Action=BOLD

Object= "parenthesis"

Dialogue Box Title = *Italics*

Subsection = <u>underline</u>

GenAlEx stands for Genetic Analysis in Excel. It is an excel add in for population genetic analysis.

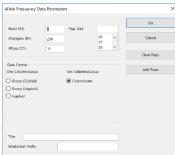
Data Data was generated from the raw data file, 'Microsatellite genotype data.xlsx', which was already in GenAlEx format. All data generated during the analysis is automatically saved as sheets in the excel workbook, and has been saved under 'microsatellite_genotype_genalex.xlsx'.

Analysis The analysis was done by working through the GenAlEx tutorials, published on the GenAlEx website, which are saved in the GenAlEx_tutorials folder.

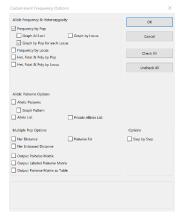
- 1) Organize the excel workbook into a readable format for the geneAlEx
- 2) Plot Allele Frequency

e.

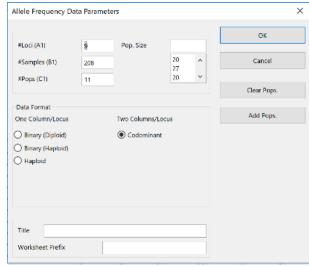
- a. **SELECT** "GenAlEx" from Ribbon
- b. **SELECT** "Frequency Based" button
- c. **SELECT** "Frequency" from GenAlEx drop down menu.
- d. Dialogue *Allele Frequency Data Parameters*
 - i. SELECT Codominant under Two Columns/Locus "OK"



- f. Dialogue Codominant Frequency Options SELECT
 - i. "Graph by Pop for Each locus"



- g. **SELECT** "OK"
- h. GenAlEx will spit a bunch of pie graphs and data sheets for us analyze showing the Allele frequency in each population by location.
- 3) Heterozygosity, F-statistics, and Allelic Patterns
 - a. SELECT "GenAlEx" from Ribbon
 - b. SELECT "Frequency Based" button
 - c. **SELECT** "Frequency" from GenAlEx drop down menu.
 - d. Dialogue Allele Frequency Data Parameters
 - i. SELECT "Codominant" under Two Columns/Locus "OK"



- e.
- f. Dialogue Codominant Frequency Options
 - i. SELECT "Frequency by Pop" under Allele Frequency and Heterozygosity.
 - ii. SELECT "Het, Fstat & Poly by Pop" under Allele Frequency and Heterozygosity.
 - iii. SELECT "Het, Fstat & Poly by Locus" under Allele Frequency and Heterozygosity.
 - iv. SELECT "Allelic Patterns" under Allelic Patterns Options
 - v. SELECT "Graph Pattern" under Allelic Patterns Options

Allele Frequency & Heterozygosity OK Frequency by Pop Graph All Loci Graph by Locus Frequency by Pop for each Locus Cancel Check All	Codominant Frequency Options	•		<
 ✓ Het, Fstat & Poly by Pop ✓ Het, Fstat & Poly by Locus Uncheck All 	Frequency by Pop Graph All Loci Graph by Pop for each Locus Frequency by Locus Het, Fstat & Poly by Pop		Cancel Check All	
Allelic Patterns Options Allelic Patterns Graph Pattern Allele List Private Alleles List	✓ Allelic Patterns ✓ Graph Pattern	Private Alleles List		
Multiple Pop Options Nei Distance Pairwise Fst Step by Step Output Pairwise Matrix Output Labeled Pairwise Matrix Output Pairwise Matrix as Table	Nei Distance Nei Unbiased Distance Output Pairwise Matrix Output Labeled Pairwise Matrix			

vi.

