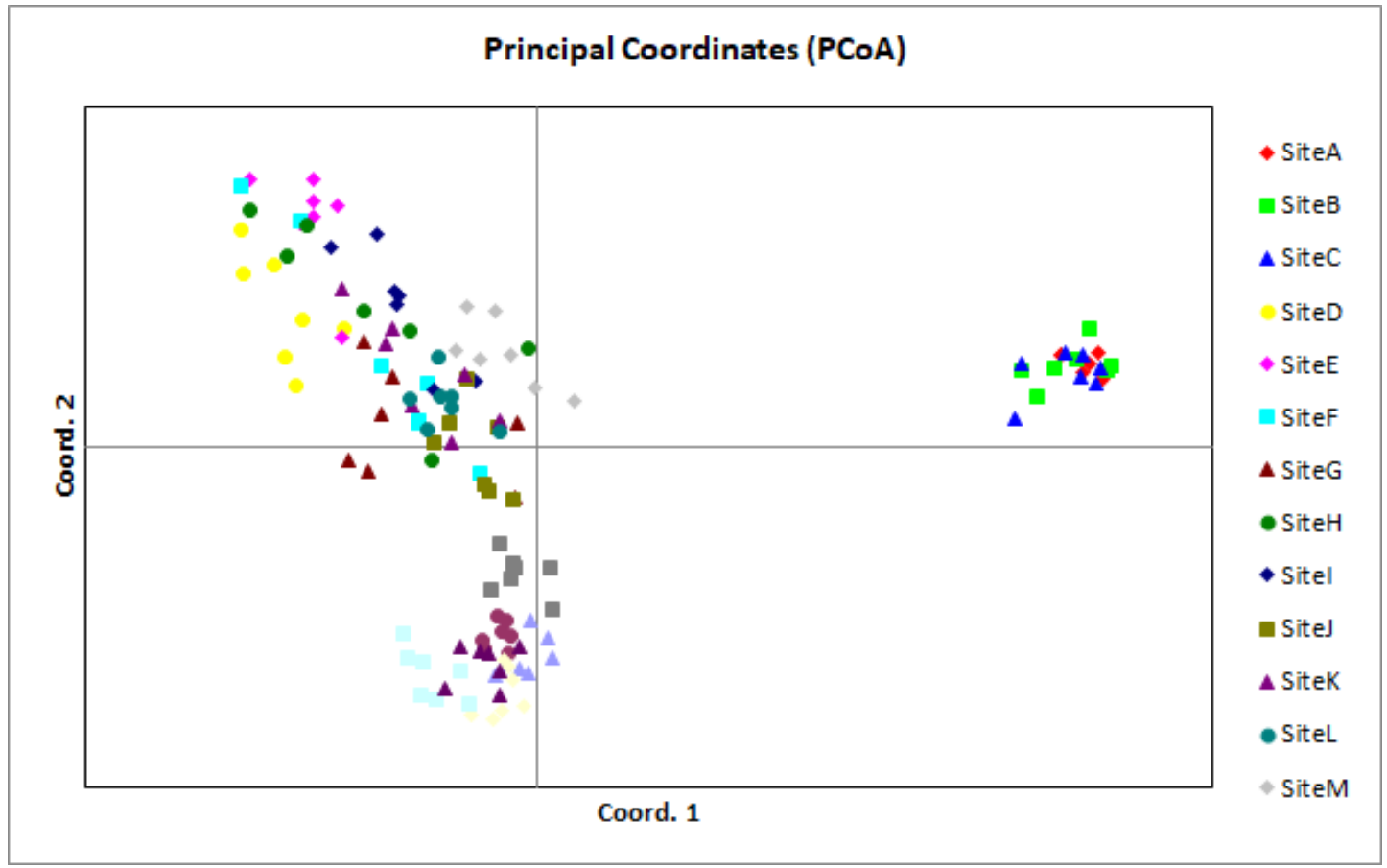
If the colour/symbol theme for the labels isn't very clear, change the style and/or colours →

