A Comprehensive Guide to:

GenAlEx

Genetic Analysis in Excel

6.5

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Australian
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http://biology.anu.edu.au/GenAlEx/

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Introduction

The current version of this guide is based on GenAlEx 6.5 beta 3. A final version of this guide will be released concurrently with GenAlEx 6.5. The full citation for Peakall and Smouse (2012) will also be available at that time. Meanwhile, you can cite the new publication as indicated below.

GenAlEx 6.5

GenAlEx 6.5 - Genetic Analysis in Excel, is written in Visual Basic for Applications (VBA) within Excel. It is designed as a user-friendly package that allows users to analyse a wide range of population genetic data within a software environment with which most users will be familiar. It can be run on both PC and Macintosh. Please refer to the Read Me file distributed with GenAlEx for up to date information on Excel version compatibility.

GenAlEx
Genetic Analysis in Excel
6.5
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Peakall R. and Smouse P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics In press. doi:10.1093/bioinformatics/bts460. Peakall R. and Smouse P.E. (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288-295.

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Proudly supported by The Australian National University
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Logo Design by GreenIdeasCreative.com

User registration and citation of GenAlEx 6.5

Please register

The GenAlEx web site (http://biology.anu.edu.au/GenAlEx/) provides an optional registration form that you are urged to complete. Registration will ensure that you will be advised via email of any updates and new versions.

Please cite both of the following when referencing GenAlEx 6.5:

Please note that from July 2012, GenAlEx has a dual citation (Peakall and Smouse 2006, 2012). Please use this dual citation whenever you reference GenAlEx. Note also that this dual citation applies for anyone using GenAlEx 6.1 onwards. This is because the new application note by Peakall and Smouse (2012) is an update that covers the features in GenAlEx that have been progressively released since the original computer note of Peakall and Smouse (2006). That is, Peakall and Smouse (2012) is not a substitute for Peakall and Smouse (2006), but rather an update to be read and cited with the original reference. Wherever possible, please update your citations and references in any existing manuscripts.

Peakall, R. and Smouse P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* In press.

First published online July 20, 2012 doi:10.1093/bioinformatics/bts460. Advanced print Epub available here

Peakall, R. and Smouse P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*. 6, 288-295.

Please also read both program notes in conjunction with this guide and other supporting documentation for GenAlEx 6.5.

GenAlEx 6.5 Installation

GenAlEx is provided as an Excel add-in, a compiled module and the associated **GenAlEx** menu. Your download file may initially be in the zipped format. Use the extract option to unzip the download and save the files to a dedicated folder of your choice. You can work with GenAlEx directly from this folder. Please refer to the Read Me file distributed with GenAlEx for detailed installation instruction for different versions of Excel on both PC and Macintosh.

About this Manual

This guide applies to GenAlEx 6.5 onwards. It assumes a level of prior knowledge of population genetics likely held by an informed graduate student. The calculations performed by GenAlEx are detailed in a separate Appendix 1: Methods and statistics in GenAlEx 6.5, by Rod Peakall and Peter Smouse. For further information on the calculations, users are advised to consult this appendix, together with the references provided therein.

The guide assumes that GenAlEx users are familiar with standard operating procedures of their computer system (PC or Macintosh). Also assumed, is a familiarity with the basic Excel working environment, including how to create and manipulate new workbooks and new worksheets within workbooks, and how to enter and manipulate data.

A main objective in releasing GenAlEx 6.5 has been to make user interaction with GenAlEx as straightforward as possible. Thus, wherever possible, the GenAlEx dialog boxes have been standardized. Options that require further explanation are described in more detail in this guide.

The guide aims to provide:

1. A description of the data types handled, and their appropriate formatting.
2. A description of the user interface, and step-by-step instructions for performing individual analyses.
3. A description of the different analysis options, where relevant, with tips to help users get the most out of GenAlEx functions.

The illustrations for the Dialog boxes used in this guide were extracted using GenAlEx in various operating systems and versions of Excel. The interface may appear slightly different on your computer.

Manual Style

The following styles have been adopted in the text when referring to the:

Menu name (eg. GenAlEx*)*

Menu option (e.g. Distance)

Menu suboption (e.g. Genetic)

Dialog box name (e.g. Genetic Distance Options)

Dialog box option (e.g. Binary)

Tips are written in italics.

Notes for users regarding procedures are written in text boxes.

Disclaimer

This guide to GenAlEx 6.5 is provided free by the authors (Blyton and Flanagan). It has been written with the consent of, and in close consultation with the program authors (Peakall and Smouse). While every care has been taken to ensure the accuracy of this text, no responsibility is taken for unintentional errors or problems that may be encountered by users. We regret that we cannot offer individualized support for users of the program.

The GenAlEx Environment

Overview

GenAlEx reads information contained in an Excel worksheet that consists of essential parameters and labels, optional labels, and the data itself.

Several options are available for users to appropriately format their data, from manual formatting of a pre-existing data worksheet, to options for the automated import, editing and formatting of data output from a genotyping / sequencing system.

In designing GenAlEx, the aim has been to make data management and analysis as efficient and as easy as possible. Nonetheless, a number of restrictions are imposed by the Excel environment. These are outlined below, along with some useful pointers to how to get the best out of GenAlEx / Excel environment.

Data Limits

GenAlEx is limited by Excel to 256 columns of data in Excel 2003 (in a .xls workbook) and to 16,384 columns in Excel 2010 (in a .xlsx, .xlsm or .xlsb workbook). This equates to 254 binary or haploid loci or 127 codominant loci in Excel 2003; while, users operating in Excel 2010 are limited to 16,382 binary or haploid loci or 8,191 codominant loci. The maximum number of samples is approximately 65,500 in 2003 and over one million in 2010. In practice, in Excel 2010 onwards, the memory limitations of your computer and the GenAlEx program itself will limit the size of the dataset you can run to far less than the number of columns or rows available.

Triangular distance matrices are limited to 254 samples in Excel 2003 and to 16,384 in Excel 2010. For larger data sets in Excel 2003, use the *Distance Matrix as Column* option.

For users of Excel 2003 with more than the maximum number of binary loci (e.g. large AFLP datasets), but with less than 254 samples, a 'Transposed' option is available (***Options*** -> ***Generic***), which allows a restricted subset of analyses to be performed.

Input

Input consists of raw data or distance matrices in appropriate GenAlEx format (see the 'Data Format in GenAlEx' section). In order to proceed with an analysis the worksheet containing the data must be activated (visible). Some analyses and procedures take several worksheets as input. Unless otherwise explained, these need to be placed starting on the left hand side (LHS) of the workbook, in the order 1 to n.

Wherever possible, GenAlEx offers two options to help keep track of data and analysis output. In the initial Data Parameter dialog box for statistical procedures, the user may provide a worksheet prefix to help identify the output of a particular analysis, and a title for the output that can provide specific details of the analysis being performed. This title will appear at the top of each output worksheet. It is strongly recommended that both these options be used.

Output

GenAlEx can generate many worksheets in routine analysis, so the ability to create and manipulate new workbooks and new worksheets within workbooks is particularly important. Each worksheet output by GenAlEx is given a name dependent on the analysis performed. This is particularly useful in analyses that have multiple worksheet outputs. In the manual worksheet names are identified using square brackets e.g. [GD]. A user-defined prefix may be added to the worksheet name for further clarity (see preceding section).

Output of GenAlEx worksheets is designed so that the raw data or other input worksheet is always at the extreme left hand side (LHS) of the workbook. Thus, output worksheets for most menu options will appear to the right hand side (RHS) of the raw data worksheet. However, Genetic Distance outputs will appear to the LHS of the raw data, as the distance matrix is used as input for subsequent analyses.

Graphs are output in standard Excel format and may need to be resized in order to see all the information. All graphs can be edited using standard Excel functions.

Note: GenAlEx outputs are optimized for a standard Excel font size of 10pts. To check and change the standard font size set in Excel, select the *Check Font* option from the *Options* menu in GenAlEx.

Note: By default GenAlEx automatically saves the active workbook at the completion of each analysis. It is strongly recommend that you save a copy of your original data in a separate workbook before manipulating or analysing that data in GenAlEx.

Standard Data Parameter Dialog Box

In order to facilitate user interaction with GenAlEx, the initial Data Parameter dialog box for the different statistical procedures has been standardized as much as possible. While the box generally has the following format, necessary adaptations have been made for some applications.

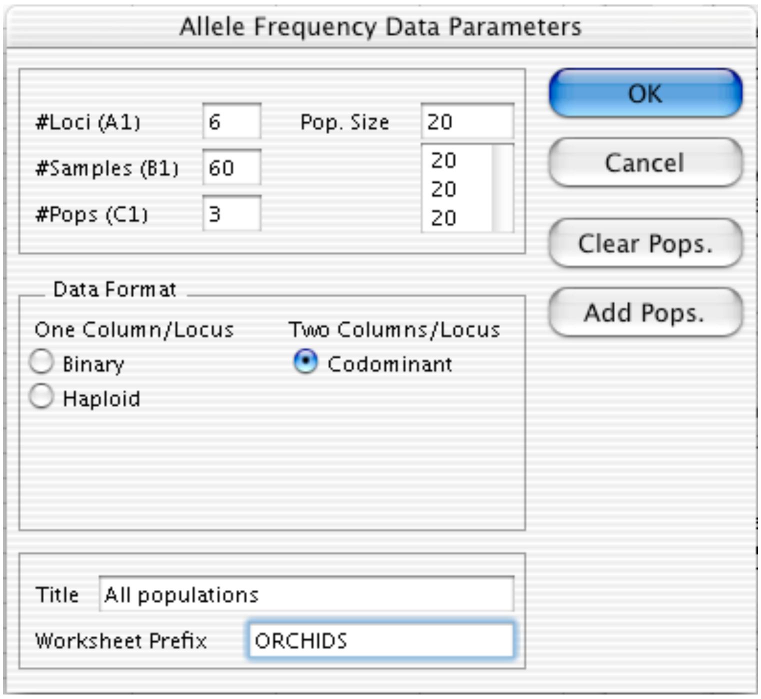
The top section of the dialog box provides edit boxes for entering the essential locus, sample and population parameters. If the parameters are present in the datasheet, they will be entered automatically (see parameters section below). If only a subset of the data is required, the parameters may be changed here.

A section is then provided in which the input data type is selected.

Finally, at the bottom of the dialog box two options are provided to help keep track of analysis output:

1. A title for the output that can provide specific details of the analysis being performed (30 characters max.). This title will appear at the top of each output worksheet, together with the name of the original data sheet used.
2. A worksheet prefix to help identify the output of a particular analysis.

An example of a Data Parameter Dialog Box for a statistical procedure



#Loci (A1) 6

#Samples (B1) 60

#Pops (C1) 3

Pop. Size 20

20
20
20

Data Format

One Column/Locus

○ Binary

○ Haploid

Two Columns/Locus

● Codominant

Title All populations

Worksheet Prefix ORCHIDS

Data Parameter Dialog Box options

Parameter Edit Boxes

Enter the number of loci, samples and pops in each box.

Add Pop. Size by entering the required size in the edit box above the list, then click the Add Pops button. Add population sizes in order from population 1 to n.

Clear Pops Use this button to clear the list of population sizes.

Data Format

Select the format appropriate to your data.

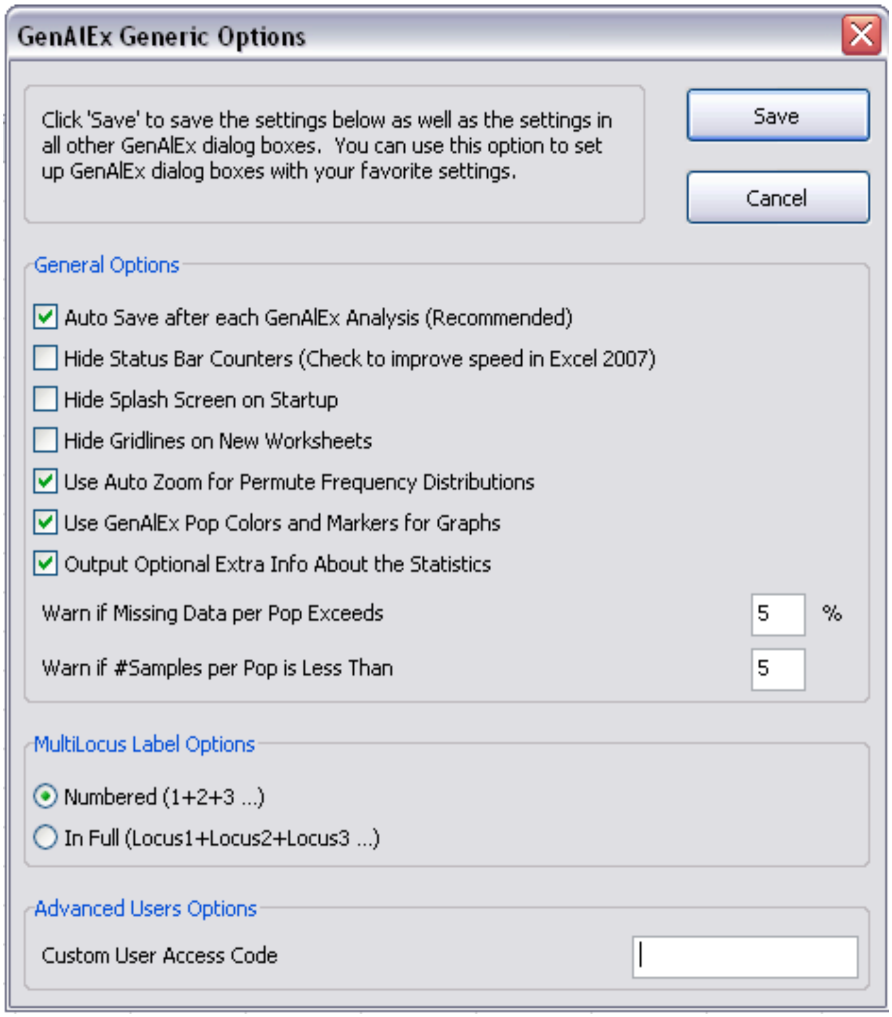
Enter Title and Worksheet Prefix.

Options

The **Options** menu contains sub-menus for customizing the GenAlEx package.

Generic: This submenu provides options for customizing the GenAlEx dialog boxes and output including graphs and worksheet labels.

Tip: This option may also be used to customize commonly used dialog boxes. For example, if you mostly work with binary datasets, you can reset the default in your usual dialog boxes to binary. After changing the dialog box options to your required settings during the course of an analysis, return to the ***Generic*** *sub-menu, and click save in the dialog box.*



GenAlEx Generic Options

Click 'Save' to save the settings below as well as the settings in all other GenAlEx dialog boxes. You can use this option to set up GenAlEx dialog boxes with your favorite settings.

General Options

☑ Auto Save after each GenAlEx Analysis (Recommended)

☐ Hide Status Bar Counters (Check to improve speed in Excel 2007)

☐ Hide Splash Screen on Startup

☐ Hide Gridlines on New Worksheets

☑ Use Auto Zoom for Permute Frequency Distributions

☑ Use GenAlEx Pop Colors and Markers for Graphs

☑ Output Optional Extra Info About the Statistics

Warn if Missing Data per Pop Exceeds 5 %

Warn if #Samples per Pop is Less Than 5

MultiLocus Label Options

● Numbered (1+2+3 ...)

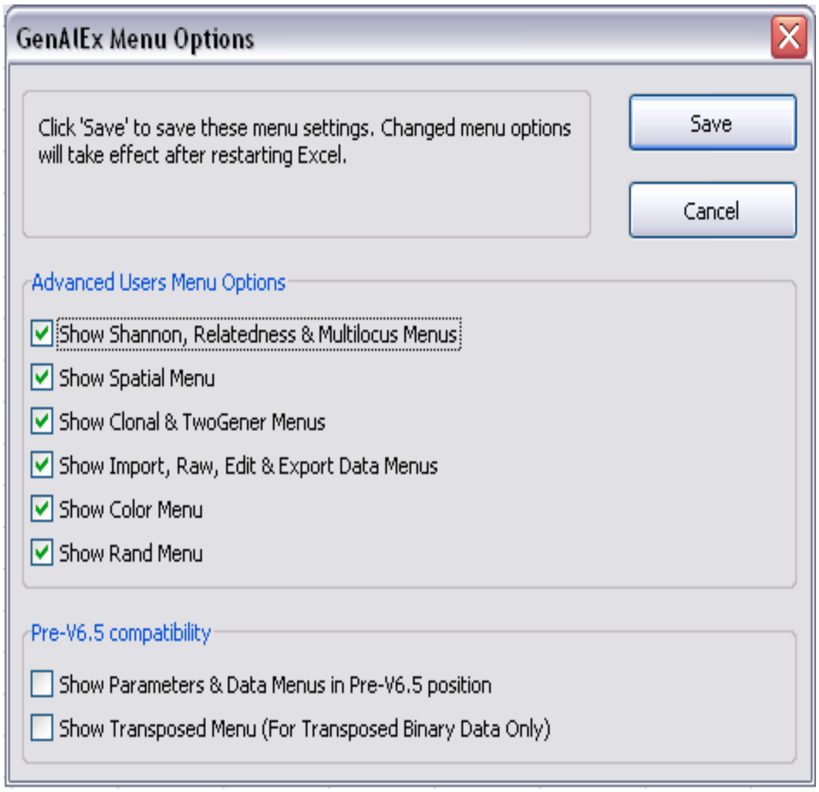
○ In Full (Locus1+Locus2+Locus3 ...)

Advanced Users Options

Custom User Access Code

Menus: This submenu provides options for customizing the GenAlEx menu.

Tip: Teachers can use this option to hide some of the advanced options from the menu.



GenAlEx Menu Options

Click 'Save' to save these menu settings. Changed menu options will take effect after restarting Excel.

Advanced Users Menu Options

☑ Show Shannon, Relatedness & Multilocus Menus

☑ Show Spatial Menu

☑ Show Clonal & TwoGener Menus

☑ Show Import, Raw, Edit & Export Data Menus

☑ Show Color Menu

☑ Show Rand Menu

Pre-V6.5 compatibility

□ Show Parameters & Data Menus in Pre-V6.5 position

□ Show Transposed Menu (For Transposed Binary Data Only)

Install: This sub-menu installs GenAlEx so that it will launch automatically when Excel is opened. Other versions of GenAlEx will be uninstalled by this process.

Uninstall: This sub-menu uninstalls GenAlEx, preventing it from automatically launching when Excel is opened.

List Add-Ins: Lists the version of GenAlEx currently installed.

Check Font: Calls a dialog box stating the current standard font size set in Excel. GenAlEx is optimized for a standard Excel font size of 10pts. This function also provides the option of changing the standard font size to 10pt.

Data format for GenAlEx

Numerical Format

GenAlEx requires all data to be coded as numbers and formatted within Excel as numeric data. Be especially careful to avoid using the text format option, and turn off all auto-formatting options. Advanced options are available for processing DNA sequence data to find polymorphisms and haplotypes and convert these to numerical format (see the 'Raw Data Editing' section).

Tip: To check your numeric values are actually treated as numeric by Excel, click the Increase or Decrease decimal buttons, under Excel's Formatting options. If Excel is unable to show decimals, your numbers are formatted as text not numeric.

Missing Data

Virtually all GenAlEx options handle missing data. Missing data can be particularly problematic for pairwise distance-based analyses such as AMOVA, Mantel and spatial autocorrelation. Therefore, a unique option for interpolating missing individual-by-individual pairwise distances is provided. This option will insert the average genetic distances for each population level pairwise contrast e.g. within Pop. 1, or between Pop. 1 and Pop. 2. Nonetheless, in order to avoid excessive bias, large numbers of missing data for individual-based distance calculations should be minimized.

Codominant and Haploid missing data are coded as '0'.

Missing Binary data are coded as '-1'.

Sample Labels

If you plan to take advantage of all the features of GenAlEx, each sample must be given a unique numerical identifier. Sample names may carry an alpha character prefix, but this must be the same (including case) for all samples in a single dataset. In this case it is important to know that when sorting on alphanumeric data, GenAlEx uses the Excel sort-order rules, sorting character by character, (e.g. A11will come after A100). For ease of sorting, we recommend that the format A001…A199 be used when using prefixes.

Note: This strict requirement for unique numerical identifiers is not essential for running most of the population genetic analyses. However, it is required for many of the useful data manipulation options.

Tip: If your samples are not in this format, it is possible to quickly create unique numerical identifiers using the ***Replace Sample code*** *option under the* ***Raw Data*** *menu in GenAlEx.*

Data Structure

For all population-based analyses within GenAlEx, the genotypes for all the samples belonging to a single population must be entered as a contiguous block of rows - one sample per row. For regional-based analyses in AMOVA, all populations belonging to a region must also be entered as a contiguous block. For ***TwoGener*** input, the genotypes of each mother and respective offspring are entered as a contiguous block, with the mother being the first individual of each block. Mother groupings are coded in the Column 2 (see **Tutorial 6** for more details).

Data Parameters and Labels

Data parameters and labels are crucial for telling GenAlEx how to read and analyze the data. GenAlEx stores all parameters and labels in rows 1, 2 and 3 of the data worksheets. For raw data, columns 1 and 2 are generally used for sample and population labels respectively; while, actual data begins in Cell C4 of a worksheet.

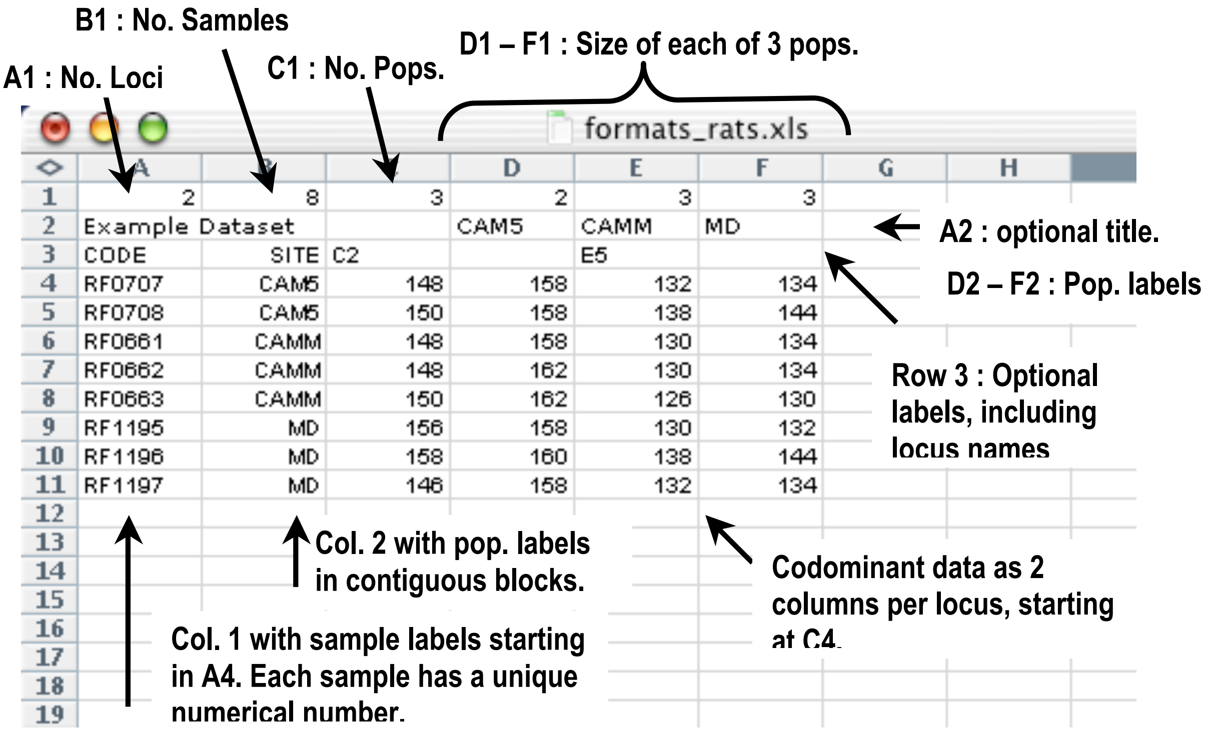
Note: When analysing your data, GenAlEx only uses the data parameters to locate and process your samples. It does not interrogate the sample or population codes in columns 1 and 2. Therefore, ensure the data parameters reflect the data format, particularly after sorting or rearranging your samples.

Data parameters and labels may be entered in GenAlEx in several ways

1. A worksheet containing data may be manually formatted to provide appropriate parameters.
2. The ***Template*** option in the **GenAlEx** menu may be used to provide parameters through a dialog box, creating a formatted worksheet into which the data are then entered (see section below for further instructions).
3. The ***Parameters*** option in the **GenAlEx** menu may be used to obtain the relevant parameter values from an existing dataset and insert them into their appropriate location (see section below for further instructions). This option requires that your data is bounded by blank columns and rows.
4. On initiating an analysis, GenAlEx prompts for the relevant parameters in a dialog box. Changing parameters in this box provides an easy way to select subsets of data for analysis.
5. If data is imported using GenAlEx options, essential parameters and labels will be inserted automatically, however labels for locus names may need to be entered manually.

Parameter locations

Essential parameters are inserted into Row 1. They are: No Loci (cell A1); No. Samples (cell B1); No Populations (cell C1); The size of each population (cell D1..to cell n1).



B1 : No. Samples

A1 : No. Loci

C1 : No. Pops.

D1 – F1 : Size of each of 3 pops.

formats_rats.xls

	A	B	C	D	E	F	G	H
1	2	8	3	2	3	3		
2	Example Dataset			CAM5	CAMM	MD		
3	CODE	SITE	C2		E5			
4	RF0707	CAM5	148	158	132	134		
5	RF0708	CAM5	150	158	138	144		
6	RF0661	CAMM	148	158	130	134		
7	RF0662	CAMM	148	162	130	134		
8	RF0663	CAMM	150	162	126	130		
9	RF1195	MD	156	158	130	132		
10	RF1196	MD	158	160	138	144		
11	RF1197	MD	146	158	132	134		
12								
13								
14								
15								
16								
17								
18								
19								

A2 : optional title.

D2 – F2 : Pop. labels

Row 3 : Optional labels, including locus names

Col. 2 with pop. labels in contiguous blocks.

Codominant data as 2 columns per locus, starting at C4.

Col. 1 with sample labels starting in A4. Each sample has a unique numerical number.

If regional information is required, the parameter for the No. of Regions is inserted into the cell immediate after the last population size, and the size of each region then follows in subsequent cells (see example under codominant data below).

Data Formats

GenAlEx accepts 4 types of numerically-coded data:

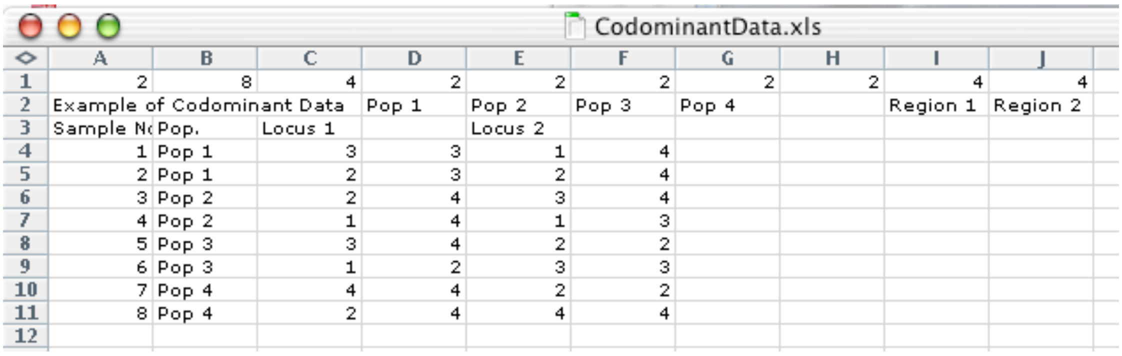
1. Codominant genotypic data with 2 columns per locus.
2. Dominant (Binary), Haploid (including Haplotypes), or Sequence data coded numerically with 1 column per locus/base.
3. Codominant and Haploid raw allele frequency data.
4. Geographic data with 2 columns for X and Y coordinates.

Tip: Examples of all GenAlEx data formats can be created via the ***Create*** *menu option. This is a useful way to explore the full range of GenAlEx options.*

Format for codominant genotypic data

Codominant genotypic data are presented as two columns per locus as in the figure below. Alleles may be simply numerically-coded (1, 2, 3 etc). Alternatively, and preferably for microsatellite data, alleles may be coded as their integer size in base pairs (bp), or as the inferred number of simple sequence repeats. These last two formats are essential for calculation of the distance measure, R_{st}. There is no limit to the number of numerically-coded alleles. Alleles coded in bp size are accepted up to a maximum allele size of 999. Codominant alleles need not be numbered consecutively.

Example of codominant, numerically-coded data, with regional parameters.

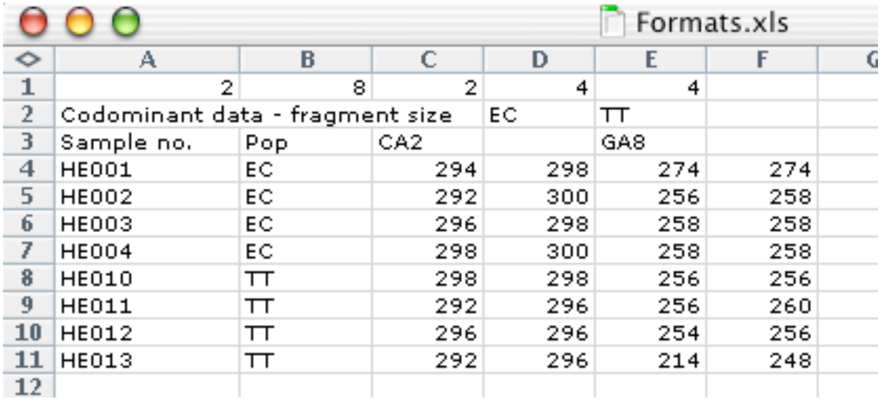


CodominantData.xls

	A	B	C	D	E	F	G	H	I	J
1	2	8	4	2	2	2	2	2	4	4
2	Example of Codominant Data			Pop 1	Pop 2	Pop 3	Pop 4		Region 1	Region 2
3	Sample N	Pop.	Locus 1		Locus 2					
4	1	Pop 1	3	3	1	4				
5	2	Pop 1	2	3	2	4				
6	3	Pop 2	2	4	3	4				
7	4	Pop 2	1	4	1	3				
8	5	Pop 3	3	4	2	2				
9	6	Pop 3	1	2	3	3				
10	7	Pop 4	4	4	2	2				
11	8	Pop 4	2	4	4	4				
12										

In this example the 4 populations are split into 2 regions with Pops 1 & 2 in Region 1 and Pops 3 & 4 in Region 2.

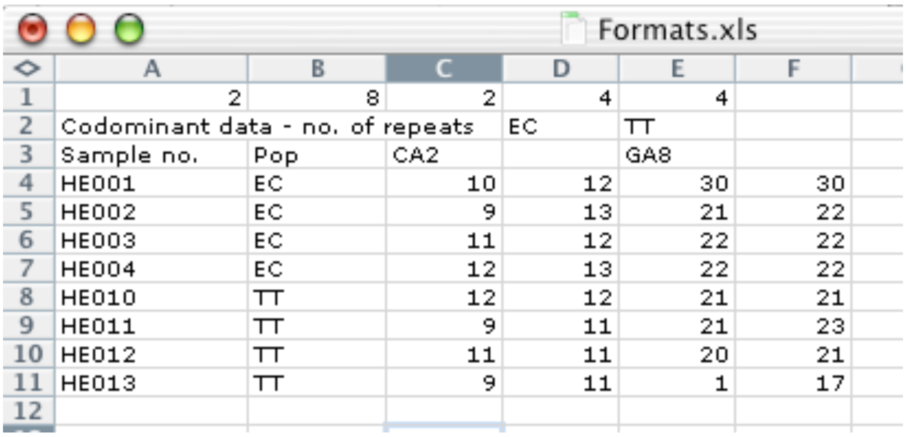
Example of codominant genotypic microsatellite data, with loci scored as fragment size.



Formats.xls

	A	B	C	D	E	F
1	2	8	2	4	4	
2	Codominant data - fragment size			EC	TT	
3	Sample no.	Pop	CA2		GA8	
4	HE001	EC	294	298	274	274
5	HE002	EC	292	300	256	258
6	HE003	EC	296	298	258	258
7	HE004	EC	298	300	258	258
8	HE010	TT	298	298	256	256
9	HE011	TT	292	296	256	260
10	HE012	TT	296	296	254	256
11	HE013	TT	292	296	214	248
12						

Example of microsatellite data, with alleles coded with the inferred number of repeats.



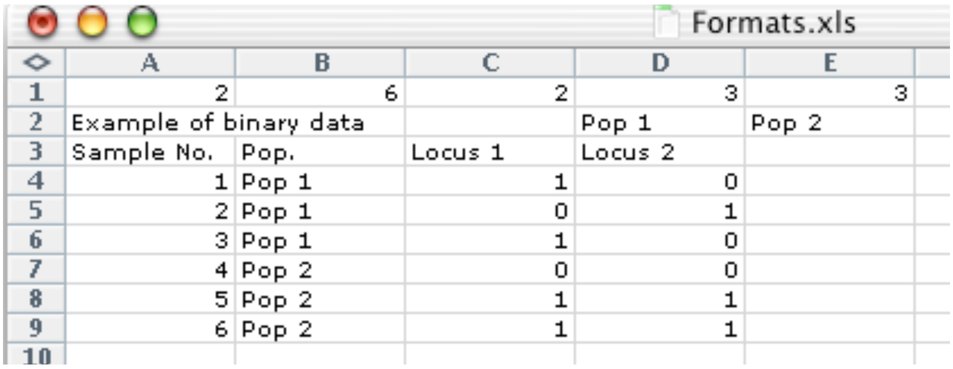
	A	B	C	D	E	F
1	2	8	2	4	4	
2	Codominant data - no. of repeats			EC	TT	
3	Sample no.	Pop	CA2		GA8	
4	HE001	EC	10	12	30	30
5	HE002	EC	9	13	21	22
6	HE003	EC	11	12	22	22
7	HE004	EC	12	13	22	22
8	HE010	TT	12	12	21	21
9	HE011	TT	9	11	21	23
10	HE012	TT	11	11	20	21
11	HE013	TT	9	11	1	17
12						

These are the same data as the previous example, for loci of 2bp simple sequence repeats.

Format for dominant (Binary), haploid or sequence data

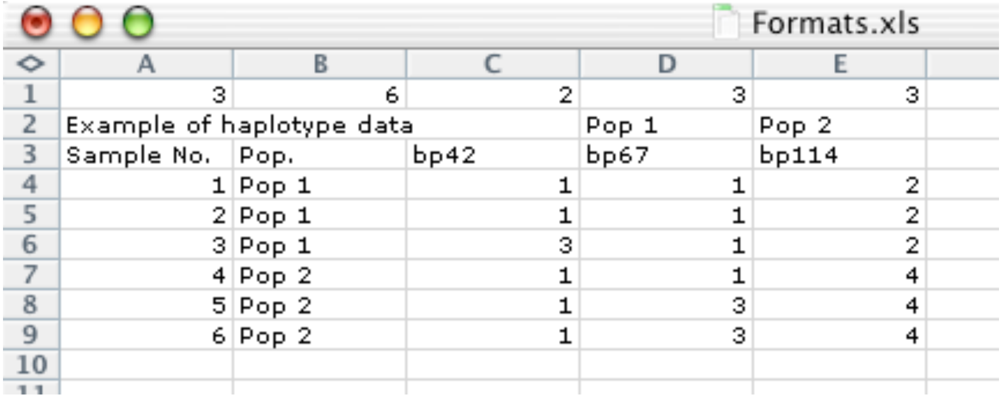
Dominant, haploid (including haplotypes) or sequence data are presented as a single column per locus. Dominant data can be coded in a binary format with one column per marker. Haploid data can be coded numerically from 1…n, or each haplotype may be represented by multiple variable sites (columns 1 … n), with multiple states. For sequence or SNP data the bases are numerically coded as follows: A=1, C=2, G=3, T=4, :=5; -=5, all other characters = 0. GenAlEx provides several options for the import of sequence data and auto conversion to numbers.

Example of dominant (binary) data.



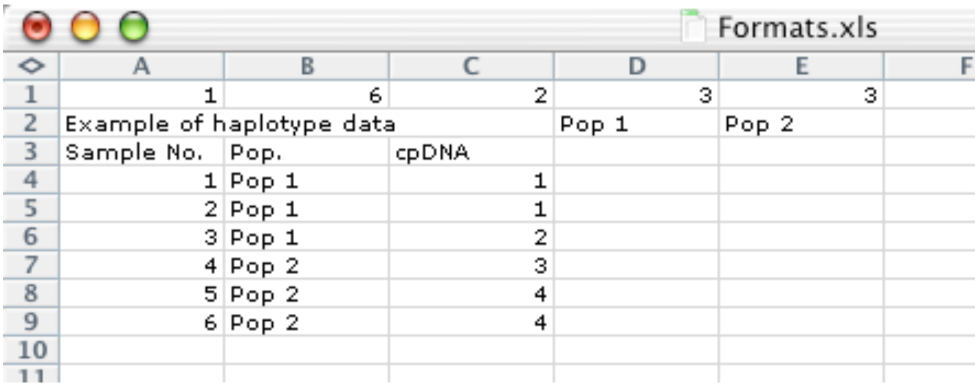
	A	B	C	D	E
1	2	6	2	3	3
2	Example of binary data			Pop 1	Pop 2
3	Sample No.	Pop.	Locus 1	Locus 2	
4	1	Pop 1	1	0	
5	2	Pop 1	0	1	
6	3	Pop 1	1	0	
7	4	Pop 2	0	0	
8	5	Pop 2	1	1	
9	6	Pop 2	1	1	
10					

Example of sequence data, coded numerically at multiple variable sites.



	A	B	C	D	E
1	3	6	2	3	3
2	Example of haplotype data			Pop 1	Pop 2
3	Sample No.	Pop.	bp42	bp67	bp114
4	1	Pop 1	1	1	2
5	2	Pop 1	1	1	2
6	3	Pop 1	3	1	2
7	4	Pop 2	1	1	4
8	5	Pop 2	1	3	4
9	6	Pop 2	1	3	4
10					

Example of haplotype data, with individual haplotypes coded numerically.



	A	B	C	D	E	F
1	1	6	2	3	3	
2	Example of haplotype data			Pop 1	Pop 2	
3	Sample No.	Pop.	cpDNA			
4	1	Pop 1	1			
5	2	Pop 1	1			
6	3	Pop 1	2			
7	4	Pop 2	3			
8	5	Pop 2	4			
9	6	Pop 2	4			
10						

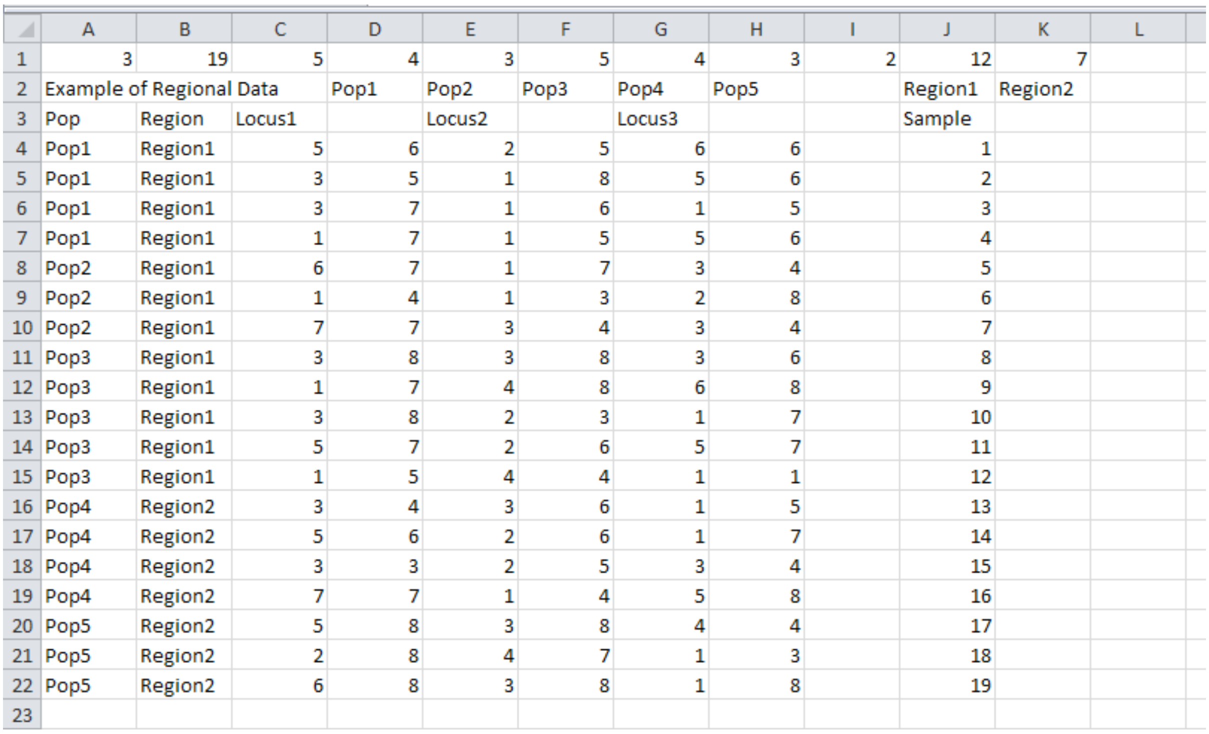
These haplotypes correspond to the sequences shown in the previous example.

Format for regional data.

For regional-based analyses, all populations belonging to a single region must be entered as a contiguous block. Region labels can be entered in columns 1 or 2 with population labels in the alternate column. The parameters for the regional data are entered in Row 1, immediately following the last population size.

Tip: In order to keep track of individual samples when performing regional analysis, enter sample labels after the genetic data with a blank intervening column.

Example of data with regional parameters.