- g. SELECT "OK"
- h. More Graphs
- 4) Shannon Indices
 - a. **SELECT** "GenAlEx" from Ribbon
 - b. **SELECT** "Frequency Based" button
 - c. **SELECT** "Shannon" from GenAlEx drop down menu.
 - d. **SELECT** "Pairwise Pops"
 - e. Dialogue Allele Frequency Data Parameters
 - i. **SELECT "**Codominant" under Two Columns/Locus "OK"
 - f. Dialogue Pairwise Pops Shannon Analysis Options

Analysis & Output Options		
Output for Total Only		✓ Step by Step
Output for Each Locus		Output Freq.
Set sHua to Zero when Less Than	0.0001	Output Single Locus
Pairwise Options		For
Output Pairwise Matrices		SH(AP)
Output Labeled Pairwise Matric	ces	sH(AP) & e^sH(AP)
Output Pairwise Matrices as Ta	ble	☐ D'(AP) & O'(AP)
Full Pairwise Multilocus Analys	is	
Optional Estimated Pop Sizes Dat None	a	
○ Worksheet	Sheet1	
sHua-sHa-sHu!	ated pop sizes data must li	matrices including: sHua, G, Nm & st pop codes in col 2 and estimated pop ill be suppressed!
	Cancel	ОК

g. SELECT "OK"

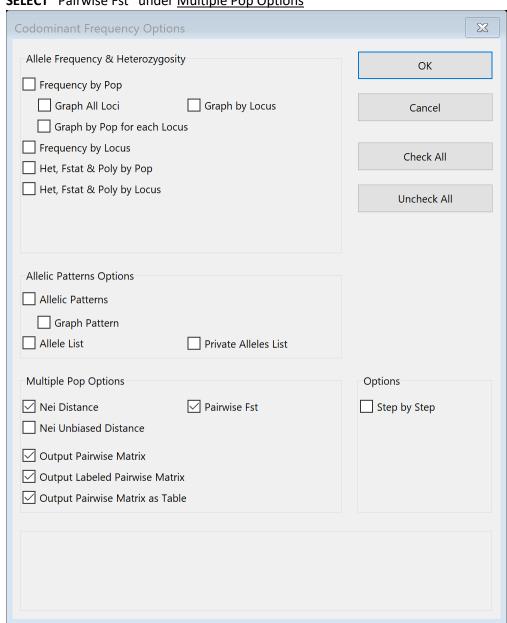
- 5) Nei Distance
 - a. **SELECT** "GenAlEx" from Ribbon
 - b. **SELECT** "Frequency Based" button
 - c. **SELECT** "Frequency" from GenAlEx drop down menu.
 - d. Dialogue Allele Frequency Data Parameters
 - i. **SELECT** Codominant under Two Columns/Locus "OK"
 - e. Dialogue Codominant Frequency Options
 - i. **SELECT** "Nei Distance" under <u>Multiple Pop Options</u>.

Codominant Frequency Options	×
Allele Frequency & Heterozygosity	OK
Frequency by Pop	
Graph All Loci Graph by Locus	Cancel
Graph by Pop for each Locus	
Frequency by Locus	Check All
Het, Fstat & Poly by Pop	
Het, Fstat & Poly by Locus	Uncheck All
Allelic Patterns Options	
Allelic Patterns	
Graph Pattern	
Allele List Private Alleles List	
Multiple Pop Options	Options
✓ Nei Distance Pairwise Fst	Step by Step
☐ Nei Unbiased Distance	
Output Pairwise Matrix	
Output Labeled Pairwise Matrix	
Output Pairwise Matrix as Table	

g. **SELECT** "OK"

- 6) Pairwise Fst and Nei Genetic Distances
 - a. **SELECT** "GenAlEx" from Ribbon
 - b. **SELECT** "Frequency Based" button
 - c. **SELECT** "Frequency" from GenAlEx drop down menu.
 - d. Dialogue Allele Frequency Data Parameters
 - i. **SELECT** Codominant under Two Columns/Locus "OK"
 - e. Dialogue Codominant Frequency Options
 - i. **SELECT** "Nei Distance" under <u>Multiple Pop Options</u>.
 - ii. **SELECT** "Output Pairwise Matrix" under <u>Multiple Pop Options.</u>
 - iii. SELECT "Output Labeled Pairwise Matrix" under Multiple Pop Options
 - iv. **SELECT** "Output Pairwise Matrix as Table" under Multiple Pop Options

v. SELECT "Pairwise Fst" under Multiple Pop Options



vi.

