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# AgeStructure (Version 1.1)

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## User's Manual

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## I. INTRODUCTION

AgeStructure is a Fortran program that implements the likelihood method (EPA) developed by Wang *et al.* (2009) to estimate the effective size ( $N_e$ ) and generation interval ( $L$ ) of a population with overlapping generations.

The method assumes that a sample of individuals is taken at random with respect to kinship from a population of overlapping generations. Each sampled individual is sexed, aged and genotyped at a number of marker loci. The sex, age and multi-locus genotypes of the sampled individuals is then used as information in a parentage assignment analysis, and the parentage assignments are used by EPA to obtain estimates of  $N_e$ ,  $L$  as well other parameters such as variance and covariance of lifetime family size, effective number of breeders of each age class. The confidence intervals of each parameter estimate are obtained by parametric bootstrapping.

In the current version, I assume a dioecious diploid population and use codominant (e.g. microsatellites or SNPs) or dominant (e.g. AFLPs), or a combination of both in parentage assignment analysis and in parameter ( $N_e$ ,  $L$ ) estimation.

For more information about the method EPA, please read the paper below.

Wang, J., Brekke, P., Huchard, E., Knapp, L. A., Cowlshaw, G. 2010. Estimation of parameters of inbreeding and genetic drift in populations with overlapping generations. *Evolution* 64: 1704-1718.

## II. INSTALLATION

The downloaded package for Windows includes 2 executable files (AgeStructure.exe, POAssignment.exe), 2 Fortran 90 source code files (AgeStructure.f90, POAssignment.f90), a brief user's guide (readme.pdf), an example dataset file (AgeStructure.dat), and an output file for the analysis of the example dataset (SimuData.Ne). The package for Linux is the same, except the 2 executable files are replaced by AgeStructure and POAssignment.

To run the program on a PC, simply unzip the downloaded zipped file in a folder on your computer's hard disc. To analyze the example dataset, just double click the executable file "AgeStructure.exe". Preferably, click "Start → Programs → Accessories → Command Prompt" to enter into a DOS window, and then use DOS command "cd " to navigate into the folder where all files of the AgeStructure program are found. Then type AgeStructure followed by the key "Enter" or "Return" will start the program.

To run the program on other platforms (Linux, Mac, ...), one needs first compile the 2 source code files using a Fortran 90/95 compiler. Alternatively for Linux, download the package for Linux and run the executable AgeStructure.

To analyse one's own data, please follow the following steps for preparing the input file.

### III. DATA INPUT FILE

A pure text file with name “AgeStructure.dat” needs to be created in the working directory where the Fortran program is found. Input the pre-processed data into the file in the following sequence and format. Items within a line (row) are separated by a comma or blank space. Therefore, blanks and commas are not allowed between digits within a number.

- (1) Dataset name (String). A string of alphanumeric characters to specify the name of the dataset. The total length is limited to 100 characters/digits. When non-alphanumeric characters (e.g. “,”, “/”) are used in the dataset name, the name must be put in double quotation marks.
- (2) Output file name (String). A string of alphanumeric characters to specify the name of the output file into which all analysis results will be directed. The total length is limited to 100 characters/digits. When non-alphanumeric characters (e.g. “,”, “/”) are used in the dataset name, the name must be put in double quotation marks.
- (3) Number of individuals (Integer). An integer specifying the number of individuals included in the sample being analysed.
- (4) Maximal age for males & females (Integer, Integer). Two integers specifying the maximum age in the time unit chosen (see below) for males and females.
- (5) Minimal age of reproduction for males & females (Integer, Integer). Two integers specifying the minimum age in the time unit chosen for males and females at which they are reproductive.
- (6) Family size distribution indicator (Binary, 0 or 1). An indicator binary variable that specifies whether (=1) or not (=0) individuals within the same age class have the same expected fertility (thus leading to Poisson distribution of family sizes).
- (7) Sampling proportion indicator (Binary, 0 or 1). An indicator binary variable that specifies whether (=1) or not (=0) the sampling proportion of each age class is known.
- (8) Male sampling proportions (Decimal numbers, optional). This line of input is required only when the sampling proportion indicator is set to 1 above. List on a single row (line) the known sampling proportions for male age classes 1, 2, 3, ... The sampling proportion of newborns (age class 0) should be omitted.
- (9) Female sampling proportions (Decimal number, optional). This line of input is required only when the sampling proportion indicator is set to 1 above. List on a single row (line) the known sampling proportions for female age classes 1, 2, 3, ... The sampling proportion of newborns (age class 0) should be omitted.
- (10) Number of bootstrapping samples (Integer). An integer ( $\geq 0$ ) specifying the number of bootstrapping samples that are used to estimate the confidence intervals of the parameter estimates. A value of 0 means no bootstrapping will be conducted and the estimator will just return a point estimate.
- (11) Number of loci (Integer).
- (12) Confidence level for parentage assignment (Integer). An integer specifying the confidence level that is to be applied in parentage assignment analysis. The accepted