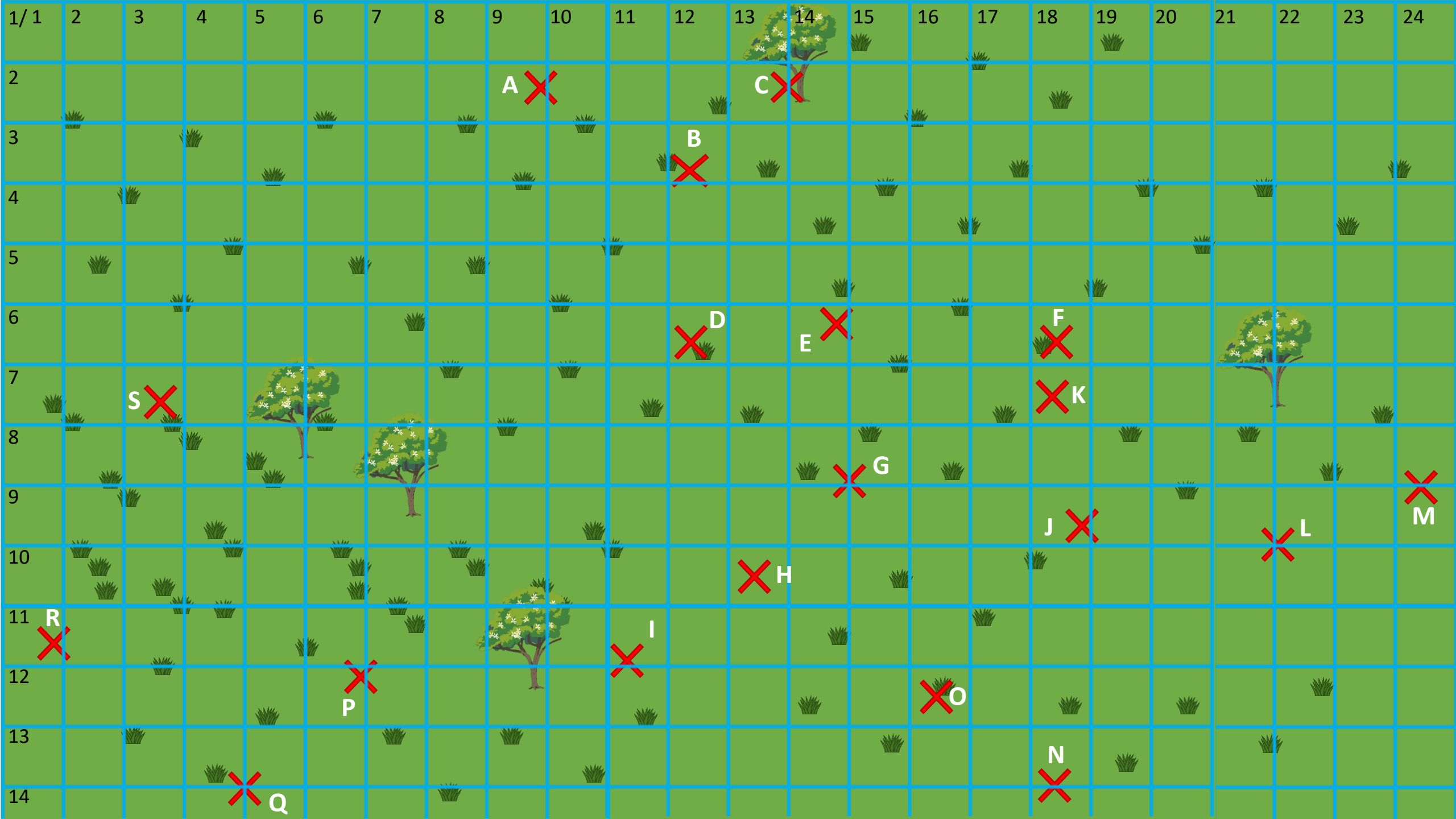


Are there any clear patterns in the PCoA?

Look at the sampling map..

How does the PCoA match with the sampling scheme?