- f. **SELECT** "OK"
- 7) Pairwise Calculations of Shannon's Indices
 - a. SELECT "GenAlEx" from Ribbon
 - b. **SELECT** "Frequency Based" button
 - c. **SELECT** "Shannon" from GenAlEx drop down menu.
 - i. "Pairwise Pops"
 - d. Dialogue Shannon Pairwise Pops Data parameter
 - i. **SELECT** "Codominant" under two <u>Columns/Locus</u>

Shannon Pairwise Pc	ps Data Pa	rameters			X
#Loci (A1)	9	Pop. Size			OK
#Samples (B1)	208		20 27	^	Cancel
#Pops (C1)	11		20	~	Clear Pops.
Data Format One Column/Locus		Two Columns/	'Locus		Add Pops.
Binary (Diploid)		Codomina			
O Binary (Haploid) O Haploid					
Title					
Worksheet Prefix					

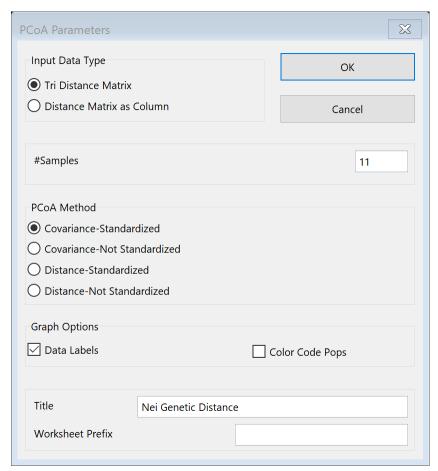
e. **SELECT** "OK"

Analysis & Output Options		
Output for Total Only		Step by Step
Output for Each Locus		Output Freq.
Set sHua to Zero when Less Than:	0.0001	Output Single Locus
Pairwise Options		For
Output Pairwise Matrices		sH(AP)
Output Labeled Pairwise Matric	es	sH(AP) & e^sH(AP)
Output Pairwise Matrices as Tal	ble	D'(AP) & O'(AP)
Full Pairwise Multilocus Analysis	S	
Optional Estimated Pop Sizes Data None	1	
○ Worksheet	Sheet1	
Check one or more Multiple Pop (Options to output pairwise	matrices including: sHua, G, Nm &
	stad man sizes data asset "	et non codes in col 2 and action at all and
The optional worksheet for estima		st pop codes in col 2 and estimated pop rill be suppressed!
size in col 3. Note that if this option		

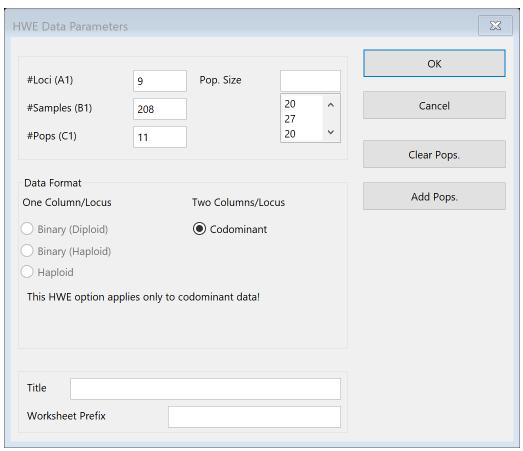
f. **SELECT** "OK"

g.

- 8) Principal Coordinate Analysis PCoA
 - a. **SELECT** "NeiP" sheet in workbook
 - b. **SELECT** "GenAlEx" button
 - c. **SELECT** "Distance Based" button
 - d. **SELECT** "PCoA" and move to Analysis
 - e. Dialogue PCoA Parameters



- f.
- g. SELECT "OK"
- 9) Hardy Weinberg Analysis
 - a. **SELECT** "GenAlEx" from Ribbon
 - b. **SELECT** "Disequil" button
 - c. **SELECT** "HWE" button
 - d. Dialogue HWE Data Parameters