values are one of {80, 85, 90, 95}, meaning to assignment parentage at the 80%, 85%, 90% and 95% confidence level respectively.

- (13) Names of loci (String). List the names (IDs) of locus 1, 2, 3, ... on a single line (row). The name (ID) of each marker locus is a string less than 21 letters.
- (14) Types of loci (Binary, 0 or 1). List the marker types of locus 1, 2, 3, ... on a single line (row). Use 0 and 1 to indicate a codominant and dominant marker locus, respectively.
- (15) Allelic dropout rate (Decimal). List the allelic dropout rates of locus 1, 2, 3, ... on a single line (row). The parentage assignment method can taken genotyping errors into account. Two kinds of genotyping errors are distinguished and separately accounted for, as described in Wang (2004) and Wang & Santure (2009).
- (16) Other error rate (Decimal). List the other error rates of locus 1, 2, 3, ... on a single line (row).
- (17) Individual ID, sex, age and genotypes (String, Integers).  
On each line, list the individual ID (name), sex, age class (=0~), the genotype at locus 1, genotype at locus 2, ... Individual ID is a string, less than 21 alphanumeric characters in length. Individual sex is indicated by an integer with values of either 1 or 2 for males or females, respectively. Individual's age class is indicated by an integer with a value between 0 and  $m$ , where  $m$  is the maximal age in the time unit chosen. For a codominant locus, the genotype is represented by 2 integers, representing the 2 alleles. For a dominant locus, the genotype is either 1 or 2, representing the dominant (band present) or recessive (band absent) phenotype respectively. A missing genotype is represented by "0 0" and "0" for a codominant and dominant locus, respectively. The sex or age of any individual is not allowed to be missing.
- (18) Candidate parent sampling proportion (Decimal, Decimal). Two decimal numbers listed on a single line specifying the proportions of candidate male and female parents that are sampled and included in the sample for a newborn individual.
- (19) Number of known paternity (Integer). An integer specifying the number of individuals in the sample that have known fathers who are also included in the sample.
- (20) List of known offspring-father dyads (String, optional). If the number of known paternity is greater than 0, then list on each line (row) the offspring ID and father ID of a known offspring-father dyad.