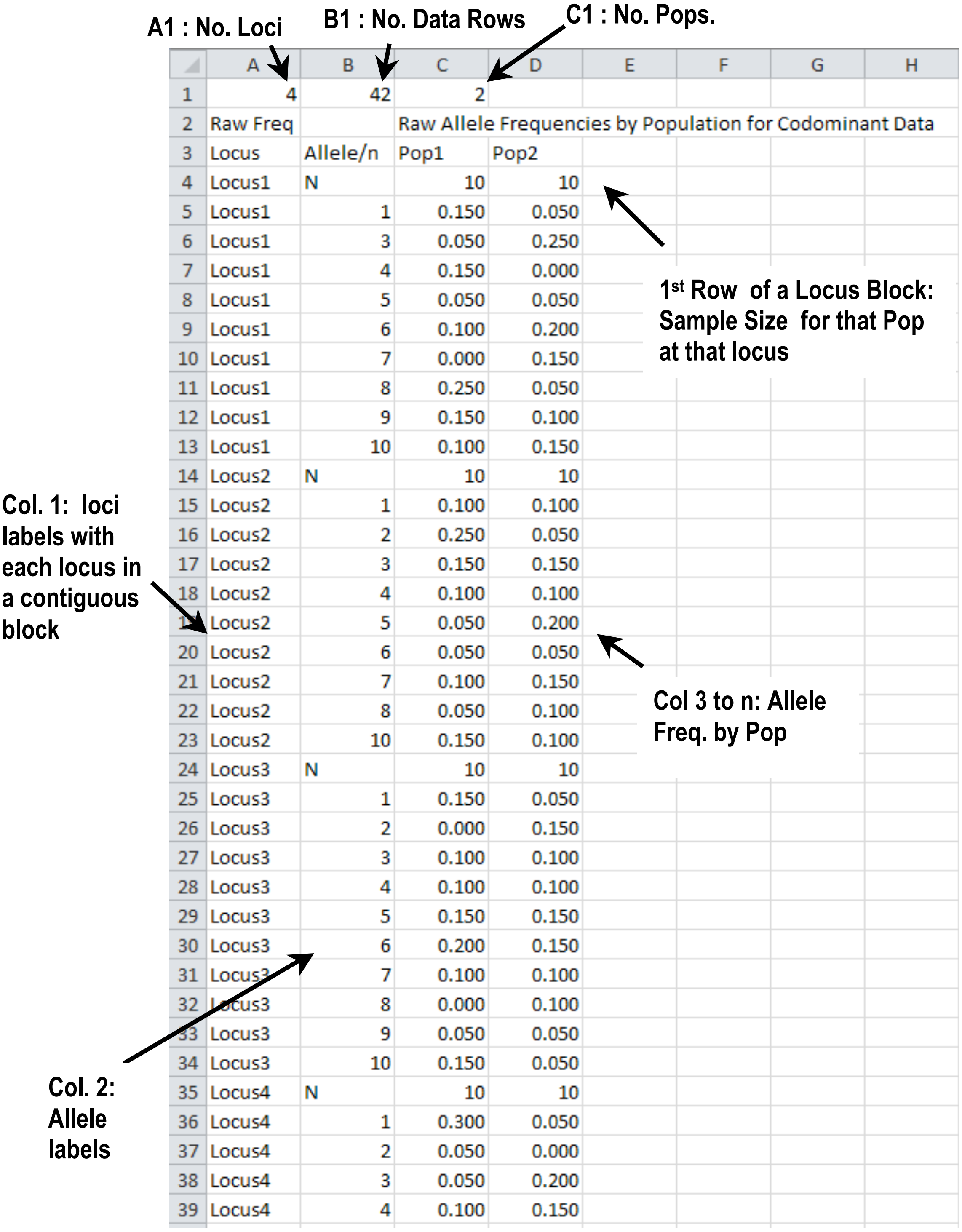
	A	B	C	D	E	F	G	H	I	J	K	L
1	3	19	5	4	3	5	4	3	2	12	7	
2	Example of Regional Data			Pop1	Pop2	Pop3	Pop4	Pop5		Region1	Region2	
3	Pop	Region	Locus1		Locus2		Locus3			Sample		
4	Pop1	Region1	5	6	2	5	6	6		1		
5	Pop1	Region1	3	5	1	8	5	6		2		
6	Pop1	Region1	3	7	1	6	1	5		3		
7	Pop1	Region1	1	7	1	5	5	6		4		
8	Pop2	Region1	6	7	1	7	3	4		5		
9	Pop2	Region1	1	4	1	3	2	8		6		
10	Pop2	Region1	7	7	3	4	3	4		7		
11	Pop3	Region1	3	8	3	8	3	6		8		
12	Pop3	Region1	1	7	4	8	6	8		9		
13	Pop3	Region1	3	8	2	3	1	7		10		
14	Pop3	Region1	5	7	2	6	5	7		11		
15	Pop3	Region1	1	5	4	4	1	1		12		
16	Pop4	Region2	3	4	3	6	1	5		13		
17	Pop4	Region2	5	6	2	6	1	7		14		
18	Pop4	Region2	3	3	2	5	3	4		15		
19	Pop4	Region2	7	7	1	4	5	8		16		
20	Pop5	Region2	5	8	3	8	4	4		17		
21	Pop5	Region2	2	8	4	7	1	3		18		
22	Pop5	Region2	6	8	3	8	1	8		19		
23												

In this example the 5 populations are split into 2 regions (Cell I1) with Pops 1, 2 & 3 in Region 1 and Pops 4 & 5 in Region 2. The first region contain 12 individuals and the second contains seven (Cells J1 & K1).

Format for codominant and haploid raw allele frequency data

Codominant and haploid raw allele frequency data are presented with each locus as a contiguous block and each allele in a separate row. The first row of each locus block must contain the sample size of each population for that locus. Locus labels are presented in column 1 and allele codes in column 2. The frequency of each allele is then entered for each population in columns 3 to n.

Example of raw allele frequency data



A1 : No. Loci

B1 : No. Data Rows

C1 : No. Pops.

1st Row of a Locus Block: Sample Size for that Pop at that locus

Col. 1: loci labels with each locus in a contiguous block

Col 3 to n: Allele Freq. by Pop

Col. 2: Allele labels

	A	B	C	D	E	F	G	H
1	4	42	2					
2	Raw Freq		Raw Allele Frequencies by Population for Codominant Data					
3	Locus	Allele/n	Pop1	Pop2				
4	Locus1	N	10	10				
5	Locus1	1	0.150	0.050				
6	Locus1	3	0.050	0.250				
7	Locus1	4	0.150	0.000				
8	Locus1	5	0.050	0.050				
9	Locus1	6	0.100	0.200				
10	Locus1	7	0.000	0.150				
11	Locus1	8	0.250	0.050				
12	Locus1	9	0.150	0.100				
13	Locus1	10	0.100	0.150				
14	Locus2	N	10	10				
15	Locus2	1	0.100	0.100				
16	Locus2	2	0.250	0.050				
17	Locus2	3	0.150	0.150				
18	Locus2	4	0.100	0.100				
19	Locus2	5	0.050	0.200				
20	Locus2	6	0.050	0.050				
21	Locus2	7	0.100	0.150				
22	Locus2	8	0.050	0.100				
23	Locus2	10	0.150	0.100				
24	Locus3	N	10	10				
25	Locus3	1	0.150	0.050				
26	Locus3	2	0.000	0.150				
27	Locus3	3	0.100	0.100				
28	Locus3	4	0.100	0.100				
29	Locus3	5	0.150	0.150				
30	Locus3	6	0.200	0.150				
31	Locus3	7	0.100	0.100				
32	Locus3	8	0.000	0.100				
33	Locus3	9	0.050	0.050				
34	Locus3	10	0.150	0.050				
35	Locus4	N	10	10				
36	Locus4	1	0.300	0.050				
37	Locus4	2	0.050	0.000				
38	Locus4	3	0.050	0.200				
39	Locus4	4	0.100	0.150				

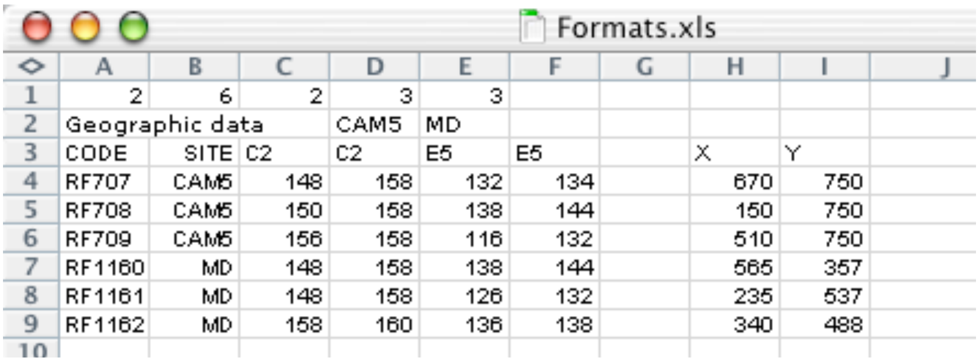
Format for geographic data.

For convenience, both geographic and genetic distances can be calculated in a single analysis. Coordinates can be entered as either integer or decimal numbers.

X and Y coordinates may be read by GenAlEx from three different formats.

1. X / Y data are located in the same worksheet as the genetic data, and separated from the genetic data by a single blank column. This format is used by GenAlEx for various analyses, including Distance and TwoGener.

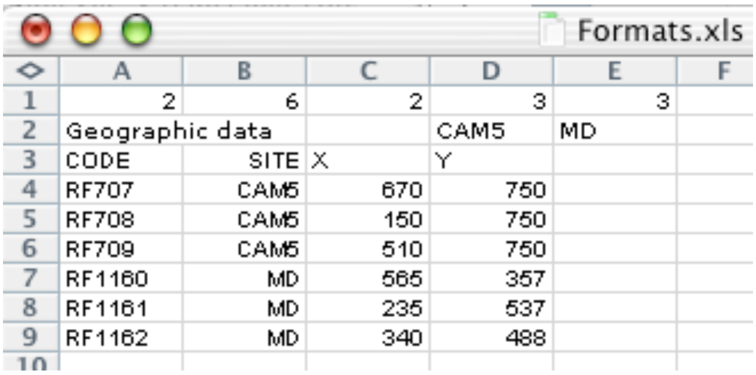
Example of geographic data after genetic data.



	A	B	C	D	E	F	G	H	I	J
1	2	6	2	3	3					
2	Geographic data			CAM5	MD					
3	CODE	SITE	C2	C2	E5	E5		X	Y	
4	RF707	CAM5	148	158	132	134		670	750	
5	RF708	CAM5	150	158	138	144		150	750	
6	RF709	CAM5	156	158	116	132		510	750	
7	RF1160	MD	148	158	138	144		565	357	
8	RF1161	MD	148	158	126	132		235	537	
9	RF1162	MD	158	160	136	138		340	488	
10										

2. In a separate worksheet, in columns 3 and 4. In this case, the labels in columns 1 & 2 will correspond exactly to those for the genetic data. This format is required for analyses such as the 2D Spatial autocorrelation.

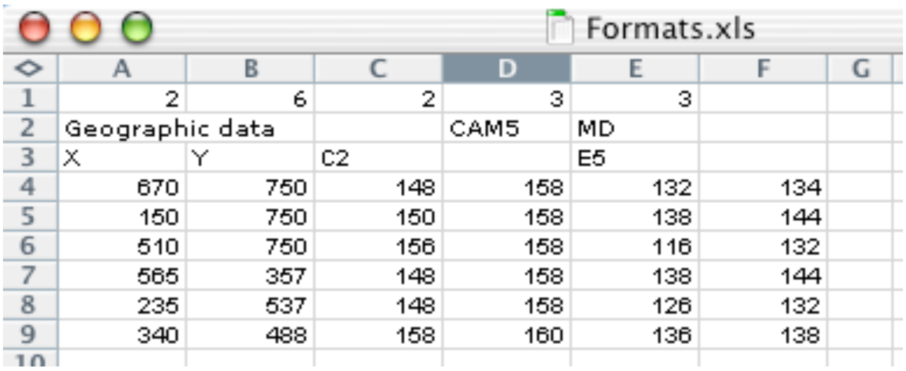
Example of geographic data in columns 3 & 4.



	A	B	C	D	E	F
1	2	6	2	3	3	
2	Geographic data			CAM5	MD	
3	CODE	SITE	X	Y		
4	RF707	CAM5	670	750		
5	RF708	CAM5	150	750		
6	RF709	CAM5	510	750		
7	RF1160	MD	565	357		
8	RF1161	MD	235	537		
9	RF1162	MD	340	488		
10						

3. Before the genetic data, in columns 1 and 2. This format is retained to allow compatibility with older GenAlEx datasets, but is no longer recommended.

Example of geographic data in columns 1 & 2.

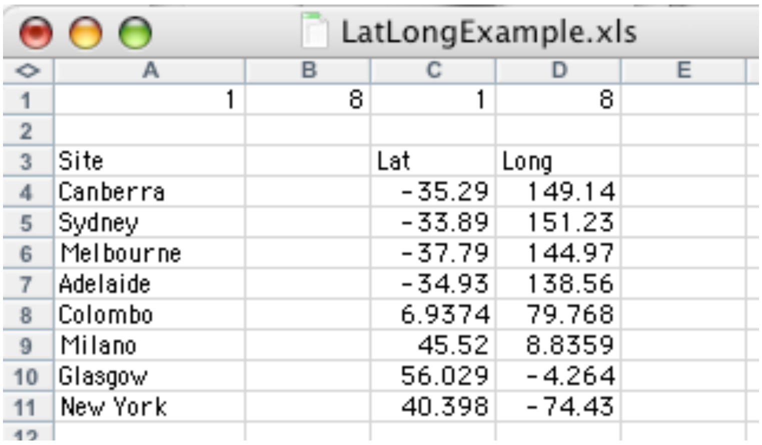


Formats.xls

	A	B	C	D	E	F	G
1	2	6	2	3	3		
2	Geographic data			CAM5	MD		
3	X	Y	C2		E5		
4	670	750	148	158	132	134	
5	150	750	150	158	138	144	
6	510	750	156	158	116	132	
7	565	357	148	158	138	144	
8	235	537	148	158	126	132	
9	340	488	158	160	136	138	

Example of Decimal Latitude / Longitude data.

Latitude / Longitude data may contain negative values. In accordance with international standards, latitude values should be presented first. These values are transformed appropriately on mapping the data (***Graph*****->** ***<u>Lat/Long</u>***). Latitude / Longitude data may be entered in any of the formats shown above.



LatLongExample.xls

	A	B	C	D	E
1	1	8	1	8	
2					
3	Site		Lat	Long	
4	Canberra		-35.29	149.14	
5	Sydney		-33.89	151.23	
6	Melbourne		-37.79	144.97	
7	Adelaide		-34.93	138.56	
8	Colombo		6.9374	79.768	
9	Milano		45.52	8.8359	
10	Glasgow		56.029	-4.264	
11	New York		40.398	-74.43	

Template

The ***Template*** option facilitates formatting a dataset for GenAlEx, by setting up a new worksheet with the appropriate parameters and labels into which your data can be entered. Refer to **Tutorial 1, Exercise 1.9** for additional assistance with this option.

Procedure

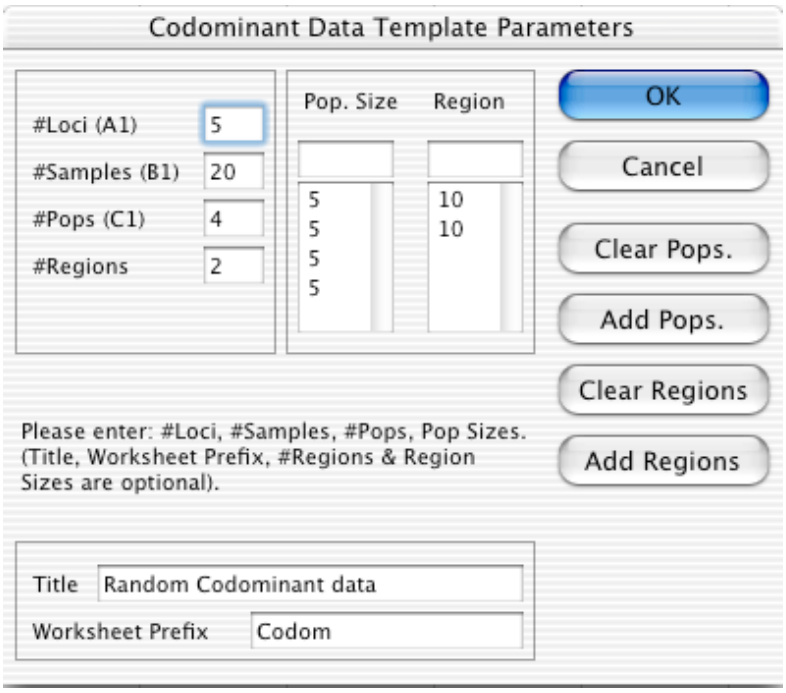
1. With a workbook open, choose the option ***Template*** from the **GenAlEx** menu, and select either ***<u>Codominant</u>*****,** ***<u>Binary</u>*** *or* ***<u>Haploid</u>*** as required.

2. In the Data Parameters dialog box enter the number (#) of loci, # samples, # populations, and, if required, # regions in the left hand side panel. These parameters are inserted into the appropriate parameter cell on the data worksheet [D]. For codominant and haploid data enter the max. # alleles required.

3. Enter the size of each pop in the edit box below ‘Pop. Size’, and add to the population list using the add Pops. option. Use the Clear Pops. Option to clear the list. Information regarding regions is similarly entered, if required.

4. Enter a Title and worksheet prefix for your data and click *Ok*. Output is to worksheet [D].

Use this template as a basis for entering your data set.



Codominant Data Template Parameters

#Loci (A1) 5

#Samples (B1) 20

#Pops (C1) 4

#Regions 2

Pop. Size	Region
5	10
5	10
5	
5	

Please enter: #Loci, #Samples, #Pops, Pop Sizes. (Title, Worksheet Prefix, #Regions & Region Sizes are optional).

Title Random Codominant data

Worksheet Prefix Codom

Create

The ***Create*** menu provides options to create random examples of all GenAlEx data formats, both Genetic and Geographic. These datasets are useful for exploring the range of GenAlEx procedures. Refer to **Tutorial 1 Exercises 1.6 to 1.8** for additional assistance with this option.

The ***Codominant*** , ***Codominant with phase*** and ***Haploid*** sub-options create data with alleles numerically encoded. ***Binary*** data is coded as 1 / 0. The two sequence data types produce DNA sequence data with Alpha-coding of nucleotides (i.e. A, C, G & T). The ***Codominant Raw Freq.*** and ***Haploid Raw Freq.*** options create a data sheet containing the frequency of each allele per locus by each population, along with a standard genotypic data sheet. The ***Raw Sequence*** sub-option will create a data sheet with the whole length of the sequence in one cell, whereas the ***Sequence*** sub-option will insert each nucleotide base of the sequence into a separate cell to a max. of 254 bp in Excel 2003 or 16, 382 in Excel 2010.The sequence data is created with a low rate of polymorphism to enable finding of haplotypes in downstream analysis.

If the advanced ***TwoGener, Clonal*** or ***Transposed*** menu options are activated via the ***Options->Menus***, the relevant ***Create*** options will also appear as submenus. The ***TwoGener*** option creates a dataset where each offspring has at least one allele from the mother, who is represented as the first sample in each 'mother group'.

Geographic data can be created at the same time as genetic data and is entered in the created worksheet after the genetic data, separated by a blank column. Alternatively, the ***XY*** and ***Lat/Long*** sub-options will create a worksheet containing geographic coordinates in columns 3 and 4.

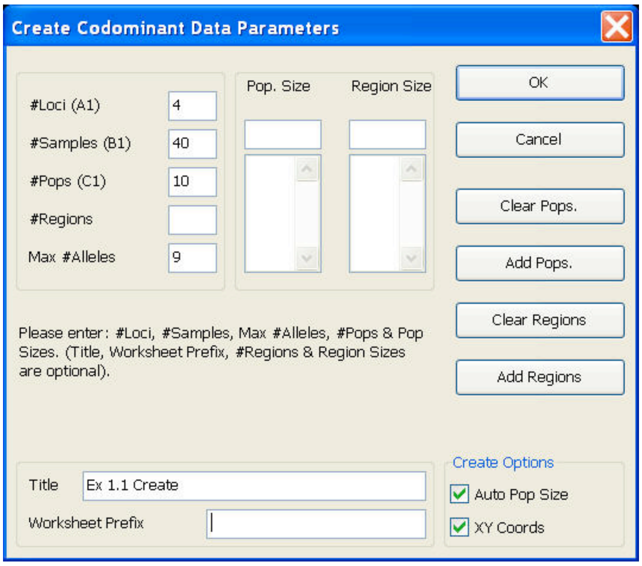
Tip: Create can be used to provide test datasets for the teaching environment. For codominant data, the genotype frequency will approximate those expected under random mating, and thus may be used to demonstrate population genetic patterns typical of random mating.

Tip: Datasets created using this option are in correct GenAlEx format and may be used to test unexpected GenAlEx errors. In this case, use Create to generate a dataset of identical size to your own, and re-test the problematic procedure. If it works, the problem must lie with your dataset.

Procedure for creating genetic data

1. With a workbook open, choose the option ***Create*** from the **GenAlEx** menu, and select the genetic data type required.
2. In the Create Data Parameters dialog box enter the number (#) of loci (which equals the number of nucleotides for sequence data), # samples, # populations, and, if required, # regions in the left hand side panel. These parameters are inserted into the appropriate parameter cell on the data worksheet [D]. For codominant and haploid data enter the max. # Alleles required.
3. Indicate the population size in one of two ways. To create even sized pops ensure the *Auto Pop Size* option is checked (if the sample size is not divisible by the number of pops GenAlEx will reduce the sample size to the nearest divisible number). To create variable sized pops, enter the size of each population in the edit box below 'Pop. Size', and add to the population list using the *Add Pops*. option. Use the *Clear Pops*. option to clear the list. Uncheck the *Auto Pop Size* option. Information regarding regions is entered as for variable population sizes, if required.
4. To create a list of geographic coordinates after the genetic data, check the *XY Coords* option.

5. Enter a Title and worksheet prefix for your data and click *Ok*. Output of genotype data is to worksheet [D], while raw frequencies created by the ***Codominant Raw Freq.*** and ***Haploid Raw Freq*** options are output to worksheet [RAFP].



Create Codominant Data Parameters

#Loci (A1)	4
#Samples (B1)	40
#Pops (C1)	10
#Regions	
Max #Alleles	9

Please enter: #Loci, #Samples, Max #Alleles, #Pops & Pop Sizes. (Title, Worksheet Prefix, #Regions & Region Sizes are optional).

Title: Ex 1.1 Create

Worksheet Prefix:

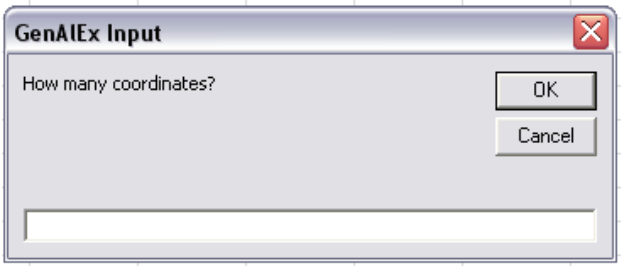
Create Options

☑ Auto Pop Size

☑ XY Coords

Procedure for creating geographic data

1. With a workbook open, choose the option ***Create*** from the **GenAlEx** menu, and select either the ***XY*** and ***Lat/Long*** sub-options.
2. In the GenAlEx input dialog box enter the number (#) of coordinates (samples) required and click *Ok*. Output is to worksheet [XY].



GenAlEx Input

How many coordinates?

Parameters

The Parameters option provides a quick means to obtain the necessary GenAlEx parameters from a pre-existing dataset, and insert them in their correct location. Unless otherwise indicated data must be in standard GenAlEx format, with data labels in column 1 and 2, and data starting in cell C4. The dataset needs to be bounded by empty rows and columns, as GenAlEx uses empty cells to define the limits of the data. All samples per population and region must have the same case sensitive population and region labels respectively, and be in a contiguous block. For each menu sub-option, GenAlEx will interrogate the chosen column(s) and insert the corresponding parameters in the correct locations. Refer to **Tutorial 1, Exercises 1.10** for additional assistance with this option.

All for Codom: When population labels are entered in column 2 this option correctly inserts loci, sample and population parameters for codominant data sets in standard GenAlEx format.

All for Haploid: When population labels are entered in column 2 this option correctly inserts loci, sample and population parameters for haploid data sets in standard GenAlEx format.

All from Raw Freq.: This option will insert the loci, sample and population parameters when the data is in the standard GenAlEx raw frequency format (see the 'Data Formats' section).

Pops from Col 2: When population labels are entered in column 2 this option correctly inserts sample and population parameters.

Pops from Col1: When the population labels are entered in column 1this option correctly inserts sample and population parameters.

Samples from Col1: When sample labels are in column 1 this option correctly inserts sample parameters. This option assumes the data only contains one population and inserts population parameters accordingly.

Loci: Inserts the correct number of loci when each locus is entered as a single column (i.e when it is Haploid, Dominant (Binary) or sequence data).

Codominant Loci: Inserts the correct number of loci when each locus is entered as two adjacent columns (i.e one column for each codominant allele).

Pops + Regions from Col1 + 2: When population and region labels are in columns 1 and 2 respectively, this option correctly inserts the sample, population and region parameters.

Regions + Pops from Col1 + 2: When region and population labels are in columns 1 and 2 respectively, this option correctly inserts the sample, population and region parameters.

Pops from Range: In the GenAlEx Input dialog box select the range that contains the population labels in contiguous blocks in a single column. This option will then insert the sample and population parameters.

Pops + Regions from Range: In the GenAlEx Input dialog box select the range that contains two columns with the population labels in the first column and the region labels in the second. This option will then insert the sample, population and region parameters.

Note: If the sample number has not been entered into B1 or the number entered does not equal the number of rows selected then GenAlEx will warn you that the range does not equal the number of samples. If you proceed then GenAlEx will determine the number of samples from the selected range.

Regions + Pops from Range: In the GenAlEx Input dialog box select the range that contains two columns with the region labels in the first column and the population labels in the second. This option will then insert the sample, population and region parameters.

Insert Header Rows and Params: Inserts two rows at the top of an active worksheet. If the data is arranged with the first row containing column labels, then this option will also correctly insert the sample parameters. This option assumes the data only contains one population and inserts population parameters accordingly.

Data

The ***Data*** menu option offers several commands for quickly manipulating your dataset.

Sort on Sample (Col1): Sorts the entire dataset on column 1(normally containing the sample labels), according to the Excel sort-order rules (see the 'Sample Labels' section). Data must be in appropriate GenAlEx format (including parameters). The sample and population parameters are automatically inserted after the data is sorted; GenAlEx assumes population codes are in column two.

Sort on Pop (Col2): Sorts the entire dataset on column 2 (normally containing the population labels), according to the Excel sort-order rules (see the 'Sample Labels' section). Data must be in appropriate GenAlEx format (including parameters). The sample and population parameters are automatically inserted after the data is sorted; GenAlEx assumes population codes are in column two.

Sort on Sample + Pop (Col1+2): First sorts the entire dataset on column 1 (normally containing the sample labels), then sorts the data within each column 1 (sample) group on column 2 (normally containing the population labels). Data must be in appropriate GenAlEx format (including parameters). The sample and population parameters are automatically inserted after the data is sorted; GenAlEx assumes population codes are in column two.

Sort on Pop + Sample (Col2+1): First sorts the entire dataset on column 2 (normally containing the population labels), then sorts the data within each column 2 (population) group on column 1 (normally containing the sample labels). Data must be in appropriate GenAlEx format (including parameters). The sample and population parameters are automatically inserted after the data is sorted; GenAlEx assumes population codes are in column two.

Select Data Rows: Enables rapid selection of all data rows. This is useful for subsequent sorting on any column using the Excel ***Sort*** option, in the **Data** menu.

Select Data Rows + Labels: Enables rapid selection of all data rows, and labels (Row 3). This is useful for subsequent sorting on any column using the Excel ***Sort*** option, in the **Data** menu.

Split by Pop: Splits data from multiple populations contained in a single dataset. Each individual population dataset is moved to a separate worksheet, labeled with the name of the population. Data must be in appropriate GenAlEx format (including parameters).

Count list from Range: Identifies the number of occurrences of each unique alphanumeric value within a specified range. When prompted by the GenAlEx Input dialog box indicate the desired range. The Output is to a specified location within the active worksheet. When prompted by the GenAlEx Input dialog box indicate the desired location for the first cell of the output table.

Comments from Range: Lists all Excel comment bubbles and the corresponding cell value for cells within a specified range. The output is to a specified location within the active worksheet. When prompted by the GenAlEx Input dialog box indicate the desired range and the location of the first cell of the output.

Row and Column No.: Returns the alphanumeric row and column value of the active cell in a dialog box.

List Worksheets [WS List]: Outputs a list of all worksheets in the active workbook together with their position in the workbook and the contents of Cells A1, B1, A2, B2 and C2.

List Data Worksheets [DWS Lists]: Outputs a list of all GenAlEx formatted data worksheets in the active workbook together with their position in the workbook and their parameters.

List Results Worksheets: Outputs a list of all GenAlEx results worksheets in the active workbook together with their position in the workbook, their title and source data sheet.

Sort Worksheets: This option sorts all work sheets in a workbook alphabetically.

Sort Selected Worksheets: Select the desired worksheets for sorting, and then select this option. This option sorts the selected worksheets alphabetically and then places them in positions 1 to n in the workbook.

Frequency Based Statistical Procedures

Frequency

This menu option provides a range of summary statistics for codominant, haploid and dominant (Binary) data. **Tutorial 1, Exercises 1.2 to 1.5** provide a guide to calculating many of these statistics by hand. **Tutorial 1, Exercises 1.11 and 1.12** provide further assistance with generating these summary statistics using GenAlEx.

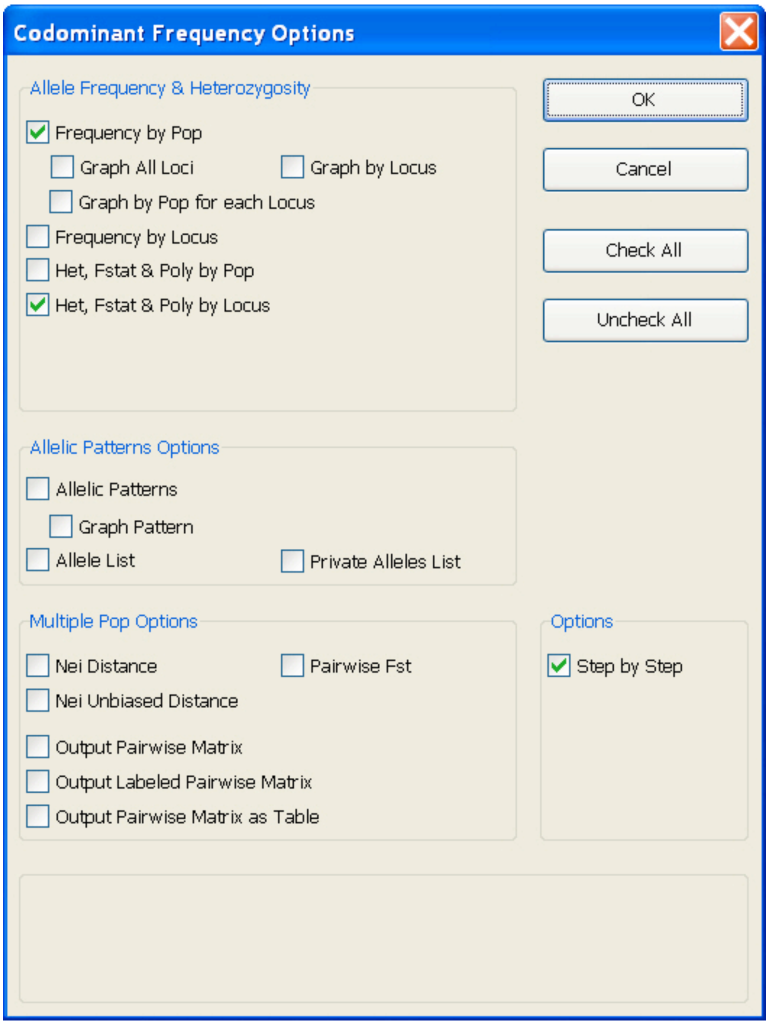
Procedure

1. Choose the option ***Frequency*** from the **GenAlEx** menu.

2. Enter all appropriate information in the Allele Frequency Data Parameters dialog box and click *Ok*.

3. Select the frequency options required from the Frequency Options dialog box (option availability depends on data type), for information about these options see below. See below for the output sheet names.

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

Frequency by Pop [AFP]: Outputs allele frequencies at each locus by population.

Graph All Loci [AGL]: Provides a single graph of locus by locus allele frequency data. For large datasets this output can take some time, and it may be preferable to skip this option

Graph by Locus [AGF]: Provides individual locus graphs of Allele Frequency Data. For large datasets this output can take some time, and it may be preferable to skip this option

Graph by Pop for each Locus[AGP]: Provides individual worksheets for each locus. Each worksheet provides an individual pie chart of allele frequencies for each population.

Frequency by Locus [AFL]: Outputs allele frequencies in each population with loci in columns. For microsatellite datasets (with alleles coded by size in base pairs), this option produces a table with the number of rows equal to the number of distinct allele sizes across the range encountered in the whole dataset. For such datasets, often with certain allele sizes missing, output can take some time.

*Tip**:** The tabled data provide a good visual indication of size distribution of alleles, and size overlap between loci, and can be a useful tool for planning the multiplexing of different loci. The Allele list is an alternative for this.*

Het, Fstat & Poly by Pop [HFP]: Outputs for each population in rows: number of samples (N), the number of alleles (Na), the effective number of alleles (N_e), the information index (I), the observed (H_o), expected (H_e) and unbiased expected heterozygosity (uH_e), and Fixation index (F). This option also outputs the mean over loci and the standard error of each statistic per population along with the grand mean. It also outputs the F statistics ($F_{is,}F_{st}$ and F_{it}) along with the number of effective migrants (Nm) for each locus and the mean across loci. The percentage of polymorphic loci is provided per population. This is the standard format for most primer note publications.

Het, Fstat & Poly by Locus [HFL]: Outputs the same information as the previous option, but with loci in columns not rows.

Allelic Patterns [APT]: Summarizes the mean and standard errors across loci for each population of the following statistics: Number of alleles (Na), Na with frequency >5%, effective number of alleles (Ne), information index (I), Number of private alleles, Number of Locally Common alleles (frequency >=5%) found in <=25% and <=50% of populations, expected heterozygosity (He) and unbiased expected heterozygosity (uHe).

Graph Pattern [APT]: Provides graphical output of the above information.

Allele list [ALI]: Tallies for each locus the occurrence of all distinct allele sizes across the range encountered in the whole dataset.

Tip: This is a useful tool for planning the multiplexing of different loci.

Private alleles list [PAS] & [PAL]: Outputs to sheet [PAS] a list of the private alleles by population, and outputs to sheet [PAL] a list of the samples containing one or more private alleles. This is in standard GenAlEx format for further analyses if required.

Nei Distance: Outputs the pairwise population Nei's Genetic Distance and Nei's Genetic Identity.

Nei Unbiased Distance: Outputs the pairwise Nei's Unbiased Genetic Distance and Nei's Genetic Identity between populations.

Pairwise Fst : Outputs the pairwise Fst values between populations.

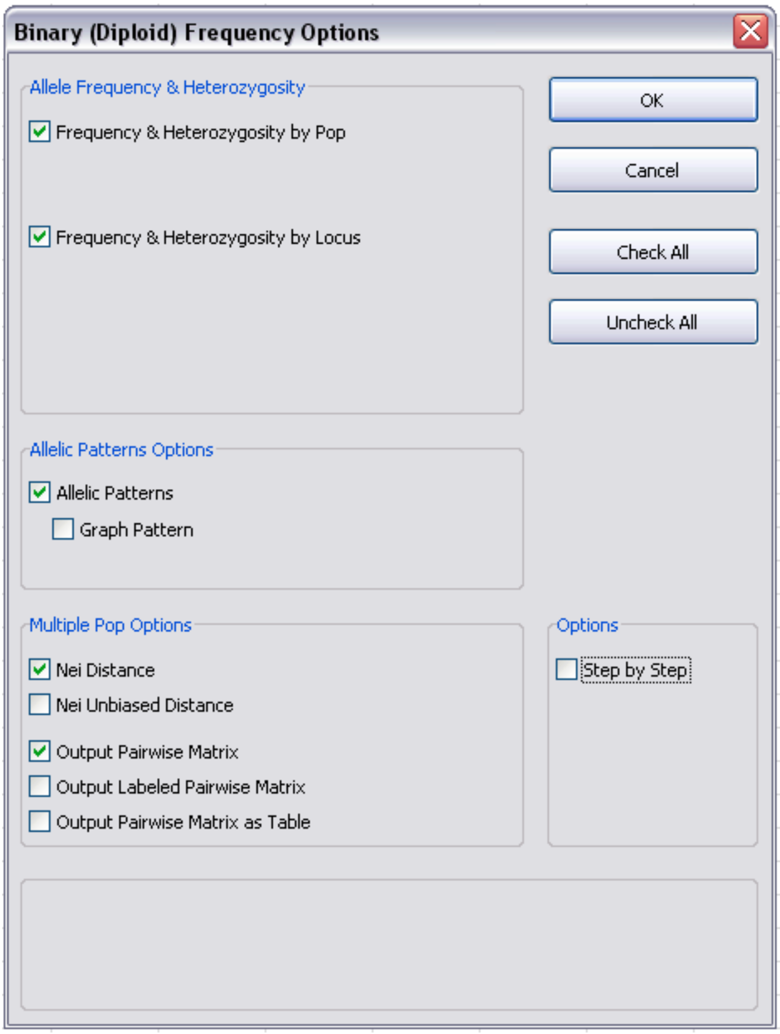
Output Pairwise Matrix: Outputs pairwise population statistics as a triangular Matrix. Output is to worksheet [NeiP] for Nei's Genetic Distance, to [uNeiP] for Nei's Unbiased Genetic Distance and to [FstP] for Pairwise Fst. This is in GenAlEx format for further analyses if required.

Output Labeled Pairwise Matrix: Outputs pairwise population statistics as a labeled triangular Matrix. Output is to worksheet [NeiL] for Nei's Genetic Distance, to [uNeiL] for Nei's Unbiased Genetic Distance and to [FstL] for Pairwise Fst.

Output Pairwise Matrix as Table: Outputs pairwise population statistics as a table. Output is to worksheet [NeiT] for Nei's Genetic Distance, to [uNeiT] for Nei's Unbiased Genetic Distance and to [FstT] for Pairwise Fst.

Step by Step: When the appropriate Multiple Pop, Allelic Patterns or Allele Frequency and Heterozygosity options are ticked this option outputs allele counts to worksheets [AFP] and [AFL]; Ht, He and Ho to worksheets [HFP] and [HFL]; per locus statistics for each population to worksheet [APT]; and the step by step calculations of Nei's Genetic Distance and Identity to worksheet [SbySN].

Binary (Diploid) frequency options



Binary (Diploid) Frequency Options

Allele Frequency & Heterozygosity

☑ Frequency & Heterozygosity by Pop

☑ Frequency & Heterozygosity by Locus

Allelic Patterns Options

☑ Allelic Patterns

□ Graph Pattern

Multiple Pop Options

☑ Nei Distance

□ Nei Unbiased Distance

☑ Output Pairwise Matrix

□ Output Labeled Pairwise Matrix

□ Output Pairwise Matrix as Table

Options

□ Step by Step

Frequency & Heterozygosity by Pop [BAFP]: Outputs in rows band frequency, (*p* & *q*), number of samples (N), number of bands (Na), the effective number of alleles (Ne), the information index (I), expected heterozygosity (H_e) and unbiased expected heterozygosity (uHe) for each locus per population, the mean over loci per population and the grand mean. At the end of the output, the % of polymorphic loci, *P*, is output for each population.

Frequency & Heterozygosity by Locus [BAFL]: Outputs the same information as the previous option, but with loci in columns not rows.

Allelic Patterns [BAPT]: Summarizes for each population the following statistics: Number of bands, Number of bands with frequency >=5%, Number of private bands, Number of Locally Common bands (frequency >=5%)found in <=25% and <=50% of populations, mean expected heterozygosity (He) and unbiased expected heterozygosity (uHe) along with their standard errors.

Graph Pattern [BAPT]: Provides graphical output of the above information.

Nei Distance: Outputs the pairwise population Nei's Genetic Distance and Nei's Genetic Identity.

Nei Unbiased Distance: Outputs the pairwise population Nei's Unbiased Genetic Distance and Nei's Genetic Identity.

Output Pairwise Matrix: Outputs pairwise population statistics as a triangular Matrix. Output is to worksheet [NeiP] for Nei's Genetic Distance and [uNeiP] for Nei's Unbiased Genetic Distance. This is in GenAlEx format for further analyses if required.

Output Labeled Pairwise Matrix: Outputs pairwise population statistics as a labeled triangular Matrix. Output is to worksheet [NeiL] for Nei's Genetic Distance and to [uNeiL] for Nei's Unbiased Genetic Distance.

Output Pairwise Matrix as Table: Outputs pairwise population statistics as a table. Output is to worksheet [NeiT] for Nei's Genetic Distance and to [uNeiT] for Nei's Unbiased Genetic Distance.

Step by Step: Outputs step by step calculations of Nei's Genetic Distance and Identity to worksheet [SbySN], when the *Nei Distance* option is ticked.

Binary (Haploid) frequency options

Frequency & Heterozygosity by Pop [BAFP]: Outputs in rows the band frequency, (*p* & *q*), number of samples (N), number of bands (Na), the information index (I), diversity (h) and unbiased diversity (uh) for each locus per population, the mean over loci per population and the grand mean. At the end of the output, the % of polymorphic loci, *P*, is output for each population.

Frequency & Heterozygosity by Locus [BAFL]: Outputs the same information as the previous option, but with loci in columns not rows.

Allelic Patterns [BAPT]: Summarizes for each population the following statistics: Number of bands, Number of bands with frequency >=5%, Number of private bands, Number of Locally Common bands (frequency >=5%) found in <=25% and <=50% of populations, mean diversity (h) and unbiased diversity (uh) along with their standard errors.

Graph Pattern [BAPT]: Provides graphical output of the above information.

Nei Distance: Outputs the pairwise Nei's Genetic Distance and Nei's Genetic Identity between populations.

Nei Unbiased Distance: Outputs the pairwise Nei's Unbiased Genetic Distance and Nei's Genetic Identity between populations.

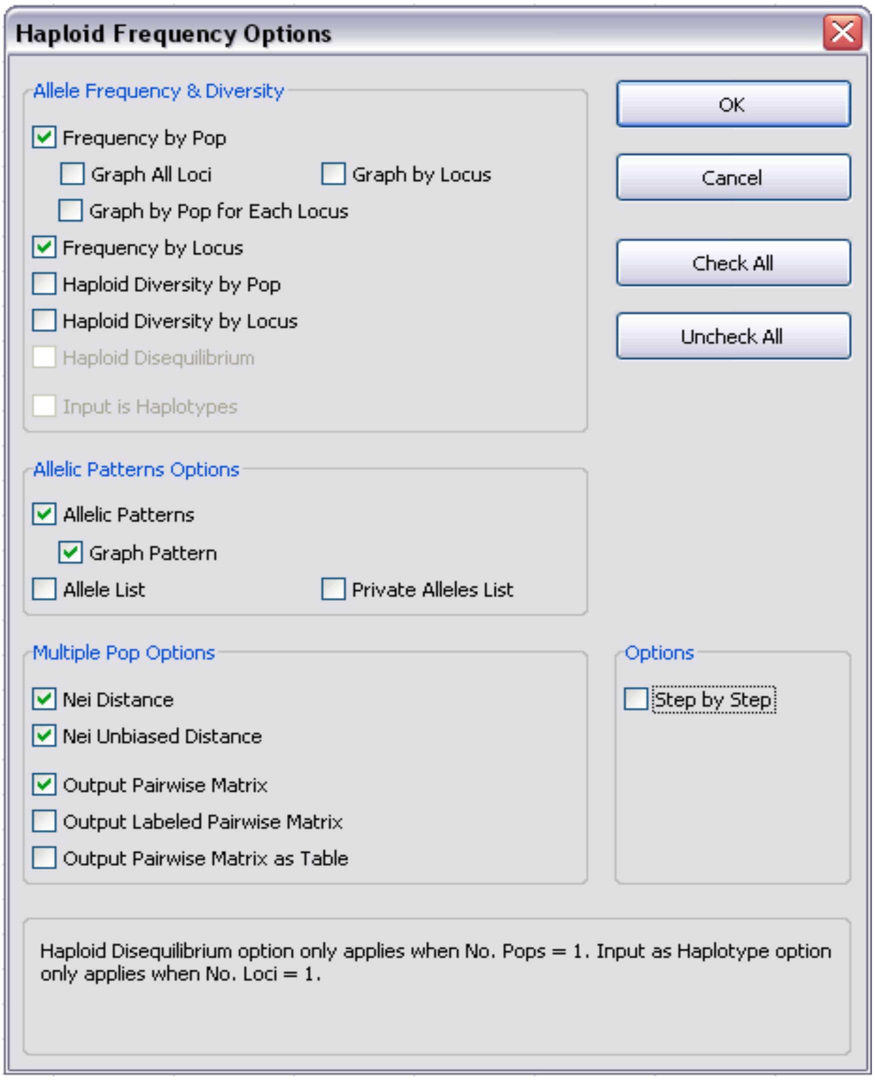
Output Pairwise Matrix: Outputs pairwise population statistics as a triangular matrix. Output is to worksheet [NeiP] for Nei's Genetic Distance and [uNeiP] for Nei's Unbiased Genetic Distance. The output is in GenAlEx format for further analyses if required.