- e. SELECT "OK"
- f. **SELECT** "OK" When next dialogue appears
- 10) Calculating Haploid Distance
 - a. **SELECT** "GenAlEx" from Ribbon
 - b. **SELECT** "Distance Based" button
 - c. **SELECT** "Genetic" from GenAlEx drop down menu.
 - d. Dialogue Genetic Distance Options
 - i. **SELECT** "Haploid" under <u>Distance Calculation</u>
 - ii. SELECT "Output Total Distance Only" under <u>Distance Output Options</u>
 - iii. SELECT "To Worksheet" under Output
 - iv. SELECT "As Tri Matrix" under Output
 - v. SELECT "Label Matrix" under Output
 - vi. **SELECT** "Pop" under Output

vii. SELECT "Labeled Opt" under Adv Output 23 Genetic Distance Options 9 208 OK #Loci #Samples Distance Calculation Cancel One Col/Locus Two Cols/Locus Output (For AMOVA-PhiPT, Spatial, Mantel, PCA) ✓ To Worksheet O Binary (Diploid) O Codom-Genotypic O Binary (Haploid) ✓ As Tri Matrix As Sq Matrix Haploid (For AMOVA-Fst and As Column O Haploid-SSR Ocodom-Allelic ✓ Label Matrix (For AMOVA-Rst and Sample Ocdom-Microsat Pop. Interpolate Missing List Missing Linear Genetic Geographic Options Distance Output Options Adv Output Output Total Distance Only ✓ Labeled Opt Output Distance All Loci Split by Pop Data by Pop

Dist by Pop

To Workbook

viii. SELECT "OK"

- 11) Calculating Codominant Genotypic Distance
 - a. **SELECT** "GenAlEx" from Ribbon

Title

Worksheet Prefix

- b. SELECT "Distance Based" button
- c. **SELECT** "Genetic" from GenAlEx drop down menu.
- d. Dialogue Genetic Distance Options
 - i. **SELECT** "Codom-Genotypic" under <u>Distance Calculation</u>
 - ii. **SELECT** "Output Total Distance Only" under <u>Distance Output Options</u>
 - iii. SELECT "To Worksheet" under Output
 - iv. SELECT "As Tri Matrix" under Output
 - v. **SELECT** "Label Matrix" under Output
 - vi. **SELECT** "Sample" under Output

ienetic Dista	nee option			
#Loci	1	#Samples	208	ОК
Distance Ca	Iculation			Cancel
One Col/Lo		Two Cols/Loc		Output
_		Spatial, Mantel, PCA		✓ To Worksheet
Binary (D		Codom-Ge	notypic	
O Binary (H	apioid)			✓ As Tri Matrix
O Haploid				As Sq Matrix
O Haploid-	SSR	(For AMOVA-Fst		As Column
		O Codom-All	elic	✓ Label Matrix
		(For AMOVA-Rsf	t and	Sample
		O Codom-Mi	crosat	O Pop.
☐ Interpola	ite Missing	List Missin	a	○ Genotype
Linear G		Geographi		
Distance Ou	ıtput Optio	ns		Adv Output
Output 1	otal Distan	ce Only		Labeled Opt
Output [
				Split by Pop
				Data by Pop
Title				Dist by Pop
Worksheet	Prefix			☐ To Workbook

vii. SELECT "OK"

12) Calculating Binary Genetic Distance

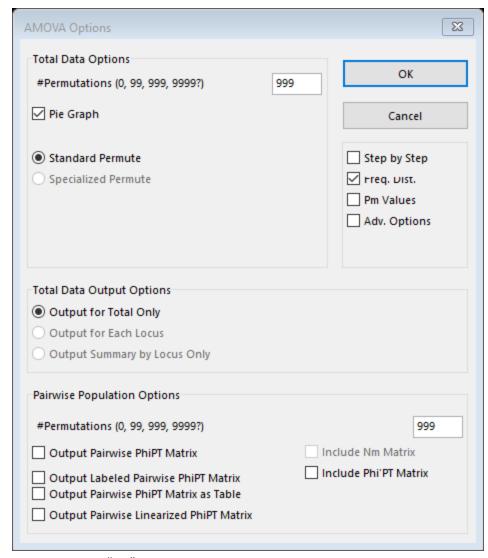
- a. **SELECT** "GenAlEx" from Ribbon
- b. **SELECT** "Distance Based" button
- c. **SELECT** "Genetic" from GenAlEx drop down menu.
- d. Dialogue Genetic Distance Options
 - i. **SELECT** "Binary (Diploid)" under <u>Distance Calculation</u>
 - ii. **SELECT** "Output Total Distance Only" under <u>Distance Output Options</u>
 - iii. **SELECT** "To Worksheet" under <u>Output</u>
 - iv. **SELECT** "As Tri Matrix" under <u>Output</u>
 - v. **SELECT** "Label Matrix" under <u>Output</u>
 - vi. **SELECT** "Sample" under <u>Output</u>

