

Action=**BOLD**

Object= “parenthesis”

Dialogue Box Title = *Italics*

Subsection = underline

GenAEx 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 GenAEx 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 GenAEx tutorials, published on the GenAEx website, which are saved in the GenAEx\_tutorials folder.

- 1) Organize the excel workbook into a readable format for the geneAEx
- 2) Plot Allele Frequency
  - a. **SELECT** “GenAEx” from Ribbon
  - b. **SELECT** “Frequency Based” button
  - c. **SELECT** “Frequency” from GenAEx drop down menu.
  - d. Dialogue *Allele Frequency Data Parameters*
    - i. **SELECT** Codominant under Two Columns/Locus “OK”

Allele Frequency Data Parameters

#Loci (A1) 9 Pop. Size 20  
#Samples (B1) 228 27  
#Popu (C1) 11 20

Data Format:  
One Column/Locus  
☐ Binary (Diploid)  
☐ Binary (Haploid)  
☐ Haploid  
☒ Codominant

Title  
Worksheet Prefix

OK  
Cancel  
Clear Pops.  
Add Pops.

- e.
- f. Dialogue *Codominant Frequency Options* **SELECT**
  - i. “Graph by Pop for Each locus”

ii.

- g. **SELECT “OK”**
  - h. GenAEx 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 “GenAEx”** from Ribbon
  - b. **SELECT “Frequency Based”** button
  - c. **SELECT “Frequency”** from GenAEx 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

### Codominant Frequency Options



#### Allele Frequency & Heterozygosity

☒ Frequency by Pop
 

☐ Graph All Loci
 ☐ Graph by Locus
 ☐ Graph by Pop for each Locus

☐ Frequency by Locus
 ☒ Het, Fstat & Poly by Pop
 ☒ Het, Fstat & Poly by Locus

#### Allelic Patterns Options

☒ Allelic Patterns
 

☒ Graph Pattern
 ☐ Allele List
 ☐ Private Alleles List

#### Multiple Pop Options

☐ Nei Distance
 ☐ Pairwise Fst
 ☐ Nei Unbiased Distance
 ☐ Output Pairwise Matrix
 ☐ Output Labeled Pairwise Matrix
 ☐ Output Pairwise Matrix as Table

#### Options

☐ Step by Step

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*

Shannon Pairwise Pops Analysis Options

Analysis & Output Options

☐ Output for Total Only

☒ Output for Each Locus

Set sHua to Zero when Less Than:

☒ Step by Step

☐ Output Freq.

☐ Output Single Locus

Pairwise Options

☐ Output Pairwise Matrices

☐ Output Labeled Pairwise Matrices

☒ Output Pairwise Matrices as Table

☐ Full Pairwise Multilocus Analysis

For

☐ sH(AP)

☐ sH(AP) & e<sup>s</sup>H(AP)

☐ D'(AP) & O'(AP)

Log Base Options

☐ Log Base e (Ln) ☒ Log Base 2 ☐ Log Base 10

Optional Estimated Pop Sizes Data

☒ None

☐ Worksheet

Check one or more Multiple Pop Options to output pairwise matrices including: sHua, G, Nm & sHua-sHa-sHu!

The optional worksheet for estimated pop sizes data must list pop codes in col 2 and estimated pop size in col 3. Note that if this option is selected G-analysis will be suppressed!

Cancel OK

i.

g. **SELECT "OK"**

## 5) Nei Distance

- SELECT "GenAlEx"** from Ribbon
- SELECT "Frequency Based"** button
- SELECT "Frequency"** from GenAlEx drop down menu.
- Dialogue *Allele Frequency Data Parameters*
  - SELECT** Codominant under Two Columns/Locus **"OK"**
- Dialogue *Codominant Frequency Options*
  - SELECT "Nei Distance"** under Multiple Pop Options.