Output Labeled Pairwise Matrix: Outputs pairwise population statistics as a labeled triangular matrix. Output is to worksheet [NeiL] for Nei's Genetic Distance and to [uNeiL] for Nei's Unbiased Genetic Distance.

Output Pairwise Matrix as Table: Outputs pairwise population statistics a table. Output is to worksheet NeiT for Nei's Genetic Distance and to uNeiT for Nei's Unbiased Genetic Distance.

Step by Step: Outputs step by step calculations of Nei's Genetic Distance and Identity to worksheet [SbySN], when the *Nei Distance* option is ticked.

Haploid frequency options



Haploid Frequency Options

Allele Frequency & Diversity

☑ Frequency by Pop

□ Graph All Loci □ Graph by Locus

□ Graph by Pop for Each Locus

☑ Frequency by Locus

□ Haploid Diversity by Pop

□ Haploid Diversity by Locus

□ Haploid Disequilibrium

□ Input is Haplotypes

Allelic Patterns Options

☑ Allelic Patterns

☑ Graph Pattern

□ Allele List □ Private Alleles List

Multiple Pop Options

☑ Nei Distance

☑ Nei Unbiased Distance

☑ Output Pairwise Matrix

□ Output Labeled Pairwise Matrix

□ Output Pairwise Matrix as Table

Options

□ Step by Step

Haploid Disequilibrium option only applies when No. Pops = 1. Input as Haplotype option only applies when No. Loci = 1.

Frequency by Pop [HAFP]: Outputs frequencies of alleles at each locus for each population.

Graph All Loci [HAGL]: Provides graphical output of the above information. For large datasets output can take some time, and it may be preferable to skip this option.

Graph by Locus [HAGF]: Provides individual locus graphs of Allele Frequency Data. For large datasets this output can take some time, and it may be preferable to skip this option.

Graph by Pop for each Locus [HAGP]: Provides individual worksheets for each locus. Each worksheet provides an individual pie chart of allele frequencies for each population.

Frequency by Locus [HAFL]: Outputs allele frequencies in each population with loci in columns.

Haploid Diversity by Pop [HDP]: Outputs in rows the number of samples (N), number of alleles (Na), the effective number of alleles (Ne), the information index (I), diversity (h) and unbiased diversity (uh) for each locus per population, the mean over loci per population and the grand mean. At the end of the output, the % of polymorphic loci, *P*, is output for each population.

Haploid diversity by Locus [HDL]: Outputs the same information as the previous option, but with loci in columns not rows.

Allelic Patterns [HAPT]: Summarizes the mean and standard errors across loci by population for the following statistics: number of alleles (N_a), N_a with frequency >=5%, effective number of alleles (N_e), Number of Locally Common alleles (frequency >=5%) found in <=25% and <=50% of populations, haploid diversity (h) and unbiased diversity (uh).

Graph Pattern [HAPT]: Provides graphical output of the above information.

Haploid disequilibrium [HDE] & [FDHDE]: Outputs the haploid disequilibrium analysis and the results of the randomization test of significance to sheet [HDE]. Also outputs the frequency distribution indicating were the data observed variance (V_o) lies within the randomly generated observed variances to sheet [FDHDE]. When prompted by the GenAlEx Input dialog box specify the desired number of randomizations for testing the significance of haploid disequilibrium (0, 99, 999). This option only applies with a single population.

Input is Haplotypes: Checking this option when a haplotype is coded as a single locus ensures that the output is annotated for haplotype data. This option is only available with single locus data.

Allele List [HALI]: Tallies the occurrence of alleles for each locus over the whole dataset. This is a useful tool for planning the multiplexing of different loci.

Private Alleles List [PAS] & [PAL]: Outputs to sheet [PAS] a list of the private alleles by population, and to output sheet [PAL] a list of the samples containing one or more private alleles. This output is in standard GenAlEx format for further analyses if required.

Nei Distance: Outputs the pairwise Nei's Genetic Distance and Nei's Genetic Identity between populations.

Nei Unbiased Distance: Outputs the pairwise Nei's Unbiased Genetic Distance and Nei's Genetic Identity between populations.

Output Pairwise Matrix: Outputs pairwise population statistics as a triangular matrix. Output is to worksheet [NeiP] for Nei's Genetic Distance and [UNeiP] for Nei's Unbiased Genetic Distance. This output is in GenAlEx format for further analyses if required.

Output Labeled Pairwise Matrix: Outputs pairwise population statistics as a labeled triangular matrix. Output is to worksheet [NeiL] for Nei's Genetic Distance and to [UNeiL] for Nei's Unbiased Genetic Distance.

Output Pairwise Matrix as Table: Outputs pairwise population statistics a table. Output is to worksheet NeiT for Nei's Genetic Distance and to UNeiT for Nei's Unbiased Genetic Distance.

Step by Step: When the appropriate Multiple Pop, Allelic Patterns or Allele Frequency options are ticked this option outputs allele counts to worksheet [HAFP] and [HAFL]; per locus statistics for each population to worksheet [HAPT]; and the step by step calculations of Nei's Genetic Distance and Identity to worksheet [SbySN].

Disquil

This menu contains two sub-menus. The ***HWE*** sub-menu tests each locus by population for despatchers from the expected genotype frequencies under Hardy-Weinburg equilibrium. The ***Paired Biallelic LD*** sub-menu tests for linkage disequilibrium between loci for each population.

HWE

The Hardy-Weinberg option only applies to codominant data.

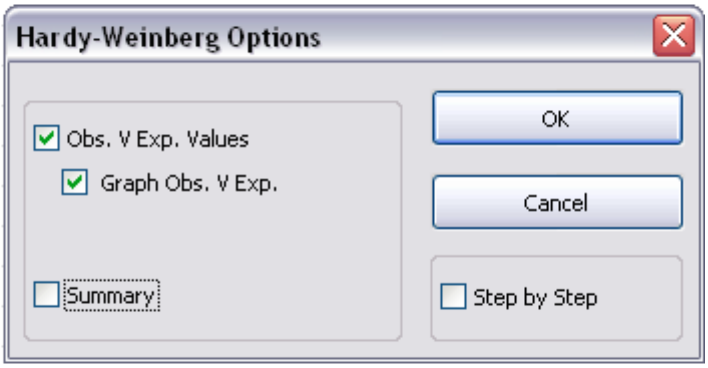
Note: The Chi-squared test of Hardy-Weinberg equilibrium offered in GenAlEx is primarily for teaching and data exploration. An alternative statistical test for assessing an overall departure from random mating is provided in GenAlEx via the AMOVA framework. Other programs provide Exact Tests that are recommended for research purposes. GenAlEx offers data export to these programs. See the GenAlEx 6.5 Appendix 1 for more details.

Procedure

1. Choose the option ***HWE*** option from the **GenAlEx** menu.

2. Enter all appropriate information in the HWE Data Parameters dialog box, click *Ok*.

3. In the subsequent Hardy-Weinberg Options dialog box choose required options (see below), click *Ok*. See options below for the output sheet names.



Hardy-Weinberg Options

☑ Obs. V Exp. Values

☑ Graph Obs. V Exp.

□ Summary

□ Step by Step

Hardy-Weinberg options

Observed vs. Expected Values [HW]: Outputs the observed and expected frequencies of each genotype, and the Chi-Square test for each locus in each population.

Graph Obs. v Exp. [HW]: Outputs graphs for above genotype data.

Summary [HWS]: Provides a summary of the Chi-Squared statistic, degrees of freedom, and probability for each locus in each population.

Step by step [HW]: Shows step by step calculations for the Chi-Squared test.

Paired Biallelic LD

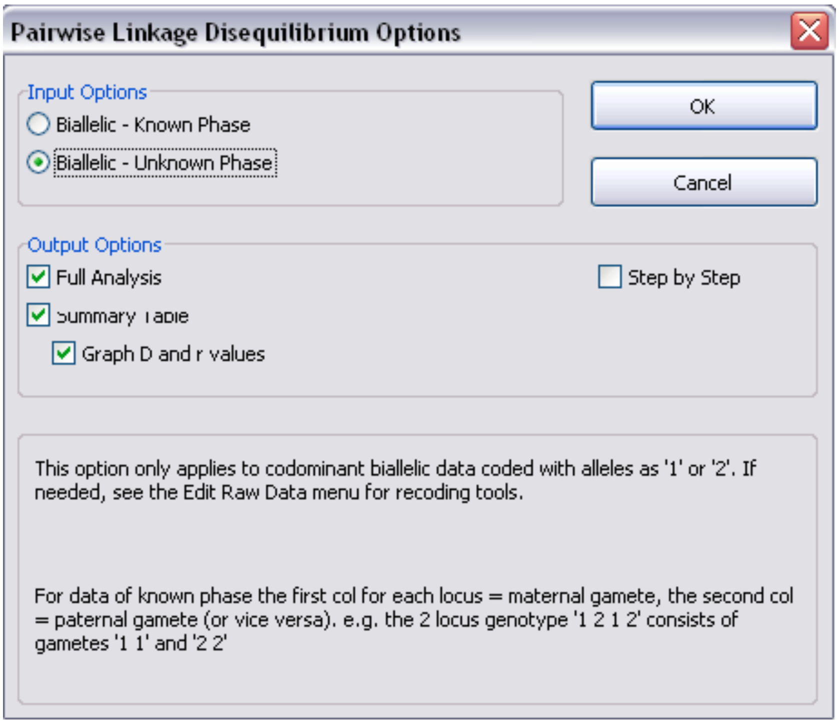
The Paired Biallelic Linkage Disequilibrium option only applies to codominant biallelic data with the two alleles at each locus coded as ‘1’ and ‘2’.

Procedure

1. Choose the ***Paired Biallelic LD*** sub-menu option from the ***Disequil*** menu.

2. Enter all appropriate information in the Paired Linkage Disequilibrium Data Parameters dialog box, click *Ok*.

3. In the subsequent Paired Linkage Disequilibrium Options dialog box choose the required options (see below), click *Ok*. See options below for the output sheet names.



Pairwise Linkage Disequilibrium Options

Input Options

○ Biallelic - Known Phase

● Biallelic - Unknown Phase

Output Options

☑ Full Analysis

☑ Summary Table

☑ Graph D and r values

□ Step by Step

This option only applies to codominant biallelic data coded with alleles as '1' or '2'. If needed, see the Edit Raw Data menu for recoding tools.

For data of known phase the first col for each locus = maternal gamete, the second col = paternal gamete (or vice versa). e.g. the 2 locus genotype '1 2 1 2' consists of gametes '1 1' and '2 2'

Pairwise Linkage Disequilibrium options

Biallelic-Known Phase: Select this option if for each locus and individual every allele can be designated as of paternal or maternal origin. For data of known phase, the maternal gamete must be entered in the first column of each loci with the paternal gamete entered in the second column (or vice versa).

Biallelic-Unknown Phase: Select this option if the origin (maternal or paternal) of the alleles is unknown.

Full Analysis: For data with known phase, this option outputs separate matrices of linkage disequilibrium, gene frequency correlation, Chi-squared statistics and probabilities for each locus pair by population to worksheet [LDK]. For data with unknown phase, this option outputs for each locus pair by population a table of disequilibrium statistics for both possible states (possible maternal/paternal gametes). Output is to worksheet [LDU].

Summary Table: Outputs a summary table of disequilibrium statistics pairwise between loci. For data of known phase, the estimated genetic linkage disequilibrium, standardized D, correlation of gene frequencies, Chi-squared statistic and corresponding probability are output to worksheet [LDKS]. For data of unknown phase, Disequilibrium estimates and chi-squared statistics both assuming and not assuming Hardy-Weinberg equilibrium are output to worksheet [LDUS].

Graph D and r values: Outputs separate graphs for the disequilibrium estimates and gene frequency correlations by each loci pair to worksheet [LDKS] or [LDUS]. If this option is selected then GenAlEx assumes *Summary Table* has been checked.

Step by Step: For data of known phase, this option outputs allele and haplotype counts along with observed and expected haplotype frequencies to worksheet [LDK]. For data of unknown phase, this option outputs allele and genotype counts along with allele frequencies and the maximum likelihood estimated frequency of gamete 11 to worksheet [LDU]. If this option is selected then GenAlEx assumes *Full Analysis* has been checked.

Note: If neither *Full Analysis* nor *Summary Table* has been checked then GenAlEx will default to Summary Table (without graphs).

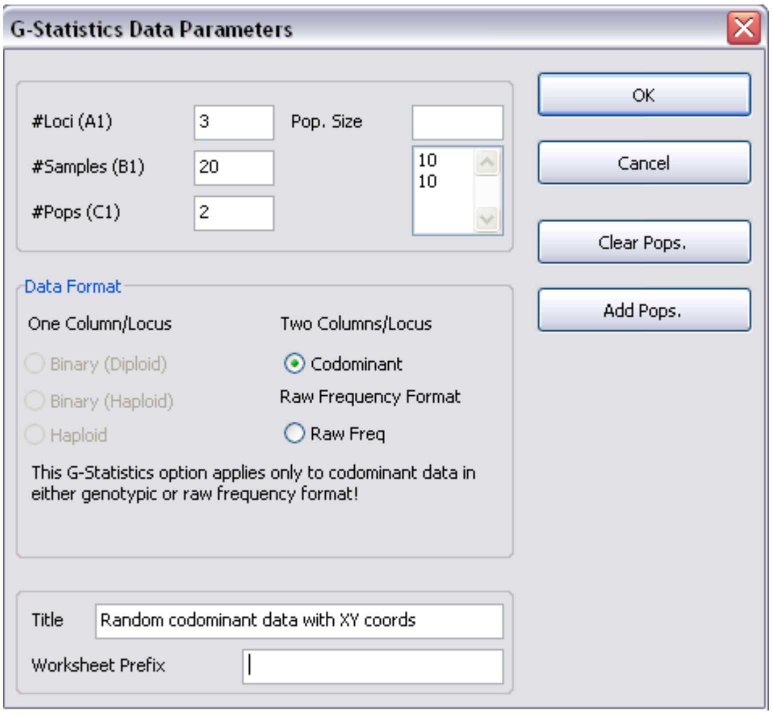
G-Statistics

This menu option calculates a range of recently developed frequency based population structure estimators for codominant data. These measures include Gst, Nei's standardized Gst, Hedrick's standardized Gst, Hedrick's further standardized Gst for small number of populations and Jost's estimate of differentiation. These estimators can be calculated across populations or pairwise. Fst can also be calculated by this menu to facilitate comparison with the newly developed statistics. Significance tests of the calculated measures, via permutation, are available for genotypic data. For further information on these recently developed statistics including formulas refer to **GenAlEx 6.5 Appendix 1**.

Procedure

1. Choose the ***G-Statistics*** sub-menu from the **GenAlEx** menu.

2. Ensure the data parameters are correct and select the appropriate data type in the G-Statistics Data Parameters dialog box, click *Ok*. If the input is raw frequency data, then a subsequent Raw Frequency Parameters dialog box will follow. Ensure the data parameters are correct, click *Ok.*



#Loci (A1) 3

#Samples (B1) 20

#Pops (C1) 2

Pop. Size
10
10

● Codominant

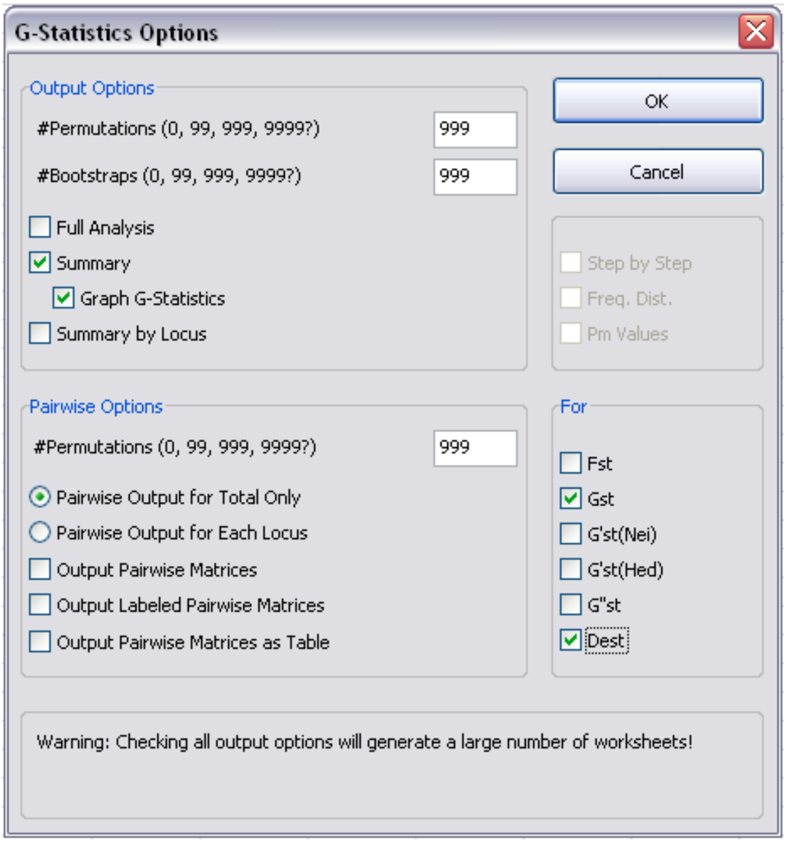
○ Raw Freq

This G-Statistics option applies only to codominant data in either genotypic or raw frequency format!

Title: Random codominant data with XY coords

Worksheet Prefix:

3. In the G-Statistics Options dialog box choose the desired options (see below), click *Ok*. See options below for the output sheet names.



G-Statistics Options

Output Options

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

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

□ Full Analysis

☑ Summary

☑ Graph G-Statistics

□ Summary by Locus

□ Step by Step

□ Freq. Dist.

□ Pm Values

Pairwise Options

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

● Pairwise Output for Total Only

○ Pairwise Output for Each Locus

□ Output Pairwise Matrices

□ Output Labeled Pairwise Matrices

□ Output Pairwise Matrices as Table

For

□ Fst

☑ Gst

□ G'st(Nei)

□ G'st(Hed)

□ G"st

☑ Dest

Warning: Checking all output options will generate a large number of worksheets!

G-Statistics Options

Output Options:

# Permutations: Enter the number of permutations desired for calculating the probabilities for the G-statistics over all populations. Permutation tests are only available for codominant genotypic data, not raw allele frequency data.

# Bootstraps: Enter the number of bootstraps desired for calculating standard errors and confidence intervals. Bootstraps only apply when more than 5 loci are being analyzed.

Full Analysis[Gst]: Outputs the following statistics combined over all populations in table format for each locus and over all loci, along with standard errors and confidence intervals: total number of samples (N), the average number of alleles (Na), the overall effective number of alleles (Ne), the average effective number of alleles (cNe), the mean observed (Ho) and expected heterozygosity (Hs), the total expected heterozygosity (Ht), the corrected mean (cHs) and total expected heterozygosity (cHt), the maximum Gst (GstM), Fis, Fst, Gis, Gst, Nei's standardized Gst (G'stN), Hedrick's standardized Gst (G'stH), Hedrick's further standardized Gst for small number of populations (G''st) and Jost's estimate of differentiation (Dest). In the case of codominant genotypic data this option also outputs a table listing the probabilities for the G-statistics.

Summary [GstG]: Outputs the following statistics combined over all populations in table format for each locus and over all loci, along with standard errors and confidence intervals: Gst, Nei's standardized Gst (G'stN), Hedrick's standardized Gst (G'stH), Hedrick's further standardized Gst for small number of populations (G''st) and Jost's estimate of differentiation (Dest). In the case of codominant genotypic data this option also outputs a table listing the probabilities for the output G-statistics.

Graph G-Statistics: Outputs a graph of the G-statistic values by each locus and overall to sheet [GstG].

Summary by Locus[GstS]: Outputs the following statistics combined over all populations in table format with loci in columns: Fis, Fst, Gis, Gst, Gst, Nei's standardized Gst (G'stN), Hedrick's standardized Gst (G'stH), Hedrick's further standardized Gst for small number of populations (G''st) and Jost's estimate of differentiation (Dest). In the case of codominant genotypic data this option also outputs a table listing the probabilities for the output statistics.

Step by Step: This option is currently not implemented.

Freq. Dist.: This option is currently not implemented.

Pm Values [GstPm]: Outputs the statistics listed under **Full Analysis** for each permutation. This option is only available for codominant genotypic data, not raw allele frequency data.

<u>**Pairwise Options:**</u>

For the pairwise population options the suffixes of the output worksheets represent the combination of options selected. For example, if **Pairwise Output for Total Only [Tot], Output Pairwise Matrices[P]** and <u>**For:**</u> **Fst [Fst]** are selected then the output worksheet suffix would be [Tot FstP].

# **Permutations:** Enter the number of permutations desired for calculating the probabilities for the pairwise G-statistics. Permutation tests are only available for codominant genotypic data, not raw allele frequency data.

Pairwise Output for Total Only [Tot]: Outputs a pairwise population matrix with the selected statistic combined across all loci below the diagonal (see below). For codominant genotypic data pairwise probabilities for the selected statistic are entered above the diagonal.

Pairwise Output for Each Locus: In addition to the output of a pairwise population matrix with the selected statistic combined across all loci, a pairwise population matrix of the selected static is output for each locus separately to a different appropriately named worksheet e.g [Locus1].

Output Pairwise Matrices[P]: Outputs a pairwise population matrix for each selected statistic in standard GenAlEx format.

Output Labeled Matrices[L]: Outputs a labeled pairwise population matrix for each selected statistic.

Output Pairwise Matrices as Table: Outputs a pairwise population matrix as a table for each selected statistic.

<u>**For:**</u> Select the desired statistics to be output in pairwise population matrices from the following: Fst [Fst]; Gst [Gst]; Nei's standardized Gst = G'st (Nei) [GstN]; Hedrick's standardized Gst = G'st (Hed) [GstH]; Hedrick's further standardized Gst for small number of populations = G''st [GstC]; and Jost's estimate of differentiation = Dest [Dest].

Shannon

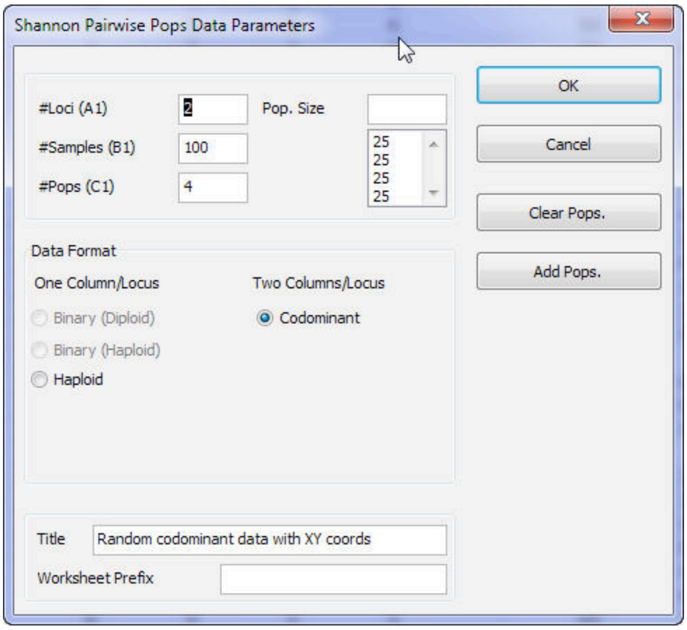
Shannon's diversity index for information theory (Shannon 1948) has been widely employed in ecology but has been less widely used in population genetics. In a recent series of studies, Sherwin et al. (2006) and Rossetto et al. (2008) have shown both by computer simulation and for real data sets that Shannon's Indices offer some ideal statistical properties for measuring biological information across multiple scales from genes to landscapes. In particular, the capacity to apply the indices at multiple scales is unique among the commonly employed population statistics. In GenAlEx Shannon indices can be calculated for codominant or haploid data via the ***Shannon*** menu options. For further assistance with calculating Shannon indices by hand and in GenAlEx refer to **Tutorial 1**, **Exercises 1.13** and **1. 15**. For formulas refer to **GenAlEx 6.5 Appendix 1.** Additional background on the application of Shannon indices to population genetics is also provided in the **Appendix** to **Tutorial 1** written by WB Sherwin.

Pairwise Pops

The ***Pairwise Pops*** sub-menu computes Shannon's mutual information index $^{S}H_{UA}$ between populations, a pairwise measure of differentiation. This option also provides a convenient chi-square based statistical test for allele frequency differences between each pairwise combination of populations through the conversion of $^{S}H_{UA}$ to the log-likelihood contingency test G statistic. This option only applies to codominant and haploid data.

Procedure

1. Activate the worksheet containing your dataset in standard GenAlEx format. Choose the option ***Shannon*** from the **GenAlEx** menu, and then select the submenu option ***Pairwise Pops***.
2. Ensure the locus and sample parameters are correct in the Shannon Pairwise Pops Data Parameters dialog box.
3. Enter Title and Worksheet Prefix then click *Ok*.



Shannon Pairwise Pops Data Parameters

#Loci (A1) 2

#Samples (B1) 100

#Pops (C1) 4

Pop. Size

25
25
25
25

Data Format

One Column/Locus

○ Binary (Diploid)
○ Binary (Haploid)
○ Haploid

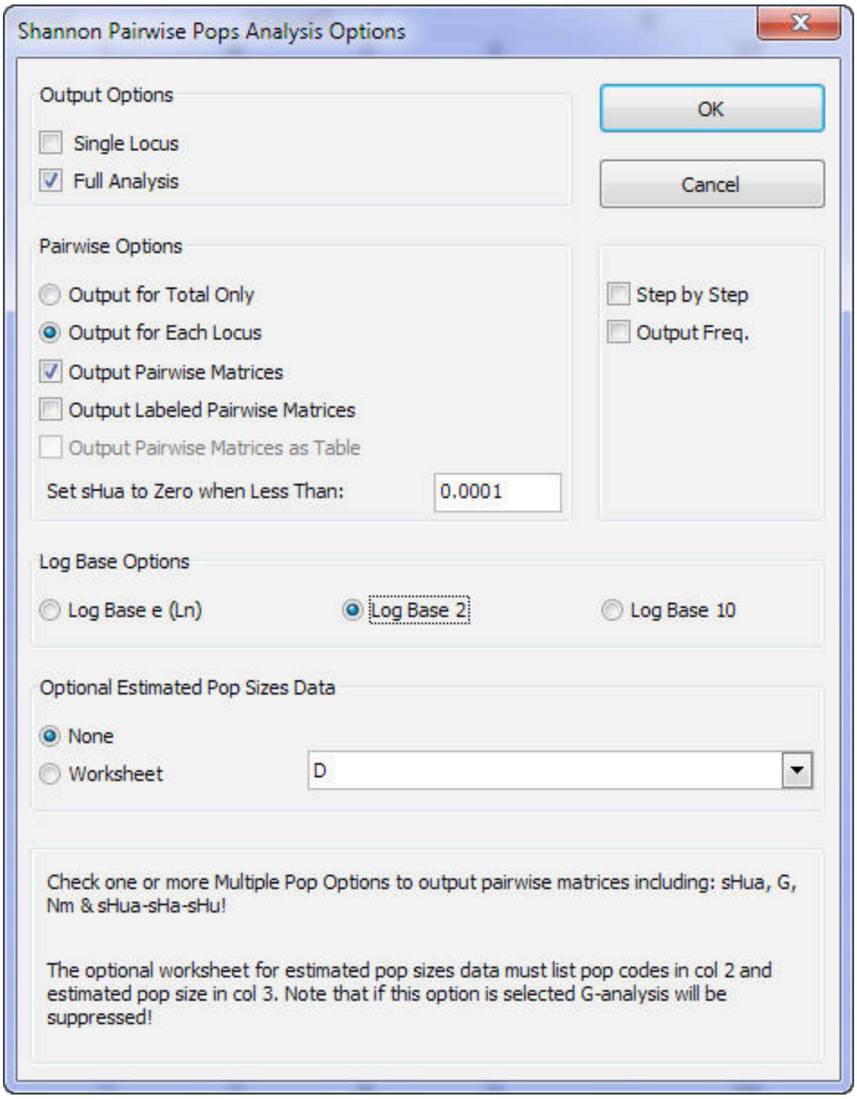
Two Columns/Locus

● Codominant

Title: Random codominant data with XY coords

Worksheet Prefix:

4. In the subsequent Pairwise Pops Shannon Analysis Options dialog box, select the options required (see below). Then Click *Ok*. Summary of the Shannon analysis, including Shannon's *mutual information* index $^{S}H_{UA}$, G statistic and Chi significance test, over loci for all pairwise population combinations is output to worksheet [SH].



Shannon Pairwise Pops Analysis Options

Output Options

□ Single Locus

☑ Full Analysis

Pairwise Options

○ Output for Total Only

● Output for Each Locus

☑ Output Pairwise Matrices

□ Output Labeled Pairwise Matrices

□ Output Pairwise Matrices as Table

Set sHua to Zero when Less Than: 0.0001

□ Step by Step

□ Output Freq.

Log Base Options

○ Log Base e (Ln) ● Log Base 2 ○ Log Base 10

Optional Estimated Pop Sizes Data

● None

○ Worksheet D

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!

Pairwise Pops Shannon Analysis Options

Single Locus: Outputs Shannon's allelic diversity index $^{S}H_{A}$ for each locus by Population to worksheet [sHa].

Full Analysis: Check this option to output the summary of Shannon analysis over loci for all pairwise Population Combinations to worksheet [SH]. Note that the summary table will reflect the pairwise options selected below. For example, if you select *Output for Total Only*, the summary table will only show the Mean over Loci.

Output for Total Only: Outputs mean values for Shannon's indices over loci for all pairwise population combinations.

Output for Each Locus: In addition to the mean values for the Shannon's indices, this option outputs the summary of the Shannon analysis for each loci to worksheet [SH]. When one of the output pairwise matrices options is selected, this option also outputs pairwise matrices for each locus to separate appropriately named worksheets e.g [Locus 4 SHuaP].

Output Freq. [AFPT]: Outputs allele frequencies and samples sizes by locus for each population.

Step by Step: Outputs step by step calculations of Shannon's *mutual information* index ${}^{S}H_{UA}$ for all pairwise population combinations at each loci to separate appropriately named worksheets e.g [Locus2 SHSbyS].

Outputs Pairwise Matrices: Outputs a series of matrices containing the pairwise Shannon indices (${}^{S}H_{A}$, ${}^{S}H_{U}$, ${}^{S}H_{UA}$), and estimated number of migrants between populations to sheet [SHaP].

Output Labeled Pairwise Matrices: Outputs labeled versions of the above described pairwise matrices to sheet [SHaL].

Output Pairwise Matrices as Table: Outputs the above described pairwise matrices as tables to sheet [SHaT].

Set sHua to Zero when Less Than: Select a cut off value below which sHua values will be converted to zero. This option prevents anomalous estimates of migrants between populations (Nm) due to very small sHua values. The default value is 0.0001.

Log Base Options:

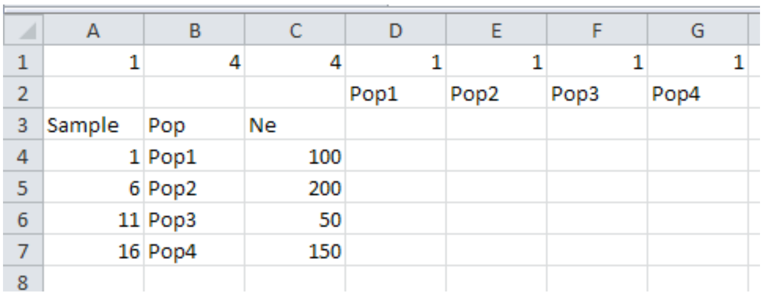
Select the log base for calculating the Shannon diversity indices. Log base 2 is recommended by Sherwin et al. (2006) as it translates to heterozygosity. However, the natural Log is commonly used in ecology and may be useful for comparison between different levels of diversity. Log base 10 is also available. The value of the Shannon indices will change with the base selected; however, the estimated number of migrants is unaffected.

Optional Estimated Pop Sizes Data:

The effective population size of each population can be used in the calculation of the Shannon Indices and in the estimation of the effective number of migrants. To do so, ensure *Worksheet* is ticked and select the appropriate worksheet from the dropdown list. The worksheet containing the effective population size data must be in GenAlEx format (with parameter and data starting in row 4) with population codes in column 2 and estimated effective population sizes in column 3. If this option is not required select *None.* Where possible this option is recommended for accurate estimation of effective migrants when effective population sizes are less than 500 for diploids or 1000 for haploids.

Note: If the estimated population sizes option is selected G-analysis is suppressed.

Example of format for effective population size data:



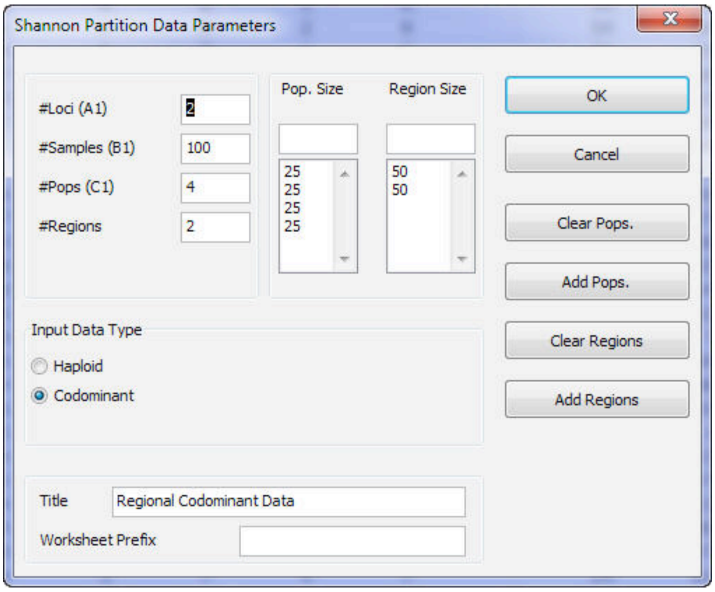
	A	B	C	D	E	F	G
1	1	4	4	1	1	1	1
2				Pop1	Pop2	Pop3	Pop4
3	Sample	Pop	Ne				
4	1	Pop1	100				
5	6	Pop2	200				
6	11	Pop3	50				
7	16	Pop4	150				
8							

Partition

The ***Partition*** sub-menu partitions genetic diversity into three levels (e.g. within populations, among populations and among regions) using Shannon indices. In addition to calculating Shannon's mutual information index this menu option derives a standardized measure of diversity that is bounded by zero and one, allowing easy comparison between studies. This option also provides a random permutation test for statistical significance in place of the G-test offered under the Shannon Pairwise option. For research purposes, statistical testing by random permutation is recommended because there are reports that the log-likelihood G-test may exhibit high type I error rates (false rejection of the null hypothesis). This option only applies to codominant and haploid data.

Procedure

1. Activate the worksheet containing your dataset in standard GenAlEx format. Choose the option ***Shannon*** from the **GenAlEx** menu, and then select the submenu option ***Partition***.
2. Ensure the parameters, including the population and region sizes, are correct in the Shannon Partition Data Parameters dialog box.
3. Select the appropriate input data type. Enter Title and Worksheet Prefix then click *Ok*.



Shannon Partition Data Parameters

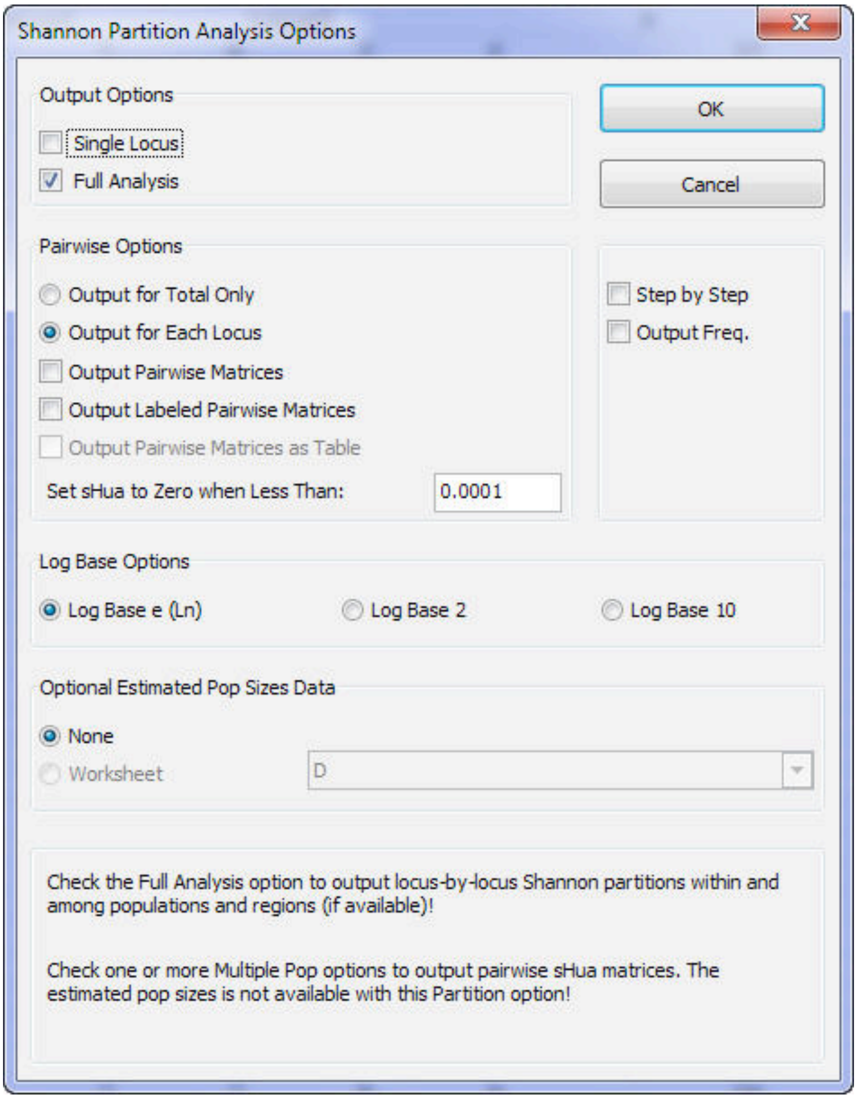
#Loci (A1) 2
#Samples (B1) 100
#Pops (C1) 4
#Regions 2

Pop. Size: 25 25 25 25
Region Size: 50 50

Input Data Type
○ Haploid
● Codominant

Title: Regional Codominant Data
Worksheet Prefix:

4. In the subsequent Shannon Partition Analysis Options dialog box, select the options required (see below). Then Click *Ok*.



Shannon Partition Analysis Options

Output Options

□ Single Locus

☑ Full Analysis

Pairwise Options

○ Output for Total Only

● Output for Each Locus

□ Output Pairwise Matrices

□ Output Labeled Pairwise Matrices

□ Output Pairwise Matrices as Table

Set sHua to Zero when Less Than: 0.0001

□ Step by Step

□ Output Freq.

Log Base Options

● Log Base e (Ln) ○ Log Base 2 ○ Log Base 10

Optional Estimated Pop Sizes Data

● None

○ Worksheet D

Check the Full Analysis option to output locus-by-locus Shannon partitions within and among populations and regions (if available)!

Check one or more Multiple Pop options to output pairwise sHua matrices. The estimated pop sizes is not available with this Partition option!

Shannon Partition Analysis Options

Single Locus [SHa]: Outputs Shannon's information index (SHa) by locus for each population, region and in total.

Full Analysis: Check this option to output locus-by-locus Shannon partitions within and among populations and regions (if available) to worksheet [Locus1 SHT] etc. Note that unlike the ***Pairwise Pops*** submenu option the summary sheet [SH] is always output. However, the summary table will reflect the pairwise options selected below. For example, if you select *Output for Total Only*, the summary table will only show the Mean over Loci.

Output for Total Only: Outputs mean values for the Shannon indices over loci for all pairwise population combinations.

Output for Each Locus: In addition to the mean values for the Shannon indices this option also outputs pairwise matrices for each locus to separate appropriately named worksheets e.g [Locus 4 SHuaP].

Output Pairwise Matrices [SHuaP]: Outputs the pairwise Shannon Mutual information index ${}^{S}H_{UA}$.

Output Labeled Pairwise Matrices [SHuaL]: Outputs labeled versions of the pairwise matrices described above.

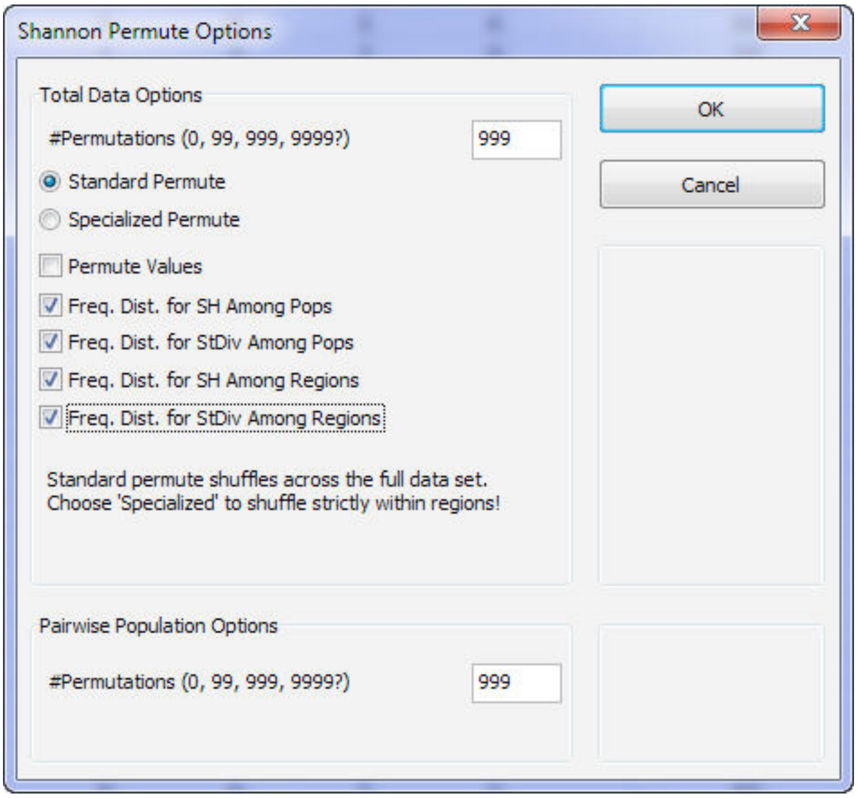
Set sHua to Zero when Less Than: Select a cut off value below which sHua values will be converted to zero. This option prevents anomalous estimates of migrants between populations (Nm) due to very small sHua values. The default value is 0.0001.