#Loci	9	#Samples	208	OK
Distance Cal	culation			Cancel
Binary (Di Binary (Ha Haploid Haploid-S	VA-PhiPT, S ploid) aploid) SSR	Two Cols/Loc Spatial, Mantel, PCA O Codom-Ge (For AMOVA-Fst O Codom-Allo (For AMOVA-Rst O Codom-Mid	notypic and elic and crosat	Output To Worksheet As Tri Matrix As Sq Matrix As Column Label Matrix Sample Pop.
Distance Out Output To	otal Distanc	ce Only		Adv Output Labeled Opt Split by Pop Data by Pop

vii.

viii. **SELECT** "OK"

- 13) AMOVA (Analysis of Molecular Variance)
 - a. **SELECT** "GenAlEx" from Ribbon
 - b. **SELECT** "Distance Based" button
 - c. **SELECT** "AMOVA" from GenAlEx drop down menu.
 - d. Dialogue Genetic Distance Options
 - e. **SELECT** "Raw Data"

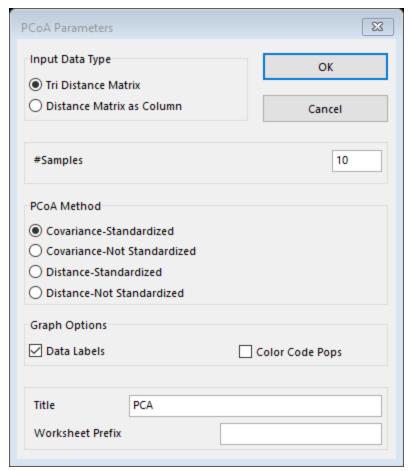


i. **SELECT** "OK"

14) More PCoA

f.

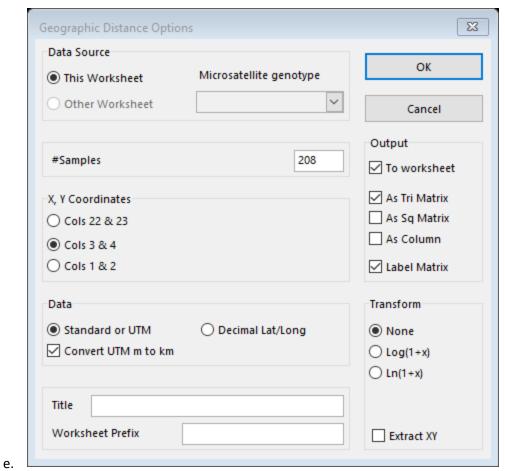
- a. **SELECT** sheet "PhiPTP"
- b. SELECT "GenAlEx" button
- c. **SELECT** "Distance Based" button
- d. **SELECT** "PCoA" and move to Analysis
- e. Dialogue PCoA Parameters



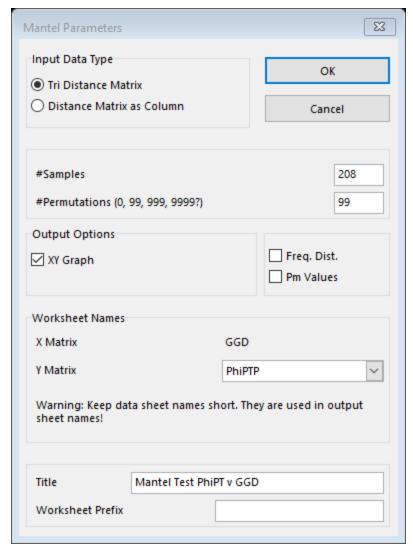
g. **SELECT** "OK"

f.