Codominant Frequency Options

Allele Frequency & Heterozygosity

☐ Frequency by Pop

☐ Graph All Loci ☐ Graph by Locus

☐ Graph by Pop for each Locus

☐ Frequency by Locus

☐ Het, Fstat & Poly by Pop

☐ Het, Fstat & Poly by Locus

Allelic Patterns Options

☐ Allelic Patterns

☐ Graph Pattern

☐ Allele List ☐ Private Alleles List

Multiple Pop Options

☒ Nei Distance ☐ Pairwise Fst

☐ Nei Unbiased Distance

☐ Output Pairwise Matrix

☐ Output Labeled Pairwise Matrix

☐ Output Pairwise Matrix as Table

Options

☐ Step by Step

OK

Cancel

Check All

Uncheck All

- f.
  - g. **SELECT "OK"**
- 6) Pairwise Fst and Nei Genetic Distances
- a. **SELECT "GenAEx"** from Ribbon
  - b. **SELECT "Frequency Based"** button
  - c. **SELECT "Frequency"** from GenAEx 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 Multiple Pop Options.
    - ii. **SELECT** "Output Pairwise Matrix" under Multiple Pop Options.
    - 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

Codominant Frequency Options

Allele Frequency & Heterozygosity

☐ Frequency by Pop

☐ Graph All Loci ☐ Graph by Locus

☐ Graph by Pop for each Locus

☐ Frequency by Locus

☐ Het, Fstat & Poly by Pop

☐ Het, Fstat & Poly by Locus

Allelic Patterns Options

☐ Allelic Patterns

☐ Graph Pattern

☐ Allele List ☐ Private Alleles List

Multiple Pop Options

☒ Nei Distance ☒ Pairwise Fst

☐ Nei Unbiased Distance

☒ Output Pairwise Matrix

☒ Output Labeled Pairwise Matrix

☒ Output Pairwise Matrix as Table

Options

☐ Step by Step

OK

Cancel

Check All

Uncheck All

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 Columns/Locus

Shannon Pairwise Pops Data Parameters ✕

#Loci (A1)	9	Pop. Size	
#Samples (B1)	208		20 27 20
#Pops (C1)	11		

Data Format

One Column/Locus      Two Columns/Locus

☐ Binary (Diploid)      ☒ Codominant

☐ Binary (Haploid)

☐ Haploid

Title

Worksheet Prefix

OK

Cancel

Clear Pops.

Add Pops.

- ii.
- e. **SELECT "OK"**

Shannon Pairwise Pops Analysis Options

**Analysis & Output Options**

☐ Output for Total Only

☒ Output for Each Locus

Set sHua to Zero when Less Than:

☐ Step by Step

☐ Output Freq.

☐ Output Single Locus

**Pairwise Options**

☐ Output Pairwise Matrices

☐ Output Labeled Pairwise Matrices

☒ Output Pairwise Matrices as Table

☐ Full Pairwise Multilocus Analysis

**For**

☐ sH(AP)

☐ sH(AP) & e<sup>s</sup>H(AP)

☐ D'(AP) & O'(AP)

**Log Base Options**

☐ Log Base e (Ln) ☒ Log Base 2 ☐ Log Base 10

**Optional Estimated Pop Sizes Data**

☒ None

☐ Worksheet

Check one or more Multiple Pop Options to output pairwise matrices including: sHua, G, Nm & sHua-sHa-sHu!

The optional worksheet for estimated pop sizes data must list pop codes in col 2 and estimated pop size in col 3. Note that if this option is selected G-analysis will be suppressed!

Cancel OK

- i.
- 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*



PCoA Parameters

Input Data Type

☒ Tri Distance Matrix

☐ Distance Matrix as Column

OK

Cancel

#Samples 11

PCoA Method

☒ Covariance-Standardized

☐ Covariance-Not Standardized

☐ Distance-Standardized

☐ Distance-Not Standardized

Graph Options

☒ Data Labels

☐ Color Code Pops

Title Nei Genetic Distance

Worksheet Prefix

- 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*

**HWE Data Parameters** [X]

#Loci (A1)	9	Pop. Size	
#Samples (B1)	208		20 27 20
#Pops (C1)	11		

**Data Format**

One Column/Locus      Two Columns/Locus

☐ Binary (Diploid)      ☒ Codominant  
☐ Binary (Haploid)  
☐ Haploid

This HWE option applies only to codominant data!

Title

Worksheet Prefix

OK  
Cancel  
Clear Pops.  
Add Pops.

