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# BOTTLENECK: A Computer Program for Detecting Recent Reductions in the Effective Population Size Using Allele Frequency Data

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BOTTLENECK (current version 1.2) is a population genetics computer program that conducts four tests for identifying populations that have recently experienced a severe reduction in effective population size ($N_e$). "Recently" is defined as within approximately the past $2N_e$–$4N_e$ generations, depending on several factors such as the severity of the bottleneck and the mutation rate of the loci being studied (Cornuet and Luikart 1996). The program runs on Windows 95™. It requires allele frequency data obtained from one sample of individuals (e.g., 20–30 diploid individuals) and at least four polymorphic loci.

Significant deviations from population mutation-drift equilibrium (e.g., bottlenecks) are important to detect because equilibrium is an assumption required for numerous analyses of population genetics data (e.g., see Nei 1987, p. 251). Bottlenecks are important to detect in conservation biology because they can increase the risk of population extinction. Founder-flush events (i.e., short but severe bottlenecks) are important to detect because they may play a role in some modes of speciation [for reviews see Harrison (1991) and Howard (1993)].

## Principle

Populations that have experienced a recent reduction of their effective population size exhibit a correlative reduction of the allele number and heterozygosity at polymorphic loci. But the allele number is reduced faster than the heterozygosity ($H_e$). Thus the $H_e$ becomes larger than the heterozygosity ($H_{eq}$) expected at mutation-drift equilibrium because $H_{eq}$ is calculated from the allele number (and the sample size; see Description below and Cornuet and Luikart 1996). Note that $H_e$ is calculated from allele frequencies (e.g., $1 - \Sigma p_i^2$, where $p_i$ is the frequency of the $i$th allele). Here both the measured heterozygosity ($H_e$) and the expected equilibrium heterozygosity ($H_{eq}$) refer to heterozygosity in the sense of Nei's (1987) gene diversity. Heterozygosity never refers to the proportion of heterozygotes observed ($H_o$). Thus we are not testing for an excess of heterozyogotes ($H_o > H_e$), but rather an excess of heterozygosity ($H_e > H_{eq}$).

Strictly speaking, heterozygosity excess has been demonstrated only for loci evolving under the infinite allele model (IAM; Kimura and Crow 1964) by Maruyama and Fuerst (1985). If the locus evolves under the strict one-step stepwise mutation model (SMM; Ohta and Kimura 1973), there can be situations where this heterozygosity excess is not observed (Cornuet and Luikart 1996). However, few loci follow the strict SMM, and as soon as loci depart slightly from the SMM toward the IAM they will exhibit a heterozygosity excess as a consequence of a genetic bottleneck. When testing for bottlenecks, the BOTTLENECK program uses both the SMM and IAM independently, because they represent two extreme models of mutation along a continuum of possible models (Chakraborty and Jin 1992). All loci will follow a mutation model somewhere in-between the two extreme models.

For selectively neutral loci in a population near mutation-drift equilibrium (i.e., a population in which $N_e$ has remained fairly constant in the past), there is approximately an equal probability that a locus will show a slight heterozygosity excess or a heterozygosity deficit. However, in recently bottlenecked populations, a majority of loci will exhibit an excess of heterozygosity (Luikart and Cornuet 1998). To determine if a population exhibits a significant number of loci with heterozygosity excess, we proposed three statistical tests: sign test, a standardized differences test (Cornuet and Luikart 1996; Luikart and Cornuet 1998), and a Wilcoxon's signed rank test (Luikart et al., submitted; Luikart 1997). We also proposed a graphical descriptor of the shape of the allele frequency distribution ("mode-shift" indicator) which can differentiate between bottlenecked and stable populations (Luikart et al. 1998).

Interpretation of output from the sign and standardized differences tests is thoroughly discussed in Cornuet and Luikart (1996) and Luikart and Cornuet (1998). Interpretation of output from the graphical descriptor is discussed in Luikart et al. (1998). Guidelines for interpreting the output from the Wilcoxon's test are less easy to find (Luikart 1997: chapter 4; Luikart et al., submitted), although this test is analogous to the sign test. The Wilcoxon's test is generally the most useful of all the tests because it is the most powerful (along with the standardized differences test), and robust (like the sign test) when used with few (<20) polymorphic loci. When testing for bottlenecks, the null hypothesis of the Wilcoxon's test is no significant heterozygosity excess (on average across loci). Thus the alternate hypothesis is significant heterozygosity excess (and thus evidence of a recent bottleneck). This is a one-tailed test that requires at least four polymorphic loci to have any possibility of obtaining a significant ($P < .05$) test result.