For example, there are 2 known offspring-father dyads in the sample. Then (19) and (20) are listed as something like

```
2
O1 M1
O2 M2
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- (21) Number of known maternity (Integer). An integer specifying the number of individuals in the sample that have known mothers who are also included in the sample.
- (22) List of offspring-mother dyads (String, optional). If the number of known maternity is greater than 0, then list on each line (row) the offspring ID and mother ID of a known offspring-mother dyad.
- (23) Number of excluded paternity (Integer). An integer specifying the number of individuals who have 1 or more excluded candidate fathers. Note that males in age class  $i$  is automatically excluded as candidate fathers for individuals in age class  $j$  if  $j-i$  is smaller than the minimal reproductive age (in the same time unit as age class) for males.

So for an individual in age class  $i$ , only excluded candidate fathers in age class  $\geq i +$  minimal reproductive age need to be counted herein.

List of excluded paternity (String). For each individual that is known to have 1 or more excluded candidate fathers, list on a single line the individual name (ID), followed by the number of excluded candidate fathers, and then the names (IDs) of each excluded candidate father. An example line is: O1 2 M1 M2

- (24) Number of excluded maternity (Integer). An integer specifying the number of individuals who have 1 or more excluded candidate mothers. Note that females in age class  $i$  is automatically excluded as candidate mothers for individuals in age class  $j$  if  $j-i$  is smaller than the minimal reproductive age (in the same time unit as age class) for females. So for an individual in age class  $i$ , only excluded candidate mothers in age class  $\geq i +$  minimal reproductive age need to be counted.
- (25) List of excluded maternity (String). For each individual that is known to have 1 or more excluded candidate mothers, list on a single line the individual name (ID), followed by the number of excluded candidate mothers, and then the names (IDs) of each excluded candidate mother.

## IV OUTPUT

When AgeStructure has finished running on the analysis of your dataset, the results will be found in a file with name specified in the 2<sup>nd</sup> line of your input file, and an extension name “.Ne”. The results include the following sections.

### 1. Parameters

This section gives the parameter values specified by you in your input file. The parameters include maximal age for males and females, minimal reproductive age for males and females, assumed family size distribution (whether individuals within an age class have the same expected fertility or not) for males and females, number of bootstrapping samples, number of loci, confidence level used to make parentage assignments.

### 2. Summary statistics

This section provides some summary statistics of the sample, such as number of individuals sampled in each sex-age class.

### 3. Estimates

This section lists the estimates for  $N_e$ , generation intervals ( $L$ ), variance and covariance of lifetime family size, effective numbers of breeders of each age class, ect.

Note that 2 other output files (with names \*.paternity and \*.maternity, where \* represents the output file name specified in the 2<sup>nd</sup> line of your data input file) are also available in the same folder as the input file. The 2 files list the paternity and maternity assignments made by the program using multi-locus genotypes. In both files, the 3<sup>rd</sup> column gives the confidence level at which the assignments were awarded to an offspring (1<sup>st</sup> column) and parent (2<sup>nd</sup> column) dyad.

## V. AN EXAMPLE DATASET

A simulated dataset is shown below. The dataset is also enclosed in the software package, in the input file “AgeStructure.dat”.

The data were simulated with parameters  $n_1=3$ ,  $n_2=4$ ,  $\{p_{r1,1}, p_{r1,2}, p_{r1,3}\} = \{0.1, 0.2, 0.2\}$ ,  $\{p_{r2,1}, p_{r2,2}, p_{r2,3}, p_{r2,4}\} = \{0.1, 0.2, 0.1, 0.1\}$ ,  $\{N_{0,1}, N_{1,1}, N_{2,1}, N_{3,1}\} = \{200, 100, 80, 60\}$ ,  $\{N_{0,2}, N_{1,2}, N_{2,2}, N_{3,2}, N_{4,2}\} = \{200, 100, 80, 60, 40\}$ , where  $r = 1, 2$ . Individuals within an age class are assumed to have the same expected fertility. The sampling proportion of each of the 0~3 age classes of males and of 0~4 age classes of females is 16%, and is assumed unknown in the analysis. Within each age class, individuals are sampled at random, and the sex and age of each sampled individual are assumed known. Each sampled individual is genotyped at 10 loci, each locus having 10 equi-frequency alleles initially in the simulation. Parentage assignments are made using the multi-locus genotypes, assuming a sampling proportion of 0.5 for both candidate fathers and candidate mothers of newborns.

From the parameter values used in simulations, we obtain the parameter values  $L=2.3$  years,  $N_e=370$ ,  $\sigma_{11}^2 = \sigma_{12}^2 = 1.23$ ,  $\sigma_{11,12} = 0.23$ ,  $\sigma_{21}^2 = \sigma_{22}^2 = 1.26$ ,  $\sigma_{21,22} = 0.26$ .

The input file reads as follows. Anything after the exclamation mark “!” on a line acts as a note and will not be read by the program.