Principal Coordinates (PCoA)
Coord. 2
Coord. 1
SiteA
SiteB
SiteC
SiteD
SiteE
SiteF
SiteG
SiteH
SiteI
SiteJ
SiteK
SiteL
SiteM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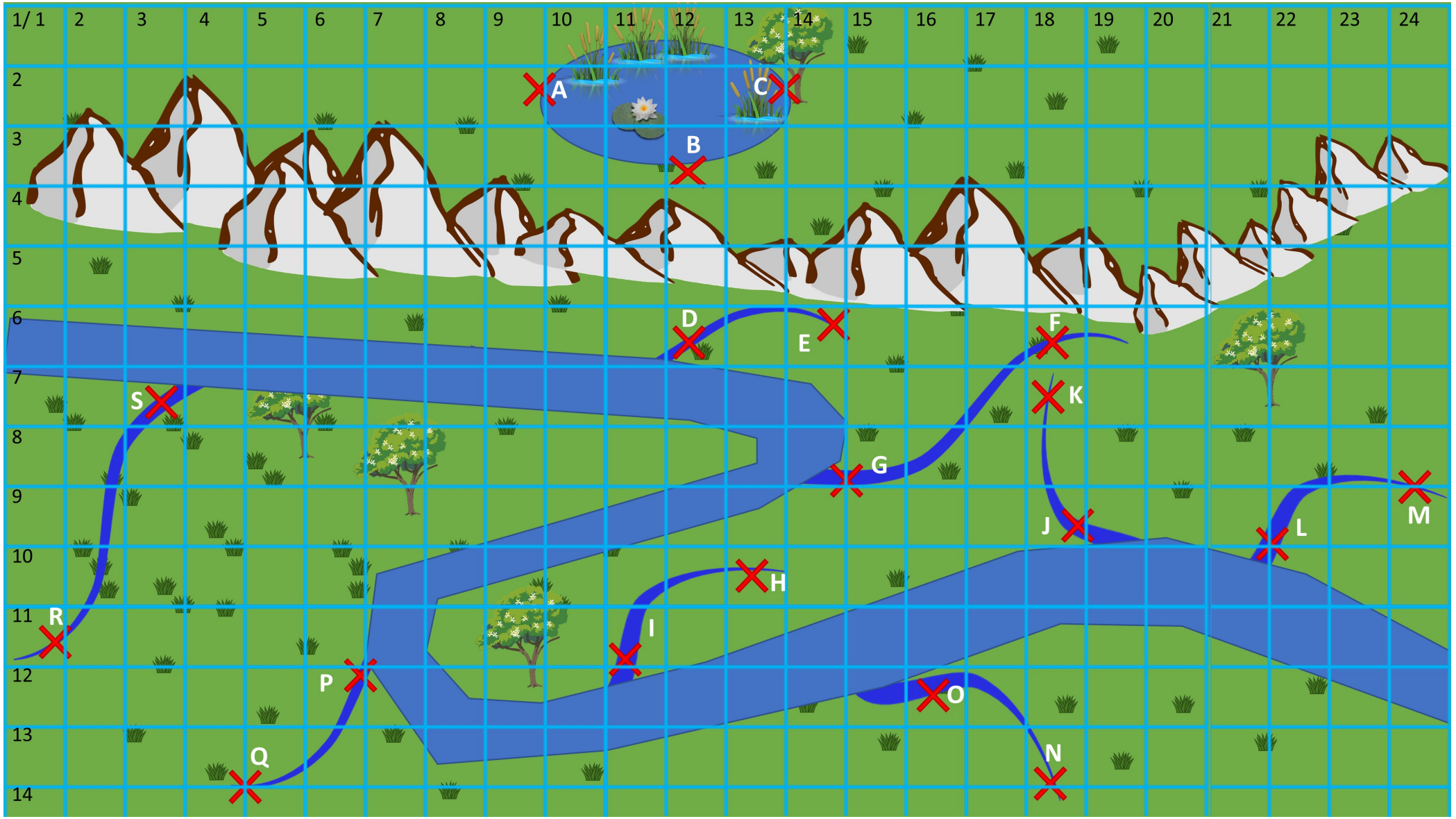
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Have another look at the Pairwise FST (FstL)

Do you see any patterns that reflect the PCoA results?

Let’s now include some landscape information

Can this explain any patterns we may have observed?

Spatial auto-correlation

Test whether there is a relationship between relatedness and geographic distance

Open the other datasets from iLearn:

Dataset 2a = North pop

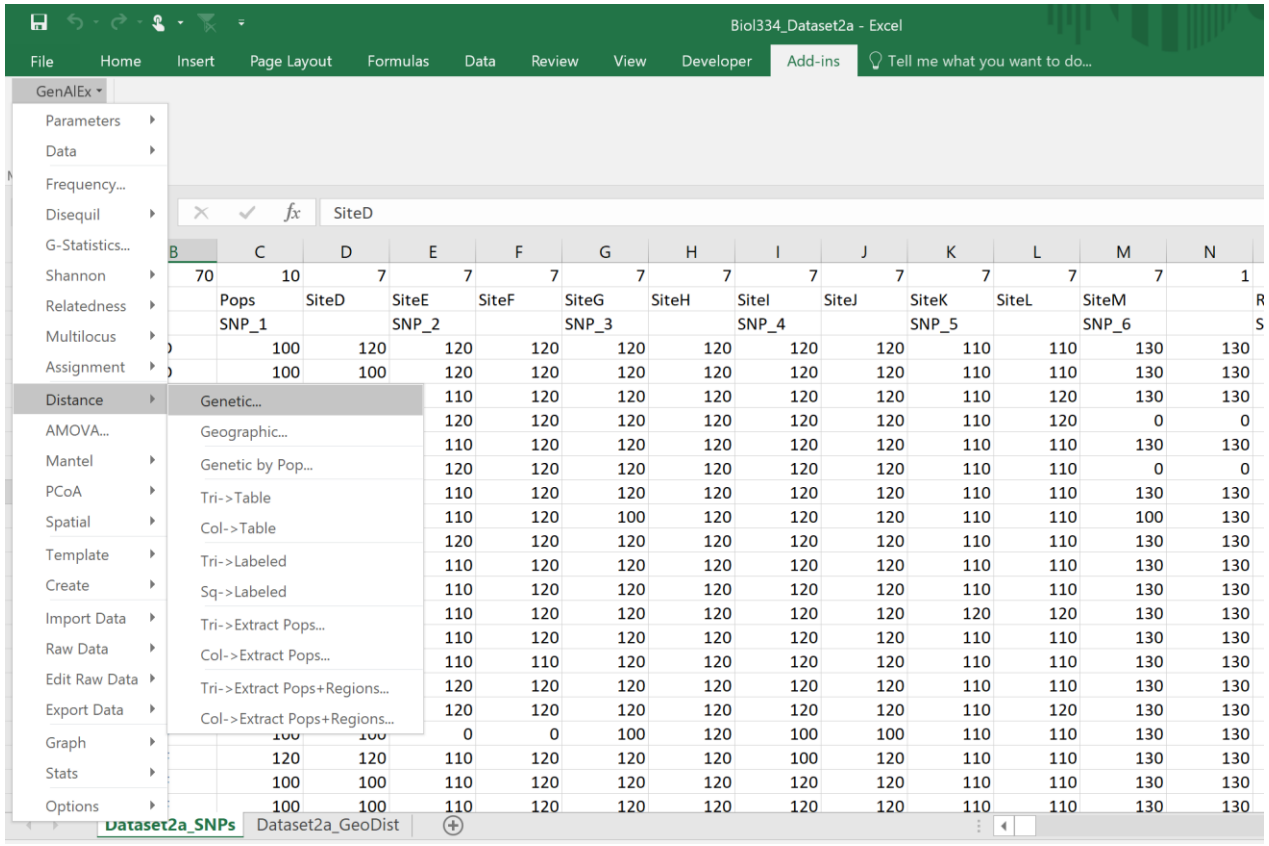
Dataset 2b = South pop

These datasets have a SNP data sheet, and GeoDist sheet with a matrix (in KM).

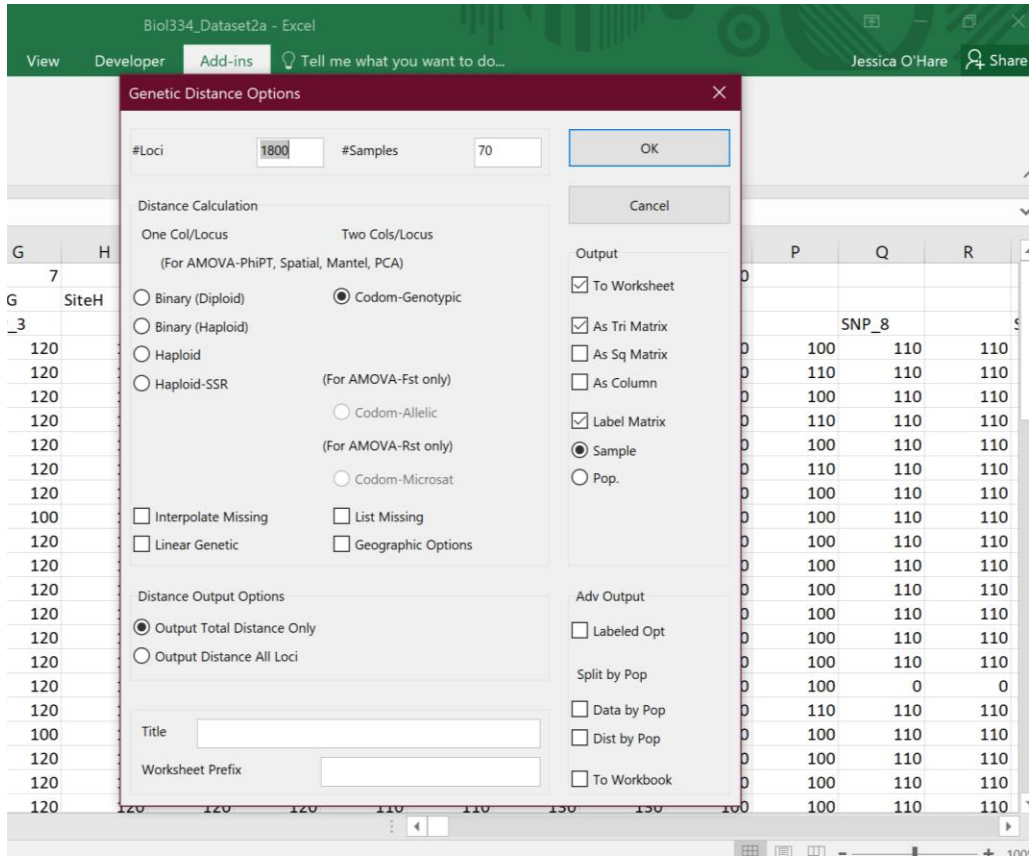
We used the grid numbers to generate geographic distance between sampling sites (“pops”)