- i.
  - 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 Distance Calculation
    - ii. **SELECT "Output Total Distance Only"** under Distance Output Options
    - 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

- viii. **SELECT** “OK”

11) Calculating Codominant Genotypic Distance

- a. **SELECT** “GenAEx” from Ribbon
- b. **SELECT** “Distance Based” button
- c. **SELECT** “Genetic” from GenAEx drop down menu.
- d. Dialogue *Genetic Distance Options*
  - i. **SELECT** “Codom-Genotypic” under Distance Calculation
  - ii. **SELECT** “Output Total Distance Only” under Distance Output Options
  - iii. **SELECT** “To Worksheet” under Output
  - iv. **SELECT** “As Tri Matrix” under Output
  - v. **SELECT** “Label Matrix” under Output
  - vi. **SELECT** “Sample” under Output

Genetic Distance Options

#Loci: 1 #Samples: 208

OK Cancel

**Distance Calculation**

One Col/Locus Two Cols/Locus  
(For AMOVA-PhiPT, Spatial, Mantel, PCA)

☐ Binary (Diploid) ☒ Codom-Genotypic

☐ Binary (Haploid)

☐ Haploid

☐ Haploid-SSR (For AMOVA-Fst and

☐ Codom-Allelic

(For AMOVA-Rst and

☐ Codom-Microsat

☐ Interpolate Missing ☐ List Missing

☐ Linear Genetic ☐ Geographic Options

**Distance Output Options**

☒ Output Total Distance Only

☐ Output Distance All Loci

Title:

Worksheet Prefix:

**Output**

☒ To Worksheet

☒ As Tri Matrix

☐ As Sq Matrix

☐ As Column

☒ Label Matrix

☒ Sample

☐ Pop.

☐ Genotype

**Adv Output**

☐ Labeled Opt

Split by Pop

☐ Data by Pop

☐ Dist by Pop

☐ To Workbook

vii.

viii. **SELECT "OK"**

## 12) Calculating Binary Genetic Distance

- SELECT "GenAlEx"** from Ribbon
- SELECT "Distance Based"** button
- SELECT "Genetic"** from GenAlEx drop down menu.
- Dialogue *Genetic Distance Options*
  - SELECT "Binary (Diploid)"** under Distance Calculation
  - SELECT "Output Total Distance Only"** under Distance Output Options
  - SELECT "To Worksheet"** under Output
  - SELECT "As Tri Matrix"** under Output
  - SELECT "Label Matrix"** under Output
  - SELECT "Sample"** under Output

Genetic Distance Options

#Loci: 9 #Samples: 208

OK Cancel

Distance Calculation

One Col/Locus Two Cols/Locus  
(For AMOVA-PhiPT, Spatial, Mantel, PCA)

☒ Binary (Diploid) ☐ Codom-Genotypic

☐ Binary (Haploid)

☐ Haploid

☐ Haploid-SSR (For AMOVA-Fst and

☐ Codom-Allelic

(For AMOVA-Rst and

☐ Codom-Microsat

☐ Interpolate Missing ☐ List Missing

☐ Linear Genetic ☐ Geographic Options

Output

☒ To Worksheet

☒ As Tri Matrix

☐ As Sq Matrix

☐ As Column

☒ Label Matrix

☒ Sample

☐ Pop.

Distance Output Options

☒ Output Total Distance Only

☐ Output Distance All Loci

Adv Output

☐ Labeled Opt

Split by Pop

☐ Data by Pop

☐ Dist by Pop

☐ To Workbook

Title

Worksheet Prefix

vii.

viii. **SELECT "OK"**

13) AMOVA (Analysis of Molecular Variance)

- SELECT "GenAlEx"** from Ribbon
- SELECT "Distance Based"** button
- SELECT "AMOVA"** from GenAlEx drop down menu.
- Dialogue *Genetic Distance Options*
- SELECT "Raw Data"**

AMOVA Options

Total Data Options

#Permutations (0, 99, 999, 9999?)

☒ Pie Graph

☒ Standard Permute  
☐ Specialized Permute

☐ Step by Step  
☒ Freq. Dist.  
☐ Pm Values  
☐ Adv. Options

OK  
Cancel

Total Data Output Options

☒ Output for Total Only  
☐ Output for Each Locus  
☐ Output Summary by Locus Only

Pairwise Population Options

#Permutations (0, 99, 999, 9999?)