```

SimuData          ! C, Dataset name, Length<=100
SimuData          ! C, Main output file name, Length<=100
    169           ! I, Number of offspring in the sample
    3    4       ! Max age for males & females
    1    1       ! Minimum age for reproduction
           1     !=1/0 for Poisson/Other dist
           0     !=1/0 =sampling proportion known for each age class
    500          !=Number of CI95 samples
    10           ! I, Number of loci
95              ! Confidence level for parentage assignment
L1 L2 L3 L4 L5 L6 L7 L8 L9 L10      ! Marker IDs
0 0 0 0 0 0 0 0 0 0               ! marker type : 0/1=codominant/dominant
0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 !Allelic dropout rate
0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 !Other error rate
M001P2P3G4G5G6G7,1,0,2,10,10,6,9,1,2,10,1,9,1,7,1,10,2,8,6,8,10,2
M008P9P10G11G12G13G14,1,0,10,1,2,10,9,1,2,9,4,7,2,8,6,5,5,1,6,1,7,7
M0015P16P17G18G19G20G21,1,0,4,6,6,9,6,5,6,5,2,2,7,3,3,5,7,9,9,9,2,5
M0022P23P24G25G26G27G28,1,0,10,2,6,7,8,2,9,3,3,3,4,1,10,2,9,4,9,9,7,9
M0029P4P30G31G32G33G34,1,0,2,8,10,1,6,5,9,2,8,5,1,3,2,4,2,3,9,2,10,9
M0035P36P37G38G39G40G41,1,0,9,1,4,5,4,7,2,6,4,9,6,4,1,7,7,5,3,4,7,10
M0042P43P44G45G46G47G48,1,0,3,2,2,10,6,5,4,7,5,5,7,7,6,9,1,1,6,8,9,5
M0049P50P51G52G53G54G55,1,0,4,4,2,8,4,4,4,4,9,10,5,8,10,3,1,6,8,6,1,2
M0056P57P58G59G60G61G62,1,0,1,2,1,9,5,6,8,7,8,2,5,5,8,7,10,9,2,5,7,4
M0063P64P65G66G67G68G69,1,0,2,8,6,2,3,3,10,10,4,3,1,1,2,10,7,7,10,5,10,8
M0070P6P71G72G53G73G74,1,0,4,8,6,1,6,6,4,5,10,5,7,4,7,7,8,1,8,9,10,2
M0075P76P77G78G79G80G81,1,0,6,1,4,3,4,5,3,6,6,5,1,8,3,3,5,5,8,4,4,6
M0082P83P84G59G85G86G87,1,0,2,6,5,5,9,2,8,8,8,9,5,2,2,2,9,6,5,7,10,8
M0088P89P90G91G92G93G94,1,0,10,9,8,7,1,4,9,4,10,5,4,6,5,3,5,7,6,8,9,4
M0095P96P97G98G99G100G101,1,0,6,9,5,4,7,3,7,1,7,3,4,7,6,6,4,1,2,9,10,4
M00102P103P104G105G106G107G108,1,0,8,6,8,10,7,3,8,5,7,8,6,5,9,1,7,5,8,10,1,6