- 15) Mantel Tests for Isolation-by-Distance
 - a. **SELECT** "GenAlEx" from Ribbon
 - b. **SELECT** "Distance Based" button
 - c. **SELECT** "Geographic Distance Options" from GenAlEx drop down menu.
 - d. Dialogue Geographic Distance Options



- f. **SELECT** "OK"
- g. **SELECT** sheet "GGD" to become x matrix
- h. **SELECT** "Distance Based" button
- i. **SELECT** "Mantel" button
- j. **SELECT** "Paired" button
- k. Dialogue Mantel Parameters

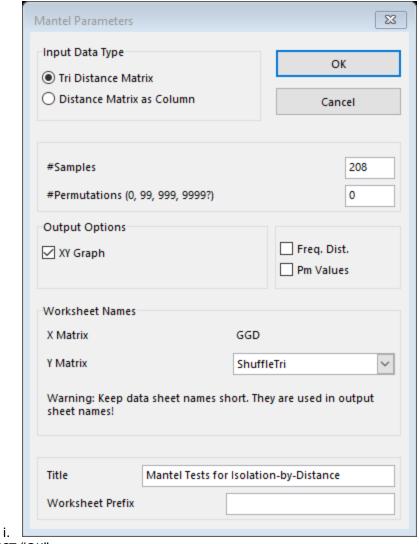


m. SELECT "OK"

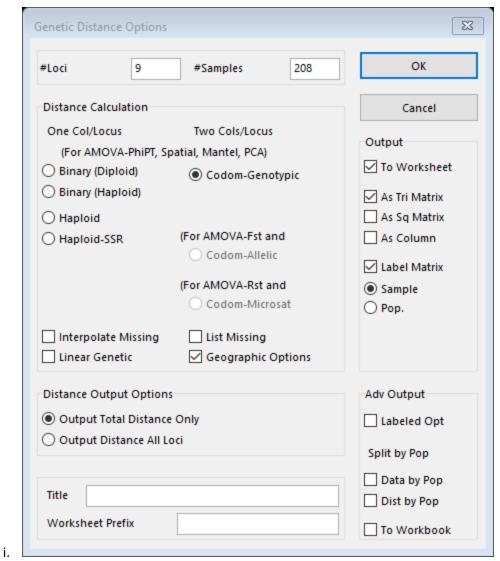
١.

16) Statistical Testing for Mantel

- a. **SELECT** "PhiPTP" sheet
- b. **SELECT** "Color Rand" button
- c. **SELECT** "Rand Data" button
- d. **SELECT** "Shuffle Tri" button
- e. **SELECT** "GGD" sheet
- f. **SELECT** "Distance-Based" button
- g. **SELECT** "Mantel" button
- h. **SELECT** "Paired" button
- i. Dialogue Mantel Parameters



- j. **SELECT** "OK"
- k. Repeat to calculate an average.
- 17) Global Spatial Autocorrelation
 - a. **SELECT** sheet "GGD"
 - b. **SELECT** "GenAlEx" from Ribbon
 - c. **SELECT** "Distance Based" button
 - d. **SELECT** "Geographic Distance Options" from GenAlEx drop down menu.
 - e. Dialogue Geographic Distance Options



- f. **SELECT** "OK"
- g. Dialogue Geographic Distance Options

This Worksheet	Microsatellite genotype	ОК
Other Worksheet	PhiPTP	Cancel
#Samples	208	
X, Y Coordinates		
 Cols 22 & 23 (After get) 	netic data)	
O Cols 3 & 4 (Other Wo	rksheet)	
O Cols 1 & 2 (Other Wo	rksheet)	
Data		Transform
Standard or UTM	O Decimal Lat/Long	None
Convert UTM m to km		O Log(1+x)
		○ Ln(1+x)
Title		

h. **SELECT** "OK"

Using this excel addon I was able to create some meaningful data analysis of previously collected and used raw data he program was simple to use and required mostly hitting buttons.