## Description

The BOTTLENECK program computes for each population sample and for each locus the distribution of the heterozygosity ($H_{eq}$) expected from the observed number of alleles ($k$), given the sample size ($n$) under the assumption of mutation-drift equilibrium. This distribution is obtained through simulating the coalescent process of $n$ genes under each of two possible mutation models, the IAM and the SMM. This distribution enables the computation of the average expected equilibrium heterozygosity ($H_{eq}$) for each locus which is compared to the Hardy–Weinberg heterozygosity ($H_e$,

i.e., gene diversity) in order to establish whether there is a heterozygosity excess or deficit at each locus. In addition, the standard deviation (SD) of the mutation-drift equilibrium distribution of the heterozygosity is used to compute the standardized difference for each locus [$(H_e - H_{eq})$/SD]. The distribution obtained through simulation also enables the computation of a *P*-value for the measured heterozygosity ($H_e$). The *P*-value is the probability of obtaining the measured $H_e$ in a sample ($n$) from an equilibrium population that has the observed number of alleles ($k$).

The way in which the coalescent process is simulated is unconventional due to conditioning by the observed number of alleles. The phylogeny of the $n$ genes is simulated as usual (Hudson 1990). Under the IAM, a single mutation is allocated at a time and the resulting number of alleles is computed. The process is repeated until the simulation reaches the number of alleles ($k$) observed in the population sample. Under the SMM, a Bayesian approach is used as explained in Cornuet and Luikart (1996). Briefly, the likelihood distribution of the parameter $\theta$ ($= 4N_e\mu$) given the number of alleles ($k$) and the sample size ($n$) is evaluated as the proportion of iterations (in the simulation process) producing exactly $k$ alleles for a varying set of $\theta$'s. As a second step, drawing random values of $\theta$ according to the likelihood distribution, the coalescent process is simulated as usual. Only heterozygosities found in iterations producing exactly $k$ alleles are considered. Once all loci in a population sample have been processed the three statistical tests are performed for each mutation model, as explained in Cornuet and Luikart (1996), and the allele frequency distribution is graphed to determine whether a bottleneck-induced mode shift has recently occurred. Note that a mode shift is a transient distortion in the distribution of allele frequencies such that the frequency of alleles at low frequency (frequency $< 0.10$) becomes lower than the frequency of alleles in an intermediate allele frequency class (see Luikart et al. 1998).

## Input File Format

Five input data file formats are accepted and automatically recognized by BOTTLENECK. All are text files. One is the GENEPOP computer program format (Raymond and Rousset 1995). The second is the GENETIX computer program format (Belkhir et al. 1996). The other three formats concern single population data and are described in the help file of the program.

## General Comments

BOTTLENECK is written in the Delphi 4 ™ (Inprise Co.) computer language. The performance of BOTTLENECK has been thoroughly evaluated using simulated datasets (Cornuet and Luikart 1996; Luikart et al. 1998) and allozyme and microsatellite datasets (Luikart and Cornuet 1998). To achieve reasonably high statistical power ($>0.80$), we recommend typing at least 10 polymorphic loci (microsatellites or allozymes) and sampling at least 30 individuals. The standardized differences test is recommended when using approximately 20 or more polymorphic loci (Cornuet and Luikart 1996). For fewer than 20 loci, the Wilcoxon's test is the most appropriate and powerful. The IAM is recommended for allozyme data and the SMM is generally more appropriate when testing microsatellite loci (i.e., dinucleotide repeat loci) (Luikart and Cornuet 1998). For most microsatellites, the TPM (two-phase model) is apparently even more appropriate than the SMM (Di Rienzo et al. 1994; Luikart G, unpublished data). The TPM was recently added as an option in BOTTLENECK. When using microsatellites we recommend the TPM with 95% single-step mutations and 5% multiple-step mutations (and a variance among multiple steps of approximately 12). When using the qualitative test for mode-shift distortion, we recommend using at least 30 individuals and 10–20 polymorphic loci to avoid unreasonably high type 1 error rates (i.e., to avoid concluding that a stable population has been recently bottlenecked).

BOTTLENECK runs on any computer with Windows 95 ™. However, we recommend a computer at least as fast as a pentium PC. A fast pentium is especially recommended for analyzing datasets containing many individuals ($>>30$) and loci with many alleles (e.g., $> 3$). Analyzing data under the SMM is far slower than analyses assuming only the IAM. On a Pentium 166 it takes about 15 minutes to analyze a dataset of 44 individuals and 7 loci (with 2–8 alleles) when using both mutation models and 1000 simulation iterations. The number of iterations influences the precision of the $H_{eq}$ estimates. A minimum of 1000 iterations is recommended. The program and example input and help files can be obtained from the World Wide Web at http://www.ensam.inra.fr/URLB.

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