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M00109P110P111G57G108G112G113,1,0,6,8,10,5,2,10,6,9,1,1,8,8,8,9,7,8,2,4,2,3  
M00114P115P116G117G118G119G120,1,0,8,8,9,1,9,10,1,1,3,5,1,5,5,4,6,7,4,1,7,7  
M00121P122P123G124G125G126G46,1,0,10,2,6,8,5,6,8,2,4,1,9,7,6,4,2,7,1,5,3,7  
M00127P128P129G130G131G132G133,1,0,1,6,3,9,2,2,6,10,5,9,3,7,10,6,4,10,10,4,9,1  
M00134P135P136G137G39G59G138,1,0,9,4,1,2,2,5,2,10,2,1,9,7,6,8,1,5,6,7,4,3  
M00139P140P141G142G143G144G145,1,0,3,6,4,6,4,7,7,6,3,4,1,9,5,6,3,6,4,6,2,3  
M00146P2P147G4G5G148G149,1,0,2,5,8,3,9,6,9,7,1,2,7,9,2,6,2,4,9,7,10,7  
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M00153P23P154G25G26G40G155,1,0,9,10,10,3,6,10,9,10,3,3,4,4,10,1,4,3,9,9,7,8  
M00156P157P158G142G141G159G160,1,0,2,8,5,3,8,10,6,9,3,4,6,5,6,7,6,6,4,9,3,9  
M00161P162P163G164G165G166G152,1,0,9,2,4,8,4,8,4,8,6,1,8,9,6,7,9,10,5,3,10,9  
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M00173P174P175G176G177G178G179,1,0,7,10,3,6,4,2,10,6,9,2,9,3,1,5,7,1,5,3,8,9  
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M00183P184P185G186G187G188G189,1,0,4,8,3,4,7,9,2,1,3,7,8,4,1,2,2,1,9,6,6,8  
M00190P191P192G193G194G195G143,1,0,6,3,4,10,2,4,7,7,5,10,2,7,6,2,4,5,7,2,6,2  
M00196P197P198G172G199G200G201,1,0,9,2,6,1,2,9,10,8,3,8,7,7,10,8,2,2,8,9,9,10  
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M00217P218P219G220G221G222G223,1,0,8,6,9,6,9,8,6,8,3,8,3,2,7,3,1,5,7,4,5,5  
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M10231P232P233G234G235G236G237,1,1,10,8,3,8,10,6,3,8,1,5,6,4,7,6,9,2,5,4,10,10  
M10238P239P240G241G242G243G244,1,1,10,5,6,3,4,10,2,1,8,7,4,8,4,1,1,3,7,8,1,9  
M10245P246P247G248G249G250G251,1,1,1,4,8,1,4,2,1,9,7,2,8,7,3,4,7,4,4,5,7,5  
M10252P253P254G68G255G256G143,1,1,4,8,4,1,3,2,9,5,2,8,4,7,8,8,6,6,5,6,9,3  
M10257P151P258G144G152G259G260,1,1,3,1,8,5,8,5,6,2,6,4,9,5,1,3,6,10,3,10,2,2  
M10261P23P262G25G26G263G264,1,1,9,1,6,1,8,3,9,8,5,6,7,2,10,2,9,7,3,9,7,4  