Output Freq. [AFPT]: Outputs allele frequencies and sample sizes by locus for each population, region and in total.

Log Base Options:

Select the log base for calculating the Shannon diversity indices. Sherwin et al. (2006) recommended Log base 2 as it translates to heterozygosity. However, the natural Log is commonly used in ecology and may be useful for comparison between different levels of diversity. Log base 10 is also available. The value of the Shannon indices will change with the base selected; however, the standardized diversities *alpha*, *beta*, *gamma*, *delta* and *omega* are not changed.

5. In the Shannon Permute Options dialog box, indicate the number of permutations required and select the desired output (see below). Then Click *Ok*. The Shannon statistics partitioned by population, region and total are output separately for each locus to an appropriately named worksheet e.g. [Locus1 SHT]. A summary of the Shannon analysis over loci is also output to worksheet [SH].


Shannon Permute Options

Total Data Options

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

● Standard Permute

○ Specialized Permute

□ Permute Values

☑ Freq. Dist. for SH Among Pops

☑ Freq. Dist. for StDiv Among Pops

☑ Freq. Dist. for SH Among Regions

☑ Freq. Dist. for StDiv Among Regions

Standard permute shuffles across the full data set.
Choose 'Specialized' to shuffle strictly within regions!

Pairwise Population Options

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

Shannon Permute Options

Total Data Options:

#Permutations: Enter the number of permutations required to test for significance. Note: For large data sets, permutation may take some time. Watch the status bar for progress. For publication purposes the number of permutations should be set to 999 or 9999.

Standard permute: Shuffles individuals between populations and regions.

Specialized permute: Only shuffles individuals within regions.

Permute Values [Pm SH]: Outputs from each standard permutation the Shannon indices and standardized diversity for within populations, between populations and among regions.

Freq. **Dist. For SH Among Pops:** Outputs the frequency distribution for the random versus observed among population Shannon statistics to worksheet [Pm FD].

Freq. **Dist. For StDiv Among Pops:** Outputs the frequency distribution for the random versus observed among population standardized diversity to worksheet [Pm FD].

Freq. **Dist. For SH Among Regions:** Outputs the frequency distribution for the random versus observed among region Shannon statistics to worksheet [Pm FD].

Freq. **Dist. For StDiv Among Regions:** Outputs the frequency distribution for the random versus observed among region standardized diversity to worksheet [Pm FD].

Pairwise Population Options:

#Permutations: Enter the number of permutations required to test for significance of the pairwise Shannon indices among populations.

Relatedness

GenAlEx provides options, under ***Pairwise***, for the calculation of several pairwise relatedness estimators that are widely used in the literature. In addition, the option ***Pop Means*** enables the calculation of the average pairwise relatedness of populations, and statistical testing by random permutation.

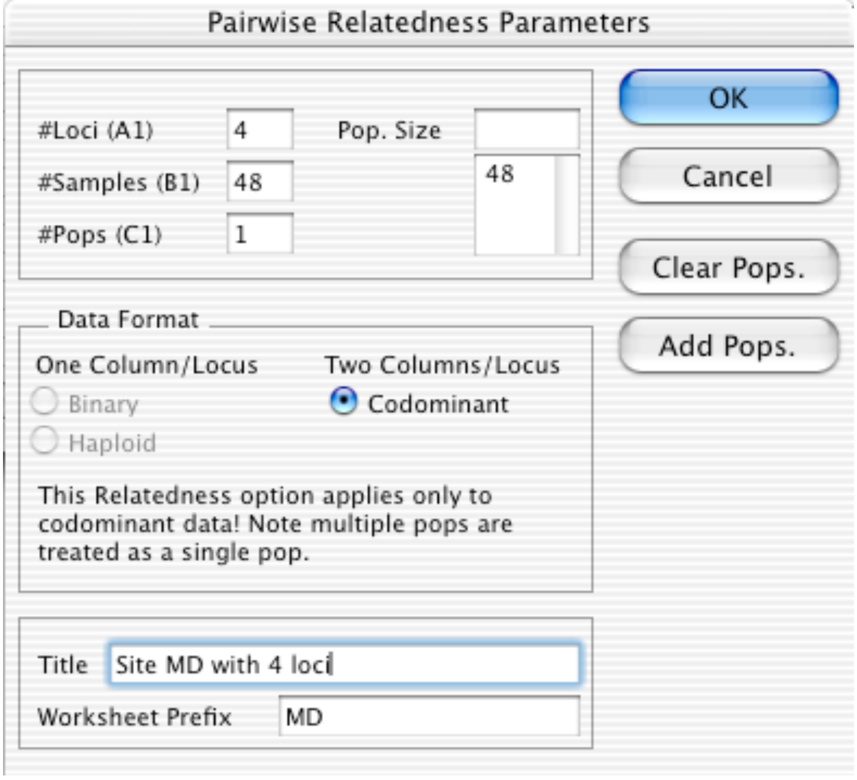
Tip: Populations in the context of relatedness may also be family groups, or sexes.

Pairwise

The ***Pairwise*** option applies only to codominant data for a single population. If multiple populations are present in the dataset, the analysis ignores the population parameters, and treats data as a single population. However, the parameters are carried over for the ***Pops Mean*** analysis. For additional assistance with this option refer to **Tutorial 4, Exercise 4.8**.

Procedure

1. Activate the worksheet containing your codominant dataset in GenAlEx format. Choose the option ***Relatedness*** from the **GenAlEx** menu, and then select the submenu option ***Pairwise***.
2. Ensure the locus and sample parameters are correct in the Pairwise Relatedness Parameters dialog box.
3. Enter Title and Worksheet Prefix then click *Ok*.



Pairwise Relatedness Parameters

#Loci (A1) 4

#Samples (B1) 48

#Pops (C1) 1

Pop. Size

48

Data Format

One Column/Locus

○ Binary

○ Haploid

Two Columns/Locus

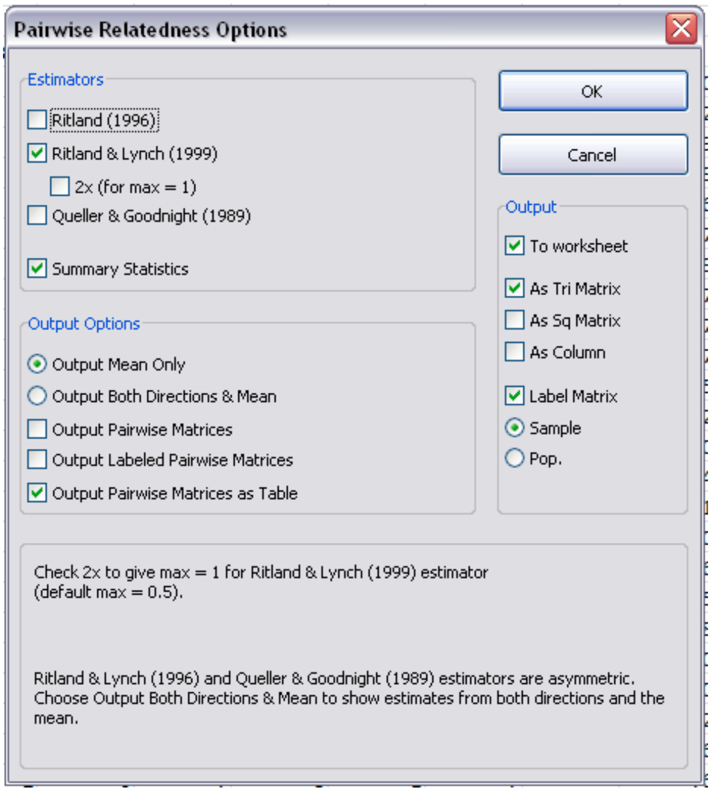
● Codominant

This Relatedness option applies only to codominant data! Note multiple pops are treated as a single pop.

Title Site MD with 4 loci

Worksheet Prefix MD

4. In the subsequent Pairwise Relatedness Options dialog box, select the options required (see below). See options below for the output sheet names.



Estimators

□ Ritland (1996)
☑ Ritland & Lynch (1999)
□ 2x (for max = 1)
□ Queller & Goodnight (1989)
☑ Summary Statistics

Output Options

● Output Mean Only
○ Output Both Directions & Mean
□ Output Pairwise Matrices
□ Output Labeled Pairwise Matrices
☑ Output Pairwise Matrices as Table

Output

☑ To worksheet
☑ As Tri Matrix
□ As Sq Matrix
□ As Column
☑ Label Matrix
● Sample
○ Pop.

Check 2x to give max = 1 for Ritland & Lynch (1999) estimator (default max = 0.5).

Ritland & Lynch (1996) and Queller & Goodnight (1989) estimators are asymmetric. Choose Output Both Directions & Mean to show estimates from both directions and the mean.

Pairwise Relatedness Options

Estimators

Ritland (1996) [RI]: Outputs the values for Ritland's (1996) RI estimator.

Ritland & Lynch (1999) [LR]: Outputs the values for Lynch & Ritland's (1999) LR estimator. This estimator has a range of -0.5 to 0.5.

2x (for max=1): Multiplies Lynch & Ritland's (1999) LR estimator by 2 to give a maximum value of 1 and minimum of -1. This standardizes LR's range with other common estimators.

Queller & Goodnight (1989) [QG]: Outputs the values for Queller & Goodnight's (1989) estimator.

Summary Statistics [RS]: Outputs the pairwise relatedness values of all selected estimators in a summary table format when the *Output Pairwise Matrices as Table* option is selected.

Output Options

Output Mean Only: Check this box to output Lynch & Ritland's and/or Queller & Goodnight's mean estimators only.

Output Both Directions & Mean: Check this box to output Lynch & Ritland's and/or Queller & Goodnight's mean estimators and both asymmetric estimators. Worksheet suffix depends on selected estimator, worksheets containing asymmetric estimators end with 1 and 2, while worksheets containing mean estimators end in 'M'.

Output Pairwise Matrices: Outputs a pairwise matrix for each relatedness estimator. Matrix form depends on selected output option (see 'output' below). Output is to worksheet with estimator suffix (described above).

Output Label Pairwise Matrices: Outputs Labeled versions of the pairwise relatedness matrices to worksheets [RIL], [RLL] and [QGL].

Output Pairwise Matrices as Table [RS]: Outputs the pairwise relatedness values for each estimator (both asymmetric and mean) as a table.

Output:

To Worksheet: Outputs the Relatedness estimators to a worksheet. Choose your desired format from the three options:

As Tri Matrix: Outputs relatedness matrix as a lower triangular matrix.

As Sq Matrix: Outputs relatedness matrix as a symmetric square matrix.

As Column: Outputs relatedness matrix as a column.

Label Matrix: Attaches sample or population labels to your matrix.

Pops Mean

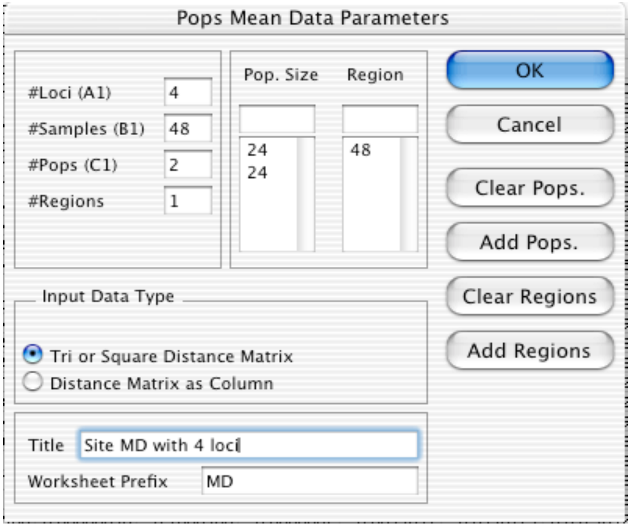
This option requires a pairwise relatedness matrix as input, with parameters specifying the populations for which GenAlEx will calculate an average within the population. If you intend to also constrain the calculation of your means within populations by distance, GenAlEx requires a geographic distance matrix, in standard GenAlEx format. For additional assistance with this option refer to **Tutorial 4, Exercise 4.9**. This option is analogous to the ***Pops as Dclass*** option available under the ***Spatial*** sub-menu option.

Tip: This option is not restricted to a relatedness matrix and can be applied to the analysis of means for any GenAlEx distance matrix.

Procedure

1. Activate the worksheet containing your pairwise relatedness matrix in GenAlEx format. Choose the option ***Relatedness*** from the **GenAlEx** menu, and then select the submenu option ***Pops Mean***.

2. Ensure the locus and sample parameters are correct in the Pops Mean Data Parameters dialog box. Select the appropriate data input type. Enter Title and Worksheet Prefix then click *Ok*.



Pops Mean Data Parameters

#Loci (A1) 4
#Samples (B1) 48
#Pops (C1) 2
#Regions 1

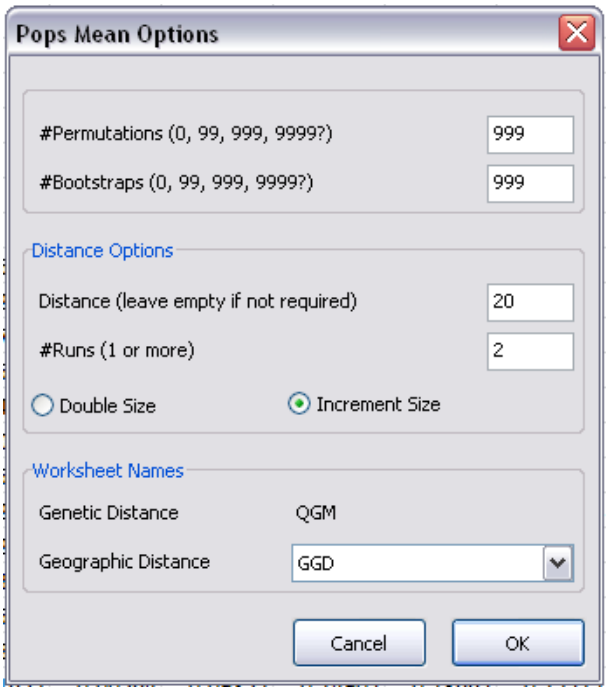
Pop. Size	Region
24	48
24	

Input Data Type
● Tri or Square Distance Matrix
○ Distance Matrix as Column

Title: Site MD with 4 loci
Worksheet Prefix: MD

3. In the subsequent Pops Mean Options dialog box, select the options required (see below).

4. If you wish to restrict the comparisons within the populations, enter the desired distance options, otherwise leave this section blank. Click *Ok*. Output is to sheet [PM1].



Pops Mean Options

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

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

Distance Options

Distance (leave empty if not required) 20

#Runs (1 or more) 2

○ Double Size ● Increment Size

Worksheet Names

Genetic Distance QGM

Geographic Distance GGD

Pops Mean Options

#Permutations: Enter the number of permutations required to test for significance. Note: For large data sets, permutation may take some time. Watch the status bar for progress. For publication purposes the number of permutations should be set to 999 or 9999.

#Bootstraps: Enter the number of bootstraps required to estimate the 95% confidence interval around *r*. For publication purposes the number of bootstraps should be set to 999 or 9999.

Distance Options

Distance: If you wish to restrict the comparisons within the populations, enter the maximum desired distance between samples to be compared. Otherwise leave blank.

#Runs: For multiple distance classes, enter the number of times you wish to restart the analysis with a new distance value. Otherwise leave blank. The first distance class will be output to [PM1], the second distance will be output to [PM2] etc.

Double Size: This option doubles the maximum distance between samples to be compared each run.

Increment Size: This option increases the maximum distance between samples to be compared by a set amount each run.

Worksheet Names

Geographic Distance: If you wish to restrict comparisons within the populations, select the worksheet containing the geographic distance matrix.

Multilocus

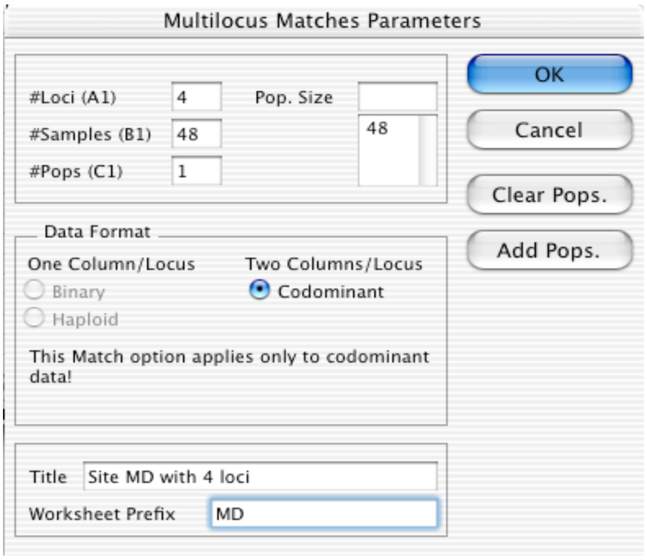
This menu option provides tools for genetic tagging. It may also be useful in other contexts, such as to locate duplicate samples in a dataset, or locate clones in plant or bacterial datasets. The menu has four sub options: ***Matches***, which automates the detection of repeated genotypes within the dataset; ***Genotype Prob***., which calculates the probability of a random match to a given specific genotype or DNA profile in the given population; ***Prob. Identity***, which provides an estimate of the average probability that two unrelated individuals drawn from the same population will have the same multilocus genotype; and ***Prob. Exclusion***, which offers three different probability estimates of paternity exclusion, depending on whether parents are known, or not. **Tutorial 4, Exercise 4.2** provides further information on genotype probability, while **Exercises 4.3** and **4.4** provide assistance on Probability of Identity. Refer to **Exercise 4.10** for assistance with Probability of Exclusion.

Procedure

1. Activate the worksheet containing your codominant dataset in GenAlEx format. Choose the option ***Multilocus*** from the **GenAlEx** menu, and then select one of four submenu options: ***Matches***, ***Genotype Prob***. ***Prob. Identity*** and ***Prob. Exclusion***. All sub options carry a standard initial Parameters dialog box.
2. Ensure the locus and sample parameters are correct in the relevant Multilocus Parameters dialog box.

Note: The *Matches* option treats all data as if it belongs to one population.

3. Enter Title and Worksheet Prefix then click *Ok*. For ***Matches*** a subsequent options dialog box appears. For ***Genotype Probability***, ***Probability Identity***, the results are output to sheet [GP] and [PI], respectively. For ***Prob. Exclusion,*** the probability of exclusion when the other parent is known is output to sheet [P1X], while, the probability of exclusion when the other parent is missing and the probability for excluding a putative parent pair are output to sheets [P2X] and [P3X] respectively.



Multilocus Matches Parameters

#Loci (A1) 4

#Samples (B1) 48

#Pops (C1) 1

Pop. Size 48

Data Format

One Column/Locus

○ Binary

○ Haploid

Two Columns/Locus

● Codominant

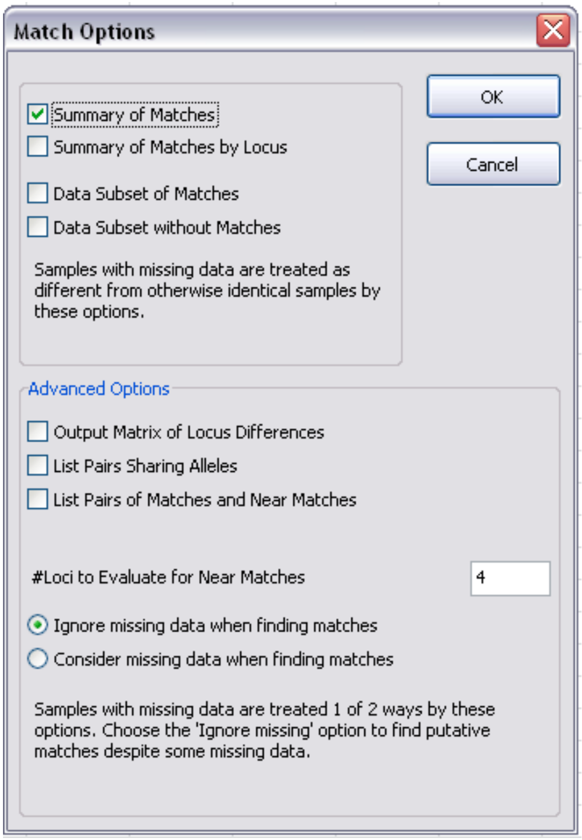
This Match option applies only to codominant data!

Title Site MD with 4 loci

Worksheet Prefix MD

Matches

In the Match Options dialog box check the options required (see below for further details), and click *Ok*. See options below for the output sheet names.



Match Options

☑ Summary of Matches
□ Summary of Matches by Locus

□ Data Subset of Matches
□ Data Subset without Matches

Samples with missing data are treated as different from otherwise identical samples by these options.

Advanced Options

□ Output Matrix of Locus Differences
□ List Pairs Sharing Alleles
□ List Pairs of Matches and Near Matches

#Loci to Evaluate for Near Matches: 4

● Ignore missing data when finding matches
○ Consider missing data when finding matches

Samples with missing data are treated 1 of 2 ways by these options. Choose the 'Ignore missing' option to find putative matches despite some missing data.

Match Options

Samples with missing data are treated as different from otherwise identical samples by the following options.

Summary of Matches [MS]: Outputs a list of the multilocus genotypes present, with the count and a label for each genotype. Samples are sorted, with the repeated genotypes shown first.

Summary of Matches by locus [MLS]: Outputs a summary of multilocus matches by locus for increasing locus combinations, with a graph plotting these results.

Note: In this option loci are added in the order they appear in the input worksheet and the output will vary with the loci order.

Tip. This option is useful for determining the minimum number of loci needed for genetic tagging, when combined with the results of the genotype prob. and prob. identity estimates.

Data Subset of Matches [MDS]: Outputs a data subset containing those individuals with matching multilocus genotypes. Output includes the number of matches and a label for each genotype.

Data Subset Without Matches [DS]: Outputs a subset of the data excluding samples possessing a repeated genotype. The last individual to carry the repeated genotype is the one retained.

Advanced Options:

For the following options, samples with missing data are treated in 1 of 2 ways. Choose '**Ignore missing data when finding matches**' to find putative matches despite some missing data. Choose '**Consider missing data when finding matches**' to treat missing data as different.

Output Matrix of Locus Differences [MLD]: Outputs a square matrix containing the number of mismatching loci.

List Pairs Sharing Alleles [MAL]: Outputs a list of pairs of samples sharing at least one allele at each locus.

List of Matches and Near Matches [ML2]: Outputs a list of pairs of samples matching at all loci, all but one locus, all but two loci and so on until the maximum number of miss match loci.

Tip: This option is particularly useful for finding genotyping errors.

# Loci to Evaluate for Near Matches: Indicates the maximum number of miss match loci to output to [ML2].

Assignment

The ***Assignment*** option provides two submenus, ***Pop Assign*** and ***Sex Bias***. These analyses only apply to codominant data.

Pop Assign

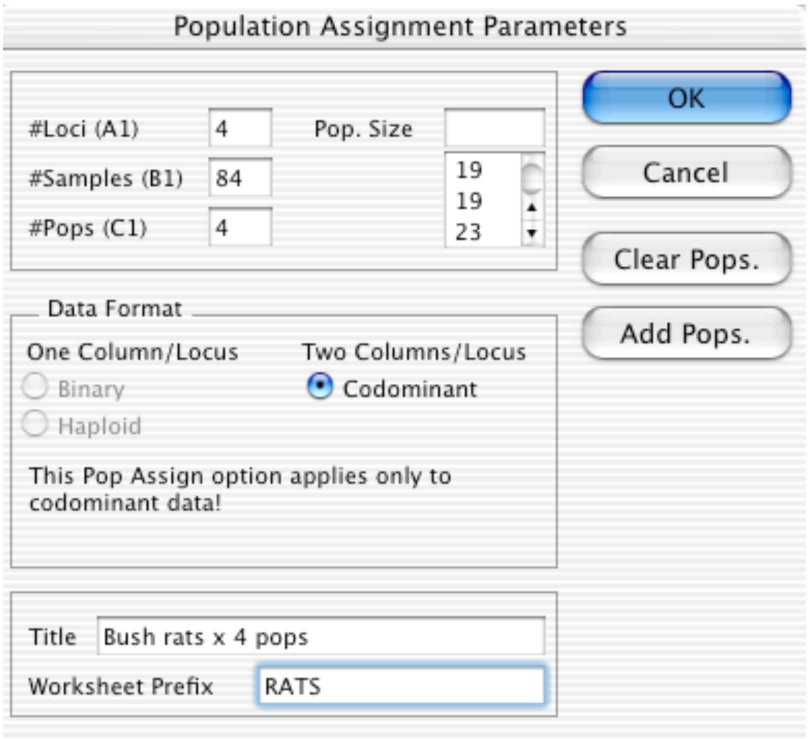
This option is provided primarily for teaching, although the unique graphical options for assignment pairwise population plots are useful for data exploration prior to analysis in other assignment analysis programs. See **GenAlEx 6.5 Appendix 1** for further information and references. For additional information refer to **Tutorial 4, Exercises 4.5 and 4.6**.

Tip: These plots provide an ideal graphical tool for assessing the power of assignment tests.

Procedure

1. Activate the worksheet containing your codominant dataset in GenAlEx format. Choose the option ***Assignment*** from the **GenAlEx** menu, and then select ***Pop Assign*** from the submenu.

2. Ensure the locus and sample parameters are correct in the Population Assignment Parameters dialog box. Enter Title and Worksheet Prefix then click *Ok.*



Population Assignment Parameters

#Loci (A1) 4

#Samples (B1) 84

#Pops (C1) 4

Pop. Size
19
19
23

Data Format

One Column/Locus
○ Binary
○ Haploid

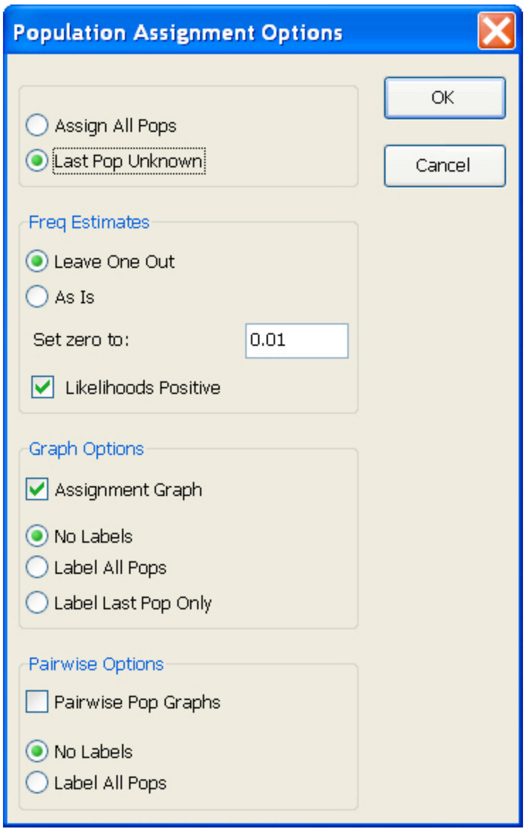
Two Columns/Locus
● Codominant

This Pop Assign option applies only to codominant data!

Title Bush rats x 4 pops

Worksheet Prefix RATS

3. In the subsequent Population Assignment Options dialog box check the options required (see below for further details), click *Ok*. Output is to sheet [ASS].



Population Assignment Options

○ Assign All Pops
● Last Pop Unknown

Freq Estimates
● Leave One Out
○ As Is
Set zero to: 0.01
☑ Likelihoods Positive

Graph Options
☑ Assignment Graph
● No Labels
○ Label All Pops
○ Label Last Pop Only

Pairwise Options
□ Pairwise Pop Graphs
● No Labels
○ Label All Pops

Population Assignment Options

Assign All Populations: Calculates assignment for all samples.

Last Population Unknown: Treats the last population as unknown samples and calculates their assignment to the preceding populations.

Freq Estimates

Leave One Out: This is the default and recommended procedure that includes the bias correction for population frequency. In this case, the individual in question is removed from the dataset before calculating the adjusted frequencies to be used in estimating the assignment likelihood.

As Is: Includes the sample in question when the frequency is calculated. This option is provided for teachers to use in class calculations, and to provide compatibility with the sex bias procedure that does not make this correction.

Set Zero to: Assignment tests cannot accept a frequency of zero, therefore a value is required. Enter a value in the range of 0.01 to 0.00001. The GenAlEx default is 0.01, following the recommendation of Paetkau et al (2004).

Likelihoods Positive: Converts log-likelihoods to positive values, where the lowest value indicates the most likely population. This is provided to facilitate presentation, and is often an easier way for students to interpret the meaning of log likelihood values.

Graph Options

Assignment Graph [ASS]: Outputs a biplot of the assignment indices for all populations based on the allele frequencies for populations 1 and 2. This is an easy initial data exploration tool.

No Labels: Individual data points (samples) are unlabeled on the assignment graph.

Label All Pops: All individual data points are labeled on the assignment graph.

Label Last Pop Only: Individual data points from the last population are labeled on the assignment graph.

Pairwise Options

Pairwise Pop Graphs [PWASS]: Outputs the separate biplots for all pairwise populations. These plots provide a visual representation of the degree of genetic separation among the populations, and are an ideal way to graphically assess the likely power of assignment tests (Paetkau et al 2004).

No Labels: Individual data points (samples) are unlabeled on the pairwise assignment graph.

Label All Pops: All individual data points are labeled on the pairwise assignment graph.

Sex Bias

This option calculates adjusted assignment indices for datasets in which the sex of the animal is identified. Comparisons of the distribution of assignment indices between the sexes allows for the detection of sex bias dispersal.

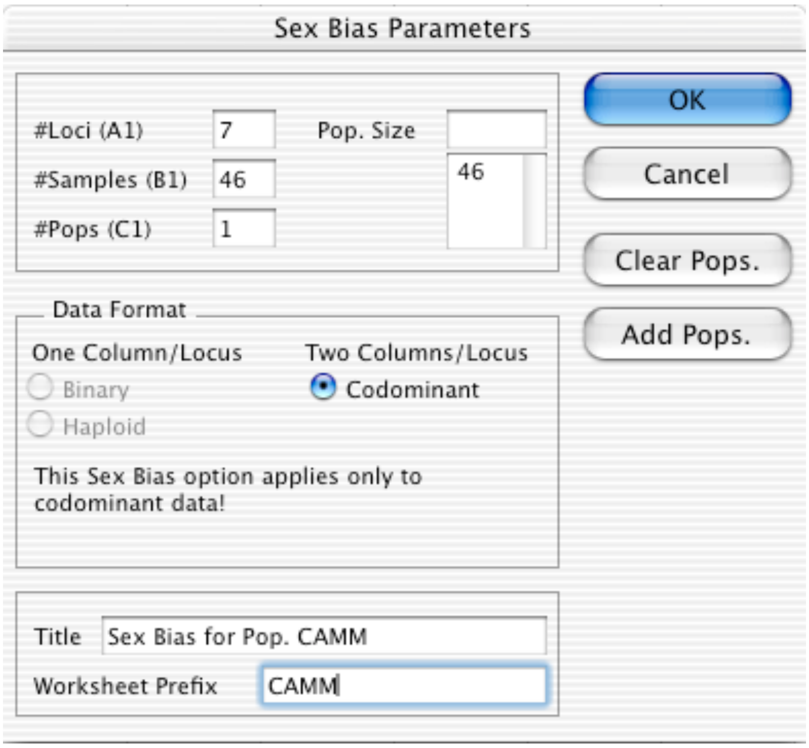
Sex bias can only be calculated for datasets representing a single population. The sex of each sample must be entered as either M or F in Column 2. There are no provisions for missing sex values, so the dataset needs to be complete. Refer to **Tutorial 4, Exercise 4.7** for additional assistance.

Procedure

1. Activate the worksheet containing your single-population, codominant dataset in GenAlEx format. Choose the option ***Assignment*** from the **GenAlEx** menu, and then select ***Sex Bias*** from the submenu.

2. Ensure the locus and sample parameters are correct in the Population Assignment Parameters dialog box. Enter Title and Worksheet Prefix then click *Ok*.

3. The analysis is output to two sheets: The population assignment values for all individuals, together with the means for males and females and U-test of significant difference between males and females are output to worksheet [SB]. The frequency distribution of the sex bias is output to worksheet [FDSB].



Sex Bias Parameters

#Loci (A1) 7

#Samples (B1) 46

#Pops (C1) 1

Pop. Size 46

Data Format

One Column/Locus

○ Binary

○ Haploid

Two Columns/Locus

● Codominant

This Sex Bias option applies only to codominant data!

Title: Sex Bias for Pop. CAMM

Worksheet Prefix: CAMM

Distance Based Statistical Procedures

Distance

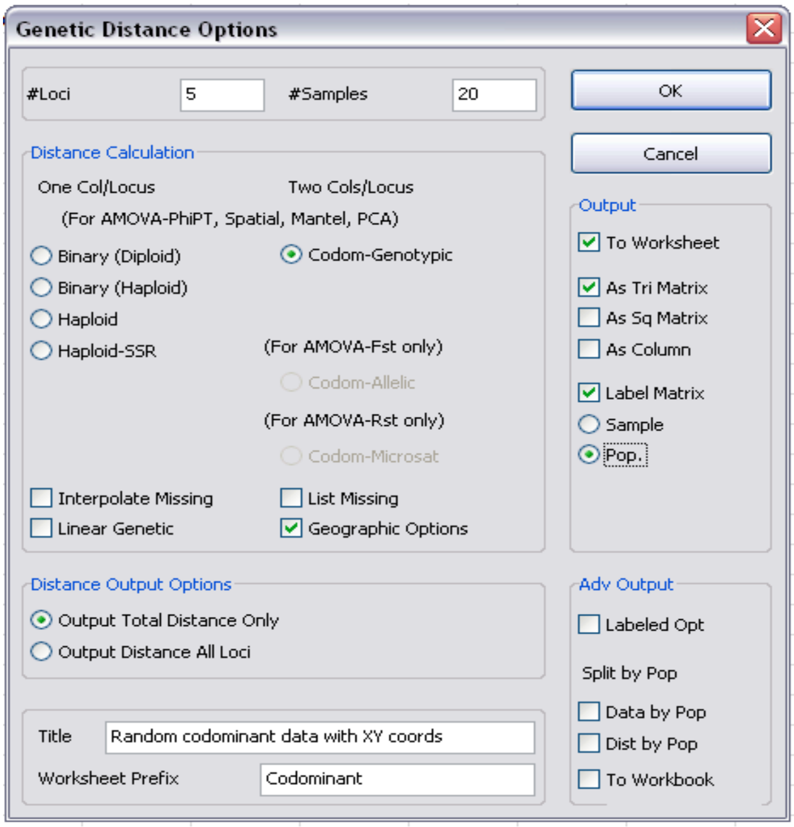
The ***Distance*** menu provides a number of calculators for pairwise genetic distance for binary, haploid and codominant data under the ***Genetic*** sub-option. The ***Genetic by Pop*** sub-option calculates the pairwise mean genetic distance between populations. In the ***Geographic*** sub-option pairwise geographic distances may be calculated from several formats. Genetic and geographic distances may be calculated simultaneously by first entering the ***Genetic*** sub-option, and checking the box *Geographic Options*. There are also a number of sub-options for manipulating the output triangular, square and column distances matrices.

Genetic Distance

This option outputs pairwise genetic distance matrices in appropriate GenAlEx format for subsequent analyses. A pairwise genetic distance matrix is a first step to a number of analyses available in GenAlEx, including Analysis of Molecular Variance (AMOVA). Formulas detailing how genetic distances are calculated are presented in **GenAlEx 6.5 Appendix 1**. For further information and step by step instructions see **Tutorial 2, Exercise 2.1 to 2.3**.

Procedure

1. Activate the worksheet containing your data
2. Choose the option ***Distance*** from the **GenAlEx** menu, and then select ***Genetic*** from the submenu.
3. Ensure the locus and sample parameters are correct in the Genetic Distance Options dialog box.
4. Select the appropriate Distance Calculation, and output options required (see below).
5. Enter Title and Worksheet Prefix then click *Ok*. Genetic distance is output to sheet [GD].



Genetic Distance Options

#Loci 5 #Samples 20

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 only)

○ Codom-Allelic

(For AMOVA-Rst only)

○ Codom-Microsat

□ Interpolate Missing □ List Missing

□ Linear Genetic ☑ Geographic Options

Distance Output Options

● Output Total Distance Only

○ Output Distance All Loci

Title Random codominant data with XY coords

Worksheet Prefix Codominant

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

Genetic Distance Options

Distance Calculation

Choose the genetic distance calculation appropriate for your data type (Binary Diploid, Binary Haploid, Haploid, Haploid-SSR or Codominant). Only one calculation for codominant data is available, with the other two being specific to AMOVA, and only accessible under that menu option. The genotypic distance available here forms the basis for many subsequent analyses, including Mantel, PCA, and the full set of spatial analyses. This genetic distance measure also facilitates comparison between codominant and haploid/binary data.

Interpolate Missing: When locus data are missing in a given individual by individual comparison, GenAlEx will interpolate the average genetic distance (calculated across all non-missing pairwise individual distances) at that locus for the relevant pairwise population contrast (e.g. within pop 1 or between pops. 1 and 2).

List missing [GDML]: Identifies the samples with missing data by locus, and provides the interpolated values for each locus with missing data.

Linear Genetic [LinGD]: Outputs linear rather than squared genetic distances. This option is only likely to be useful when attempting to correlate genetic and geographic distances (Mantel test). For all other purposes be sure to leave this option unchecked.

Geographic Options: Calls the geographic distance options dialog box.

Distance Output Options

Output Total Distance Only: Produces a genetic distance matrix summed over all loci. For all 3 codominant methods, distance matrices for each locus are summed across loci under the assumption of independence.

Output Distance All Loci: Produces a genetic distance matrix for each locus individually, plus a total genetic distance matrix, each on a separate worksheet. This option is useful for further locus-specific analyses and for the Multiple locus Spatial Autocorrelation option.

Output

To Worksheet: Outputs a distance matrix to a worksheet. This is required for all subsequent analyses requiring data as distance matrix such as Mantel and Spatial analyses.

As Tri Matrix: Outputs genetic distance matrix as a lower triangular matrix. This is the recommended option.

As Sq Matrix: Outputs genetic distance matrix as a symmetric square matrix. This is useful for other programs that require a square matrix.

As Column: Outputs genetic distance matrix as a column.

Label Matrix: Attaches appropriate labels to your matrix. Sample and population labels are useful for subsequent graphical output, such as a PCoA.

Tip: If the dataset consists of single locus data, the option to label the matrix with genotypes appears. This is a useful teaching tool.

Advanced Output

Labeled Opt [LGD]: Outputs a labeled pairwise matrix in the form (tri or sq.) indicated in the output section. Labels reflex selection under ***Label Matrix***.

Split by Pop: Outputs options by each population.

Data by Pop: Splits input data into separate worksheets for each population. The original data sheet is retained.

Dist by Pop: In addition to the total genetic distance matrix separate genetic distance matrices are output for each population.

To Workbook: When this option is ticked worksheets generated from the ***Split by Pop*** options are output to a new workbook. Save the new workbook to the desired location when prompted by GenAlEx.

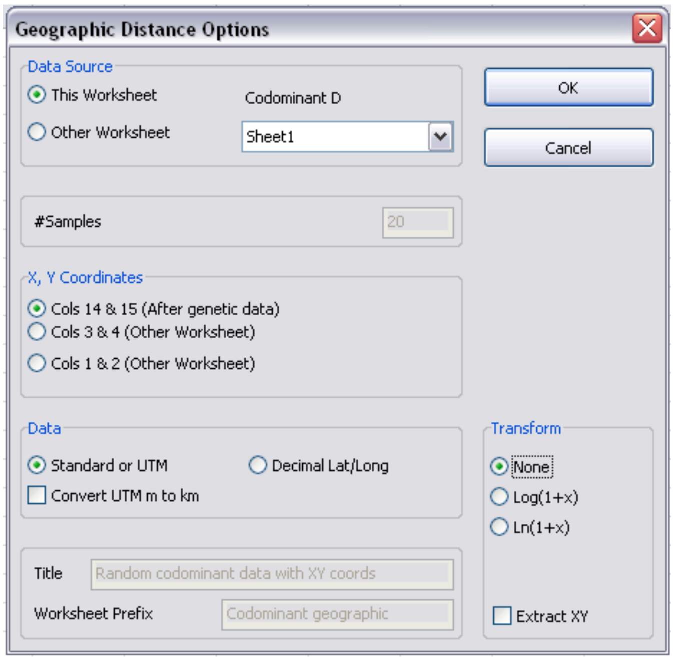
Geographic Distance

The options for calculating geographic distances can be accessed directly through the ***Distance*** -> ***Geographic*** sub-option or by checking the *Geographic Options* box in the Genetic Distance Options dialog box. For further information see **Tutorial 3, Box 3.2**.

Procedure for geographic with genetic distance

1. Make sure the *Geographic options* box in the Genetic Distance Options dialog box is checked.

2. Enter all appropriate information in the Geographic Distance Options dialog box (for more information on options see below). Pairwise geographic distances are output to sheet [GGD].



Geographic Distance Options

Data Source
● This Worksheet
○ Other Worksheet
Codominant D
Sheet1

#Samples 20

X, Y Coordinates
● Cols 14 & 15 (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)
□ Extract XY

Title: Random codominant data with XY coords
Worksheet Prefix: Codominant geographic

Geographic with Genetic Distance Options

<u>**Data Source**</u>

This Worksheet: Check this option if your geographic data are in the same sheet as the genetic data.

Other Worksheet: Check this option if your geographic data are in another worksheet. Select the sheet from the pull-down menu.

X, Y Coordinates

Col 14 & Col 15 (After Genetic Data): Check this if your X, Y data come after your genetic data in the same worksheet. There must be one blank column between the genetic and the XY data. In the illustration above the XY data are in columns 14 & 15, where the genetic data ends in column 12.

Cols 3 & 4 (Other Worksheet): Check this if your XY data are in columns 3 & 4 of a separate worksheet.

Cols 1 & 2 (Other Worksheet): Check this if your XY data are in columns 1 & 2 of a separate worksheet. This format is not recommended, but is maintained from previous versions of GenAlEx to ensure the compatibility of old datasets.

Data

Choose the appropriate data type:

Standard or UTM: For Universal Transverse Mercator Grid values in metres, or other map grid coordinates. These values should be used for fine scale genetic analysis.

Convert UTM m to km: Converts UTM values in metres to kilometres, as large datasets extending over kms can be unwieldy in graphical outputs.