Calculate Genetic Distance for both Datasets

GenAlEx > Distance > Genetic



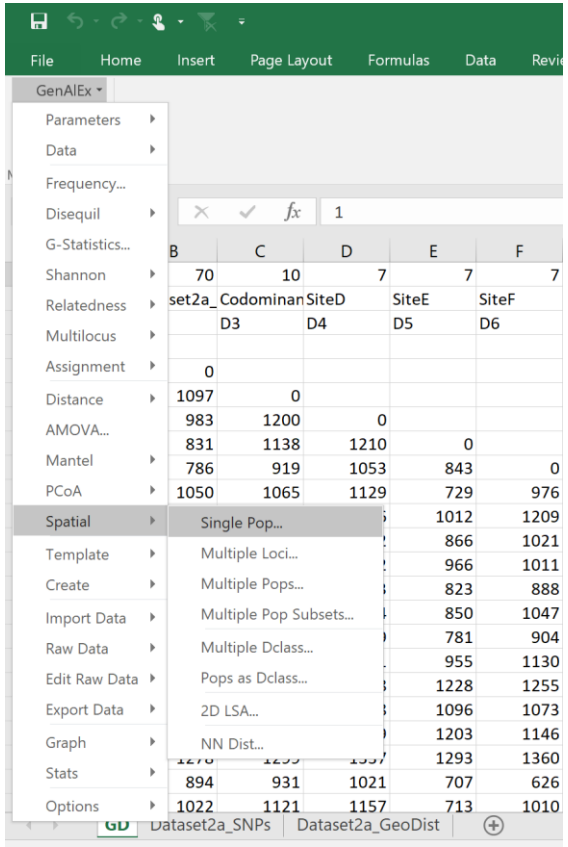
Leave defaults

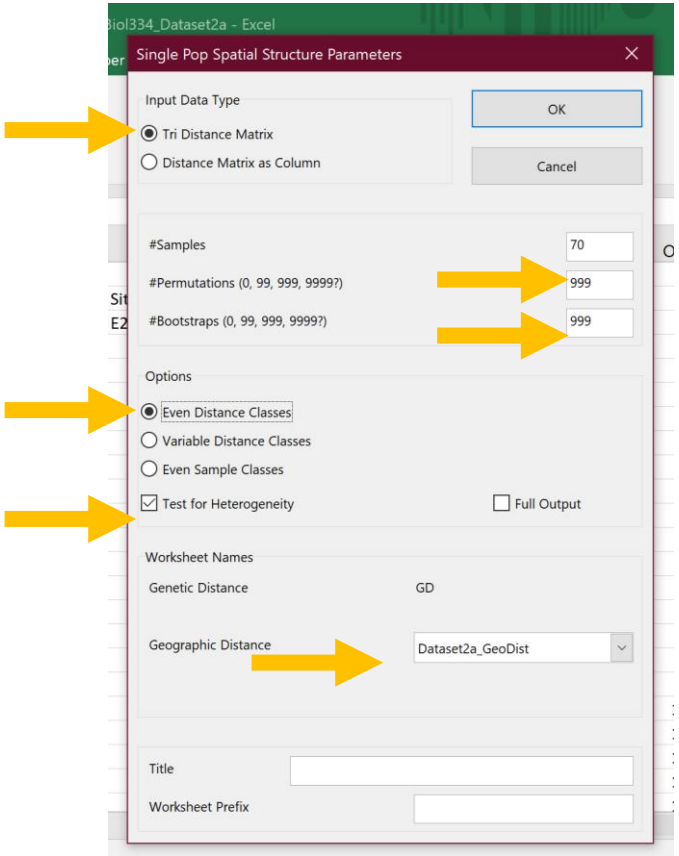


Compare Genetic and Geographic Distance

*From **within** the **GD** sheet:*

GenAlEx > Spatial > Single-pop





Tri-distance Matrix

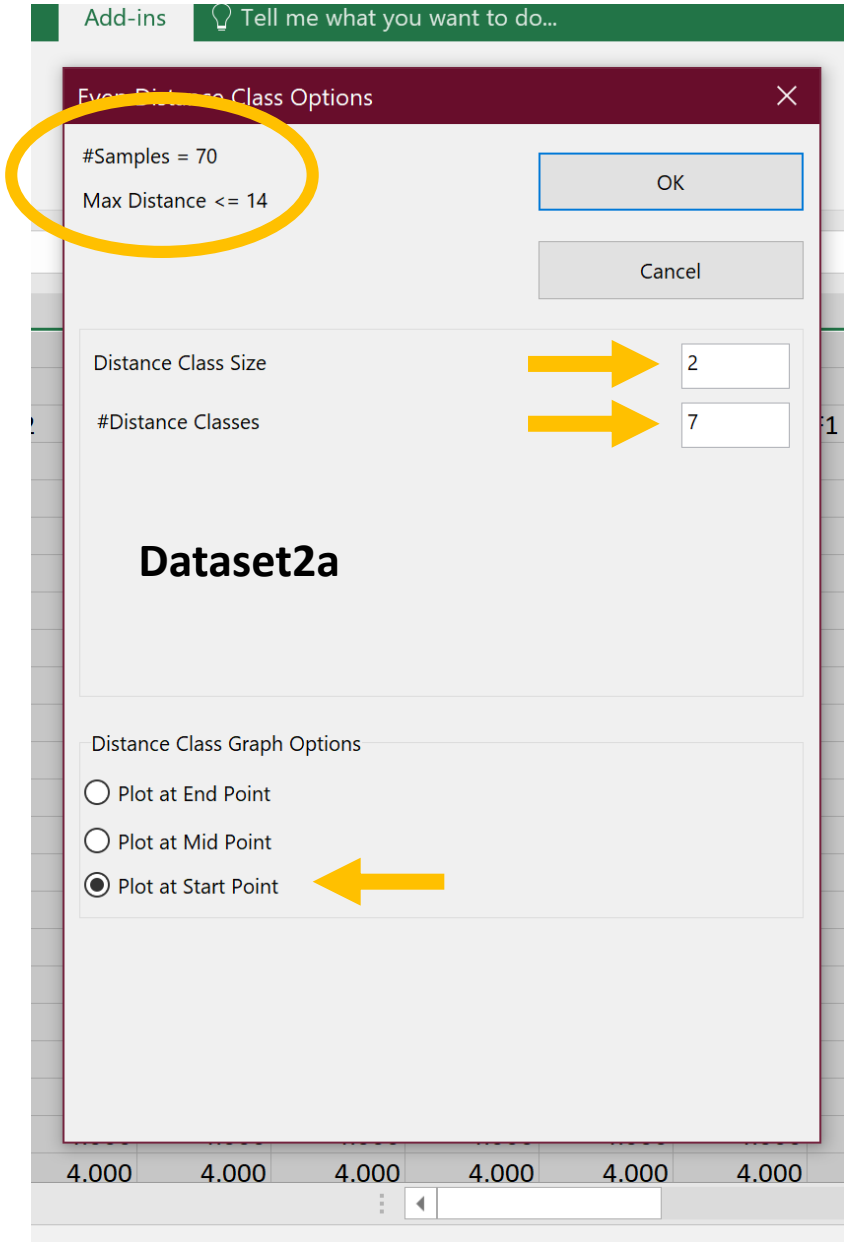
999 Permutations
999 Bootstraps

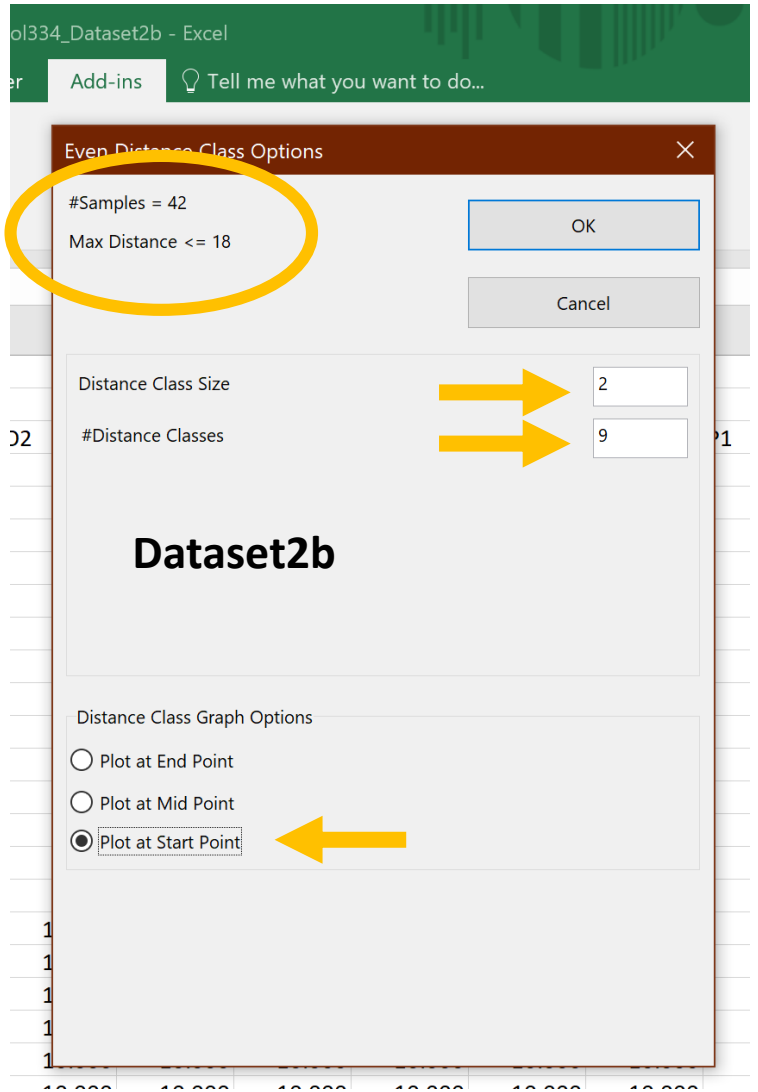
Even Distance Classes

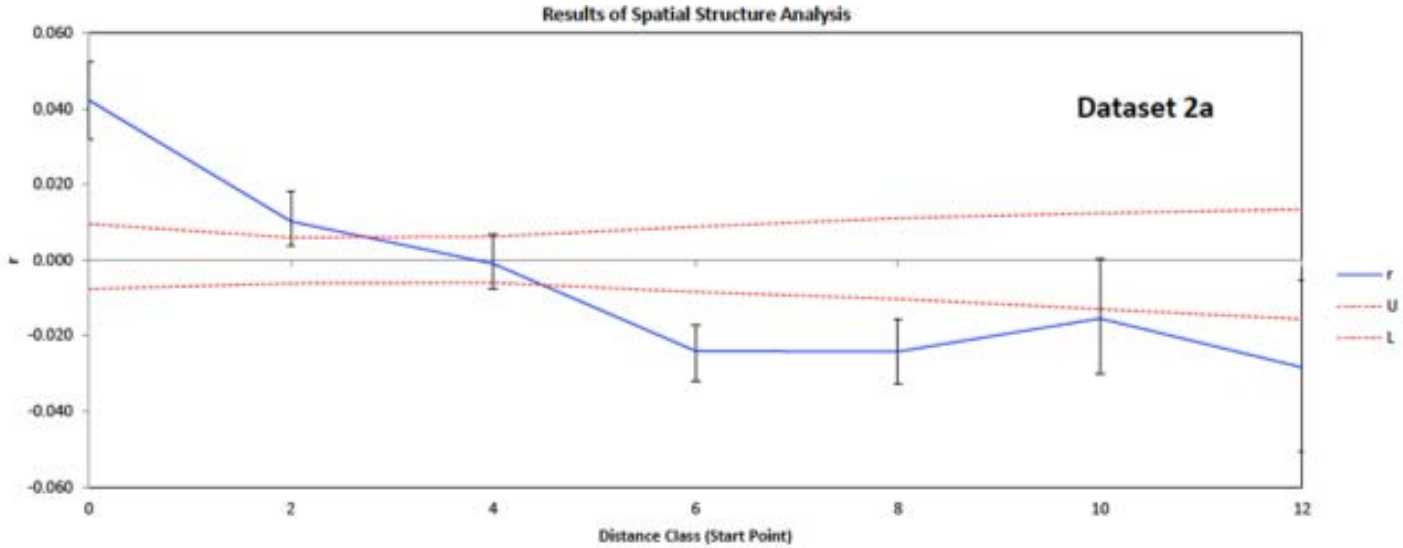
Test for Heterogeneity

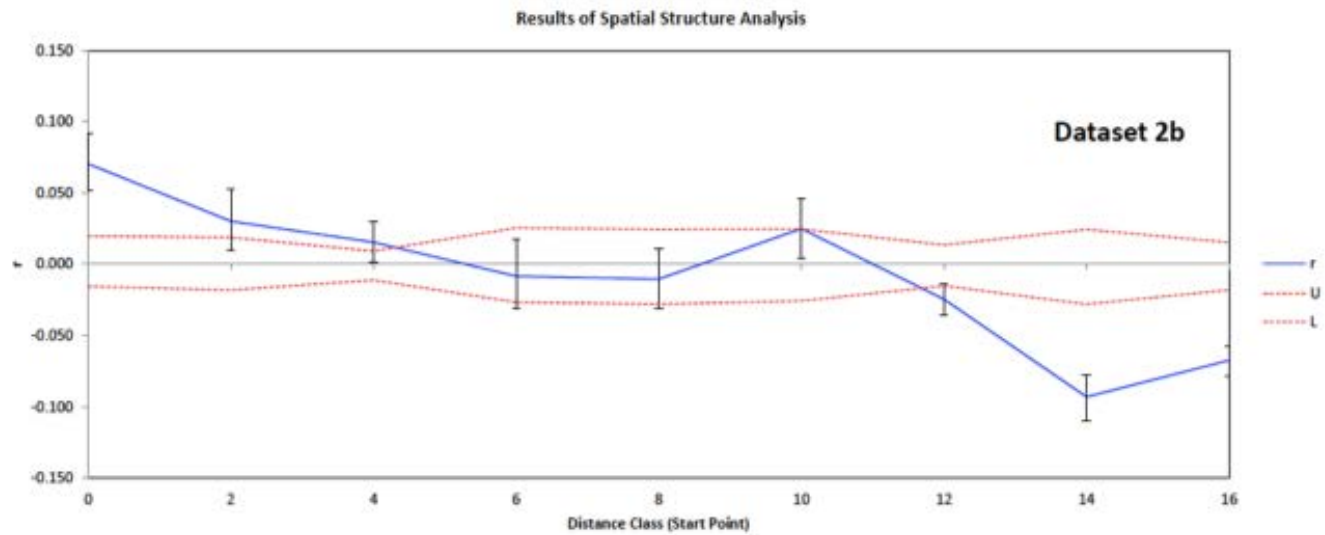
Match Geo Dist with the correct Geo Dist Sheet