M10265P266P267G268G269G270G271,1,1,2,1,1,6,1,7,9,7,4,4,2,10,10,10,2,3,7,8,6,5  
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M10276P277P278G279G280G281G282,1,1,5,8,4,7,3,4,1,2,5,10,5,5,7,4,2,1,5,8,8,5  
M10283P23P284G25G26G285G92,1,1,9,8,6,8,6,3,10,5,5,7,7,4,7,1,4,7,3,10,2,5  
M10209P40P53G286G237G287G288,1,1,5,4,3,6,4,3,10,8,1,1,4,5,6,7,5,8,4,8,8,1  
M109P11P12G289G290G291G292,1,1,3,10,2,10,4,9,2,9,4,8,2,9,5,6,6,5,6,9,7,4  
M10293P294P295G296G297G298G299,1,1,10,5,9,9,6,7,3,4,4,3,10,3,10,4,6,3,8,2,7,9  
M20300P301P302G303G304G305G306,1,2,9,2,10,6,6,6,10,3,7,6,7,2,6,10,7,10,5,2,1,6  
M20307P308P233G309G310G236G237,1,2,10,5,3,8,3,6,8,8,5,8,6,4,1,6,10,5,4,4,8,10  
M20311P72P136G207G312G59G138,1,2,10,2,9,3,6,8,8,10,10,9,10,8,1,8,8,5,4,7,9,6  
M20313P314P315G316G317G318G288,1,2,9,2,10,6,2,1,6,8,7,6,8,10,6,5,2,1,4,8,10,1  
M20319P320P321G256G322G323G324,1,2,2,4,7,9,4,6,8,8,2,7,10,8,3,6,6,3,8,8,2,10  
M20325P326P19G327G328G309G329,1,2,5,4,3,7,5,6,5,7,9,9,5,7,6,3,5,10,9,5,8,2  
M20330P289P331G332G333G334G255,1,2,6,4,2,9,6,3,9,4,4,2,9,9,2,2,5,7,6,1,4,9  
M2089P91P92G335G315G336G337,1,2,1,10,9,8,1,3,8,9,10,3,10,4,5,1,5,7,6,10,9,1  
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M20341P342P304G343G94G344G345,1,2,4,1,4,3,1,6,7,8,1,9,5,7,8,3,4,10,9,3,10,7  
M20346P347P348G98G165G349G194,1,2,6,8,4,9,7,4,7,8,4,3,4,1,8,7,10,10,8,7,1,9  
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M20374P375P376G377G378G379G380,1,2,2,2,5,2,2,5,3,7,8,9,1,8,7,1,4,10,7,10,8,4  
M20381P382P331G383G92G334G255,1,2,1,2,1,6,6,5,3,4,3,1,3,9,9,2,10,6,3,9,9,9  
M20110P57P108G59G60G384G385,1,2,9,6,1,10,5,2,8,6,1,3,8,9,8,1,5,7,5,2,2,7  
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0.5000 0.5000 !sampling proportion for males & females
0 !number of known paternity
0 !number of known maternity
0 !number of excluded paternity
0 !number of excluded maternity

```

The output from the analysis of the above example dataset can be found in the file “SimuData.Ne”, and is listed below.