Decimal Lat/Long: For Decimal latitude / Longitude values.

Transform:

When performing downstream analyses such as a Mantel test of isolation by distance at the population level certain transformations of the Geographic distance matrix may be useful.

None [GGD]: Outputs standard untransformed geographic distance matrix.

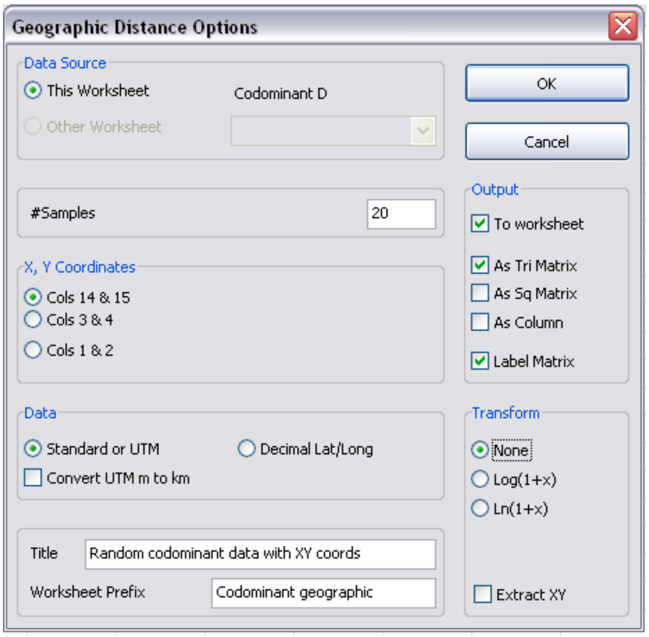
Log(1+x) [Log(1]: Outputs a Log transformed geographic distance matrix.

Ln(1+x) [Ln(1+]: Outputs the natural Log transformed geographic distance matrix.

Extract XY [XY]: When the geographic data appears after the genetic data this option outputs the XY coordinates from the input data sheet to columns 3& 4 of a new worksheet with the corresponding population and sample labels in columns 1&2.

Procedure for geographic distances only

1. Activate the worksheet containing your XY data. This data may be located after the genetic data, separated by an intervening column or in either Cols 1 & 2, or Cols. 3 & 4 of a different worksheet.
2. Choose the option ***Distance*** from the **GenAlEx** menu, and then select ***Geographic*** from the submenu.
3. Enter all appropriate information in the Geographic Distance Options dialog box (for more information on options see below). Pairwise geographic distances are output to sheet GGD.



Geographic Distance Options

Data Source
● This Worksheet Codominant D
○ Other Worksheet

#Samples 20

X, Y Coordinates
● Cols 14 & 15
○ Cols 3 & 4
○ Cols 1 & 2

Data
● Standard or UTM ○ Decimal Lat/Long
□ Convert UTM m to km

Title Random codominant data with XY coords
Worksheet Prefix Codominant geographic

Output
☑ To worksheet
☑ As Tri Matrix
□ As Sq Matrix
□ As Column
☑ Label Matrix

Transform
● None
○ Log(1+x)
○ Ln(1+x)

□ Extract XY

Geographic Distance Options

Data Source

This Worksheet: Your geographic data should be in the activated sheet.

X, Y Coordinates

Col 14 & Col 15: Check this if your X, Y data come after your genetic data in the same worksheet. There must be one blank column between the genetic and the XY data. In the illustration above the XY data are in columns 14 & 15, where the genetic data ends in column 12.

Cols 3 & 4: Check this if your XY data are in columns 3 & 4.

Cols 1 & 2: Check this if your XY data are in columns 1 & 2. This format is not recommended, but is maintained from previous versions of GenAlEx to ensure the compatibility of old datasets.

Data

Choose the appropriate data type:

Standard or UTM: For Universal Transverse Mercator Grid values in metres, or other map grid coordinates.

Convert UTM m to km: Converts UTM values in metres to kilometres, as large datasets extending over kms can be unwieldy in graphical outputs.

Decimal Lat/Long: For Decimal latitude / Longitude values.

Output

To Worksheet: Outputs a distance matrix to a worksheet. This is required for all subsequent analyses requiring data as distance matrix such as Mantel and Spatial analyses.

As Tri Matrix: Outputs geographic distance matrix as a lower triangular matrix.

As Sq Matrix: Outputs geographic distance matrix as a symmetric square matrix. This is useful for other programs that require a square matrix.

As Column: Outputs geographic distance matrix as a column.

Label Matrix: Attaches sample labels to your matrix.

Transform:

When performing downstream analyses such as a Mantel test of isolation by distance at the population level certain transformations of the Geographic distance matrix may be useful.

None [GGD]: Outputs standard untransformed geographic distance matrix.

Log(1+x) [Log(1]: Outputs a Log transformed geographic distance matrix.

Ln(1+x) [Ln(1+]: Outputs the natural Log transformed geographic distance matrix.

Extract XY [XY]: When the geographic data appears after the genetic data this option outputs the XY coordinates from the input data sheet to columns 3& 4 of a new worksheet with the corresponding population and sample labels in columns 1&2.

Genetic by Pop

The ***Distance->*** ***Genetic by Pop*** sub-option calculates the pairwise mean genetic distance between populations. The output is a square distance matrix labeled by population.

Procedure

1. Activate the worksheet containing your data
2. Choose the option ***Distance*** from the **GenAlEx** menu, and then select ***Genetic by Pop*** from the sub-menu.
3. Ensure the locus and sample parameters are correct in the Genetic Distance Options dialog box.
4. Select the appropriate distance calculation required.
5. Enter Title and Worksheet Prefix then click *Ok*. Genetic distance is output to sheet [PopGD].

Matrix Manipulation

Tri -> Table: Converts a triangular pairwise distance matrix into table format. Make sure the worksheet containing the tri matrix of interest is activated, and in GenAlEx format. The table is output to worksheet [TB].

Col -> Table: Converts pairwise distances in column format into a table. Make sure the worksheet containing the pairwise distances is activated, and in GenAlEx format. The table is output to worksheet [TB].

Tri -> Labeled: Converts a triangular or square pairwise distance matrix into a triangular distance matrix labeled along all four edges. If the input distance matrix is unlabeled the output matrix is labeled 1 to n. Make sure the worksheet containing the matrix of interest is activated, and in GenAlEx format. The output is to worksheet [LGD].

Sq -> Labeled: Converts a triangular or square pairwise distance matrix to a square distance matrix labeled along all four edges. If the input distance matrix is unlabeled the output matrix is labeled 1 to n. Make sure the worksheet containing the matrix of interest is activated, and in GenAlEx format. The output is to worksheet [LGD].

Tri -> Extract Pops: Starting with a triangular distance matrix this option separates the distances from within population pairs from the pairwise distances from among population comparisons. The separated genetic distances are output as columns to worksheet [TBE]. The frequency distribution of the within population distances and the among population distances are also output to worksheet [MFD]. Make sure the worksheet containing the tri matrix of interest is activated, and in GenAlEx format.

Col -> Extract Pops: Produces the same output as above but starts with a distance matrix as a column.

Tri -> Extract Pops+Regions: Starting with a triangular distance matrix this option separates the distances from within population comparisons, among population within region comparisons and among region comparisons. The separated genetic distances are output as columns to worksheet [TBE]. The frequency distributions of the within population, among population (within region) and among region distances are output to worksheet [MFD]. The outcomes of U tests comparing the within population distances to the among population distances and the among region distances (WPvAP and WPvAR) as well as the among population distances to the among region distances (APvAR) are also output to sheet [UT]. Make sure the worksheet containing the tri matrix of interest is activated, and in GenAlEx format.

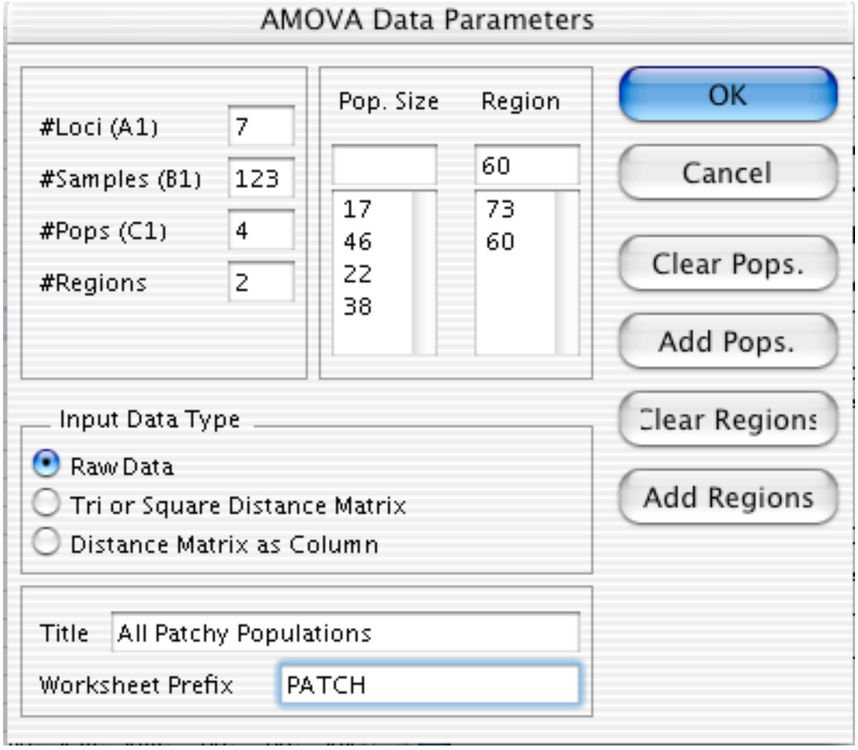
Col -> Extract Pops+Regions: Produces the same output as above but starts with a distance matrix as a column.

AMOVA

The Analysis of Molecular Variance may be performed using either raw data or a previously calculated distance matrix. For raw data, a number of calculators are provided for the generation of pairwise genetic distances from binary, haploid or codominant data. Formulas detailing how AMOVA is calculated are presented in **GenAlEx 6.5 Appendix 1**. For additional information refer to **Tutorial 2, Exercises 2.4 to 2.6**.

Procedure

1. With the worksheet containing your data active, choose the option ***AMOVA*** from the **GenAlEx** menu.
2. Enter all requested data parameters in the AMOVA Data Parameters dialog box, including population and regional sizes.
3. Select input data type. Distance matrices obtained previously may be input at this point.
4. Enter a Worksheet Title and Prefix, then click *Ok*.



AMOVA Data Parameters

#Loci (A1) 7

#Samples (B1) 123

#Pops (C1) 4

#Regions 2

Pop. Size	Region
	60
17	73
46	60
22	
38	

Input Data Type

● Raw Data

○ Tri or Square Distance Matrix

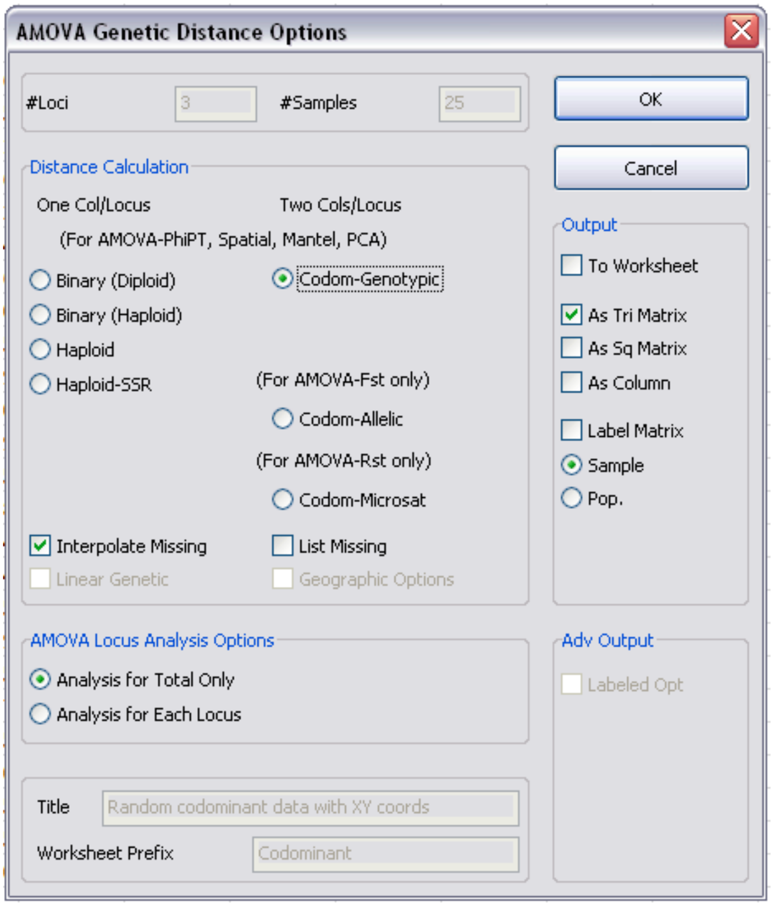
○ Distance Matrix as Column

Title All Patchy Populations

Worksheet Prefix PATCH

5. In the AMOVA Genetic Distance Options dialog box select the appropriate Distance Calculation and output options (see below for further details) and Click *Ok*.

Note: Calculation of the Genetic Distance matrix may take some time for larger datasets, before GenAlEx proceeds to the AMOVA options dialog box.



AMOVA Genetic Distance Options

#Loci 3 #Samples 25

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 only)

○ Codom-Allelic

(For AMOVA-Rst only)

○ Codom-Microsat

☑ Interpolate Missing ☐ List Missing

☐ Linear Genetic ☐ Geographic Options

AMOVA Locus Analysis Options

● Analysis for Total Only

○ Analysis for Each Locus

Title: Random codominant data with XY coords

Worksheet Prefix: Codominant

Output

☐ To Worksheet

☑ As Tri Matrix

☐ As Sq Matrix

☐ As Column

☐ Label Matrix

● Sample

○ Pop.

Adv Output

☐ Labeled Opt

AMOVA Genetic Distance Options

<u>Distance Calculation</u>

Choose the genetic distance calculation required.

Three different calculations are available for codominant data:

Codom - Genotypic: Outputs PhiPT, a measure facilitating comparison between codominant and haploid/binary data. This measure does not consider the intra-individual variation.

Codom - Allelic: Estimates standard Fst values and F'st values (which are corrected by the maximum Fst achievable given the input marker panel).

Codom - Microsat: Calculates Rst, an estimator of genetic differentiation for microsatellite loci that assumes a stepwise mutation model.

Two different calculations are available for haploid data:

Haploid: Outputs PhiPT.

Haploid SSR: Outputs a PhiPT value analogous to Rst for codominant data, which is based on genetic distance estimates that assume a stepwise mutation model.

Interpolate Missing: When locus data are missing in a given individual by individual comparison, GenAlEx will interpolate the average genetic distance (calculated across all non-missing pairwise individual distances) at that locus for the relevant pairwise population contrast (e.g. within pop 1 or between pops. 1 and 2).

List missing [GDML]: Identifies the samples with missing data by locus, and provides the interpolated values for each locus with missing data.

AMOVA Locus Analysis Options

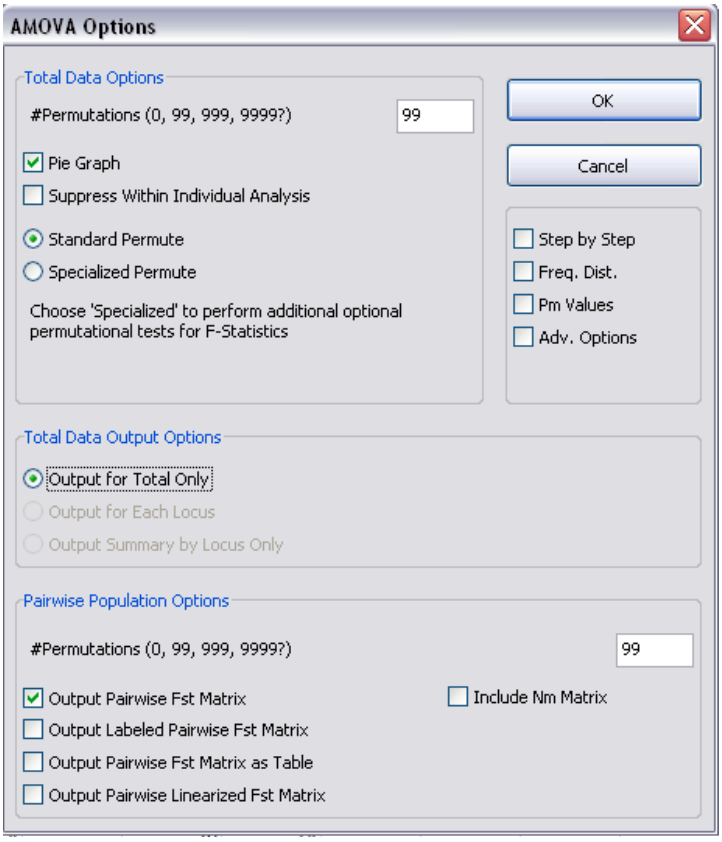
Analysis for Total Only: Calculates AMOVA from genetic distances summed over all loci. For all methods, distance matrices for each locus are summed across loci under the assumption of independence.

Analysis for Each Locus: Calculates AMOVA for each locus separately, as well as for the genetic distances summed over all loci.

Output

The output of the Allelic and Microsat distance matrices is only recommended for advanced users wanting to interrogate this data. This output is not required by GenAlEx to perform the AMOVA. If *Output to worksheet* is selected, genetic distances will be output to sheet [GD], [GDA] or [GDM], for Genotypic, Allelic and Microsat distances respectively.

6. At the AMOVA Options dialog box select required options (see below for details) and click *Ok*. The overall AMOVA analysis across all loci is output to sheet [PhiPT] for Binary, Haploid and Codominant Genotypic data, to [Fst] for Codominant Allelic data and to [Rst] for Codominant Microsatellite data.



AMOVA Options

Total Data Options

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

☑ Pie Graph

□ Suppress Within Individual Analysis

● Standard Permute

○ Specialized Permute

Choose 'Specialized' to perform additional optional permutational tests for F-Statistics

□ Step by Step

□ Freq. Dist.

□ Pm Values

□ Adv. Options

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?) 99

☑ Output Pairwise Fst Matrix

□ Include Nm Matrix

□ Output Labeled Pairwise Fst Matrix

□ Output Pairwise Fst Matrix as Table

□ Output Pairwise Linearized Fst Matrix

AMOVA Options

Total Data Options

#Permutations: Enter the number of permutations required to test for significance. Note: For large data sets, permutation may take some time. Watch the status bar for progress. For publication purposes the number of permutations should be set to 999 or 9999.

Pie Graph: Outputs a pie chart illustrating the distribution of variance.

Suppress Within Individual Analysis: This option is only available when codom-allelic is selected and suppresses within individual variation.

Standard permute: Shuffles individuals between populations and regions.

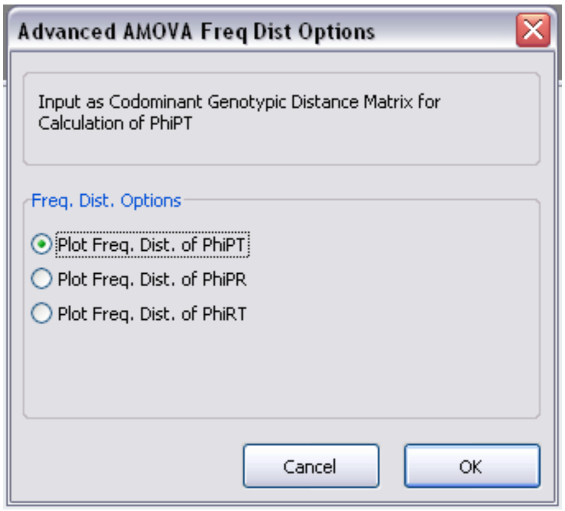
Specialized permute: Performs additional permutations that only shuffle individuals within populations to calculate the probability of Fis and within regions to calculate the probability of Fsr/PhiPR.

Step by Step [SSAM]: Outputs pairwise distances along with relevant population labels. This information can be used to manually calculate AMOVA. This option is only available when *Analysis for Total Only* is selected in the AMOVA Genetic Distance Options dialog box.

Freq. Dist.: Outputs the frequency distribution of permuted PhiPT/Fst/Rst values vs the observed value to sheet [PhiPTFD], [FstFD] or [RstFD] respectively.

Pm Values: Outputs the differentiation values from each standard permutation to sheet [PhiPTPV], [FstPV] or [RstPV]. This option is only available when *Analysis for Total Only* is selected in the AMOVA Genetic Distance Options dialog box.

Adv. Options: Calls the Advance AMOVA Freq Dist Options dialog box allowing the frequency distribution of other measures besides PhiPT/Fst/Rst values to be plotted. Select the desired measure to be plotted and click *Ok*. Output sheet suffix varies with measure selected but always end with “FD”.


Advanced AMOVA Freq Dist Options

Input as Codominant Genotypic Distance Matrix for Calculation of PhiPT

Freq. Dist. Options

● Plot Freq. Dist. of PhiPT
○ Plot Freq. Dist. of PhiPR
○ Plot Freq. Dist. of PhiRT

Total Data Output Options

Output for Total Only: Outputs selected analyses for AMOVA across all loci. Also outputs summery of differentiation statistics by locus and their corresponding probabilities to sheet [PhiPTS],[FstS]or[RstS] if *Analysis for Each Locus* is selected in the AMOVA Genetic Distance Options dialog box.

Output for Each Locus: Outputs selected analyses for AMOVA across all loci and for AMOVA of each locus separately. Also outputs a summary of the differentiation statistics by locus and their corresponding probabilities to sheet [PhiPTS],[FstS]or[RstS]. This option is only available when *Analysis for Each Locus* is selected in the AMOVA Genetic Distance Options dialog box.

Output Summary by Locus Only: Only outputs a summary of the differentiation statistics by locus and their corresponding probabilities to sheet [PhiPTS],[FstS]or[RstS]. This option is only available when *Analysis for Each Locus* is selected in the AMOVA Genetic Distance Options dialog box.

Pairwise Population Options

#Permutations: Enter the number of permutations required to test for pairwise significant differentiation between populations. Note: For large data sets, permutations may take some time. Watch the status bar for progress. For publication purposes the number of permutations should be set to 999 or 9999.

Output Pairwise PhiPT/Fst/Rst Matrix [PhiPTP],[FstP]or[RstP]: Outputs pairwise PhiPT/Fst/Rst values among all pairs of populations as a tri-matrix with probability values shown above the diagonal.

Output Labeled Pairwise PhiPT/Fst/Rst Matrix [PhiPTL],[FstL]or[RstL]: Outputs Labeled version of [PhiPTP], [FstP] or [RstP] matrices.

Output Pairwise PhiPT/Fst/Rst Matrix as Table: Outputs pairwise PhiPT/Fst/Rst values among all pairs of populations in table format to worksheet [PhiPTT],[FstT]or[RstT].

Output Pairwise Linearized PhiPT/Fst/Rst Matrix: Outputs linearized pairwise PhiPT/Fst/Rst values as a tri-matrix to sheet [LinPhiPT],[LinFst]or[LinRst] and in table format to worksheetsheet [PhiPTT],[FstT]or[RstT] if the *Output Pairwise PhiPt/Fst/Rst Matrix as Table* option is selected.

Include Nm Matrix: Outputs the effective number of migrants among all pairs of populations as a tri-matrix to sheet [PhiPTP],[FstP]or[RstP] and in table format to sheet [PhiPTT],[FstT]or[RstT] if the *Output Pairwise PhiPt/Fst/Rst Matrix as Table* option is selected.

Mantel

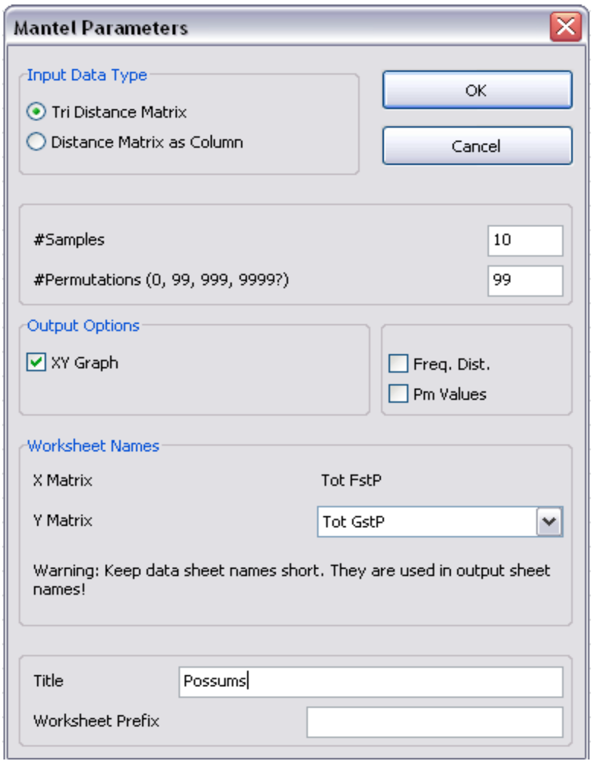
Mantel is a versatile non-parametric test that assesses the relationship between the elements of any two matrices with matching entries. Therefore to perform a Mantel test in **GenAlEx**, you require matrices in standard GenAlEx format as input. The ***Mantel*** menu contains three sub-menus. The ***Paired*** sub-menu tests the relationship between two matrices. The ***Multi*** sub-menu tests the relationship pairwise between multiple input matrices, while the ***Compare*** sub-menu tests the relationship between the first matrix and all other input matrices.

Paired

This menu can be used to test for isolation by distance within or between populations. The input for such an analysis is a genetic distance matrix and a corresponding geographic distance matrix. For individual by individual analyses make sure that the genetic distance matrix is linear (LinGD), and not squared (see under ***Distance*** above). For further instructions and information on paired Mantel tests in GenAlEx refer to **Tutorial 3, Exercises 3.2 to 3.5**.

Procedure

1. First calculate appropriate matrices via the **GenAlEx** menu.
2. Make sure the worksheet containing your X distance matrix (e.g. Geographic distance matrix) is activated. Choose the ***Paired*** option under the ***Mantel*** menu in **GenAlEx**.
3. In the Mantel Parameters dialog box ensure the data type and sample numbers are correct. Select the desired output options (for more information on output options see below).
4. Specify a worksheet for the Y distance matrix (e.g. Genetic distance) and enter output worksheet title and prefix. Enter either 0, 99, 999, or 9999 for the number of permutations, then click *Ok*. Output is to worksheet [MT].



Input Data Type

● Tri Distance Matrix

○ Distance Matrix as Column

#Samples 10

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

Output Options

☑ XY Graph

□ Freq. Dist.

□ Pm Values

Worksheet Names

X Matrix Tot FstP

Y Matrix Tot GstP

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

Title Possums

Worksheet Prefix

Output Options

XY Graph [MT]: Check this option to show an XY plot of the data.

Freq. Dist. [FDMT]: Check this option to output the frequency distribution of permuted Rxy values vs the observed Rxy value.

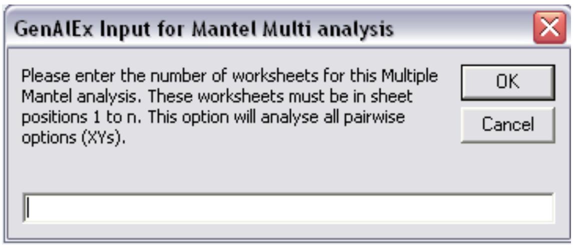
Pm Values [PVMT]: Check this option to output the Mantel values from each permutation.

Multi

This option is a useful tool for testing the correlation between multiple statistical measures, such as the various frequency based population structure estimators output via the ***G-Statistics*** menu in **GenAlEx**.

Procedure

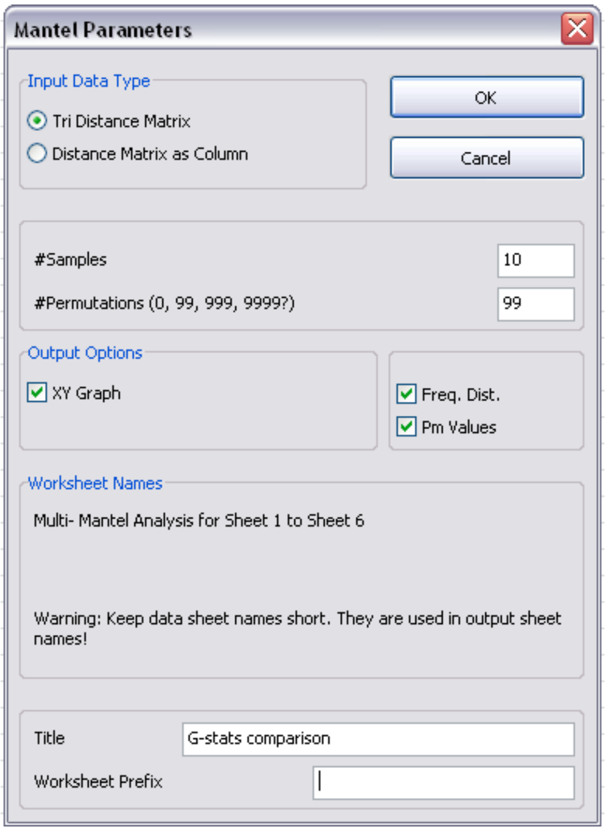
1. First calculate appropriate matrices via the **GenAlEx** menu. Each matrix must be in a separate worksheet within a single workbook in positions 1 through n.
2. Make sure the worksheet containing your first matrix is activated. Choose the ***Multi*** option under the ***Mantel*** menu in **GenAlEx**.
3. Enter the number of matrices you wish to compare in the GenAlEx Input for Mantel Multi analysis dialog box, click *Ok*.



GenAlEx Input for Mantel Multi analysis

Please enter the number of worksheets for this Multiple Mantel analysis. These worksheets must be in sheet positions 1 to n. This option will analyse all pairwise options (XYs).

4. In the subsequent Mantel Parameters dialog box ensure the data type and sample numbers are correct. Select the desired output options (for more information on output options see below).
5. Enter output worksheet title and prefix. Enter either 0, 99, 999, or 9999 for the number of permutations then click *Ok*. Output for each pairwise matrix comparison is to an appropriately named worksheet e.g. [GstvFst MT].



● Tri Distance Matrix
○ Distance Matrix as Column

#Samples 10
#Permutations (0, 99, 999, 9999?) 99

☑ XY Graph
☑ Freq. Dist.
☑ Pm Values

Multi- Mantel Analysis for Sheet 1 to Sheet 6

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

Title G-stats comparison
Worksheet Prefix

Output Options

XY Graph: Check this option to output an XY plot of the data to each pairwise comparison worksheet e.g [GstvFst MT].

Freq. Dist. [FDMT]: Check this option to output for each pairwise matrix comparison a frequency distribution of permuted Rxy values vs the observed Rxy value.

Pm Values [PVMT]: Check this option to output for each pairwise matrix comparison the Mantel values from each permutation.

Compare

This option can be useful for comparing a particular statistical measure, such as Fst, to a number of other statistics, such as the G statistics output via the ***G-Statistics*** menu in **GenAlEx**.

Procedure

Each matrix must be in a separate worksheet within a single workbook in positions 1 through n. The matrix to which all other matrices are to be compared must be in position 1. See ***Mantel -> Mutli*** for procedure details.

PCoA

The Principal Coordinates Analysis (PCoA) is a multivariate technique that allows one to find and plot the major patterns within a multivariate dataset e.g. multiple loci and multiple samples. The ***Analysis*** option in the ***PCoA*** menu will find the relationship between the distance matrix elements based on their first three principal coordinates. While, the ***Axes 1vs 2***, ***Axes 1vs 3*** and ***Axes 2vs 3*** options enable different axes combinations to be plotted. All distance matrices produced within GenAlEx are accepted. Refer to **Tutorial 3, Exercise 3.1** for additional information.

Procedure

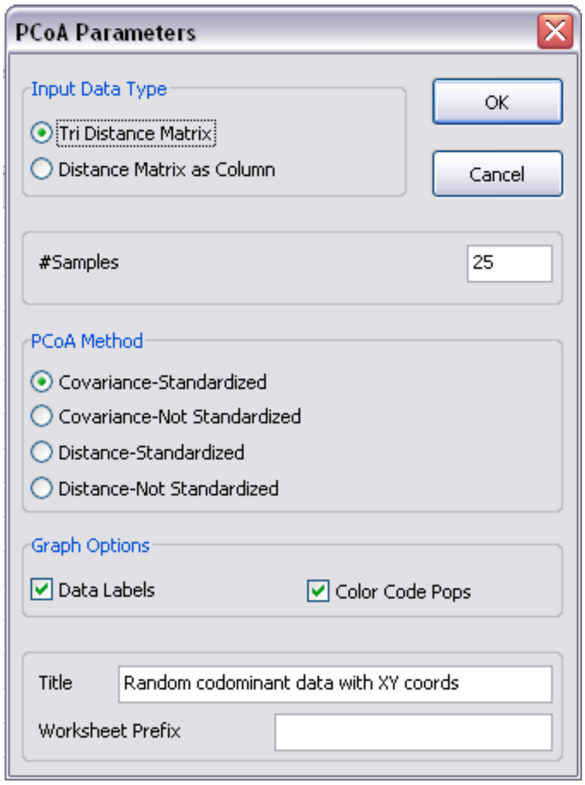
1. First calculate the appropriate distance matrix via one of the following options: ***Distance***, ***AMOVA, G-statistics***, or *Nei's Distance/Unbiased Nei's Distance* (under ***Frequency***). If your matrix has labels, these will be plotted onto the PCoA plot.

2. Make sure the worksheet containing your Genetic distance matrix is activated. Choose the ***Analysis*** sub-option from the ***PCoA*** menu.

3. At the PCoA Parameters dialog box, select the appropriate input Data Type.

4. Select your preferred method, and required output options (see below for further details)

5. Enter an optional Title and Worksheet Prefix and click *Ok*. A scatter plot of the first two coordinates will be output to worksheet [PCoA].


PCoA Parameters

Input Data Type
● Tri Distance Matrix
○ Distance Matrix as Column

#Samples 25

PCoA Method
● Covariance-Standardized
○ Covariance-Not Standardized
○ Distance-Standardized
○ Distance-Not Standardized

Graph Options
☑ Data Labels
☑ Color Code Pops

Title Random codominant data with XY coords
Worksheet Prefix

Note: PCoA is an iterative procedure that may take some time for larger data sets. Watch the status bar for progress.

PCoA method

Four different, but related PCoA methods are provided as options. All methods produce essentially the same patterns, but may resolve some clusters better than others depending on the underlying data. You might like to experiment with the options. The first two methods are based on the covariance matrix and latter two on the distance matrix. Refer to **GenAlEx 6.5 Appendix 1** for further details.

Graph Options

Data labels: checking this option will output the sample labels to the graph.

Color Code Pops: checking this option will color code populations on the graph.

Plotting First vs Third and Second vs Third coordinates

Excel does not yet provide the option to plot a 3D scatter plot. However, GenAlEx options allow plots of the second and third, and first and third coordinates.

1. Select the worksheet containing an appropriate PCoA output.

2. Choose the required submenu (*Axes 1 vs 2, Axes 1 vs 3* or *Axes 2 vs 3*).

Spatial Autocorrelation

About the Spatial Autocorrelation method

GenAlEx offers a wide range of options for spatial autocorrelation analysis, employing multivariate procedures developed by the authors of GenAlEx. Refer to **GenAlEx 6.5 Appendix 1** and **Tutorial 3** for an overview of the statistical procedures. **Appendix 1** also provides a reference list for further information.

Global spatial analyses offered by GenAlEx are: ***Single Pop...***, for the analysis of a single population using a single genetic distance matrix; ***Multiple Loci***… for the separate analysis of multiple genetic distance matrixes (from multiple loci) with a single geographic matrix; ***Multiple Pops...*** for autocorrelation analysis over multiple populations; ***Multiple Pops Subsets...*** for autocorrelation analysis over multiple subsets, where each subset contains multiple populations; ***Multiple Dclass...*** for autocorrelation analysis over multiple distance class sizes for multiple populations: ***Pops as Dclass...*** for comparing the genetic autocorrelation between multiple populations.

Also provided is an option for 'local' spatial analysis, the 2 Dimensional Local Spatial Analysis (***2D LSA).*** The option ***NN Dist...***, is available as a complement to the ***2D LSA***, by providing a summary of the Nearest Neighbors and their distance from each sample, up to a user specified number of Nearest Neighbors.

With the exception of ***Pops as Dclass*** and the ***NN Dist*** submenu options, all other menu suboptions require standard GenAlEx genetic distance [GD] and geographic distance [GGD] matrices in separate worksheets as input. In the case of the submenu options ***Multiple loci***, ***Multiple pops***, ***Multiple Pops Subsets*** and ***Multiple Dclass***, multiple genetic distance matrices are required. In all cases, matrix formats and parameter settings must be in GenAlEx format. These can be generated via the ***Distance*** option in GenAlEx.

For some of the spatial analyses in GenAlEx the input sheets must be in a specific order. This is detailed below. Where possible, GenAlEx will automatically place these sheets in their correct order when generating the distance matrices.

Single Pop…

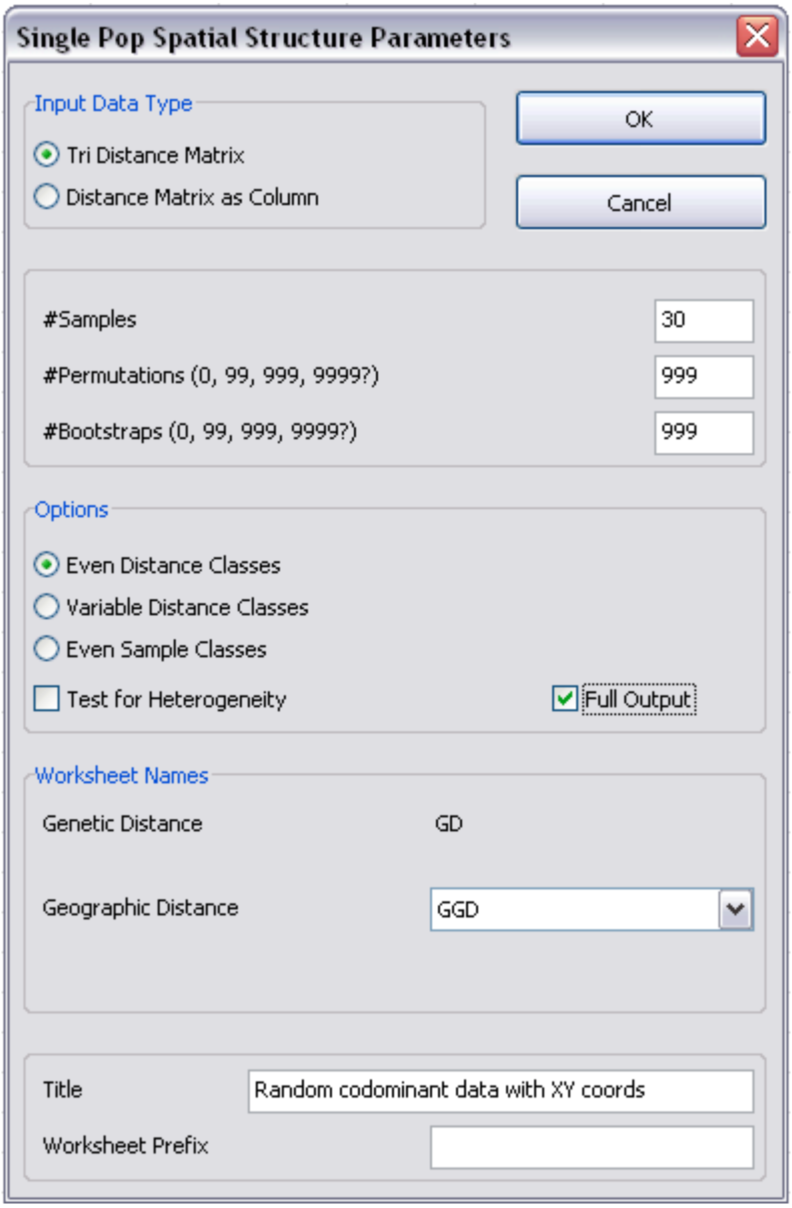
Use this option to perform a Spatial Autocorrelation for a single population. Input consists of a single genetic distance matrix, typically representing the total genetic distance over multiple loci, and the matching geographic distance matrix for the same set of samples. For further assistance with this option refer to **Tutorial 3, Exercises 3.6 to 3.8**.

Procedure

1. First calculate appropriate Genetic [GD] and Geographic Distance [GGD] matrices via the ***Distance*** option in the **GenAlEx** menu. If your data is codominant, ensure the GD matrix is for genotypic distances.
2. It is recommended that the geographic distance worksheet [GGD] is in the first position in the workbook, followed by the genetic distance worksheet [GD] in the second position.
3. Activate the worksheet containing your genetic distance matrix [GD]. Choose the option ***Spatial*** from the **GenAlEx** menu, and then select ***Single Pop...*** from the submenu.

4. At the Single Spatial Structure Parameters dialog box, select the appropriate input data format, then enter the number of samples, the number of permutations (0, 99, 999, 9999) and the desired options (see below for details).

5. Specify a worksheet for the geographic distance matrix. Enter a Worksheet Title and Prefix, then click *Ok*. The spatial analysis will be output to a worksheet [R].



Single Pop Spatial Structure Parameters

Input Data Type

● Tri Distance Matrix

○ Distance Matrix as Column

#Samples 30

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

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

Options

● Even Distance Classes

○ Variable Distance Classes

○ Even Sample Classes

□ Test for Heterogeneity ☑ Full Output

Worksheet Names

Genetic Distance GD

Geographic Distance GGD

Title Random codominant data with XY coords

Worksheet Prefix

Note: this analysis may take a few moments with larger datasets as GenAlEx reads the GGD matrix and calculates information from the data. Watch the status bar for progress.

Single Pop. Spatial Structure options

#Samples: Enter the number of samples. This is automatically inserted if the input distance matrices are in GenAlEx format.

#Permutations: Enter the number of permutations required to test for significance.

Note: For large data sets, permutation may take some time. Watch the status bar for progress. For publication purposes the number of permutations should be set to 999 or 9999.

#Bootstraps: Enter the number of bootstraps required to estimate the 95% confidence interval around *r.* For publication purposes the number of bootstraps should be set to 999 or 9999.

Options:

GenAlEx offers 3 different genetic class options, which provide flexibility for defining the size and boundaries of the distance classes.

Even Distance Classes: This option will create geographic distance classes of equal size. When this option is selected the spatial analysis will consider all samples that are represented by a distance greater than the previous distance class, and less than or equal to the upper distance class. For example, with a distance class size of '1' and a No. of distance classes of '8', all samples with a geographic distance of >1 and ≤8 are included. The only exception to this rule is that the first distance class includes a distance of zero up to a value ≤ the first distance class value. Selecting this option calls the Even Distance Class Options dialog box (see below).

Variable Distance Classes: This option may be used to manually create geographic distance classes of unequal sizes. As for the even distance classes, the spatial analysis will consider all samples that are represented by a distance greater than the previous distance class and less than or equal to the upper distance class, with the exception of the first distance class. Selecting this option calls the Variable Distance Class Options dialog box (see below).