☐ Output Pairwise PhiPT Matrix  
☐ Output Labeled Pairwise PhiPT Matrix  
☐ Output Pairwise PhiPT Matrix as Table  
☐ Output Pairwise Linearized PhiPT Matrix

☐ Include Nm Matrix  
☐ Include Phi'PT Matrix

f.

i. **SELECT "OK"**

#### 14) More PCoA

- SELECT** sheet "PhiPTP"
- SELECT** "GenALEX" button
- SELECT** "Distance Based" button
- SELECT** "PCoA" and move to Analysis
- Dialogue *PCoA Parameters*

PCoA Parameters

Input Data Type

☒ Tri Distance Matrix

☐ Distance Matrix as Column

OK

Cancel

#Samples 10

PCoA Method

☒ Covariance-Standardized

☐ Covariance-Not Standardized

☐ Distance-Standardized

☐ Distance-Not Standardized

Graph Options

☒ Data Labels ☐ Color Code Pops

Title PCA

Worksheet Prefix

- f.
  - g. **SELECT "OK"**
- 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*

Geographic Distance Options

**Data Source**

☒ This Worksheet      Microsatellite genotype

☐ Other Worksheet     

**#Samples**     

**X, Y Coordinates**

☐ Cols 22 & 23

☒ Cols 3 & 4

☐ Cols 1 & 2

**Data**

☒ Standard or UTM      ☐ Decimal Lat/Long

☒ Convert UTM m to km

**Title**     

**Worksheet Prefix**     

**Output**

☒ To worksheet

☒ As Tri Matrix

☐ As Sq Matrix

☐ As Column

☒ Label Matrix

**Transform**

☒ None

☐ Log(1+x)

☐ Ln(1+x)

☐ Extract XY

OK

Cancel

- e.
- 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*



Mantel Parameters

Input Data Type

☒ Tri Distance Matrix

☐ Distance Matrix as Column

OK

Cancel

#Samples 208

#Permutations (0, 99, 999, 9999?) 99

Output Options

☒ XY Graph

☐ Freq. Dist.

☐ Pm Values

Worksheet Names

X Matrix GGD

Y Matrix PhiPTP

Warning: Keep data sheet names short. They are used in output sheet names!

Title Mantel Test PhiPTP v GGD

Worksheet Prefix

- l.
  - m. **SELECT "OK"**
- 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*

Mantel Parameters

Input Data Type

☒ Tri Distance Matrix

☐ Distance Matrix as Column

OK

Cancel

#Samples 208

#Permutations (0, 99, 999, 9999?) 0

Output Options

☒ XY Graph

☐ Freq. Dist.

☐ Pm Values

Worksheet Names

X Matrix GGD

Y Matrix ShuffleTri

Warning: Keep data sheet names short. They are used in output sheet names!

Title Mantel Tests for Isolation-by-Distance

Worksheet Prefix

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

Genetic Distance Options

#Loci: 9 #Samples: 208

OK

Cancel

Distance Calculation

One Col/Locus Two Cols/Locus  
(For AMOVA-PhiPT, Spatial, Mantel, PCA)

☐ Binary (Diploid) ☒ Codom-Genotypic

☐ Binary (Haploid)

☐ Haploid

☐ Haploid-SSR (For AMOVA-Fst and

☐ Codom-Allelic

(For AMOVA-Rst and

☐ Codom-Microsat

☐ Interpolate Missing ☐ List Missing

☐ Linear Genetic ☒ Geographic Options

Distance Output Options

☒ Output Total Distance Only

☐ Output Distance All Loci

Title

Worksheet Prefix

Output

☒ To Worksheet

☒ As Tri Matrix

☐ As Sq Matrix

☐ As Column

☒ Label Matrix

☒ Sample

☐ Pop.

Adv Output

☐ Labeled Opt

Split by Pop

☐ Data by Pop

☐ Dist by Pop

☐ To Workbook

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

Geographic Distance Options

**Data Source**

☒ This Worksheet      Microsatellite genotype

☐ Other Worksheet      PhiPTP

**#Samples**      208

**X, Y Coordinates**

☒ Cols 22 & 23 (After genetic data)

☐ Cols 3 & 4 (Other Worksheet)

☐ Cols 1 & 2 (Other Worksheet)

**Data**

☒ Standard or UTM      ☐ Decimal Lat/Long

☒ Convert UTM m to km

**Transform**

☒ None

☐ Log(1+x)

☐ Ln(1+x)

**Title**

**Worksheet Prefix**

☐ Extract XY

OK

Cancel

- i.
- 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.