```

*****
AgeStructure Analysis Results for Dataset SimuData
*****
Time Started: D=17/08/2009 T=27/25/14
Time Finished: D=17/08/2009 T=29/52/14

```

# 1. Parameters

Parameter	Males	Females
Maximal Age	3	4
Minimal Parental Age	1	1
Assumed Family Size Distr.	Poisson	Poisson
Number bootstrapping samples	500	
Number of Loci	10	
Parentage confidence level	95	

# 2. Summary statistics

Number of individuals sampled in each sex-age class

AgeClass	Males	Females
0	37	42
1	14	19
2	20	14
3	8	12
4		3

Number of males & females of age class j (column 1) whose paternity is assigned to age class i (row 1)

AgeClass	1	2	3
0	2	7	2
1	0	1	1
2	0	0	0
3	0	0	0

Number of males & females of age class j (column 1) whose maternity is assigned to age class i (row 1)

AgeClass	1	2	3	4
0	1	8	4	2
1	0	2	3	0
2	0	0	1	0
3	0	0	0	0

Number of male parents of age class j (column 1) who each have i offspring assignments (row 1)

AgeClass	0	1	2	3
1	12	2	0	0
2	15	3	1	1
3	5	3	0	0

Number of female parents of age class j (column 1) who each have i offspring assignments (row 1)

AgeClass	0	1	2
1	18	1	0
2	7	4	3
3	6	4	2
4	1	2	0

### 3. Estimates

Estimates of generation effective size (Ne) & generation intervals (GI)

	Ne	GI Paternal-GI Maternal-GI		
Estimate	359	2.30	1.86	2.74
CI95L	70	1.78	1.49	1.86
CI95U	544	2.60	2.45	3.12

Estimates of variances and covariances of lifetime family size

	Var(mm)	Var(mf)	Cov(mm,mf)	Var(fm)	Var(ff)	Cov(fm,ff)
Estimate	1.52	0.31	0.14	5.52	1.13	0.49
CI95L	1.00	0.14	0.00	0.95	1.00	0.00
CI95U	2.45	1.75	0.45	49.62	7.29	14.98

Estimates of parameter P

AgeClass	Males			Females		
	Est	CI95L	CI95U	Est	CI95L	CI95U
1	0.1625	0.0266	0.3023	0.0496	0.0127	0.1855
2	0.2439	0.1081	0.4123	0.1915	0.0962	0.3607
3	0.0936	0.0000	0.2717	0.1000	0.0000	0.2317

Estimates of parameter N

AgeClass	Males			Females		
	Est	CI95L	CI95U	Est	CI95L	CI95U
1	223.0	78.2	465.5	60.4	40.7	201.6
2	111.8	64.2	228.5	55.7	37.5	113.2
3	57.7	18.2	169.7	52.6	31.6	103.9
4				37.0	1.0	89.8

Estimates of parameter S

AgeClass	Males			Females		
	Est	CI95L	CI95U	Est	CI95L	CI95U
1	0.0763	0.0335	0.2214	0.3235	0.0929	0.4801
2	0.2023	0.0873	0.3432	0.2600	0.1013	0.4282
3	0.1732	0.0328	0.4363	0.2366	0.0982	0.4312
4				0.1636	0.0000	0.5771

Estimates of parameter effective size of each age class

AgeClass	Males			Females		
	Est	CI95L	CI95U	Est	CI95L	CI95U
1	223.0	78.2	465.5	60.4	40.7	201.6
2	111.8	64.2	228.5	55.7	37.5	113.2
3	57.7	18.2	169.7	52.6	31.6	103.9
4				37.0	1.0	89.8

## VI. SOME NOTES

### 1. Parameter denotations

$\text{Var}(xy)$ : The variance of the number of offspring of sex  $y$  per parent of sex  $x$ , with  $x, y = m$  for males and  $f$  for females.

$\text{Cov}(xm, xf)$ : The covariance between the numbers of sons and daughters per parent of sex  $x$ , with  $x = m$  for male and  $f$  for female parents.

$P$ : Age specific reproductive contribution of males and females.

$N$ : Number of individuals in each age class of each sex.

$S$ : Sampling proportion of individuals in each age class of each sex.

### 2. Age class

In principle, age classes can be defined in any time unit, such as 2 months, half a year, 1 year, 2 years. However, for accurate estimation results, time unit should be defined by considering the length of a breeding season, the maximal age of males and females, and the sample size of each age.

For a given sample, a too small time unit will lead to numerous age classes and a substantial number of the age classes are empty with no individuals. This leads not only an increase in computational time, but also a decrease in accuracy of the parameter estimates. On the other hand, a too large time unit will lead to too few age classes, and in each age class there are a large number of individuals of various ages. This could also result in inaccurate estimates of  $L$  and  $N_e$ . In practice, the time unit should be the length of at least one breeding season, and usually needs to be the length of multiple breeding seasons to reduce the number of empty age classes when sample size is small relative to population size.

### **3. Confidence level**

The method used in Cervus (Marshall *et al.* 1998) is adopted in AgeStructure to assign parentage at a certain confidence level. The full likelihood method that is used to assign parentage and sibship jointly can be more accurate, but is too time consuming and is not suitable for parametric bootstrapping.

The suggested confidence level for parentage assignment is 95%. However, when the number of marker loci is small, sometimes this high level of confidence precludes any parentage assignments. Setting a lower confidence level (say, 80%) allows more assignments to be made.

### **4. Known parentage or excluded parentage**

If some individuals have known parentage (e.g. from behavioural observation) and the known parents are also included in the sample, the known offspring-parent relationship could be included in the input file for better estimate of  $N_e$  and other parameters. Similarly, information on some individuals being known to be excludable as parents of an individual can be included in the input file for more accurate estimation of the parameters.

### **Literature**

- Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998 Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**, 639–655.
- Wang, J. L. 2004 Sibship reconstruction from genetic data with typing errors. *Genetics* **166**, 1963–1979.
- Wang, J. L. & Santure, A. W. 2009 Parentage and sibship inference from multi-locus genotype data under polygamy. *Genetics* **181**, 1579–1594.