Even Sample Classes: This option selects distance classes by attempting to choose integer classes that provide as equal a number of samples in each distance class as possible (within the constraints of the integer class sizes). This is particularly useful for reducing noisy confidence limits when sample sizes are very uneven. Selecting this option calls the Even Sample Class Options dialog box (see below).

Test for Heterogeneity: Outputs the heterogeneity test for overall correlogram significance.

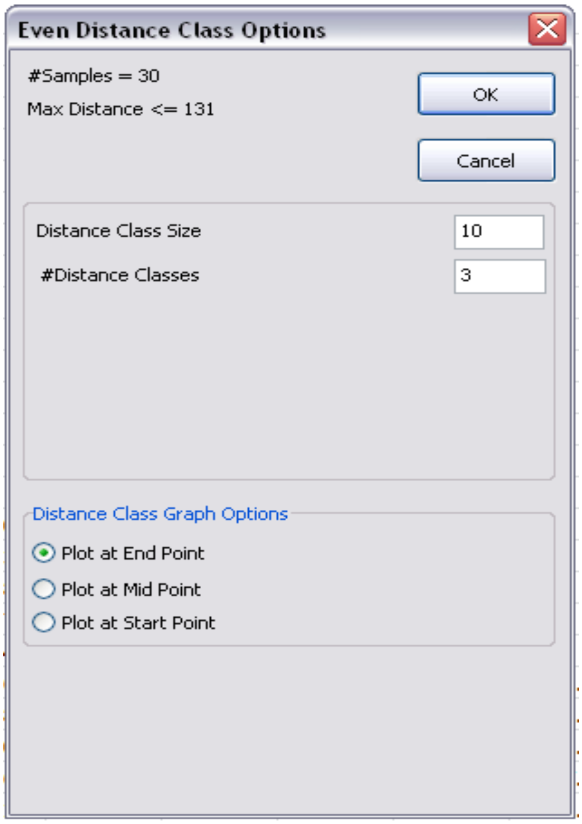
Note: This option is only recommended for advanced users. Following Banks and Peakall (2012), significance of the Heterogeneity Test is declared when P< 0.01)

Full output: Outputs the full statistics, including the results of the permutation and bootstrap analyses.

Distance class options

Even Distance Class Procedure

1. In the Even Distance Class Options dialog box enter a numeric value for the distance class size, the number of distance classes and the desired distance class graph option (see below). Use the provided information on the maximum distance in the matrix to guide you choices. Then Click *Ok.*



#Samples = 30
Max Distance <= 131

Distance Class Size: 10
#Distance Classes: 3

● Plot at End Point
○ Plot at Mid Point
○ Plot at Start Point

Distance Class Graph Options:

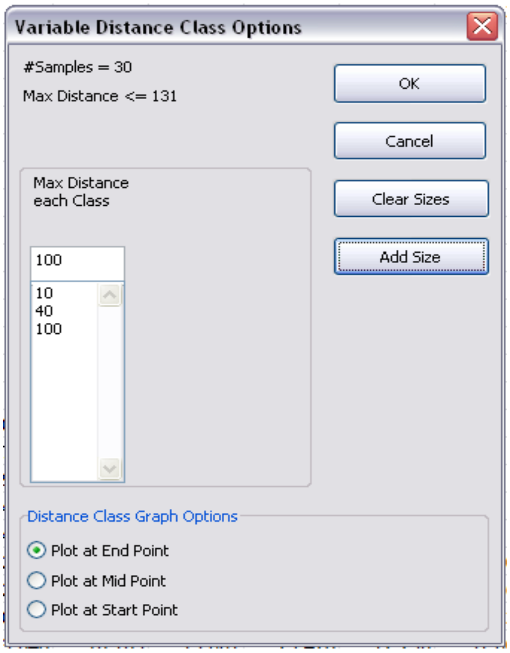
Plot at End Point: plots the autocorrelation *r* values for each distance class against the maximum pairwise geographic distance value for that class.

Plot at Mid-Point: plots the autocorrelation *r* values for each distance class against the middle pairwise geographic distance value for that class.

Plot at Start Point: plots the autocorrelation *r* values for each distance class against the minimum pairwise geographic distance value for that class.

Variable Distance Class Procedure

1. In the Variable Distance Class Options dialog box enter a desired distance class size in the edit box, and click the *Add Size* button. Repeat until all desired distance classes have been added. Decimal values may be used.
2. Select the desired distance class graph option (see even distance class procedure above), then Click *Ok.*



#Samples = 30
Max Distance <= 131

Max Distance each Class

100

10
40
100

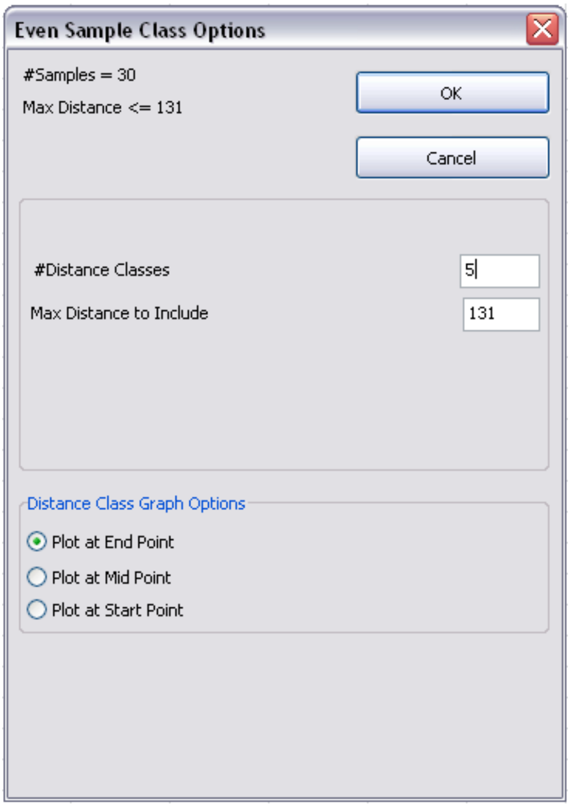
Distance Class Graph Options

● Plot at End Point
○ Plot at Mid Point
○ Plot at Start Point

Even Sample Class Procedure

1. In the Even Sample Class Options dialog box provide the number of distance classes and the maximum distance class to include.

2. Select the desired distance class graph option (see even distance class procedure above), then Click *Ok.*



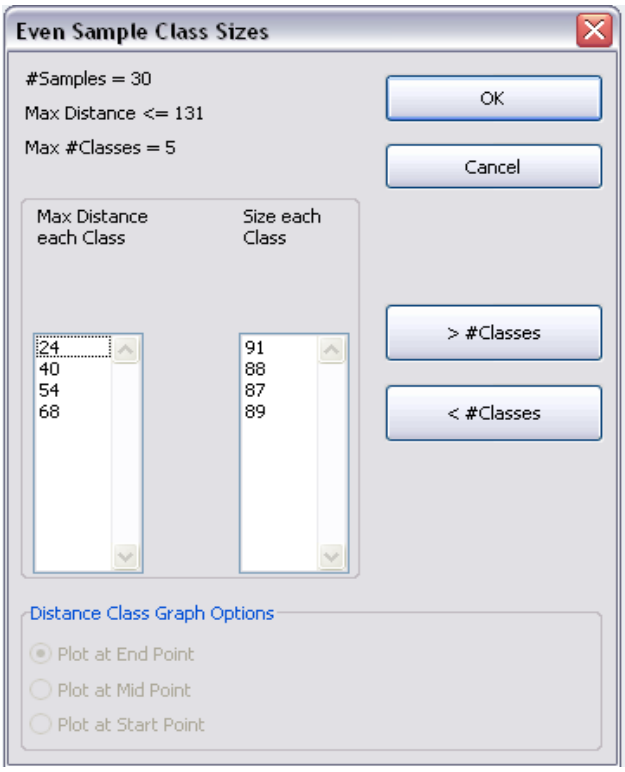
#Samples = 30
Max Distance <= 131

#Distance Classes 5
Max Distance to Include 131

Distance Class Graph Options

● Plot at End Point
○ Plot at Mid Point
○ Plot at Start Point

2. The subsequent Even Sample Class Sizes dialog box shows a list of the number of pairwise comparisons within each distance class. Click the buttons to increase or decrease the number of distance classes as required, to optimise the sample size within each class.



#Samples = 30

Max Distance <= 131

Max #Classes = 5

Max Distance each Class	Size each Class
24	91
40	88
54	87
68	89

Distance Class Graph Options

● Plot at End Point

○ Plot at Mid Point

○ Plot at Start Point

Multiple loci…

This option is designed for the automatic analysis of multiple loci from the same set of samples. Input consists of multiple genetic distance matrixes, each derived from a single locus, with one matching geographic distance matrix. Output includes a convenient correlogram with the results of each locus overlaid. Separate correlograms for each individual locus may also be obtained, if the full output option is selected. For further assistance with this option refer to **Tutorial 3, Exercise 3.9**.

Procedure

1. First calculate appropriate Genetic [GD] and Geographic Distance [GGD] matrices via the ***Distance*** option in the **GenAlEx** menu. If your data is codominant, ensure the GD matrix is for genotypic distances. Check the *Output Distance All Loci* option in the Distance Options dialog box to output individual genetic distance matrices for each locus.

2. Ensure the genetic distance worksheets for loci 1 to n are in worksheet positions 1 to n. The geographic distance matrix should be located after these genetic distance worksheets.

3. Activate the worksheet containing your first genetic distance matrix [GD]. Choose the option ***Spatial*** from the **GenAlEx** menu, and then select ***Multiple loci*** from the sub-menu.

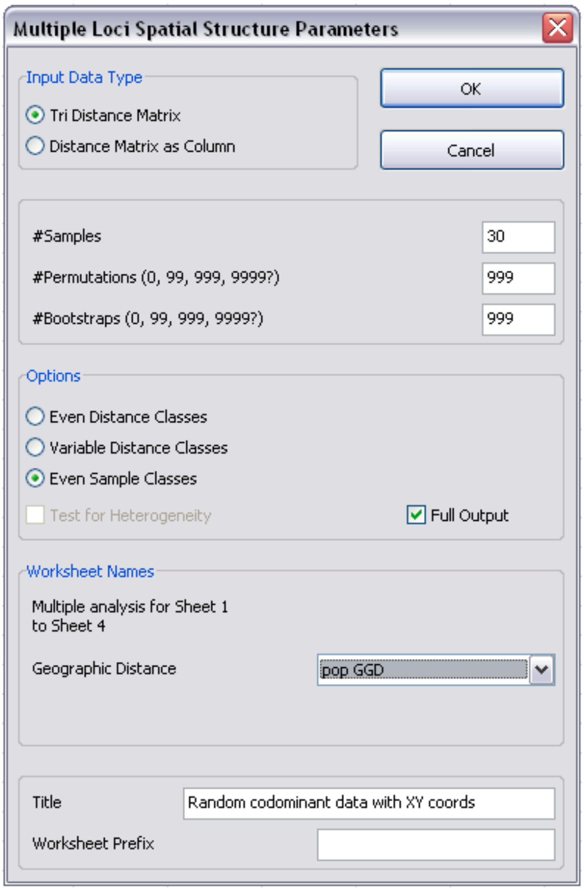
4. When prompted by the GenAlEx Input for Spatial Multiple Loci dialog box, enter the number of genetic distance worksheets you wish to analyze (normally this will equal the number of loci + 1 for the total genetic distance matrix).

5. At the Multiple Loci Spatial Structure Parameters dialog box, select the appropriate input data format then enter the number of samples, the number of permutations (0, 99, 999, 9999), the number of bootstraps and the distance class option desired (as per ***Single Pop.*** for details see under Single Pop. Spatial Structure options).

6. Select *Full Output* if desired (see below).

7. Specify a worksheet for the geographic distance matrix. Enter a worksheet title and prefix, and then click *Ok*. The combined spatial analysis will be output to a worksheet [RML].

Note: with larger data sets and many loci the analysis may take some time. Watch the status bar for progress.



Input Data Type

● Tri Distance Matrix

○ Distance Matrix as Column

#Samples 30

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

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

Options

○ Even Distance Classes

○ Variable Distance Classes

● Even Sample Classes

□ Test for Heterogeneity ☑ Full Output

Worksheet Names

Multiple analysis for Sheet 1 to Sheet 4

Geographic Distance pop GGD

Title Random codominant data with XY coords

Worksheet Prefix

Multiple Loci Spatial Structure options

Full output: Outputs the full statistics, including the results of the permutation and bootstrap analyses and correlogram for individual loci to separate worksheets. Outputs are to appropriately named worksheets e.g [Locus1 R].

Multiple Pops…

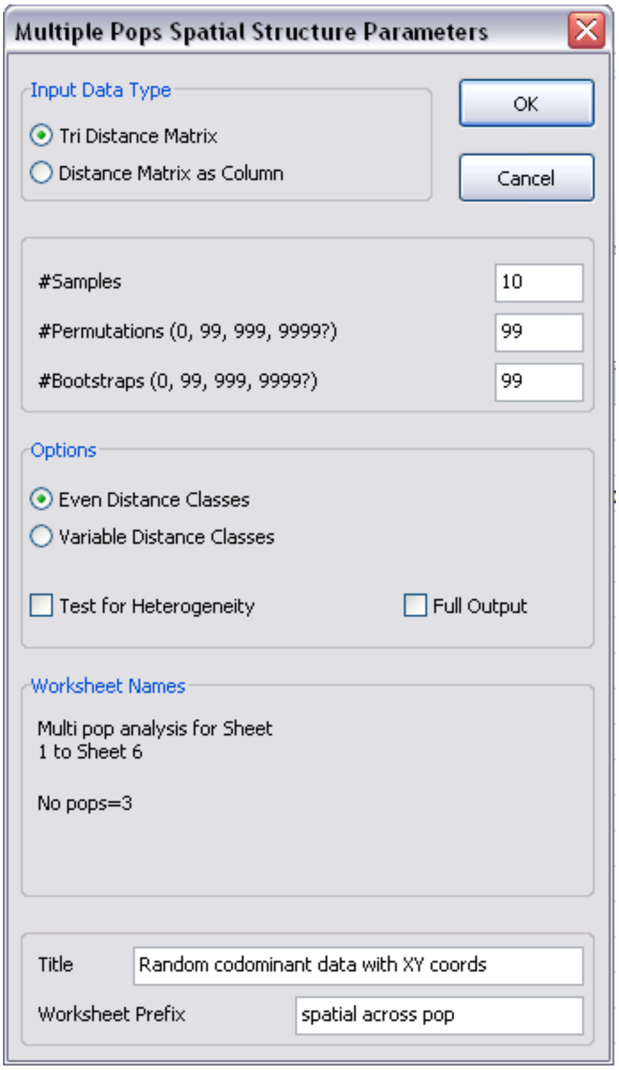
This option allows you to perform autocorrelation over multiple populations. This analysis will detect whether a common process is generating structure in different populations. Additionally, sample sizes are improved giving statistical power to detect even subtle structure, if it exists.

Tip: You can generate separate genetic and geographic distance matrices for each population (required for this analysis) by selecting Dist by Pop *in the* Genetic Distance Options *dialog box, under* ***Distance*****:** ***Genetic.***

Procedure

1. First calculate appropriate genetic [GD] and geographic distance [GGD] matrices for each population via the ***Distance*** option in the GenAlEx menu. If your data is codominant, ensure the GD matrix is for genotypic distances.
2. These sheets must be ordered within a single workbook in the following manner: the geographic matrix (GGD) for Pop 1, followed by genetic distance (GD) matrix for Pop 1, GGD Pop 2, GD Pop 2 … GGD Pop n, GD Pop n. GenAlEx does not check that your sheets are in the correct order, so be sure to double check before analysis.
3. Activate the worksheet containing your first genetic distance matrix [GD]. Choose the option ***Spatial*** from the **GenAlEx** menu, and then select ***Multiple Pops*** from the sub-menu.
4. When prompted by the Input for Spatial Multiple Pops dialog box, enter the number of populations you wish to analyze. Each population requires a GGD and a GD matrix in separate consecutive sheets, as described above.
5. At the Multiple Pops Spatial Structure Parameters dialog box, select the appropriate input data format then enter the number of samples, the number of permutations (0, 99, 999, 9999), the number of bootstraps and the desired distance class option (as per ***Single Pop*** *,* for details see under Single Pop. Spatial Structure options).
6. Select *Test for Heterogeneity* and *Full Output* if desired (see below)
7. Enter a worksheet title and prefix, then click *Ok*. The spatial structure analysis for each population is output to the worksheet [RMP], and the combined analysis across populations is output to worksheet [RC].

Note: with larger data sets and many loci the analysis may take some time. Watch the status bar for progress.



Multiple Pops Spatial Structure Parameters

Input Data Type

● Tri Distance Matrix

○ Distance Matrix as Column

#Samples 10

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

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

Options

● Even Distance Classes

○ Variable Distance Classes

□ Test for Heterogeneity

□ Full Output

Worksheet Names

Multi pop analysis for Sheet
1 to Sheet 6

No pops=3

Title: Random codominant data with XY coords

Worksheet Prefix: spatial across pop

Multiple Pops Spatial Structure options

Test for Heterogeneity: Outputs: 1. statistical tests for heterogeneity in spatial patterns between populations to sheet [MPOS]; 2. the squared paired-sample t-test for heterogeneity between populations at each distance class to sheet [MPTS]; and 3. for the combined population spatial analysis, the heterogeneity test for overall correlogram significance [RC].

Note: This option is only recommended for advanced users. Following Banks and Peakall (2012), significance of the Heterogeneity Tests are declared when P< 0.01)

Full output: Outputs: 1. the full statistics, including the sums of squares for calculating R, for the spatial structure analysis of each population to the worksheet [RMP]; 2. the full statistics for the spatial analyses of each individual population to separate appropriately named worksheets e.g. [Pop1 R]; and 3. a summary of the spatial analyses for each population at Distance Class 1 [DC]. When the *Test for Hetrogeneity* option is selected this option also outputs: 1. the frequency distribution of random omegas versus the observed omega for each pair of populations to separate appropriately worksheets e.g. [Pop1vPop2 OFD]; 2. the list of random squared paired-sample t-statistics between populations for each distance class [MPT]; and 3. the random omega values for the combined spatial analysis [RC].

Multiple Pop Subsets ...

This option allows you to perform autocorrelation over multiple subsets, with each subset containing multiple populations. This analysis will detect whether a common process is generating structure across the different populations within each subset. Additionally, it will test for heterogeneity in spatial patterns between subsets.

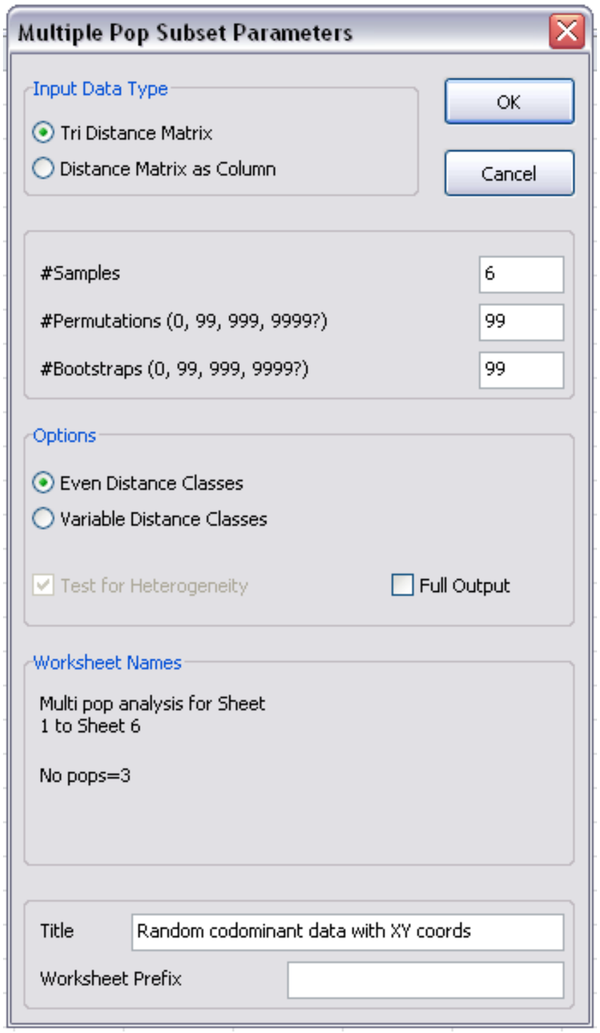
Note: This option is only recommended for advanced users.

Tip: You can generate separate genetic and geographic distance matrices for each population (required for this analysis) by selecting Dist by Pop *in the* Genetic Distance Options *dialog box, under* ***Distance***: ***Genetic.***

Procedure

1. First calculate appropriate genetic [GD] and geographic distance [GGD] matrices for each population via the ***Distance*** option in the GenAlEx menu. If your data is codominant, ensure the GD matrix is for genotypic distances.
2. These sheets must be ordered within a single workbook in the following manner: the geographic matrix (GGD) for Pop 1, followed by genetic distance (GD) matrix for Pop 1, GGD Pop 2, GD Pop 2 … GGD Pop n, GD Pop n. Populations within the same subset must be grouped consecutively. GenAlEx does not check that your sheets are in the correct order, so be sure to double check before analysis.
3. Activate the worksheet containing your first genetic distance matrix [GD]. Choose the option ***Spatial*** from the **GenAlEx** menu, and then select ***Multiple Pop Subsets*** from the submenu.
4. When prompted by the Input for Multiple Pop Subsets dialog box, enter the number of populations you wish to analyze. Each population requires a GGD and a GD matrix in separate consecutive sheets, as described above.
5. At the Multiple Pop Subset Parameters dialog box, select the appropriate input data format then enter the number of samples, the number of permutations (0, 99, 999, 9999), the number of bootstraps and the desired distance class option (as per ***Single Pop..*** ,for details see under Single Pop. Spatial Structure options).
6. Select *Full Output* if desired (see below for details).
7. Enter a Worksheet Title and Prefix, then click *Ok*.

Note: with larger data sets and many loci the analysis may take some time. Watch the status bar for progress.



Multiple Pop Subset Parameters

Input Data Type

● Tri Distance Matrix

○ Distance Matrix as Column

#Samples 6

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

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

Options

● Even Distance Classes

○ Variable Distance Classes

☑ Test for Heterogeneity □ Full Output

Worksheet Names

Multi pop analysis for Sheet 1 to Sheet 6

No pops=3

Title: Random codominant data with XY coords

Worksheet Prefix:

8. When prompted by the Input for Multiple Pop Subsets dialog box, enter the number of populations in each subset. Subsets must be grouped as contiguous populations, as described above. Click *Ok*. Output of statistical tests for heterogeneity in spatial patterns between subsets is to sheet [MPOS]. Output of the squared paired-sample t-test for heterogeneity between subsets at each distance class is to [MPTS]. The combined spatial analyses across populations for each subset, including the heterogeneity test for overall correlogram significance, are output to appropriately named sheets e.g. [RCSS1].

Note: Following Banks and Peakall (2012), significance of the Heterogeneity Tests are declared when P< 0.01)

Multiple Pop Subsets options

Full output: Outputs: 1. the frequency distribution of random omegas versus the observed omega for each pair of subsets to separate appropriately named worksheets e.g. [Subset1vSubset2 OFD]; 2. the list of random squared paired-sample t-statistics between subsets for each distance class [MPT];and 3. the list of random omega values for the combined spatial analysis of each subset to the appropriate worksheet e.g. [RCSS1].

Multiple Dclass …

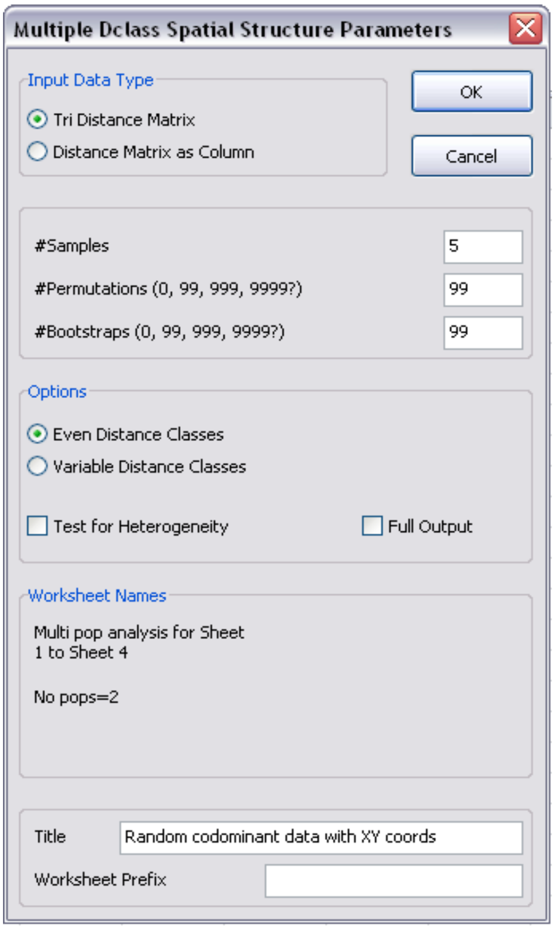
This option performs a spatial autocorrelation over multiple distance class sizes for a single or multiple populations. The analysis differs from the standard spatial analysis in that multiple analyses are performed with automatically increasing distance size classes. Thus, the analysis is equivalent to repeatedly restarting a single or multiple population spatial analysis with a differing distance classes. The output *rc* (*combined r*) for each distance class has a small correction for bias that varies between each run. If you wish to compare the output to standard autocorrelations, be sure to use the uncorrected *rc* values in the table below the autocorrelogram (not the *r* value plotted, which has the correction applied).

This analysis allows exploration of the interplay between sample size and distance class size, allowing one to determine the extent of detectable genetic structure. For further assistance with this option refer to **Tutorial 3, Exercise 3.10**.

Procedure

1. First calculate appropriate genetic [GD] and geographic distance [GGD] matrices via the ***Distance*** option in the **GenAlEx** menu. If your data is codominant, ensure the GD matrix is for genotypic distances.
2. These sheets must be ordered within a single workbook in the following manner: the geographic matrix (GGD) for Pop 1, followed by genetic distance (GD) matrix for Pop 1, GGD Pop 2, GD Pop 2 … GGD Pop n, GD Pop n. GenAlEx does not check that your sheets are in the correct order, so be sure to double check before analysis.
3. Activate the worksheet containing your first genetic distance matrix [GD]. Choose the option ***Spatial*** from the **GenAlEx** menu, and then select ***Multiple Dclass*** from the sub-menu.
4. When prompted by the Input for Spatial Multiple Dclass dialog box, enter the number of populations you wish to analyze. Each population requires a GGD and a GD matrix in separate consecutive sheets, as described above.
5. At the Multiple Dclass Spatial Structure Parameters dialog box, select the appropriate input data format then enter the number of samples, the number of permutations and bootstraps required (0, 99, 999, 9999) (as per ***Single Pop***, for details see under Single Pop. Spatial Structure options).
6. Select the desired distance class option and check *Full Output* if desired (see below for details).
7. Enter a worksheet title and prefix, then click *Ok*. The spatial structure analysis (or combined spatial structure analysis in the case of multiple populations) over multiple distance classes is output to the worksheet [MDC].

Note: with larger data sets and many loci the analysis may take some time. Watch the status bar for progress.



Input Data Type

● Tri Distance Matrix

○ Distance Matrix as Column

#Samples 5

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

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

Options

● Even Distance Classes

○ Variable Distance Classes

□ Test for Heterogeneity □ Full Output

Worksheet Names

Multi pop analysis for Sheet
1 to Sheet 4

No pops=2

Title Random codominant data with XY coords

Worksheet Prefix

Multiple Dclass Spatial Structure options

Even Distance Classes: This option produces the Even Distance Class Options dialog box, which prompts for the base distance class size. Subsequent distance class sizes for this analysis are calculated as *run no * base distance class size* for the number of runs you specified. For example, if you set the base size to 50 m, for 3 runs, the first analysis will calculate *rc* (*rc* = *r* for a combined population analysis) based on all those pairwise comparisons with a geographic distance of 0 to 50 m. In the second analysis, *rc* will be calculated for all those pairwise comparisons with a geographic distance of 0 to 100 m. For the third analysis *rc* will be calculated for all those pairwise comparisons with a geographic distance of 0 to 150 m.

Variable Distance Classes: This option allows you to manually set the distance class sizes for each run.

Full output: In addition to the multiple distance class analysis output to worksheet [MDC], this option outputs the analyses for separate populations for each distance class to appropriately named worksheets [e.g. 0-100 DC].

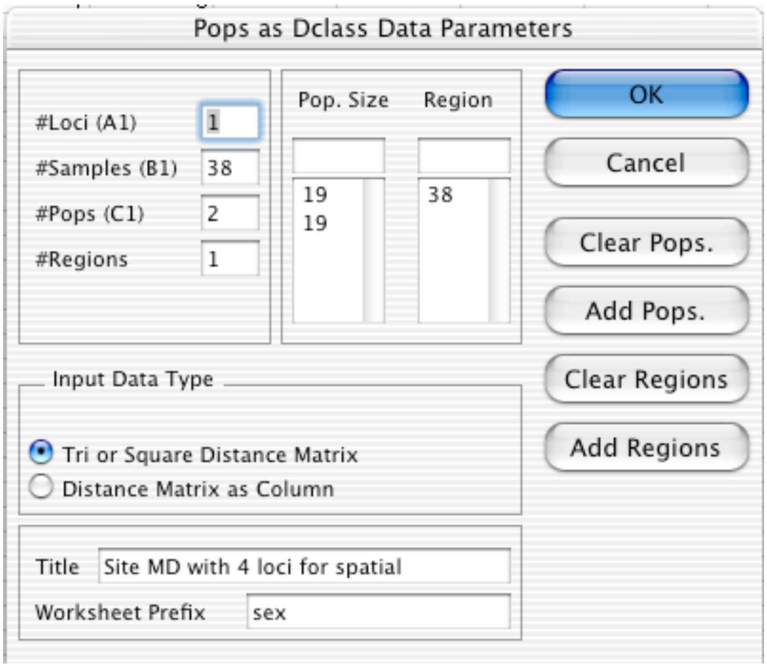
Pops as Dclass ...

This option performs the equivalent of the ***Multiple Dclass*** option, but with the classes defined as populations, rather than geographic distances. Comparisons may also be delimited by the distance among samples. In this case, this option requires both genetic and geographic distance matrices as input. Note that the values of *r* obtained in this analysis may differ from those yielded by other options such as the ***Single Pop*** or ***Multiple Pops***, which use geographic distance classes.

Tip: This option is useful for quickly producing a graph that compares r values between males and females in a single population. In this case, the two sexes would be defined as two different populations.

Procedure

1. First calculate appropriate genetic [GD] and geographic distance [GGD] matrices via the ***Distance*** option in the **GenAlEx** menu. Ensure that the GD matrix includes the population parameters.
2. These sheets must be ordered within a single workbook with the geographic matrix [GGD] in the first position, followed by genetic distance [GD] matrix. GenAlEx does not check that your sheets are in the correct order, so be sure to double check before analysis.
3. Activate the worksheet containing the genetic distance matrix [GD]. Choose the option ***Spatial*** from the **GenAlEx** menu, and then select ***Pops as Dclass*** from the sub-menu.
4. At the Pops as Dclass Data Parameters dialog box, the sample and population parameters should be entered automatically. Enter the appropriate input data type. Enter a Worksheet Title and Prefix, then click *Ok*.



Pops as Dclass Data Parameters

#Loci (A1): 1
#Samples (B1): 38
#Pops (C1): 2
#Regions: 1

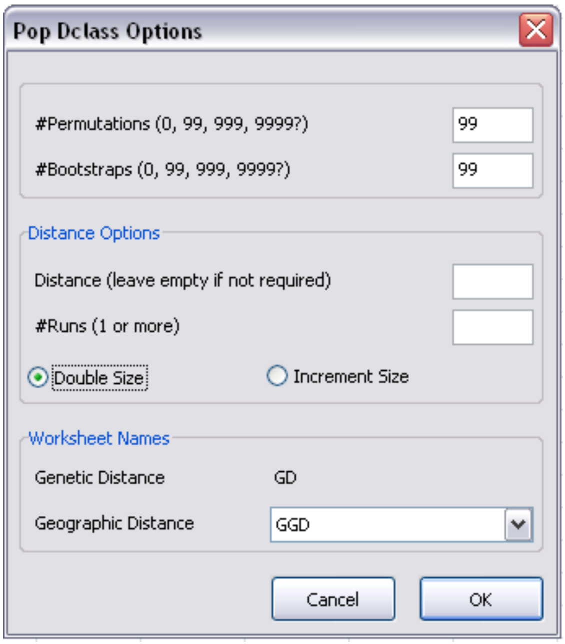
Pop. Size	Region
19	38
19	

Input Data Type
● Tri or Square Distance Matrix
○ Distance Matrix as Column

Title: Site MD with 4 loci for spatial
Worksheet Prefix: sex

5. At the Pop Dclass Options dialog box enter the number of permutations and bootstraps required (0, 99, 999, 9999).

6. If you wish to restrict the comparisons within specified populations, enter the desired distance in the edit box provided. For multiple runs with different maximum distances for restricting comparisons, enter the number of runs and select the method for increasing the distance over these runs – doubling of size each run, or incrementing by the nominated distance.



Pop Dclass Options

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

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

Distance Options

Distance (leave empty if not required)

#Runs (1 or more)

● Double Size ○ Increment Size

Worksheet Names

Genetic Distance GD

Geographic Distance GGD

7. Select the worksheet containing the appropriate geographic distance matrix and click ok. Each run of the analysis is output to a separate worksheet [RP].

2D Local Spatial Analysis (2D LSA)

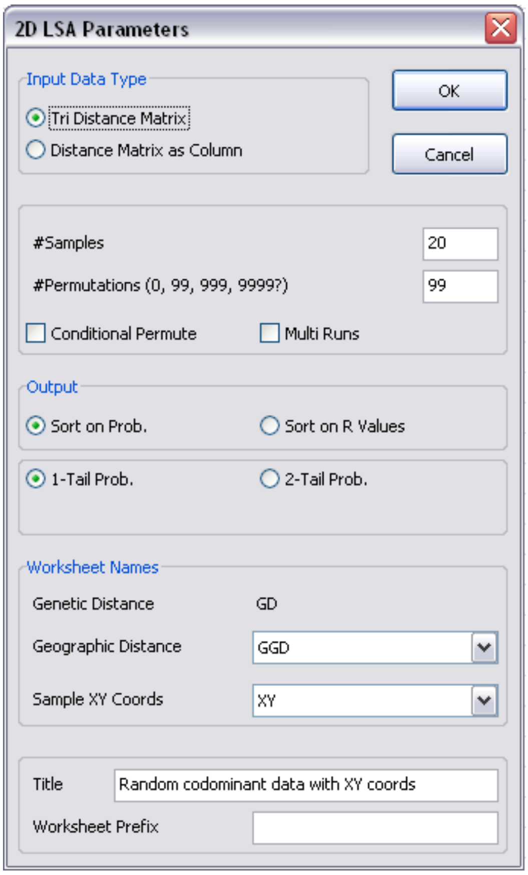
This procedure performs two-dimensional local spatial autocorrelation analyses. Refer to **Appendix 1** and **Tutorial 3, Exercise 3.11** for further information on this option.

In addition to the genetic and geographic distance matrices, the analysis requires a third worksheet containing the XY coordinates in the same order as for the samples used to generate the distance matrices. This worksheet must be in GenAlEx format, with the coordinates contained in columns 3 & 4.

Tip: You can generate a separate worksheet containing the XY coordinates when generating the genetic and geographic distance matrices by selecting Extract XY in the Geographic Distance Options *dialog box, under* ***Distance*: *Genetic.***

Procedure

1. First calculate appropriate genetic [GD] and geographic distance [GGD] matrices via the ***Distance*** option in the **GenAlEx** menu. Ensure that the [GD] matrix includes the population parameters. Prepare a third sheet containing the XY data corresponding to the same samples in the distance matrices.
2. These sheets must be in standard GenAlEx format, and be contained within a single workbook with the geographic matrix [GGD] in the first position, followed by genetic distance [GD] matrix, then the XY data.
3. Activate the worksheet containing the genetic distance matrix [GD]. Choose the option ***Spatial*** from the **GenAlEx** menu, and then select ***2D LSA*** from the sub-menu.
4. At the 2D LSA Parameters dialog box, select the appropriate input data type. Enter the sample number and number of permutations required. Select the conditional permute or multi runs (see below for more details).
5. Select required output options (see below for more details).
6. Select the appropriate worksheets for the analysis. Enter a Worksheet Title and Prefix, then click *Ok*.


2D LSA Parameters

Input Data Type
● Tri Distance Matrix
○ Distance Matrix as Column

#Samples 20
#Permutations (0, 99, 999, 9999?) 99
□ Conditional Permute □ Multi Runs

Output
● Sort on Prob. ○ Sort on R Values
● 1-Tail Prob. ○ 2-Tail Prob.

Worksheet Names
Genetic Distance GD
Geographic Distance GGD
Sample XY Coords XY

Title Random codominant data with XY coords
Worksheet Prefix

2D LSA options

Conditional Permute: Check this for the conditional permute option.

Multiple Runs: Check this to enable multiple runs with increasing numbers of Nearest Neighbors. Checking this option will alter the subsequent dialog box (see below).

Sort on Prob.: Sorts data from the smallest to the largest probability values. i.e places the smallest probability values at the top. This generally corresponds with the largest *r* values being sorted to the top, barring sample size effects.

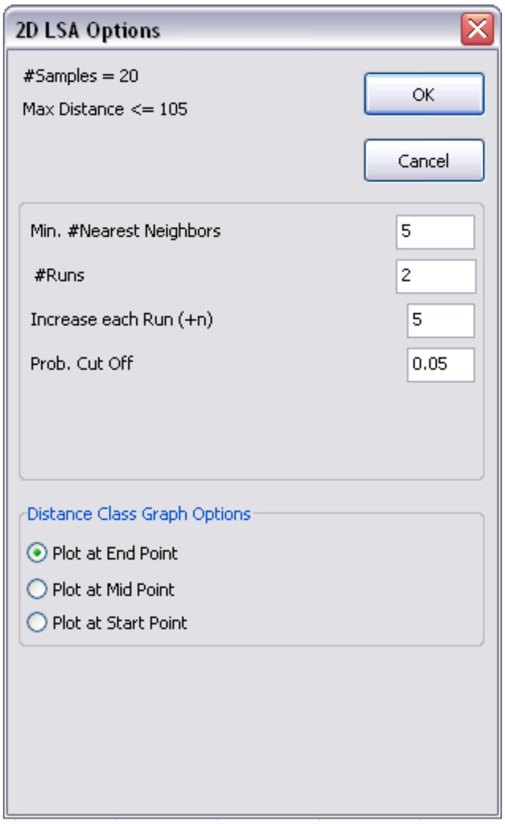
Sort on R Values.: Sorts by r values, moving the largest r values to the top.

1-Tail Prob. Choose this option to label on the graph the values greater than or equal to the specified probability cutoff (see below).

2-Tail Prob. Choose this option to label on the graph the values greater than or equal to the specified probability cutoff divided by two, for both positive and negative values.

7. In the subsequent 2D LSA Options dialog box enter the number of Nearest Neighbors, the required probability cut off (see below for details) and the desired distance class graph option (as per ***Single Pop..*** ,for details see under Single Pop. Spatial Structure options).

8. If you selected *Multiple Runs*, in the previous 2D LSA Parameter dialog box you will also need to enter the number of runs and the number of neighbors to increase each run by (see below for details). Output is to an appropriately named worksheet e.g. [R2D1] for the 1st run.



#Samples = 20

Max Distance <= 105

Min. #Nearest Neighbors 5

#Runs 2

Increase each Run (+n) 5

Prob. Cut Off 0.05

Distance Class Graph Options

● Plot at End Point

○ Plot at Mid Point

○ Plot at Start Point

2D LSA Options

No of Nearest Neighbors: Enter a value for a meaningful number of Nearest Neighbors in your data set.

Tip: It may be helpful to use the Nearest Neighbor Distance (NN Dist) option prior to this analysis to guide you with this value.

No of Runs: Enter the number of runs. For each run, GenAlEx will increase the number of Nearest Neighbors by the value entered below.

Increase each run (+n): Enter a value for increasing the number of Nearest Neighbors in each analysis.

Prob. cut off: Enter your desired Probability cut off, less than or equal to 0.05.

Nearest Neighbor Distance (NN Dist)

This option is provided to complement the 2D LSA results by providing a summary of the Nearest Neighbors and their distances from each other sample, up to the user specified number of Nearest Neighbors.

Tip: This option is useful to identify a meaningful range for the numbers of Nearest Neighbors before performing the 2D LSA.

Procedure

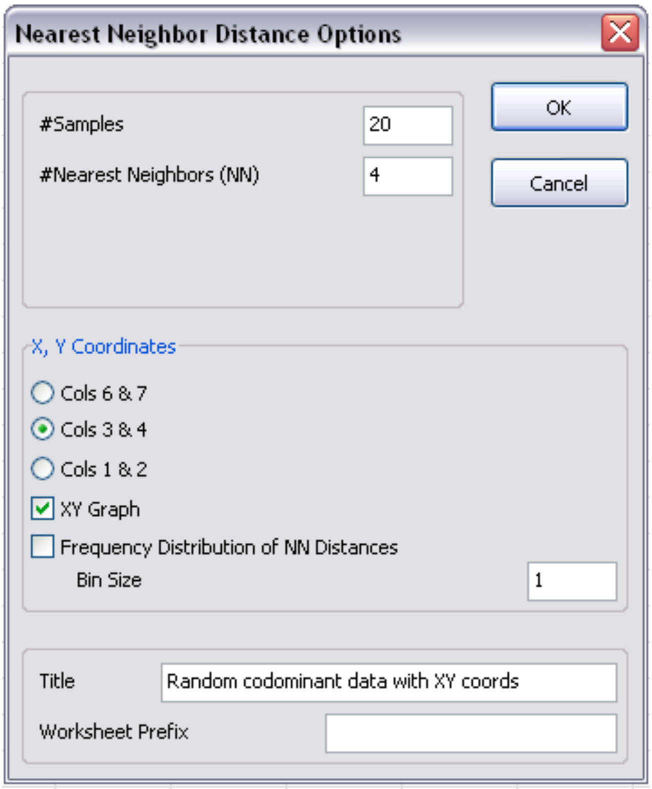
1. Activate the sheet containing your XY data. This data is most conveniently located in Columns 3 & 4 with sample labels in Column 1.

3. Choose the option ***Spatial*** from the **GenAlEx** menu, and then select ***NN Dist*** from the submenu.

4. At the Nearest Neighbor Distance Options dialog box, enter the number of samples, the number of nearest neighbors to interrogate, select the location of your XY data.

5. Select required output options (see below for more details).

6. Enter a Worksheet Title and Prefix, then click *Ok*. Output is to worksheet [NND].


Nearest Neighbor Distance Options

#Samples: 20

#Nearest Neighbors (NN): 4

X, Y Coordinates

○ Cols 6 & 7

● Cols 3 & 4

○ Cols 1 & 2

☑ XY Graph

□ Frequency Distribution of NN Distances

Bin Size: 1

Title: Random codominant data with XY coords

Worksheet Prefix:

Nearest Neighbor Distance Options

XY Graph: Outputs a plot of the samples geographic locations to [NND].