Compare Genetic and Geographic Distance






Results of Spatial Structure Analysis
Dataset 2a
r
0.060
0.040
0.020
0.000
-0.020
-0.040
-0.060
0
2
4
6
8
10
12
Distance Class (Start Point)
r
U
L


Results of Spatial Structure Analysis
Dataset 2b
r
0.150
0.100
0.050
0.000
-0.050
-0.100
-0.150
0
2
4
6
8
10
12
14
16
Distance Class (Start Point)
r
U
L

Compare Genetic and Geographic Distance

What can you conclude from the correlogram (spatial autocorrelation plot)?

What are the red dashed lines?

What does the heterogeneity test tell us? What is the null hypothesis?

What does a significant result indicate?

Which distance classes are significantly different from what we expect under a null model?

Possible organism?

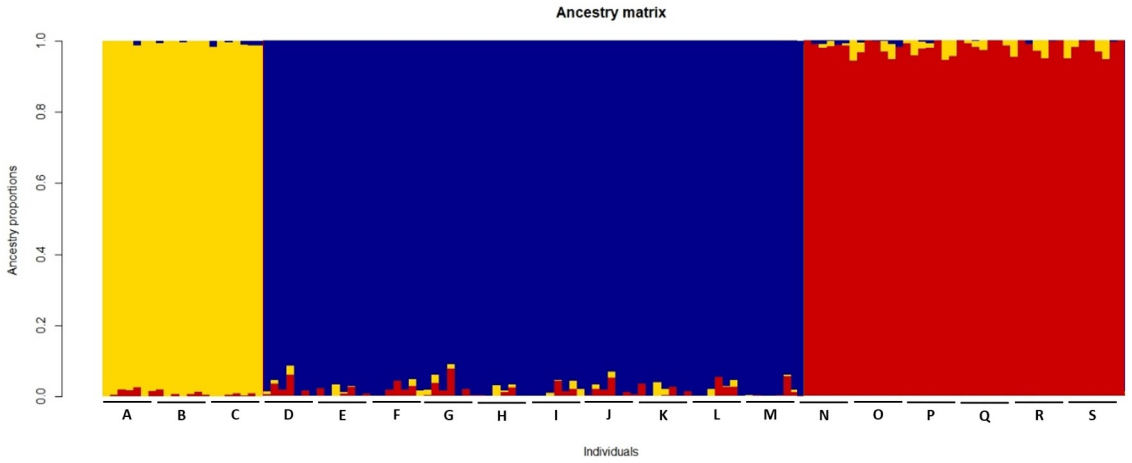
- Given the landscape patterns and genetic structure observed, what traits do you expect this organism to have?

Possible future work

- What are some other approaches that could help us better understand how environmental variables shape genetic variation?

- These can be suggested in the Discussion

The table
Calculate using linkage disequilibrium
As the population gets smaller, the incidence of linkage disequilibrium increases
The mountain group is isolated by mountain range - Elevated homozygosity - effective pop size is lower than the other 2 groups



3 genetic groups
Those groups are evident in PCoA and Fst
Admixture - group individuals into genetic group based on HW
Genetic approach
Look at genotypes that would be expected in HW
3 different colours representing 3 different groups - Proportion of genetic heritage that can be associated with those 3 groups
Individuals from each different group are pretty much linked to the group and not related with another group
Blue top - yellow middle - red bottom

Make sure you have saved all your results!!

Good luck preparing your Practical Report ☺