Frequency Distribution of NN Distances: Check this option to output a series of frequency distributions of the distance to each nearest neighbor. The distribution of the distance to the 1st nearest neighbor is output to [NN1].

Bin Size: Enter a required bin value for the frequency distributions.

Clonal

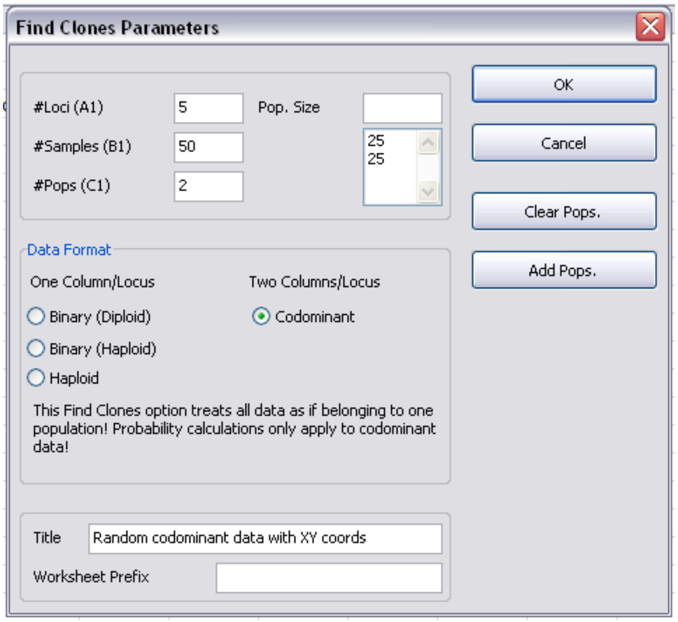
This menu option provides tools for the analysis of clonal structure. For codominant data probability estimates for inferring clonality can also be calculated. The menu has two sub options: ***Find Clones***, which automates the detection of repeated genotypes within the dataset, and calculates probabilities; and ***Clone size***, which estimates the size of putative clones.

Find Clones Procedure

1. Activate the worksheet containing your codominant dataset in GenAlEx format.
2. Choose the option ***Clonal*** from the **GenAlEx** menu, and then select the ***Find Clones*** submenu option. Ensure the locus and sample parameters are correct in the Find Clones Parameters dialog box. Select the data format.

Note: This analysis treats all data as if it belongs to one population

3. Enter Title and Worksheet Prefix then click *Ok*.


Find Clones Parameters

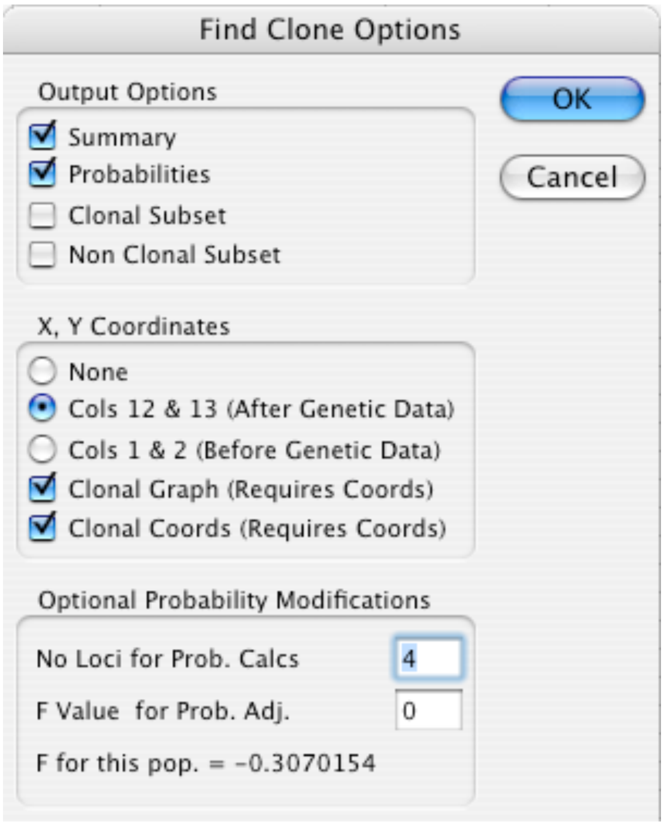
#Loci (A1) 5
#Samples (B1) 50
#Pops (C1) 2
Pop. Size
25
25

Data Format
One Column/Locus
○ Binary (Diploid)
○ Binary (Haploid)
○ Haploid
Two Columns/Locus
● Codominant

This Find Clones option treats all data as if belonging to one population! Probability calculations only apply to codominant data!

Title Random codominant data with XY coords
Worksheet Prefix

4. In the subsequent Find Clone Options dialog box, check the options required (see below for further details), and click *Ok*. See options below for the output sheet names.



Output Options

☑ Summary
☑ Probabilities
□ Clonal Subset
□ Non Clonal Subset

X, Y Coordinates

○ None
● Cols 12 & 13 (After Genetic Data)
○ Cols 1 & 2 (Before Genetic Data)
☑ Clonal Graph (Requires Coords)
☑ Clonal Coords (Requires Coords)

Optional Probability Modifications

No Loci for Prob. Calcs: 4

F Value for Prob. Adj.: 0

F for this pop. = -0.3070154

Find Clones Options

Summary [CL]: Outputs a list of the putative clones based on repeated multilocus genotypes with the count and a label for each genotype. Samples are sorted, with the repeated genotypes shown first.

Probabilities [CLP]: Outputs a range of probability estimates for putative clonal genotypes. This option is only available for codominant data. See **Appendix 1** for formulas and method explanations.

Clonal Subset [MDS]: Outputs a data subset containing those individuals with matching multilocus genotypes (clones). Output includes the number of matches and a label for each genotype.

Non Clonal Subset [DS]: Outputs a subset of the data excluding samples possessing a repeated matching genotype. The last individual to carry the repeated genotype is the one retained.

X, Y Coordinates

Specify the location of your XY data, and select required output:

Clonal Graph [CL]: Outputs an X,Y plot of all samples, with the repeated genotypes labelled.

Clonal Coords [CLC]: Outputs the XY coordinates of putative clones to a separate worksheet. This is the required input for the Clone Size analysis.

Optional Probability Modifications

These options only apply to codominant data

No. of loci for Prob. Calcs: This option allows you to choose the loci that GenAlEx will use for probability calculations, from locus 1 to n.

Tip: if haploid data are coded as if codominant, they may be used to find clones. This option will then facilitate the exclusion of the data from probability calculations.

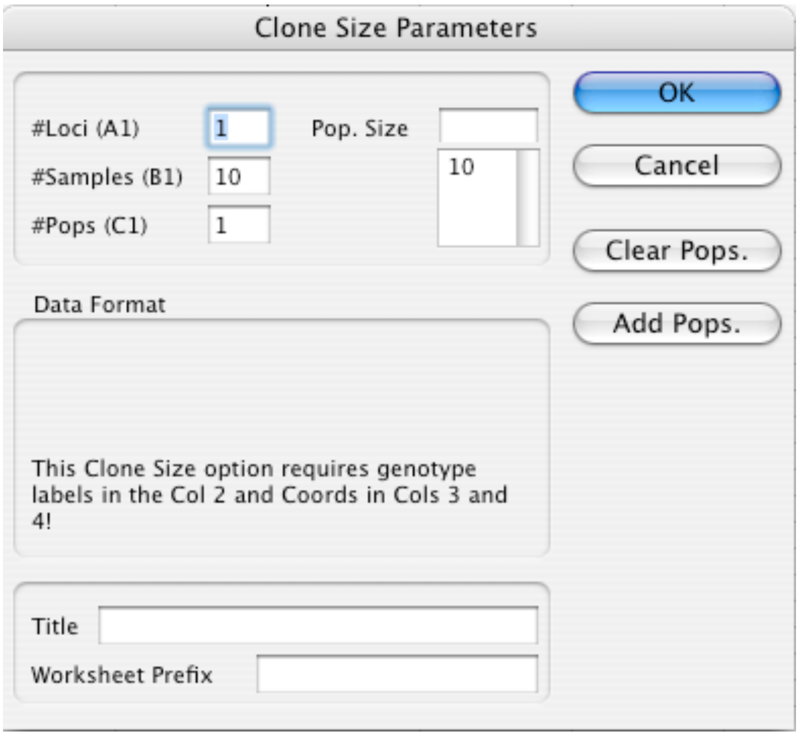
F value for Prob. Adj.: This option allows for an adjustment to the probabilities, taking into account the inbreeding coefficient, F. If the F-value provided is calculated from data including clones it may not be appropriate.

Clone Size Procedure

1. Activate the worksheet containing data formatted with genotype labels in column 2, and XY coordinates in columns 3 and 4. This format is outputted in worksheet [CLC] (see above.

2. Choose the option ***Clonal*** from the **GenAlEx** menu, and then select the ***Clone Size*** submenu option. Ensure the locus and sample parameters are correct in the Clone Size Parameters dialog box.

3. Enter Title and Worksheet Prefix then click *Ok*. Output includes a frequency distribution for maximum clone size [CLMS] and for minimum distance among clones [CLMD].



Clone Size Parameters

#Loci (A1) 1

#Samples (B1) 10

#Pops (C1) 1

Pop. Size

10

Data Format

This Clone Size option requires genotype labels in the Col 2 and Coords in Cols 3 and 4!

Title

Worksheet Prefix

TwoGener

The central idea of ***TwoGener*** is to sample paternal contributors to the seed crops of different maternal parents. Where paternity analysis is actually practical, we recommend it, but where the challenges are too great, ***TwoGener*** forsakes direct paternal delineation, concentrating instead on estimating two derivative constructs, the effective number of pollen donors per average maternal parent (N_{ep}) and the average distance of pollen dispersal (δ). **Tutorial 6** provides detailed background information on the TwoGener analyses and step by step instructions on performing those analyses in GenAlEx.

Raw Data Editing

Import Data

GenAlEx offers several options to facilitate the import of genetic data into Excel. Single files of tab- or space-delimited text can be imported directly. Alternatively, multiple files of either Genotype or Sequence data may be imported simultaneously when contained within a single folder. This facilitates the extraction of data from genotyping / sequencing systems.

GenAlEx also offers the option for importing formatted files from the population genetic analysis program, GenePop, and sequence alignment files in Nexus format.

If neither the tab- nor space-delimited data formats are suitable for your needs, you can also use the text import wizard provided by Excel. Simply choose ***Open*** under the Excel **File** menu.

Genotypes

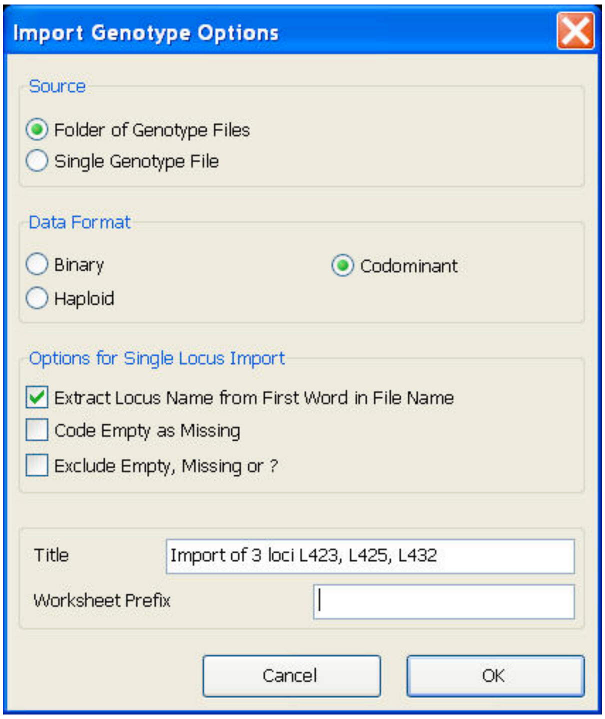
This option imports genotype data from tab-delimited text files. Each file is imported into GenAlEx format in a separate worksheet within a single Excel workbook. An option for importing a single genotype file is also provided. For further assistance with this option refer to **Tutorial 5, Exercise 5.3**.

Tip: To facilitate rapid downstream processing in GenAlEx, it is recommended that options are used within the genotyping software, such as GeneMapper (AB) to prepare a file consisting of: Column 1 - Sample ID; Col 2 – additional information such as locus name; Col 3 – Allele 1 for locus 1; Col 4 – Allele 2 for locus 1.

Procedure

1. Choose the option ***Import Data*** from the **GenAlEx** menu, and then select ***Genotypes*** from the submenu.

2. In the Import Genotypes Options dialog box select the source of your data files. Select the correct data format and check desired options for single locus data (see below). Enter a Title and Prefix for your output worksheet(s) and click *Ok*. Output is to sheet [IG].


Import Genotype Options

Source

● Folder of Genotype Files
○ Single Genotype File

Data Format

○ Binary
○ Haploid
● Codominant

Options for Single Locus Import

☑ Extract Locus Name from First Word in File Name
□ Code Empty as Missing
□ Exclude Empty, Missing or ?

Title: Import of 3 loci L423, L425, L432

Worksheet Prefix:

Import Genotype Options

Source

Folder of Genotype Files: Select this if you have multiple individual genotype files contained within a single folder. You will be prompted to identify the folder containing your files. To do so you need to select a file within the folder from the subsequent dialog box, before clicking 'Open'. Each file is imported into a separate worksheet in a new workbook. Imported data are in GenAlEx format.

Single Genotype File: Select this if you have a single genotype file to import. The data are imported in GenAlEx format into a single worksheet.

Data Format: Indicate whether your genotypes are binary, haploid or codominant.

Options for Single Locus Import:

Extract Locus Name from first Word in File Name: Inserts the first word of the file name into cells C3 and D3 of the output worksheet.

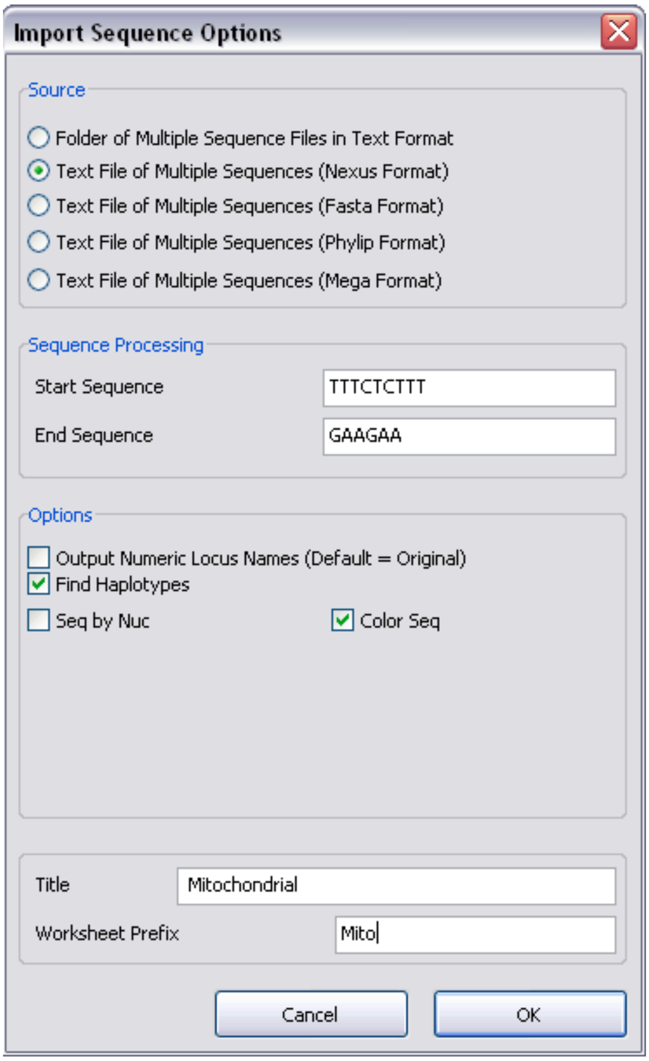
Sequences

This option imports multiple sequences into a single Excel worksheet in GenAlEx format. Options are provided to automate the downstream processing of the sequence data. Sequence data may be upper or lower case, with gaps, and ambiguous base codings. As GenAlEx will only function with numerical data, alpha characters are converted to numeric in the following way: A=1, C=2, G=3, T=4, :=5, -=5, others=zero. For further assistance with this option refer to **Tutorial 5, Exercise 5.1**.

Procedure

1. Choose the option ***Import Data*** from the **GenAlEx** menu, and then select ***Sequences*** from the submenu.

2. In the Import Sequences Options dialog box select the Source of your data files. Select desired options (see below) and data for sequence processing, if required. Enter a Title and Prefix for your output worksheet(s) and click *Ok*. See below for the output sheet names.



Import Sequence Options

Source

○ Folder of Multiple Sequence Files in Text Format
● Text File of Multiple Sequences (Nexus Format)
○ Text File of Multiple Sequences (Fasta Format)
○ Text File of Multiple Sequences (Phylip Format)
○ Text File of Multiple Sequences (Mega Format)

Sequence Processing

Start Sequence: TTTCTCTTT
End Sequence: GAAGAA

Options

□ Output Numeric Locus Names (Default = Original)
☑ Find Haplotypes
□ Seq by Nuc ☑ Color Seq

Title: Mitochondrial
Worksheet Prefix: Mito

Import Sequence Options

Source

Folder of Multiple Sequence Files in Text Format: Select this if you have multiple individual sequence files in ‘text only’ format contained within a single folder. You will be prompted to identify the folder containing your files. To do so you need to select a file within the folder from the subsequent dialog box, before clicking ‘Open’.

Text Files of Multiple Sequences: Select the appropriate format (Nexus, Fasta, Phylip, Mega) of a single text file of multiple aligned sequences that you wish to import.

Sequence Processing

Start Sequence: Sequences will only be imported starting at the specified nucleotide sequence.

Tip: This is useful for trimming unwanted ends from sequences, in order to obtained aligned sequences in GenAlEx.

End Sequence: Sequence subsequent to the specified nucleotide sequence will not be imported.

Tip: this is useful for trimming unwanted ends from sequences.

Options:

Output Numeric Locus Names: Renames imported loci (base positions) numerically one to n. If this option is not selected the loci are labeled as indicated in the import file.

Find haplotypes: Checking this option will process the data to find haplotypes, yielding various outputs to separate worksheets:

> **Polymorphic Sites [PS]:** Outputs a subset of the data, including only polymorphic sites.
>
> *Tip: This is a quick way to output a table of variable sites for a sequence dataset.*
>
> **Polymorphic Numerical [PN]:** Outputs the Polymorphic Sites subset, converted to numerical codes as GenAlEx will only function using numerical data. (A=1, C=2, G=3, T=4, :=5, -=5, others=zero).
>
> **Haplotype [HA]:** Provides a haplotype code for each individual sequence, together with its haplotype as both alpha and numerical characters. For the latter, ‘h’ is added to end of the haplotype so that it is not treated as a number by Excel.
>
> **Haplotype Count [HC]**: Provides a count of each haplotype together with their numerical codes.
>
> **Haplotype List [HL]:** Provides a list of haplotypes together with the numerical coding of their polymorphic sites. Also provided is an example individual and population that contains each haplotype.

Color Seq: Colors the imported sequences in sheets [SQ] and [PS] by nucleotide.

Output:

Imported Sequence [IS]: Imports each raw, unprocessed sequence into a single cell in the worksheet.

Sequence [SQ]: Imports each sequence with a single nucleotide per cell. GenAlEx uses the length of the first sequence as a guide for processing subsequent sequences. As such, if a subsequent sequence is longer than the first one, the extra bases will not be processed.

For uses of Excel 2003: If the full sequence consists of more than 254 nucleotides, this option is not completed due to the maximum of 256 columns in an Excel worksheet.

Sequence warnings [WS]: This sheet outputs all warnings associated with the sequence import. These warnings will include detection of alpha codes other than A,C,T,G, : & -, to facilitate further checking of ambiguous base calls.

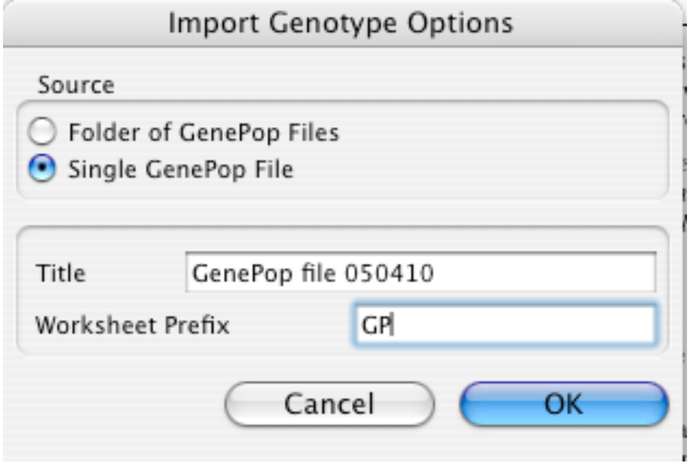
GenePop file

This option will import a GenePop file exactly matching the GenePop format exported by GenAlEx.

Procedure

1. Choose the option ***Import Data*** from the **GenAlEx** menu, and then select ***GenePop*** from the submenu.

2. In the Import Genotypes Options dialog box select the source of your data files. Enter a Title and Prefix for your output worksheet(s) and click *Ok*. Output is to worksheet [genepop].



Import Genotype Options

Source

○ Folder of GenePop Files
● Single GenePop File

Title: GenePop file 050410
Worksheet Prefix: GP

Import GenePop Options

Source

Folder of Genotype Files: Select this if you have multiple individual GenePop files contained within a single folder. You will be prompted to identify the folder containing your files. To do so you need to select a file within the folder from the subsequent dialog box, before clicking 'Open'. Each file is imported into a separate worksheet in a new workbook with the name of the imported file. Imported data are in GenAlEx format.

Single Genotype File: Select this if you have a single GenePop file to import. The data are imported in GenAlEx format into a single worksheet with the name of the imported file.

Tab Delimited

Imports a tab delimited file as a single worksheet in an Excel workbook.

For further work in GenAlEx, imported files should be saved as Excel workbooks, and formatted appropriately for GenAlEx (options are available under the ***Raw Data*** option in the **GenAlEx** menu to automate some of these tasks).

Space Delimited

Imports a space delimited file as a single worksheet in an Excel workbook. For further work in GenAlEx, imported files should be saved as Excel workbooks, and formatted appropriately for GenAlEx (options are available under the ***Raw Data*** option in the **GenAlEx** menu to automate some of these tasks).

Folder Nexus Alignments

Select this option if you have multiple alignment files in Nexus format contained within a single folder. You will be prompted to identify the folder containing your files. To do so you need to select a file within the folder from the subsequent dialog box, before clicking ‘Open’. Each file is imported as a separate worksheet in an Excel workbook.

Raw data

GenAlEx offers several options to assist in assimilating and formatting datasets imported from genotyping/sequencing systems. These options are particularly useful for the manipulation of large datasets.

Sorts on Col 3: This option sorts the dataset on Locus 1. It first sorts the GenAlEx dataset (starting in Row 4) on column 3, and then sorts the data within each column 3 group on column 4.

Exclude Empty at Col 3: This option excludes samples containing an empty cell in column 3, and reformats the GenAlEx parameters. Extracted samples are moved to below the end of the input dataset on the same worksheet, with an intervening blank row meaning that GenAlEx will not use these samples in analyses.

Exclude Missing at Col 3: This option excludes samples containing missing data (coded as '0'or '-1') or an empty cell in column 3, and reformats the GenAlEx parameters. Extracted samples are moved to below the end of the input dataset on the same worksheet, with an intervening blank row meaning that GenAlEx will not use these samples in analyses.

Exclude ? at Col 3: This option excludes samples containing a '?' or an empty cell in column 3 and reformats the GenAlEx parameters. Extracted samples are moved to below the end of the input dataset on the same worksheet, with an intervening blank row meaning that GenAlEx will not use these samples in analyses.

Combine Data

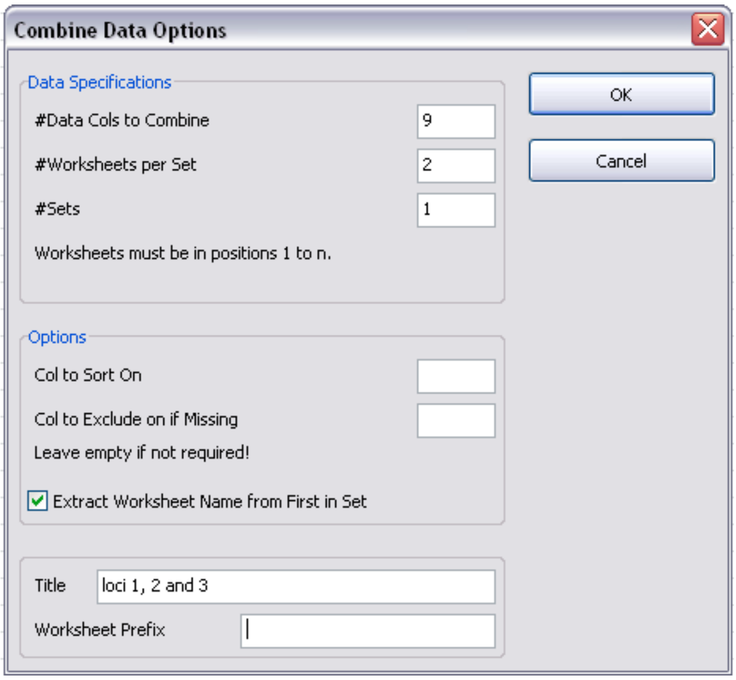
This option combines into one worksheet data for the same loci held in several worksheets in a single workbook (e.g. output from different genotyping runs). Sets of equal numbers of worksheets for different loci groups may be combined simultaneously by entering the appropriate information in the dialog box. Geographic data and other data pertaining to the samples, such as sex etc, may also be combined.

Procedure

1. Ensure that the worksheets containing your data for each loci group are in positions 1 to n, with data in GenAlEx format (sample code in Col. 1, optional population data in Col. 2, and genetic data starting in Row 4). If data for more than one loci group are to be combined, the worksheets for each group need to be together in the workbook.

2. Choose the option ***Raw Data*** from the **GenAlEx** menu, and then select ***Combine Data*** from the submenu.

2. In the Combine Data Options dialog box enter the required information (see below for details). Enter a Title and Prefix for your output worksheet(s) and click *Ok*. Output is to sheet [CBD].



Combine Data Options

Data Specifications

#Data Cols to Combine: 9

#Worksheets per Set: 2

#Sets: 1

Worksheets must be in positions 1 to n.

Options

Col to Sort On:

Col to Exclude on if Missing:

Leave empty if not required!

☑ Extract Worksheet Name from First in Set

Title: loci 1, 2 and 3

Worksheet Prefix:

Combine data options.

Data Specifications

No. Data Cols to Combine: Enter the number of columns to be combined, starting with column 1.

Tip: A subset of the loci present in the worksheets may be combined by choosing only the required columns. Conversely, if you wish to include additional columns containing, for example XY data, make sure these are counted in the number of columns selected.

No. Worksheets per Set: Enter the number of worksheets to be combined per loci group (set). When combining multiple sets, the number of sheets per set must be the same, with all sheets for the same set in a contiguous block.

No sets: Enter the number of sets of worksheets (1 set per loci group) that you wish to combine.

Options

Leave these boxes empty if not required.

Col. No to Sort On: Enter a column number here to simultaneously sort all combined data on the column specified.

Col. No to Exclude On if Missing: Enter a column number here to simultaneously exclude samples with data absent from the column specified. Empty cells, or cells with ? are considered as absent data. Cells containing ? are converted to empty cells.

Extract Worksheet Name from First in Set: Check this option if desired.

Check for Dups.

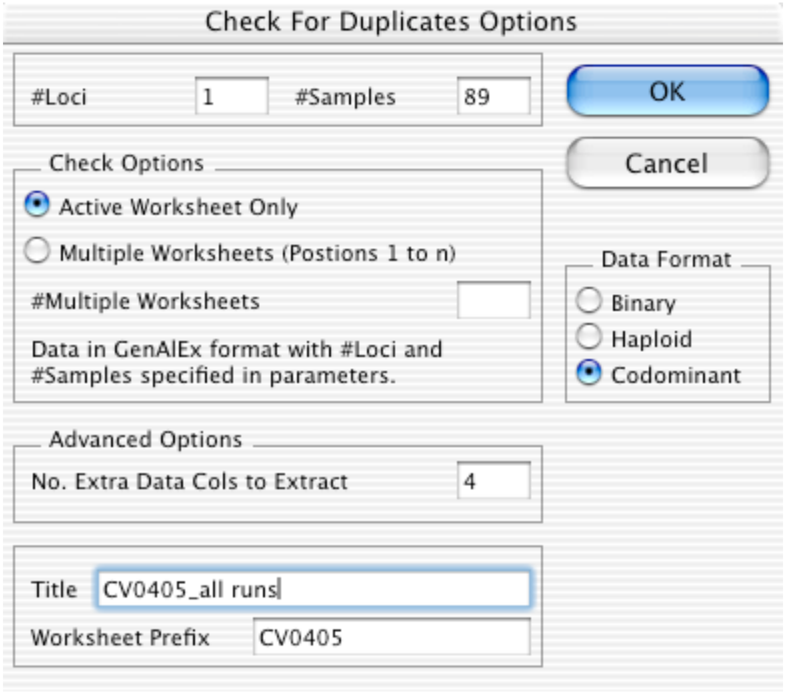
This option processes data and checks for duplicate sample names. For further assistance with this option refer to **Tutorial 5, Exercise 5.4**.

Tip: This function is useful for removing duplicate control samples, or detecting repeat samples over several genotyping runs.

Procedure

1. With the worksheet containing your data in GenAlEx format activated, choose the option ***Raw Data*** from the **GenAlEx** menu, and then select ***Check for Dups*** from the submenu.
2. In the Check For Duplicates Options dialog box enter the required information (see below for details). Enter a title and prefix for your output worksheet(s) and click *Ok*.

Output: to sheet [DS], comprises a data subset with duplicate samples removed, and a record of the number of matching samples found. The sample parameter is adjusted accordingly. In two further columns, separated from the data by a blank column, information is given on the number of duplicate matches found for each sample name (No. Matches), and whether or not the genotypes of the duplicates match (Match). If two samples with the same name have differing genotypes, the duplicate is retained and a note 'Dup does not match' attached to the sample.


Check For Duplicates Options

#Loci 1 #Samples 89

Check Options

● Active Worksheet Only

○ Multiple Worksheets (Postions 1 to n)

#Multiple Worksheets

Data in GenAlEx format with #Loci and #Samples specified in parameters.

Advanced Options

No. Extra Data Cols to Extract 4

Title CV0405_all runs

Worksheet Prefix CV0405

Data Format

○ Binary

○ Haploid

● Codominant

Check for Duplicates options

Check options

Active worksheet only: Checks for duplicates in data held on a single worksheet.

Multiple Worksheets (Positions 1 to n): Checks for duplicates in data held on multiple worksheets. Ensure these worksheets are in positions 1 to n, and insert the number of sheets to be checked in the edit box.

Advanced options

No. of Extra Data cols to Extract: Enter the number of columns of extra data you wish to carry on to the subsequent worksheets. These columns must be separated from the data by a single blank column.

Data format: Select the appropriate format for your data.

Merge Loci

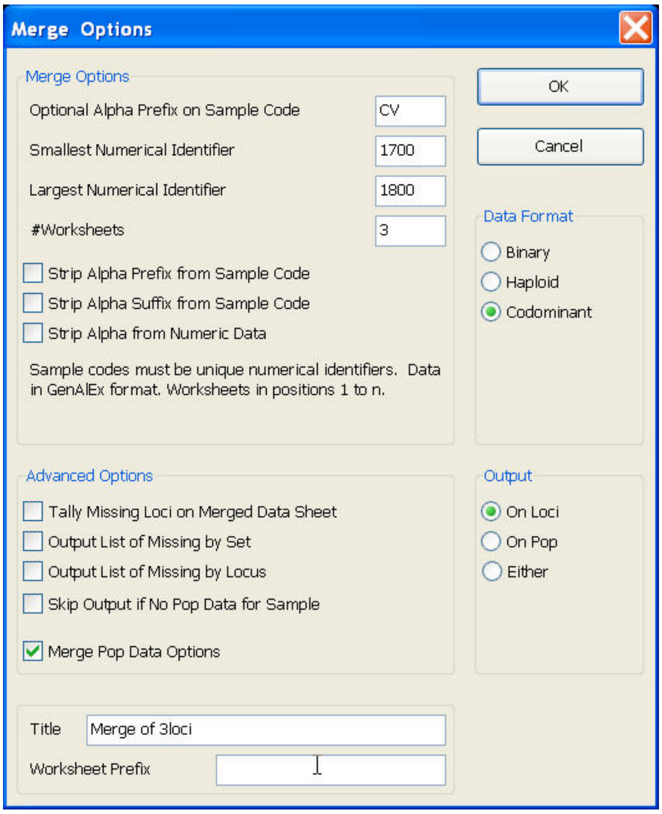
This option merges data from different loci held in separate worksheets within a single workbook. Optionally, population data held in a specified worksheet may also be merged. For further assistance with this option refer to **Tutorial 5, Exercise 5.5**.

Tip: To facilitate data management locus labels should be inserted into Row 3 in the input datasheets.

Procedure

1. Ensure the multiple worksheets containing your data are in GenAlEx format, and located in positions 1 to n. Choose the option ***Raw Data*** from the **GenAlEx** menu, and then select ***Merge Loci*** from the submenu.
2. In the Merge Loci Options dialog box enter the required information (see below for details). Enter a Title and Prefix for your output worksheet(s) and click *Ok*.

Output: to worksheet [MGL] consists of the merged data, with appropriate locus and sample parameters. Where data for one locus contains samples not present in other locus datasets, this missing data is entered as 0.



Merge Options

Merge Options

Optional Alpha Prefix on Sample Code: CV

Smallest Numerical Identifier: 1700

Largest Numerical Identifier: 1800

#Worksheets: 3

□ Strip Alpha Prefix from Sample Code

□ Strip Alpha Suffix from Sample Code

□ Strip Alpha from Numeric Data

Sample codes must be unique numerical identifiers. Data in GenAlEx format. Worksheets in positions 1 to n.

Data Format

○ Binary

○ Haploid

● Codominant

Advanced Options

□ Tally Missing Loci on Merged Data Sheet

□ Output List of Missing by Set

□ Output List of Missing by Locus

□ Skip Output if No Pop Data for Sample

☑ Merge Pop Data Options

Output

● On Loci

○ On Pop

○ Either

Title: Merge of 3loci

Worksheet Prefix:

Merge loci options.

<u>**Merge options**</u>

Optional Alpha Prefix on Sample Code: Enter the alpha character prefix of the sample labels, if applicable. This prefix must be the same for all samples. If the data contains an alpha prefix but it is not entered here, then the combined samples will not be output unless the 'strip Alfa prefix' option is selected below.

Smallest Numerical Identifier: Enter the value for the smallest unique numerical sample identifier (label).

Largest Numerical Identifier: Enter the value for the largest unique numerical sample identifier.

Tip: The sample labels need not be continuous, but the range defined by these two items must include the minimum and maximum value (unless a subset of the data is required).

# Worksheets: Enter the number of loci you wish to merge. Data for each locus is on a separate worksheet in position 1…n.

Strip Alpha Prefix from Sample Code: Strips all alphabetical character prefixes from the output sample codes that are not the Alpha prefix specified above. If a sample code retains any alpha characters that prevent the code from being recognized by GenAlEx as a number, that sample will not be output.

Strip Alpha Suffix from Sample Code: Strips all alphabetical character suffixes from the output sample codes. If a sample code retains any alpha characters that prevent the code from being recognized by GenAlEx as a number, that sample will not be output.

Strip Alpha from Numeric Data: Strips Alpha characters from all allele codes.

Advanced Options

Tally missing loci on merged datasheet [MGL]: This option tallies the missing data across all samples for each locus, and the total missing per sample across all loci.

Output list of missing by set [MISS]: This option outputs a list of missing samples for each locus to a single worksheet.

Tip: This option is useful to quickly produce a list of samples that might need repeating.

Output list of missing by locus [locus name_MISS]: This option outputs a list of missing samples for each locus to a separate worksheet.

Skip Output if No Pop Data for Sample: Check this option to remove those samples from the output that do not have population data.

Merge Pop Data Options: This option enables population data held in a separate worksheet to be merged with the genetic data. Checking this option will call a subsequent dialog box. See below for further details.

Data format: Select the appropriate format for your data.

Output

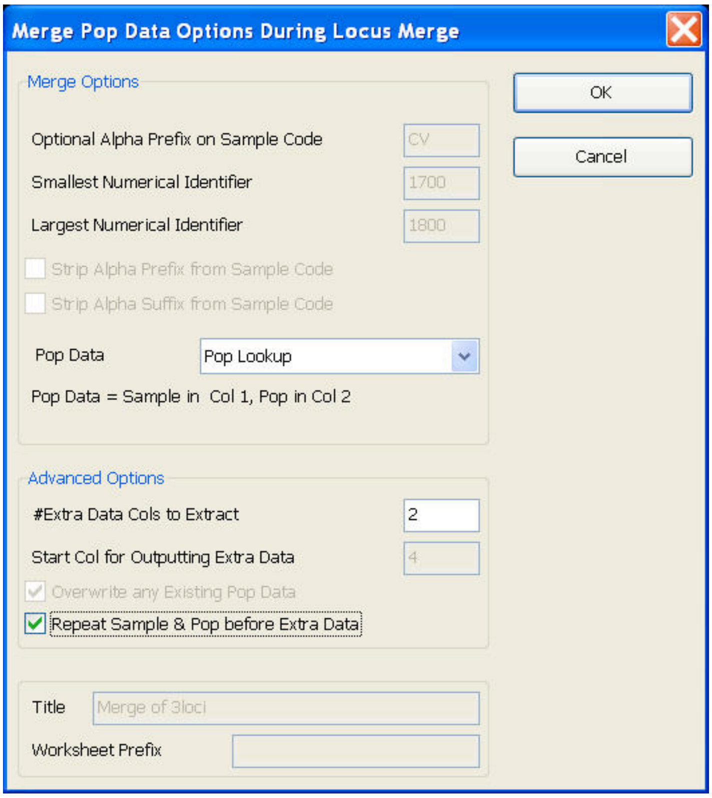
On loci: Will merge data for which locus information is available i.e. a sample that is listed in the population data, but is not on any of the locus worksheets will not appear in the output.

On pop: Will merge data for which pop information is available. i.e. a sample for which locus information is available, but that is not listed in the population data will not appear in the output. Conversely, if population data is available but no locus data, the sample will be listed. This is a useful option for locating samples that need to be genotyped.

Either: Will merge all data for which either locus or population data are available.

Merge Pop data with loci

This option enables population data held in a separate worksheet to be merged with the genetic data. Select the worksheet containing the population data from the pull-down “Pop Data’ menu. Population data must be in the format with Sample labels in column 1 and population labels in column 2, with the correct sample parameter in Cell B1. If this option is checked, the merged data output is to sheet [MGLP].



Merge Pop Data Options During Locus Merge

Merge Options

Optional Alpha Prefix on Sample Code: CV

Smallest Numerical Identifier: 1700

Largest Numerical Identifier: 1800

□ Strip Alpha Prefix from Sample Code

□ Strip Alpha Suffix from Sample Code

Pop Data: Pop Lookup

Pop Data = Sample in Col 1, Pop in Col 2

Advanced Options

#Extra Data Cols to Extract: 2

Start Col for Outputting Extra Data: 4

☑ Overwrite any Existing Pop Data

☑ Repeat Sample & Pop before Extra Data

Title: Merge of 3loci

Worksheet Prefix:

Advanced Options

# Extra Data Cols to Extract: Enter the number of columns of extra data, from column 1 to column n, that you wish to carry on to the output worksheets. These columns must be separated from the data by a single blank column.

Tip: this option allows further data to be extracted from a worksheet containing the population data (e.g. XY coordinates).

Start Col for Outputting Extra Data: Enter the column on the output sheet where you wish the extra data to be inserted.

Overwrite any existing pop data: Overwrites any pre-existing population data on the datasheets to be merged.

Repeat Sample & Pop before Extra Data: Repeats the sample and population labels before any extra data. This may be convenient for management of very large genetic datasets.

Unmerge Loci

This option will produce separate worksheets for individual loci from a multi-locus dataset in GenAlEx format held in an activated worksheet. Output worksheets are named according to locus names. The original data sheet is left intact.

Procedure

1. With the sheet containing your genetic data activated, choose the option ***Raw Data*** from the **GenAlEx** menu, and then select ***Unmerge Loci*** from the submenu.

2. In the Unmerge Loci Data Parameters dialog box ensure all parameters are correct. Select the appropriate data format. Enter a Title and Worksheet Prefix and click *Ok*.

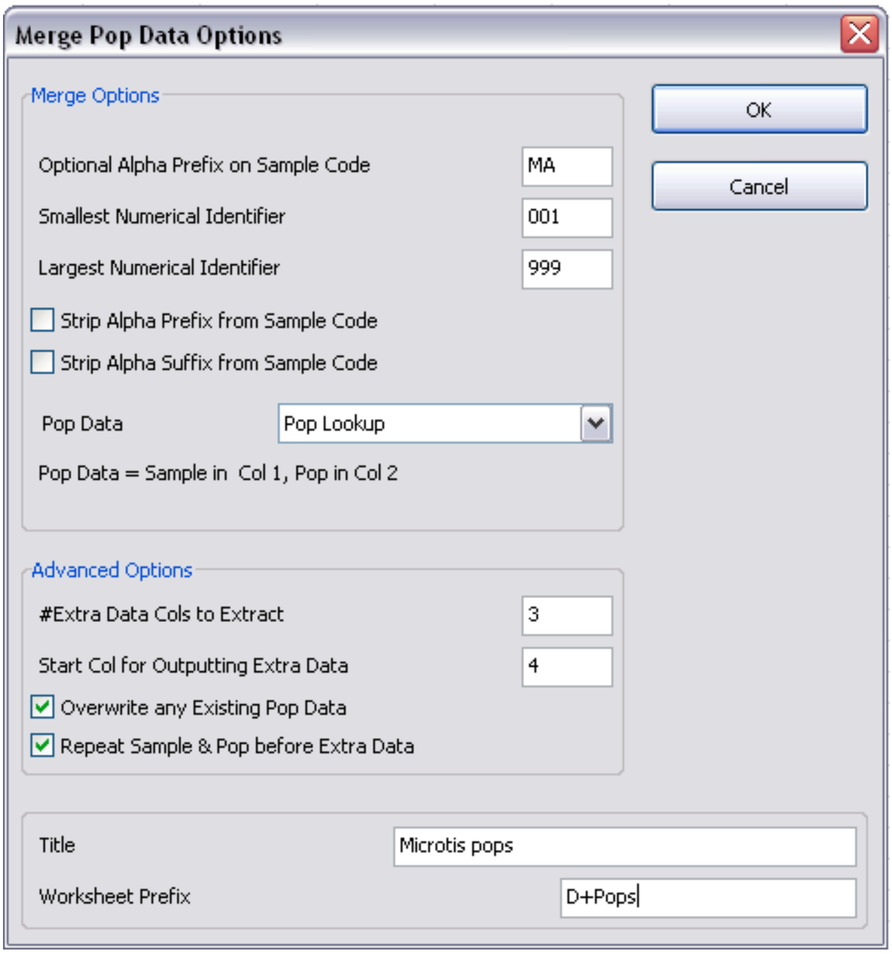
Output: Data for each locus is inserted in a separate, appropriately formatted GenAlEx worksheet, with the name of the locus as the worksheet name.

Merge Pops

This option enables population data held in a separate worksheet to be merged with the genetic data. The population data must be in standard GenAlEx format, with the sample code in column 1 and population code in column 2, and correct sample parameters.

Procedure

1. With the sheet containing your genetic data activated, choose the option ***Raw Data*** from the **GenAlEx** menu, and then select ***Merge Pops*** from the submenu.
2. In the Merge Pop Data Options dialog box enter the required information (see below for details).
3. Select the worksheet containing the population data from the pull-down "Pop data' menu. Population data must be in the format with Sample labels in column 1, and population labels in column 2.
4. Enter a Title and Prefix for your output worksheet(s) and click *Ok*. Output is to sheet [MGLP].



Merge Pop Data Options

Merge Options

Optional Alpha Prefix on Sample Code: MA

Smallest Numerical Identifier: 001

Largest Numerical Identifier: 999

□ Strip Alpha Prefix from Sample Code

□ Strip Alpha Suffix from Sample Code

Pop Data: Pop Lookup

Pop Data = Sample in Col 1, Pop in Col 2

Advanced Options

#Extra Data Cols to Extract: 3

Start Col for Outputting Extra Data: 4

☑ Overwrite any Existing Pop Data

☑ Repeat Sample & Pop before Extra Data

Title: Microtis pops

Worksheet Prefix: D+Pops

Merge options

Optional Alpha Prefix on Sample Code: Enter the alpha character prefix for your sample labels, if applicable. This prefix must be the same for all samples. If the data contains an alpha prefix but it is not entered here, then the population codes will not be output.

Smallest Numerical Identifier: Enter the value for the smallest unique numerical sample identifier (label).

Largest Numerical Identifier: Enter the value for the largest unique numerical sample identifier.

Tip: The sample labels need not be continuous, but the range defined by these two items must include the minimum and maximum value (unless a subset of the data is required).

Strip Alpha Prefix from Sample Code: Strips all alphabetical character prefixes from the output sample codes that are not the Alpha prefix specified above. If a sample code retains any alpha characters that prevent the code from being recognized by GenAlEx as a number, that sample will not be output.

Strip Alpha Suffix from Sample Code: Strips all alphabetical character suffixes from the output sample codes. If a sample code retains any alpha characters that prevent the code from being recognized by GenAlEx as a number, that sample will not be output.

Advanced Options

# Extra Data Cols to Extract: Enter the number of columns of extra data you wish to carry on to the output worksheets. These columns must be separated from the data by a single blank column.

Tip: this option allows further data to be extracted from a worksheet containing the population data (e.g. XY coordinates).

Start Col for Outputting Extra Data: Enter the column on the output sheet where you wish the extra data to be inserted.

Overwrite any existing pop data: Checking this option will overwrite any pre-existing population data on the datasheets to be merged.

Repeat Sample & Pop before Extra Data: This option will insert the sample and population labels before any extra data.

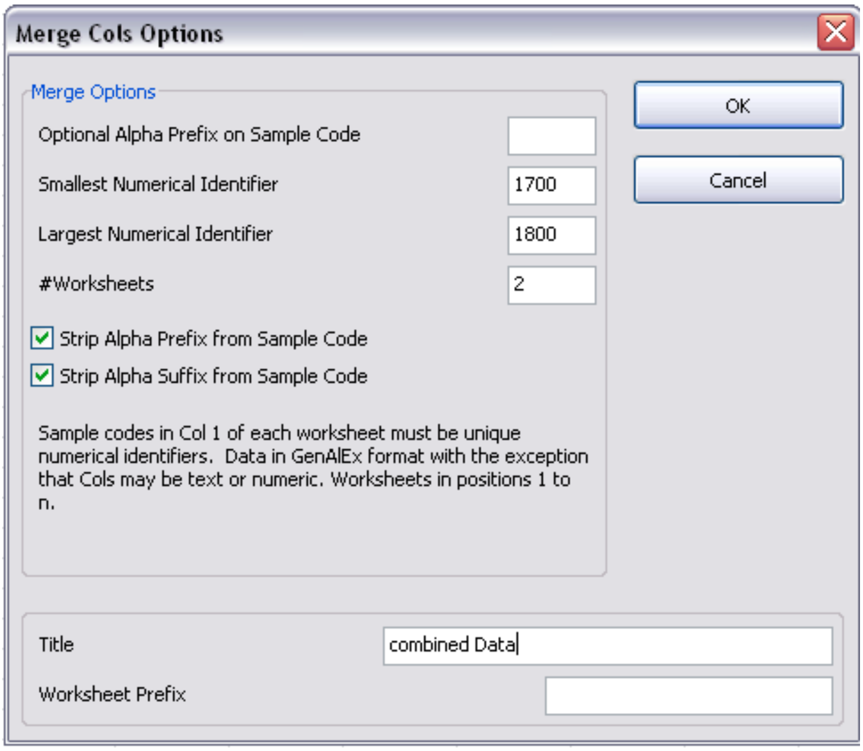
Merge Cols

This option merges data columns from different worksheets, in a single workbook, into adjacent columns in a single worksheet. Unlike the ***Merge Loci*** option, the column values can be either text or numeric. Sample and population codes from different worksheets are merged into columns one and two of the output.

Procedure

1. Ensure the multiple worksheets containing your data columns are in GenAlEx format with appropriate parameters, and located in positions 1 to n. Choose the option ***Raw Data*** from the **GenAlEx** menu, and then select ***Merge Cols*** from the submenu.

2. In the Merge Cols Options dialog box enter the required information (see below for details). Enter a Title and Prefix for your output worksheet(s) and click *Ok*. Output is to worksheet [MGC].



Optional Alpha Prefix on Sample Code

Smallest Numerical Identifier 1700

Largest Numerical Identifier 1800

#Worksheets 2

☑ Strip Alpha Prefix from Sample Code

☑ Strip Alpha Suffix from Sample Code

Sample codes in Col 1 of each worksheet must be unique numerical identifiers. Data in GenAlEx format with the exception that Cols may be text or numeric. Worksheets in positions 1 to n.

Title combined Data

Worksheet Prefix

Merge Cols options.

Merge options

Optional Alpha Prefix on Sample Code: Enter the alpha character prefix of the sample labels, if applicable. This prefix must be the same for all samples. If the data contains an alpha prefix but it is not entered here, then the combined columns will not be output unless the 'strip Alfa prefix' option is selected below.

Smallest Numerical Identifier: Enter the value for the smallest unique numerical sample identifier (label).

Largest Numerical Identifier: Enter the value for the largest unique numerical sample identifier.

Tip: The sample labels need not be continuous, but the range defined by these two items must include the minimum and maximum value (unless a subset of the data is required).

# **Worksheets**: Enter the number of worksheets you wish to merge.

Strip Alpha Prefix from Sample Code: When this option is selected sample codes containing Alpha prefixes and the corresponding merged columns values are output. If a sample code contains an Alpha prefix and this option is not selected, then that sample will not be output.

Strip Alpha Suffix from Sample Code: When this option is selected sample codes containing Alpha suffixes and the corresponding merged columns values are output. If a sample code contains an Alpha suffix and this option is not selected, then that sample will not be output.

Replace Sample Code

This option can be used to quickly replace idiosyncratic sample codes in one or more worksheets with unique numerical identifiers. There is no auto save on competition of this option.

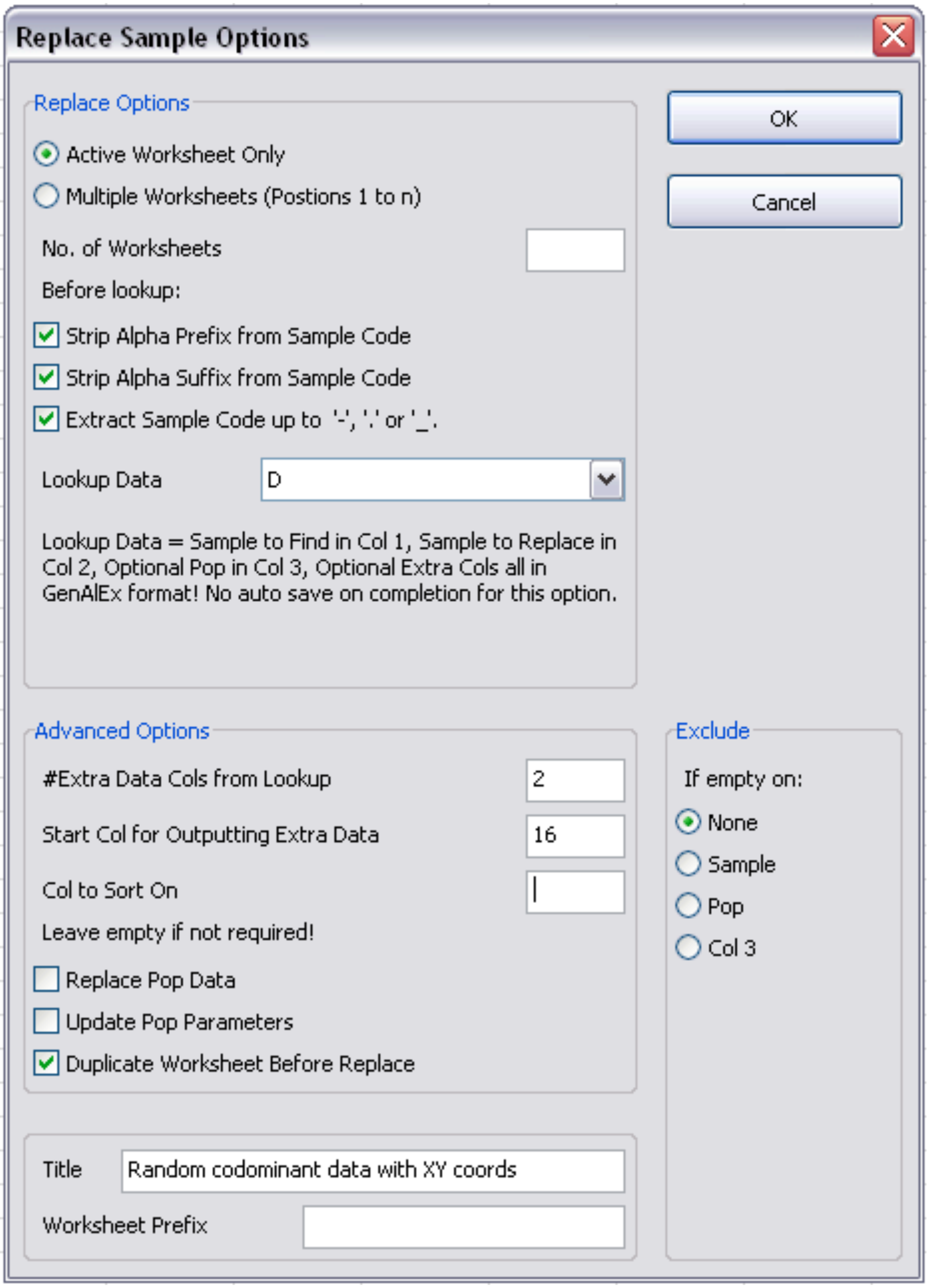
Procedure

1. All worksheets to be changed should be located in positions 1 to n in the workbook. Each worksheet must be in **GenAlEx** format. Activate the first worksheet to be changed, choose the option ***Raw Data*** from the **GenAlEx** menu, and then select ***Replace Sample Code*** from the submenu.

2. In the Replace Sample Options dialog box indicate if one or multiple worksheets are to be changed. If multiple worksheets are to be changed, enter the number. Select required "Before lookup' options (see below for details).

3. Select the worksheet containing the replacement sample codes from the pull-down "Lookup Data' menu. Lookup data must be in the following format: original sample labels in column 1, replacement sample labels in column 2, optional population labels in column 3 and extra data in columns 4 to n.

4. Enter a Title and Prefix for your output worksheet and click *Ok*. If a sample code in the input worksheet does not match any values in column 1 of the lookup data worksheet than the code is not replaced. Output is to sheets [REP].



Replace Options

● Active Worksheet Only

○ Multiple Worksheets (Postions 1 to n)

No. of Worksheets

Before lookup:

☑ Strip Alpha Prefix from Sample Code

☑ Strip Alpha Suffix from Sample Code

☑ Extract Sample Code up to '-', '.' or '_'.

Lookup Data: D

Lookup Data = Sample to Find in Col 1, Sample to Replace in Col 2, Optional Pop in Col 3, Optional Extra Cols all in GenAlEx format! No auto save on completion for this option.

Advanced Options

#Extra Data Cols from Lookup: 2

Start Col for Outputting Extra Data: 16

Col to Sort On

Leave empty if not required!

□ Replace Pop Data

□ Update Pop Parameters

☑ Duplicate Worksheet Before Replace

Title: Random codominant data with XY coords

Worksheet Prefix

Exclude

If empty on:

● None

○ Sample

○ Pop

○ Col 3

Replace options: Before lookup

Strip Alpha Prefix from Sample Code: Strips all Alfa prefixes from the input sample codes before they are compared to the values in column 1 of the lookup data worksheet.

Strip Alpha Suffix from Sample Code: Strips all Alfa suffixes from the input sample codes before they are compared to the values in column 1 of the lookup data worksheet.

Extract Sample Code up to '-', '.' Or '_': Strips '-', '.', '_' and all subsequent characters from the input sample codes before they are compared to the values in column 1 of the lookup data worksheet.

Advanced Options

# Extra Data Cols to Extract: Enter the number of columns of extra data you wish to carry on to the output worksheets, if applicable. These columns must be columns 4 to n.

Tip: this option allows further data to be extracted from a worksheet containing the lookup data (e.g. XY coordinates).

Start Col for Outputting Extra Data: Enter the column on the output sheet where you wish the extra data to be inserted, if applicable.

Col to Sort on: Enter the column of the output worksheet that you wish to sort your dataset on, if applicable.

Replace Pop Data: Checking this option will replace any pre-existing population data with the values located in column 3 of the lookup worksheet.

Tip: this option allows idiosyncratic or missing population codes to be replaced at the same time as the sample codes.

Update Pop Parameters: Checking this option will update the population parameters in the output worksheet.

Duplicate Worksheet before replacement: This option creates a copy of the original worksheets before replacement. If this option is not selected replacement codes and extra data will be output to the original worksheets not to [REP].

Exclude

If a sample is blank for the selected field (sample, pop or col 3) it will be exclude from the output dataset. Excluded samples will be placed below the dataset, separated by a blank row.

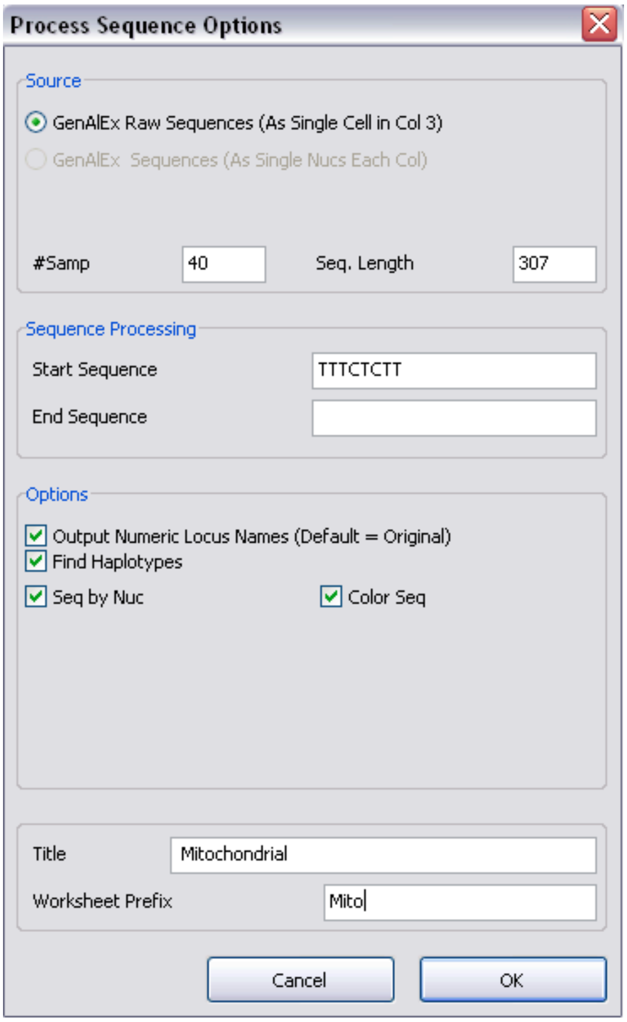
Process Seqs

This option will process sequence data contained in a single Excel worksheet in GenAlEx format, in order to detect haplotypes. These functions are also available when importing raw sequence data via the sub-menu ***Import Data -> Sequences***. For further assistance with this option refer to **Tutorial 5, Exercise 5.2**.

Sequence data may be upper or lower case, with gaps, and ambiguous base coding. As GenAlEx will only function with numerical data, alpha characters are converted to numeric in the following way: A=1, C=2, G=3, T=4, :=5, -=5, others=zero.

Procedure

1. Choose the option ***Raw Data*** from the **GenAlEx** menu, and then select ***Process Seqs*** from the sub-menu.
2. In the Process Sequences Options dialog box ensure the source of your data files, number of samples and sequence length are correct. Enter data for sequence processing, if required, and check required options. Enter a Title and Prefix for your output worksheet(s) and click *Ok*. See options below for the output sheet names.



Source

● GenAlEx Raw Sequences (As Single Cell in Col 3)

○ GenAlEx Sequences (As Single Nucs Each Col)

#Samp 40 Seq. Length 307

Sequence Processing

Start Sequence TTTCTCTT

End Sequence

Options

☑ Output Numeric Locus Names (Default = Original)

☑ Find Haplotypes

☑ Seq by Nuc ☑ Color Seq

Title Mitochondrial

Worksheet Prefix Mito

Process Sequence Options

Source

GenAlEx will interrogate your data to determine whether your sequence data is held in a single cell, or if each nucleotide is in a separate cell. GenAlEx will then automatically select the appropriate data source and enter the number of samples and sequence length.

Sequence Processing

Start Sequence: Sequences will be trimmed to start at the specified nucleotide sequence.

Tip: This is useful for obtaining aligned sequences in GenAlEx.

End Sequence: Sequence subsequent to the specified nucleotide sequence will be trimmed.

Options

Output Numeric Locus Names: Renames loci (base positions) numerically one to n. If this option is not selected the loci are labeled as indicated in the input worksheet.

Seq by Nuc [SQ]: Outputs each sequence with a single nucleotide per cell.

For uses of Excel 2003: If the full sequence consists of more than 254 nucleotides, this option is not completed due to the maximum of 256 columns in an Excel worksheet.

Find haplotypes: Check this option to process the data to find haplotypes, yielding various outputs to separate worksheets:

Polymorphic Sites [PS]: Outputs a subset of the data, including only polymorphic sites.

Tip: This is a quick way to output a table of variable sites for a sequence dataset.

Polymorphic Numerical [PN]: Outputs the Polymorphic Sites subset, converted to numerical codes as GenAlEx will only function using numerical data. (A=1, C=2, G=3, T=4, :=5, -=5, others=zero).

Haplotype [HA]: Lists the haplotype for each sample, as both alpha and numerical characters, and provides a code for each haplotype. A 'h' is added to end of the haplotype so that it is not treated as a number by Excel.

Haplotype Count [HC]: Provides a count of each haplotype together with their numerical codes.

Haplotype List [HL]: Provides a list of haplotypes together with the numerical coding of their polymorphic sites. Also provided is an example individual and the population that contains each haplotype.

Color Seq: Colors the sequences in sheets [SQ] and [PS] by nucleotide.

Output:

Sequence [SQ]: If the input consists of raw sequence data in a single cell, the sequences are processed so that each nucleotide is contained in a single cell. GenAlEx uses the length of the first sequence as a guide for processing subsequent sequences. As such, if a subsequent sequence is longer than the first one, the extra bases will not be processed.

For Excel 2003 users: If the full sequence consists of more than 254 nucleotides, this option is not completed due to the maximum of 256 columns in an Excel worksheet.

Sequence warnings [WS]: This sheet outputs all warnings associated with the sequence data. These warnings will include detection of alpha codes other than A,C,T,G, : & -, to facilitate further checking of ambiguous base calls.

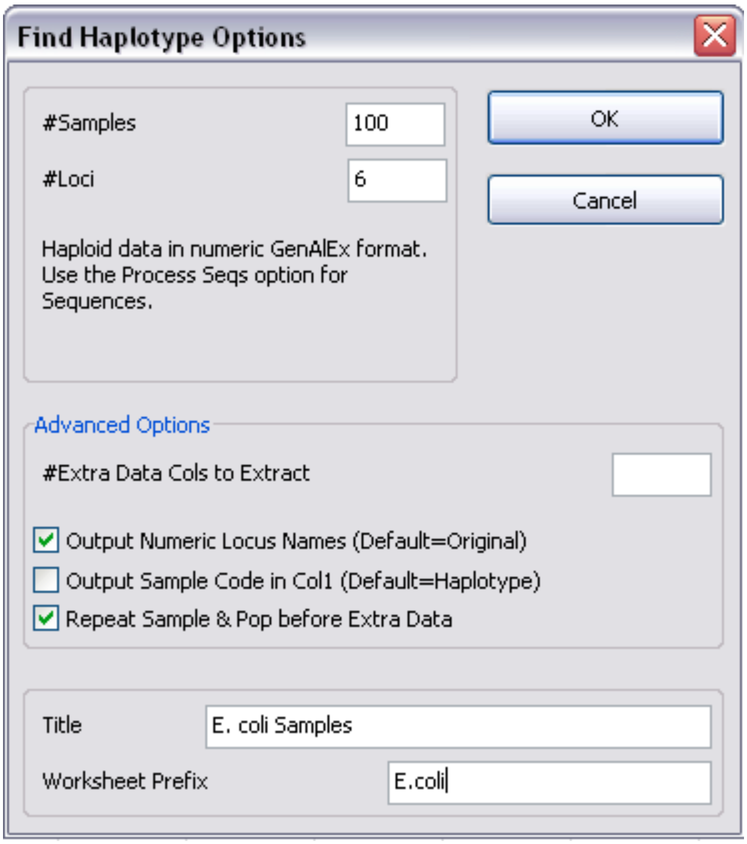
Find Haplotypes

This option applies to haploid data in numeric GenAlEx format. For sequence data similar functions are performed in ***Process Sequences*** under ***Raw Data***, or by the *Processing* options in the ***Import ->Sequences*** sub-menu.

Procedure

1. Activate the worksheet containing your haploid dataset in GenAlEx format (one nucleotide per cell and coded numerically). Choose the option ***Raw Data*** from the **GenAlEx** menu, and then select ***Find Haplotypes*** from the sub-menu.
2. Ensure the locus and sample parameters are correct in the Find Haplotypes Options dialog box. Select the desired advanced options (see below for details).

3. Enter Title and Worksheet Prefix then click *Ok.* See options below for the output sheet names.



Find Haplotype Options

#Samples 100

#Loci 6

Haploid data in numeric GenAlEx format. Use the Process Seqs option for Sequences.

Advanced Options

#Extra Data Cols to Extract

☑ Output Numeric Locus Names (Default=Original)

□ Output Sample Code in Col1 (Default=Haplotype)

☑ Repeat Sample & Pop before Extra Data

Title E. coli Samples

Worksheet Prefix E.coli

Advanced Options:

# Extra Data Cols to Extract: Enter the number of extra data columns (positioned after the genetic data separated by an empty column) to be extracted. Extracted column values for the last sample carrying a haplotype will be entered after the genetic data in worksheet [HL].

Output Numeric Locus Names: Renames loci (base positions) numerically one to n. If this option is not selected the loci are labeled as indicated in the input worksheet.

Output Sample Code in Col1: Enters the sample code of the last sample carrying a haplotype in the dataset in column 1 of worksheet [HL]. If this option is not selected the haplotype number is entered in column 1.

Repeat Sample &Pop before Extra Data: If this option is selected, then the sample and population codes of the last sample carrying a haplotype will be entered after the genrtic data and before any extra data in worksheet [HL].

Output:

Polymorphic [PN]: Outputs the Polymorphic Positions only.

Haplotype [HA]: Lists the haplotype for each sample, and provides a code for each haplotype. A 'h' is added to end of the haplotype so that it is not treated as a number by Excel.

Haplotype Count [HC]: Provides a count of each haplotype together with their numerical codes.

Haplotype List [HL]: Provides a list of haplotypes together with the polymorphic positions. Also provided is an example sample and the population that contains each haplotype.

Data to Raw Freq

The ***Data to Raw Freq*** option provides a convenient way to convert genotypic datasets into standard GenAlEx raw frequency format for input into appropriate analyses.

Procedure

1. Activate the worksheet containing your dataset in standard GenAlEx genotype format. Choose the option ***Raw Data*** from the **GenAlEx** menu, and then select the submenu option ***Data to Raw Freq***.
2. Ensure the locus and sample parameters are correct in the Data Parameters dialog box.
3. Enter Title and Worksheet Prefix then click *Ok*. Output is to worksheet [RAFP].

Edit Raw Data

The ***Edit Raw Data*** menu option provides a series of options for manipulating and editing your raw data and for preparing it for export via Excel functions to other programs. GenAlEx will prompt you for a data selection where appropriate.

Tip: Some of these may be useful outside the context of genetic analysis.

Empty > Zero: This option converts empty cells to zero.

? > Zero: This option converts all cells containing a ? to 0.

Empty > Number: This option converts empty cells to a user specified number.

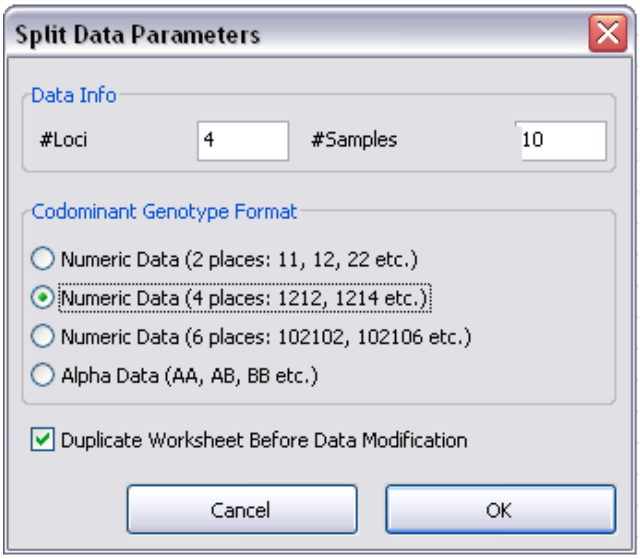
Empty > Text: This option converts empty cells to a user specified string (text).

Zero > Empty: This option empties all cells containing a zero.

Text > Num: This option converts values that appear as numbers, but are treated by Excel as text into true numeric format for GenAlEx.

Num > Bin: This option converts numeric data to binary format. All empty cells are converted to zero, and all other numeric values > 0 to 1. Zero values are not altered.

Split Codom: This option splits codominant alleles contained in a single column into two adjacent columns (one allele per column). Activate the desired spreadsheet. In the Split Data Paramters dialog box ensure the number of codominant loci and samples are correct. Indicate the genotype format. For example, select numeric data 4 places, if each column in the input worksheet contains 4 numeric characters to be split into two columns, each with 2 characters. If a cell contains less than the indicated number of places, ‘0’s will be added before the characters. Select ‘Duplicate worksheet before modification’ if desired.



Split Data Parameters

Data Info

#Loci 4 #Samples 10

Codominant Genotype Format

○ Numeric Data (2 places: 11, 12, 22 etc.)

● Numeric Data (4 places: 1212, 1214 etc.)

○ Numeric Data (6 places: 102102, 102106 etc.)

○ Alpha Data (AA, AB, BB etc.)

☑ Duplicate Worksheet Before Data Modification

Recode Codom: This option recodes the alleles at each locus as 1 to n, where n is the number of different alleles observed at that locus. The dataset with recoded alleles is output to worksheet [REC]. A list of the original allele codes and the corresponding new codes for each locus are output to worksheet [RECT].

Alpha Codom > Numeric [REC]: This option recodes alpha coded codominant data in GenAlEx format as numeric codominant data. a=1, b=2, c=3….z=26, all other characters =0.

Rev Comp: Outputs the reverse compliment of an alpha coded DNA sequence, contained in a single cell, to a specified cell in the worksheet.

Export Data

GenAlEx offers options to export formatted data to a series of other programs listed in the menu. For all export options a standard Export Parameters dialog box is provided, showing the data formats available for export, and any specific options relevant to the destination software. Brief notes and instructions are also provided for users familiar with the formatting options and requirements of the target program.

Depending on the export option chosen, GenAlEx provides output either directly to a text file, or to an Excel worksheet, which then needs to be manually saved as a tab-delimited text file. In both cases users may be required to make further modifications for analysis in the intended software.

Additional Features

Color Data

The ***Color Data*** menu option in GenAlEx offers options for coloring data sets in standard GenAlEx formats by various parameters. This menu was primarily designed for teaching. When used in conjunction with the ***Rand Data*** menu these options allows students to explore the principles of random permutation and bootstrapping tests used in analyses such as AMOVA, Mantel and Spatial Autocorrelation. For suggestions on how these menus can be utilized refer to **Tutorial 2**, **Exercise 2.5** and **Tutorial 3**, **Exercises 3.4** and **3.7**.

Tip: This menu may also be a useful tool for quickly locating information in large data sets.

by Pop [CbyP]: Outputs the selected data sheet colored by the population (indicated by the population parameters). A key is output below the data set. The input datasheet must be in standard GenAlEx format.

by Allele[CbyA]: Outputs the selected datasheet colored by allele number. Alleles from different loci with the same allele number will be colored the same. The input datasheet must be in standard GenAlEx format.

by Seq [CbyS]: Outputs the selected sequence datasheet colored by nucleotide base (G = yellow, C= blue, T= red and A= green). The input datasheet must contain alpha coded sequence data with each position in the sequence entered in a separate column, starting in column 3. Ensure Haploid is selected in the Data Parameters dialog box.

Tri by Pop [TriCbyP]: Outputs the selected tri matrix datasheet colored by the pairwise population comparison (e.g Pop 1 vs Pop2). The matrix is labeled by sample number and a key is output below the data set. The input datasheet must be in standard tri matrix GenAlEx format.

Sq by Pop [SqCbyP]: Outputs the selected square matrix datasheet colored by the pairwise population comparison (e.g Pop 1 vs Pop2). The matrix is labeled by sample number and a key is output below the data set. The input datasheet must be in standard square matrix GenAlEx format.

Rand Data

The ***Rand Data*** menu in GenAlEx generates permuted or bootstrapped data from input data sets in standard GenAlEx formats. When used in combination with the ***Color Data*** menu these options provide useful teaching tools (see ***Color Data*** above for more information).

Shuffle[Shuffle]: Outputs the selected data sheet with the samples shuffled within and between populations, i.e each sample is randomly assigned to a population. The original number of samples within each population is retained. The original sample and population labels are output in two columns after the genetic data to facilitate sample tracking.

Shuffle by Pop [ShuffleByPop]: Outputs the selected data sheet with the sample order shuffled within populations. The original sample and population labels are output in two columns after the genetic data to facilitate sample tracking.

Shuffle Tri [ShuffleTri]: Outputs the selected tri matrix data sheet with the samples shuffled within and between populations, resulting in the shuffling of the matrix elements. The matrix is labeled with the original sample codes to facilitate sample tracking.

Shuffle Sq [ShuffleSq]: Outputs the selected square matrix data sheet with the samples shuffled within and between populations, resulting in the shuffling of the matrix elements. The matrix is labeled with the original sample codes to facilitate sample tracking.

Color Shuffle [Shuffle]: Performs the same action as ***Shuffle*** when the input data set is first colored using one of the ***Color Data*** menu options. The original colors and key are retained in the output to facilitate sample tracking.

Color Shuffle by Pop [ShuffleByPop]: Performs the same action as ***Shuffle by Pop*** when the input data set is first colored using one of the ***Color Data*** menu options. The original colors and key are retained in the output to facilitate sample tracking.

Color Shuffle Tri by Pop [ShuffleTri]: Performs the same action as ***Shuffle Tri*** when the input data set is first colored using one of the ***Color Data*** menu options. The original colors and key are retained in the output to facilitate sample tracking.

Color Shuffle Sq by Pop [ShuffleSq]: Performs the same action as ***Shuffle sq*** when the input data set is first colored using one of the ***Color Data*** menu options. The original colors and key are retained in the output to facilitate sample tracking.

Bootstrap [BStrap]: Samples are randomly selected from the input dataset with replacement and assigned to a population. The output dataset contains the same number of samples per population as the original data set. The original sample and population labels are output in two columns after the genetic data to facilitate sample tracking.

Bootstrap by Pop [BStrapByPop]: For each population in the selected input dataset the samples are randomly selected with replacement and assigned to that same population in the output dataset (bootstrap within each population). The output dataset contains the same number of samples per population as the original dataset. The original sample and population labels are output in two columns after the genetic data to facilitate sample tracking.

Color Bootstrap [BStrap]: Performs the same action as ***Bootstrap*** when the input data set is first colored using one of the ***Color Data*** menu options. The original colors and key are retained in the output to facilitate sample tracking.

Color Bootstrap by Pop [BStrapByPop]: Performs the same action as ***Bootstrap by Pop*** when the input data set is first colored using one of the ***Color Data*** menu options. The original colors and key are retained in the output to facilitate sample tracking.

Graph

The ***Graph*** menu option in GenAlEx offers options to create and manipulate a labeled graph of geographic coordinate data.

XY: Outputs a labeled graph of geographic positions from XY coordinate data located in columns 3 and 4. The input datasheet must be in standard GenAlEx format, with sample labels in column 1. Optional axis labels should be in cells C3 and D3.

XY from Range: Outputs a graph of geographic positions from XY coordinate data located in adjacent columns. The first row of the selection should contain axis labels.

Re-Label XY: Re-labels an existing labeled graph with labels specified by the range selected.

Lat/Long: Outputs a labeled graph of geographic positions from Lat/Long coordinate data located in columns 3 and 4, with longitude shown as the X-axis. The input datasheet must be in standard GenAlEx format, with sample labels in column 1.

Remove Error Caps: This option removes the error caps from error bars.

Stats

GenAlEx offers a series of tools for statistics and data transformation which users may find useful both for manipulating GenAlEx datasets and for wider use in any Excel worksheet.

Sum: Outputs the sum of all values in a specified range to a selected worksheet location.

Mean: Outputs to a selected worksheet location the number of values, sum, mean, variance, standard deviation, standard error, minimum and maximum for a specified range.

Means of Cols: Calculates for each column in a specified block the number of values, sum, mean, median, standard deviation, standard error, minimum and maximum. Column labels are indicated in the first row of the selection. Output is to a selected worksheet location.

Means of Rows: Calculates for each row in a specified block the number of values, sum, mean, median, standard deviation, standard error, minimum and maximum. Column labels are indicated in the first column of the selection. Output is to a selected worksheet location.

Freq Dist: Creates a frequency distribution with user specified bin sizes from a selected range consisting of only positive values in a single column. The minimum and maximum values for the frequency distribution can also be specified. Frequency distribution and summary statistics are output to worksheet [FD].

Freq Dists of Cols: Creates a separate frequency distribution, with user specified bin sizes, of each column in a selected block consisting of only positive values. The minimum and maximum values for the frequency distribution can also be specified. Column labels are indicated in the first row of the selection and are used to identify the output distributions. Frequency distributions along with summary statistics are output to worksheet [MFD].

Freq Dists of Rows: Creates a separate frequency distribution, with user specified bin sizes, of each row in a selected block consisting of only positive values. The minimum and maximum values for the frequency distribution can also be specified. Row labels are indicated in the first column of the selection and are used to identify the output distributions. Frequency distributions along with summary statistics are output to worksheet [MFD].

Freq Dist (-1 to +1): Creates a frequency distribution, from a selected block of data in one column containing values in the range of -1 to +1. The Frequency distribution and summary statistics are output to worksheet [SFD].

Freq Dists Paired (-1 to +1): Creates two overlaid frequency distributions, from the first two adjacent columns in a selected block of data (one distribution for each column). Data values must be in the range of -1 to +1. The Frequency distributions and summary statistics are output to worksheet [PFD].

Regression: Calculates the linear regression equation (slope and intercept) and the R square value for user selected x and y variables.

U-test by Col: Calculates the U-test between two groups when data values are located in a single column with group labels in a second corresponding column. Output includes the U values, the two tailed probability and both the lower and upper tailed probabilities. Statistics are output to a user specified worksheet location.

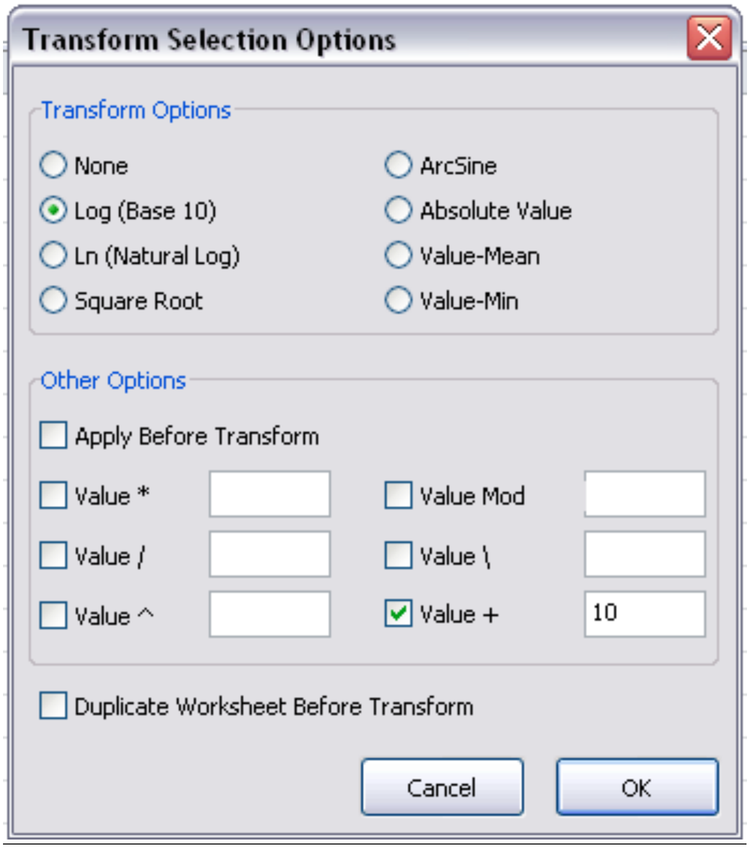
U-test as 2 Cols: Calculates the U-test between two groups when the data for each group is located in a different column. Data columns must be adjacent. Column labels are indicated in the first row of the selection. Output includes the U values, the two tailed probability and both the lower and upper tailed probabilities. Statistics are output to a user specified worksheet location.

G-test 1xC: Calculates the G-test 'goodness of fit' of a set of observed values entered in a single row to a user specified ratio (entered in a single corresponding row). Output includes the G-test statistic, degrees of freedom and probability both with and without the William's correction. Statistics are output to a user specified worksheet location.

G-test RxC: Calculates the G-test 'goodness of fit' of a set of observed values entered in a contingency table to that expected from the row and column totals. Output includes the G-test statistic, degrees of freedom and probability. Statistics are output to a user specified worksheet location.

Transform: Transforms a user specified block of data (see dialog box below for available transformations). The original un-transformed data can also be retained by selecting *Duplicate Worksheet Before Transformation* in the Transform Selection Options dialog box.

Tip: This option is particularly useful for transforming triangular matrices as blank cells are unaffected by the transformation.



Transform Selection Options

Transform Options

○ None ○ ArcSine
● Log (Base 10) ○ Absolute Value
○ Ln (Natural Log) ○ Value-Mean
○ Square Root ○ Value-Min

Other Options

□ Apply Before Transform

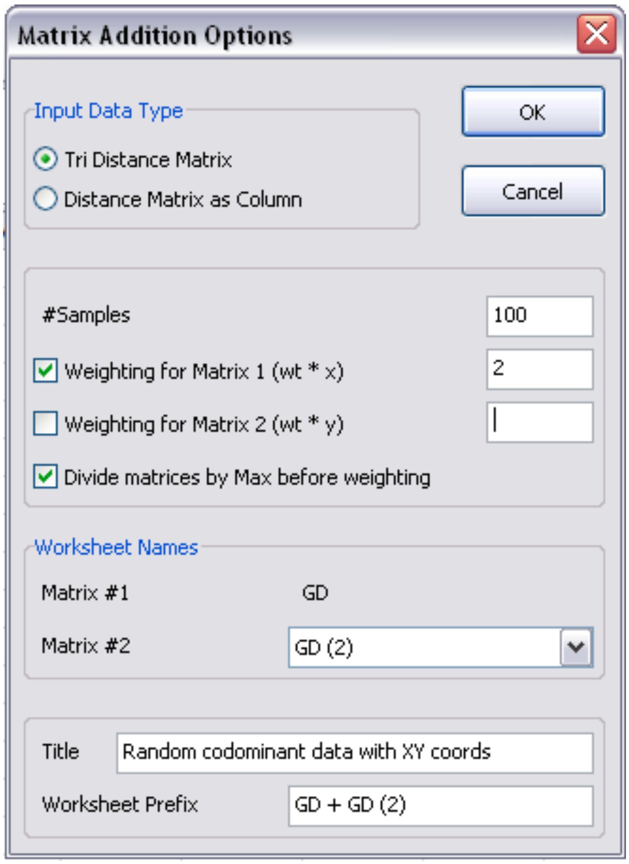
□ Value * □ Value Mod
□ Value / □ Value \
□ Value ^ ☑ Value + 10

□ Duplicate Worksheet Before Transform

Diagonal: Transforms the diagonal values of a matrix in either GenAlEx format or in a specified range. Diagonal values can be cleared, converted to 1 or 0. The original un-transformed data can also be retained by selecting *Duplicate Worksheet Before Transformation* in the Transform Diagonal Options dialog box.

Tip: This option is particularly useful for returning the diagonals of a genetic or geographic distance matrix to 0 after transforming that matrix using the ***Transform*** *option.*

Matrix Addition: Takes two matrices from separate worksheets in standard GenAlEx format (either tri-matrices or matrices as columns) and adds them together i.e the value in cell 1:1 of matrix 1 is added to the value in cell 1:1 of matrix 2 and so on until cell i:j. If desired each matrix may be weighted before addition. The matrices may also be divided by the matrix maximum before weighting. The resulting matrix is output to sheet [MX]. The original matrices are retained.



Matrix Addition Options

Input Data Type
● Tri Distance Matrix
○ Distance Matrix as Column

#Samples 100
☑ Weighting for Matrix 1 (wt * x) 2
□ Weighting for Matrix 2 (wt * y)
☑ Divide matrices by Max before weighting

Worksheet Names
Matrix #1 GD
Matrix #2 GD (2)

Title Random codominant data with XY coords
Worksheet Prefix GD + GD (2)